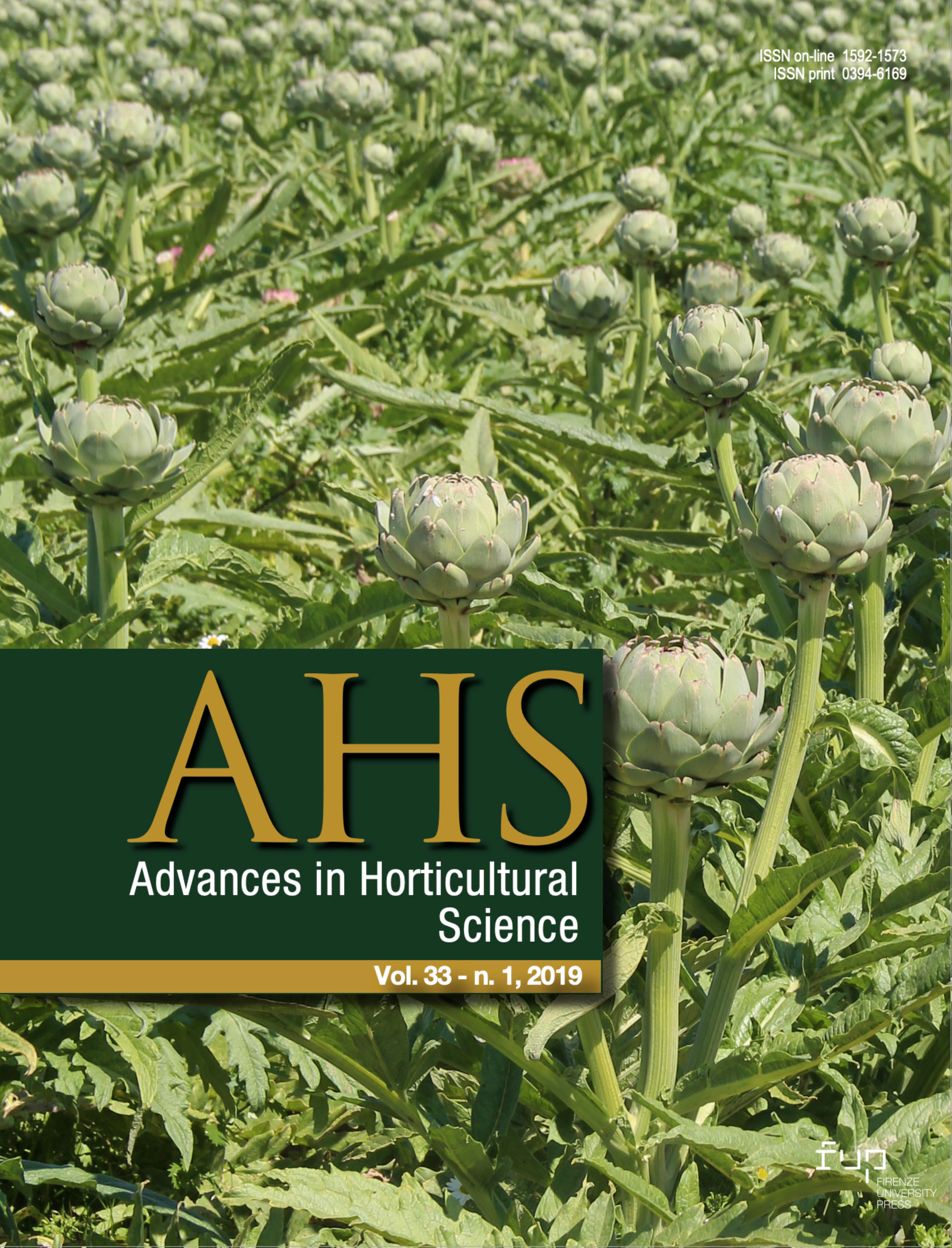

ISSN on-line 1592-1573
ISSN print 0394-6169

AHS

Advances in Horticultural Science

Vol. 33 - n. 1, 2019

FIRENZE UNIVERSITY PRESS

Advances in Horticultural Science
Published by **Firenze University Press** - University of Florence, Italy
Via Cittadella, 7 - 50144 Florence - Italy
http://www.fupress.com/ahs

Direttore Responsabile: **Francesco Ferrini**, University of Florence, Italy.

ADVANCES IN HORTICULTURAL SCIENCE

Formerly Rivista dell'Ortoflorofrutticoltura Italiana
founded in 1876 and issued by University of Florence, Italy

supported by ENTE CASSA DI RISPARMIO DI FIRENZE

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Advances in Horticultural Science is published by the Department of Agri-Food Production and Environmental Sciences, University of Florence, Viale delle Idee, 30, 50019 Sesto Fiorentino (FI), Italy
Phone +39-055-4574021-22, Fax +39-055-4574910, E-mail: advances@dispaa.unifi.it

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Adv. Hort. Sci., 2019 33(1): 3-11 DOI: 10.13128/ahs-22935

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The effect of cutting type, leaf area, leaf number, putrescine and indole-3-Butyric acid on the rooting of Ficus cuttings (*Ficus elastica* Roxb. ex Hornem.)

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Key words: auxin, cutting, plant propagation, polyamine, rubber fig.

Abstract: In order to study the importance of lateral or apical buds and also the possibility of replacing the role of bud and leaf with Putrescine (Put) and auxin, three experiments were conducted using leaf-bud cuttings with intact leaf blade (full blade) or halved-blade, and terminal cuttings having 1 to 4 leaves of *Ficus elastica*. Treatments included IBA (0, 2000 mg/l) and Put (0, 1000, 2000, 4000 mg/l). Comparing the lateral cuttings with intact or halved-blade showed that the rooting of the cuttings with intact leaf blade was better. The longest root length and the best rooting index were observed in cuttings treated with 1000 mg/l Put + 2000 mg/l IBA. The highest root number resulted from the IBA treatment. In cuttings with halved-leaf blade, Put along with IBA improved the indices of rooting in comparison with the control. The rooting of leaf-bud cutting was better in comparison with apical cuttings and the application of Put with IBA increased rooting indices in both types of cuttings and there was no significant difference between the different concentrations of Put. Results showed that terminal cuttings with three and four leaves had the longest, heaviest and most abundant roots, and that the rooting index resulted in the highest value. The rooting of leaf-bud cutting with intact leaf blade was better than that of one-leaf apical cutting. In general, the experiment showed that in the one-leaf terminal cutting, the apical bud has a negative effect on rooting and the increase in the leaf area or the application of Put with Auxin improves rooting.

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Citation:
GHASEMI GHEHSAREH M., KOSH-KHUI M., 2019 - *The effect of cutting type, leaf area, leaf number, putrescine and indole-3-Butyric acid on the rooting of Ficus cuttings (Ficus elastica Roxb. ex Hornem.)*. - Adv. Hort. Sci., 33(1): 3-11

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 23 March 2018
Accepted for publication 7 August 2018

1. Introduction

Rooting of cuttings is a method of vegetative propagation and is one of the most important methods of clonal propagation for many plants. Stimulating the formation of adventitious roots in stem cuttings with the application of Auxin is well-known (Altman, 1972; Bolat, 1995). However, other factors do also play a role and may be restrictive under certain circumstances. Inhibitors (Hess, 1969), rooting co-factors (Hess, 1969; Heaser and Hess, 1972), Auxin antagonists (Heaser and Hess, 1972; Batten and Goodwin, 1981) and nutrients (Nanda *et al.*, 1971; Heaser and Hess, 1972) are specified to have a role in regulating the process of rooting. It has been found that cuttings without buds do not produce roots even when they are treated with Auxin. This suggests that another factor other than Auxin which is likely to be produced in the bud is essential for root formation (Hartmann *et al.*, 2011). It has also been specified that the presence of leaves on cuttings has a stimulatory effect on rooting (Reuveni and Raviv, 1980). The stimulatory effect of the leaves on rooting in stem cuttings has been well-specified in an experiment on Avocado (Reuveni and Raviv, 1980). The cuttings of hard rooting cultivars soon lost their leaves under the mist system, while the leaves remained on rooted cuttings for a long time (9 months). After five weeks, it was observed that the amount of starch found in the planting bed of easy-rooting Avocado cuttings was five times more than that of the beginning of the experiment. In Hibiscus, the maintenance of leaves on cuttings increased rooting (Van Overbeek *et al.*, 1946). Carbohydrates that are transmitted from the leaves are important for root development. But the effects of improving leaves and bud on rooting are likely to be related to other factors (Hartmann *et al*., 2011). It has been reported that carbohydrates and nitrogen affect the onset of adventitious roots. Using tomato cuttings, researchers explained the interactions between carbohydrates and nitrogen in regulating root and shoot growth and adventitious root formation (Kraus and Kraybill, 1918; Starring, 1923; Reid, 1924 a, b). Cuttings from plants grown under conditions of high carbohydrates availability (i.e., high light intensity) relative to nitrogen availability produced roots that grew vigorously (Reid, 1924 a, b; Schrader, 1924). Van Overbeek *et al*. (1946) reported that the rooting stimulation effect of leaves in Red hibiscus cuttings could be replaced with sucrose and nitrogen. In the study of the effect of lateral and apical buds on rooting of Avocados under *in vitro* conditions, it was reported that the apical buds had the highest rooting percentage, root number and root length, but lateral buds had no significant effect on these indices (Zulfiqar *et al.*, 2009).

The effect of Polyamines on the rooting of woody plants varies. Put, Spermidine (Spd), and Spermine (Spm), along with IBA, improved the rooting of hazel cuttings (Rey *et al.*, 1994). On the contrary, higher levels of endogenous Put, Spd and Spm were observed at maturity stage (difficult to root) compared to the juvenile stage (easy to root) (Ballester *et al.*, 1999). The rooting of micro cuttings of olives was increased with the use of Polyamines and NAA, but did not increase in almonds, pistachios, Chestnut, Jujuba, apricots and walnuts (Reuveni and Raviv, 1980). *In vitro* studies showed an increase in Polyamine levels, especially Put, with root formation by culturing leaf explants of the *Datura innoxia* (Chriqui *et al.*, 1986) and *Passiflora alta-caerulea* (Desai and Mehta, 1985). Investigating the role of Polyamines during adventitious root formation with the evaluation of the de-bladed petiole rooting in juvenile stage (easy-to-root) and maturity stage (difficult-to-root) in *Hedera helix* showed that Auxin stimulated the root formation at the juvenile stage (easy to root), but did not have a positive effect on the mature phase. Adding Put, Spd and Spm with or without NAA, did not affect the rooting in juvenile or mature phase. But a significant increase was observed in endogenous levels of Put and Spd in cuttings treated with Auxin (Geneve and Kester, 1991). Thus, the Polyamines may have the role of a secondary messenger for rooting (Hartmann *et al*., 2011). Ghasemi Ghehsareh and Khosh-Khui (2016) showed that Put along with Auxin improved rooting and increased the quality of roots in Ficus leaf-bud cuttings.

According to previous studies regarding the positive role of buds, leaves, glucose, nitrogen, auxins and polyamines in rooting of cuttings, this experiment was conducted to examine the importance of lateral and apical buds, as well as the possibility of replacing the role of buds and leaves with Put and auxin. For this purpose, three separate experiments were carried out using leaf-bud cuttings with intact leaf blade or leaf blade trimmed to 50%, and terminal cuttings of the rubber fig (*Ficus elastica* Roxb. ex Hornem.) having 1 to 4 leaves with IBA and Put treatments.

2. Materials and Methods

Experiment conditions

The experiments were carried out using ficus cuttings in the greenhouse of the Department of Horticultural sciences of Shiraz University. The medium containing washed sand was equipped with a bottom heat system at a temperature of about 22°C.

An intermittent mist system was employed to provide moisture, and the misting was carried out daily at 10, 13 and 16 hours, and each session lasting 1 minute. The greenhouse temperature was between 21 to 25°C during daytime and 15 to 18°C at night.

First experiment

Effect of leaf size, auxin and put. Healthy and uniform leaf-bud cuttings included leaf blade, petiole and a part of the stem with a length of 3 to 4 cm with lateral buds from the mid-section of the annual stems were taken in January. In half of the cuttings, the leaf blade was halved, and the rest remained intact. The cuttings were kept in a Benomyl fungicide solution with a concentration of 2000 ppm for five minutes and then were washed with distilled water. After removing the surface moisture, the cuttings were treated with Put [zero (distilled water), 1000, 2000, and 4000 mg/l)], Indole-3-butyric acid (IBA) (zero and 2000 mg/l, the best concentrations resulting from the previous experiment) (Ghasemi Ghehsareh and Khosh-Khui, 2016) and their combinations by dipping the stems in the solutions for 10 seconds and then planted in the bed.

Second experiment

Effect of leaf-bud or terminal cutting, Auxin and Put. In this experiment, leaf-bud and terminal cuttings with one leaf were used. Growth regulators were used and other conditions were similar to the first experiment.

Third experiment

Effect of leaf-bud or terminal cutting, leaf area and leaf number. In this experiment, the rooting of different cuttings including leaf-bud cuttings with halved blade, leaf-bud cuttings with intact blade and terminal cuttings with 1, 2, 3 and 4 leaves were compared. All cuttings were treated with IBA at a concentration of 2000 mg/l. Other conditions were similar to the first experiment.

In all three experiments, after 2 months, the cuttings were removed from the bed and after isolating the medium form the roots, root fresh weight, root length (length of the longest root), root number were measured. To determine the quality of the roots, rooting index was calculated. For this purpose the cuttings were visually grouped into five groups including cuttings with heavy, medium and weak rooting, rootless but alive cuttings and dead cuttings. For each group, a coefficient was considered as the weight of rooting. Number 5 was for heavy rooting, 4 for medium rooting and 3 for weak rooting. A coefficient value of 2 was used for rootless but alive cuttings, and a coefficient value of 1 was used for dead cuttings (Criley, 2011; Ghasemi Ghehsareh and Khosh-Khui, 2016).

Statistical design and data analysis

The first and second experiments were conducted as factorial in a completely randomized design with three replications. Each replication included 10 cuttings. The third experiment was conducted in a completely randomized design with three replications, each with 6 cuttings. Statistical analysis of the data was performed using SAS 9.13 software and the mean values were compared using LSD (P≤0.05).

3. Results

First experiment

The results of analysis of data variance showed that the treatments had a significant effect on the root length, root number, root fresh weight and root quality. Maximum root length (8.9 cm) was caused by IBA 2000 mg/l + Put 1000 mg/l treatment in full-blade cuttings, but did not show any significant difference with IBA 2000 mg/l + Put 2000 or 4000 mg/l on full-blade cuttings. Furthermore, no significant difference was observed between the mentioned results and the result of treatment with IBA 2000 mg/l + Put 1000 or 2000 mg/l on halved blade leaves. The lowest root length was observed in cuttings with halved leaf blade, and generally, root length decreased with a decrease in leaf area. In terms of the number of main roots, the highest number (13.3) was obtained by IBA 2000 mg/l on full- blade, and the lowest number of leaves was obtained with halved blade cuttings, without growth regulators. The application of Put did not significantly affect the number of main roots. The highest root fresh weight was observed by the application of Put 4000 mg/l with IBA treatment in full-blade cuttings, which did not have significant difference with other concentrations in full leaves and Put 4000 mg/l with auxin in cuttings with halved blade leaves. In fact, by increasing Put concentration, the root fresh weight increased in both types of cuttings and the amount of increase was more in cuttings with halved-blade leaves (Table 1).

In terms of root quality, the highest rooting index (4.7) was observed by treatment with Put (1000 mg/l) + IBA (2000 mg/l) in full-blade cuttings. The highest rooting index in halved-blade cuttings (3.8) was observed by Put 1000 mg/l + IBA treatment. The lowest rooting index (2.3) was observed in control treatment with halved-blade cuttings. In general, cuttings with leaf blade trimmed to 50% had roots with lower quality compared to cuttings with full leaf. With increasing the Put concentration, the quality of the roots first increased and then decreased (Table 2).

Second experiment

The results showed that the interaction of cutting types (leaf-bud or terminal cutting) with growth regulators had a significant effect on rooting characteristics. The terminal cuttings were not rooted without the application of growth regulators, but rooting improved with the application of rooting growth regulators. The longest root length (9.7 cm) was found in leaf-bud cuttings, and there was not a significant difference between different treatments in this cutting. In the terminal cuttings, the use of Put + auxin in comparison with the control treatment and auxin alone significantly increased the root length, but there were no significant differences between different concentrations of Put. The highest main root number (14.8) was observed in the leaf-bud cutting treated with Put 4000 mg/l + IBA 2000 mg/l. The application of IBA as well as increased Put concentration increased the number of roots. In the terminal cuttings, the highest number of roots (6.4) was observed by treatment with 2000 mg/l of Put along with IBA, which did not differ significantly in different concentrations (Table 3).

The highest root fresh weight was observed in leaf-bud cuttings. The highest fresh weight (2.9 g) was obtained by using auxin alone, and by Put at 2000 mg/ml + IBA, which did not differ significantly with other concentrations of Put. In the terminal cuttings, the application of Put and its increased concentration increased the fresh weight of the root.

The best root quality (4.0) was observed in the leaf-bud cuttings treated with Put (2000 mg/l) +IBA. In the terminal cuttings, the application and increase of the concentration of Put improved the quality of the roots as compared with other treatments (Table 4).

Table 1 - Effect of leaf size, Auxin and Put on root length, root number and root fresh weight of Ficus leaf bud cutting

Leaf size	Treatments					Mean
	control	aux2000	aux2000+put1000	aux2000+put2000	aux2000+put4000	
Root length						
Intact leaf blade	3.6 c *	6.5 ab	8.917 a	8.417 ab	6.917 ab	6.87 A
Halved-blade	0.833 d	5.833 bc	6.75 ab	8.333 ab	5.917 bc	5.5333 B
Mean	2.2167 C	6.1667 B	7.8333 AB	8.375 A	6.416 7 B	
Root number						
Intact leaf blade	1.333 e	13.333 a	8.167 b-d	8.5 bc	10.333 b	8.3333 A
Halved-blade	0.5 e	7.167 cd	5.833 d	6.0 d	6.667 cd	5.2333 B
Mean	0.9167 C	10.25 A	7.0 B	7.25 B	8.5 AB	
Root fresh weight						
Intact leaf blade	0.475 d	1.6533 a	1.5233 ab	1.7533 a	1.93 a	1.467 A
Halved-blade	0.4833 d	0.7033 cd	0.83 b-d	0.9067 b-d	1.2167 a-c	0.828 B
Mean	0.4792 B	1.1783 A	1.1767 A	1.33 A	1.5733 A	

* Means with similar letters (lowercase letters for whole means and capital letters for means of rows and columns) are not significant at 5% level of probability using LSD.

Table 2 - Effect of leaf size, Auxin and Put on root quality (rooting index) of Ficus leaf bud cutting

Leaf size	Treatments	High (×5)	Medium (×4)	Low (×3)	Alive (×2)	Sum of weights	Rooting index
Intact leaf blade	control	0	0	20	10	80	2.7
	aux2000	13	13	4	0	129	4.3
	aux2000+put1000	20	10	0	0	140	4.7
	aux2000+put2000	20	10	0	0	140	4.7
	aux2000+put4000	20	5	5	0	135	4.5
Halved-blade	control	0	0	10	20	70	2.3
	aux2000	0	17	13	0	107	3.6
	aux2000+put1000	0	25	5	0	115	3.8
	aux2000+put2000	5	10	15	0	110	3.7
	aux2000+put4000	5	7	18	0	107	3.6

Third experiment

By comparing the rooting of leaf-bud cuttings having either full or halved-leaf blade, and terminal cuttings having 1, 2, 3 or 4 leaves, it was observed that by increasing the leaf area, the rooting was also increased. Accordingly, the longest roots were obtained in the terminal cuttings with 4 leaves (13.1 cm), followed by terminal cuttings with 3 leaves (9.75 cm). The shortest roots (1.47 cm) were obtained in leaf-bud cuttings with halved-blade. Terminal cuttings with 4 leaves produced the highest main root number (6.4) which did not have a significant difference with two and three leaf cuttings. The highest root fresh weight (1.72 g) was observed in four leaf cuttings. The lowest root number, root fresh weight and rooting index were observed in one-leaf terminal cuttings (Table 5). In terms of rooting index, the highest rate (3.6) was observed in three and four leaf cuttings (Table 6). Generally, the experiment showed that the best rooting was obtained in the three or four leaf terminal cuttings and it was weaker in one-leaf terminal cuttings compared to other cuttings.

Table 5 - Effect of cutting type, leaf area and leaf number on root indices of *Ficus elastica*

Leaf area/cutting type	Root length	Root No.	Root fresh weight
Half leaf/leaf bud	1.47 c *	3.83 bc	0.49 cd
Full leaf/leaf bud	2.25 c	4.6 ab	0.83 bc
1 leaf/terminal	1.92 c	2.33 c	0.22 d
2 leaf/terminal	6.92 b	3.33 ab	0.68 bc
3 leaf/terminal	9.75 a	4.25 ab	0.93 b
4 leaf/terminal	13.0 a	6.42 a	1.72 a

* Means with similar letters (lowercase letters for whole means and capital letters for means of rows and columns) are not significant at 5% level of probability using LSD.

Table 3 - Effect of leaf bud or terminal cutting, Auxin and Put on root indices of Ficus cutting

Cutting type	Treatments					Mean
	control	aux2000	aux2000+put1000	aux2000+put2000	aux2000+put4000	
Root length						
Terminal	0.000 c *	0.900 c	4.200 b	4.300 b	4.170 b	2.7140 B
Leaf bud	9.718 a	8.518 a	8.532 a	9.582 a	8.750 a	9.0200 A
Mean	4.8590 BC	4.7090 C	6.3660 AB	6.9410 A	6.4600 A	
Root number						
Terminal	0.000 c	1.200 c	4.000 b	6.400 b	5.600 b	3.4400 B
Leaf bud	4.167 b	10.833 a	11.667 a	13.333 a	14.833 a	10.9666 A
Mean	2.083 C	6.017 B	7.833 AB	9.867 A	10.217 A	
Root fresh weight						
Terminal	0.0000 c	0.2300 c	0.9460 b	1.3120 b	1.4790 b	0.7934 B
Leaf bud	1.2000 b	2.9000 a	2.4550 a	2.9032 a	2.4318 a	2.3780 A
Mean	0.6000 B	1.5650 A	1.7005 A	2.1076 A	1.9554 A	

* Means with similar letters (lowercase letters for whole means and capital letters for means of rows and columns) are not significant at 5% level of probability using LSD.

Table 4 - Effect of leaf bud or terminal cutting, Auxin and Put on root quality (rooting index) of Ficus cutting

Cutting type	Treatments	High (×5)	Medium (×4)	Low (×3)	Alive (x2)	Sum of weights	Rooting index
Terminal	0	0	0	0	30	60	2.0
	aux2000	2	3	4	21	76	2.5
	aux2000+put1000	1	2	24	3	91	3.0
	aux2000+put2000	3	7	18	2	101	3.4
	aux2000+put4000	2	6	19	3	97	3.2
Leaf bud	0	0	2	24	4	88	2.9
	aux2000	1	18	11	0	110	3.7
	aux2000+put1000	2	22	6	0	116	3.9
	aux2000+put2000	6	17	7	0	119	4.0
	aux2000+put4000	5	14	11	0	114	3.8

Table 6 - Effect of cutting type, leaf area and leaf number on root quality (rooting index) of *Ficus elastica*

Leaf area/cutting type	High (×5)	Medium (×4)	Low (×3)	Alive (×2)	Sum of weights	Rooting index
Half leaf /leaf bud	0	5	8	5	54	3
Full leaf/leaf bud	2	6	5	5	59	3.44
1 leaf/terminal	0	5	4	8	48	2.89
2 leaf/terminal	0	9	6	3	60	3.33
3 leaf/terminal	2	9	5	2	65	3.61
4 leaf/terminal	3	12	1	0	66	3.67

4. Discussion and Conclusions

First experiment

Our test results showed that the application of Put along with auxin increased the main root length and root fresh weight, which is consistent with the results of other researchers (Cristofori *et al*., 2010; Birlanga *et al.*, 2015; Ghasemi Ghehsareh and Khosh-Khui, 2016). The studies of root formation in the mung bean showed that in the rooting phase stimulated with auxin, the endogenous polyamines increase (Friedman *et al.*, 1982; Jarvis *et al.*, 1983). Desai and Mehta (1985) and Chriqui *et al*. (1986) studied the rooting of leaf explants *in vitro* which showed that at the time of root formation the amount of polyamines, especially Put, increases. They showed that the activity of Ornithine Decarboxylase as a key enzyme in Put biosynthesis increased in auxin-treated explants, but the use of polyamines in an auxin-free environment did not lead to rooting. The increase in endogenous polyamines during root formation by *in-vitro* auxin is reported to stimulate the formation of callus, leaf discs and de-bladed petiole explants (Malfatti *et al.*, 1983; Desai and Mehta, 1985; Chriqui *et al*., 1986; Tiburcio *et al.*, 1989; Geneve and Kester, 1991). This increase in Put in the formation and extension of the roots shows that Put is a biochemical marker for root differentiation (Tiburcio *et al*., 1989).

Researchers have shown that Put, Spm and Spd inhibit root formation (Friedman *et al*., 1982; Jarvis *et al*., 1983; Palavan-Ünsal, 1987). Also, Schwartz *et al.* (1986) reported that the activities of Ornithine Decarboxylase (ODC) and Arginine de-carboxylase (ADC) enzymes of Put biosynthesis pathway increase during lateral root formation in *Zea mays*. The biosynthesis of the polyamines is associated with cell division in the organogenesis process. In most cases, polyamines have not been able to stimulate root formation in the absence of auxin (Birlanga *et al.*, 2015), and numerous studies have shown that the simultaneous application of auxins and polyamines increases rooting (Sankhla and Upadhyaya, 1988; Birlanga *et al*., 2015). Biondi *et al.* (1990) showed that the polyamines do not directly influence the induction of adventitious root in the *Prunus avium* micro-cuttings, but do play a role in the later stages of root development and elongation. However, Put improved the quality of roots in our experiment.

In this experiment, the decrease in leaf area caused a decrease in rooting, which is consistent with Akinyele (2010) results in *Bachholzia coriaria*. It was reported that leaf size had a very significant effect on rooting, and that the rooting of full-leaf cuttings was better than half-leaf ones.

Our results showed that the highest number of main roots was obtained by auxin treatments alone and the combination of Put along with auxin reduced the number of main roots, but increased root length and weight. On the other hand, rooting index increased in treatments containing both auxin and putrescine in full-blade cuttings. It seems that Put, along with auxin, increases root weight by increasing the number of root branches and also the root length, and in comparison with using auxin alone, it reduced the number of thick roots, thereby improving the quality of the roots. Based on our results, the simultaneous application of auxin and Put has been shown to improve rooting, and to some extent it has been able to compensate for the decrease in leaf area in cuttings with halved-blades. This was observed in a manner that no significant difference existed among the indices of root length and root weight, even when comparing cuttings with full and half blades treated with Put 2000 or 4000 mg/l. This suggests that perhaps a part of the positive role of the leaf blade in rooting is related to polyamines and their derivatives.

Second experiment

Results of comparisons between one-leaf terminal and leaf-bud cuttings showed that rooting rate in terminal cuttings was zero without using growth regulators and treatment of cuttings with Put and IBA significantly increased rooting in comparison to the use of auxin alone, thereby confirming the results of the first experiment and indicating that part of the positive role of leaf in rooting is related to Put. On the other hand, the rooting conditions of the leaf-bud cuttings is better than that of the terminal cuttings, which indicates that the apical buds have had a negative effect on rooting. The results of this experiment are consistent with previous reports by Al-Zebari and Al-Brifkany (2015), where it was observed that the rooting of Citron (*Citrus medica*) cuttings was mostly successful in cuttings obtained from the middle of the stem and the least rate of rooting was in the apical cuttings. This indicates that the apical bud is not only a strong source of auxin, compared to the lateral bud (Thimann and Skoog, 1933; Thimann *et al.*, 1934), but also a strong consumer which cannot play a positive role in rooting without the presence of a leaf. Concerning the inhibitory effect of the apical buds on the growth of lateral buds, it has been reported that the apical bud is a strong consumer of auxin and sucrose. In this regard, Thimann (1937) suggested that the optimal auxin concentration for stem elongation is more than the concentration necessary for the development of lateral buds. There is also evidence that the apical bud is a strong consumer which limits access to sugars for lateral buds (Taiz *et al.*, 2015). This strong sink effect of the apical bud can also have a negative effect on rooting.

A study on *in vitro* rooting of avocado showed that the apical buds had a better rooting than the lateral buds (Zulfiqar *et al.*, 2009). On the other hand, rooting is a highly critical and energy-demanding process that is influenced by the complex interaction between sucrose and hormonal levels (Birlanga *et al.*, 2015). Roberts and Fuchigami (1973) studied the effect of seasonal changes on auxin and rooting of the Douglas-Fir stem cuttings. They showed that at the time of removing cuttings from the beds, rooted cuttings showed a certain activity in buds, but most of the cuttings with very active buds had not produced roots. Lanphear and Meahl (1963) observed that rooting occurred more actively at or before the bud break, but it decreased sharply with increasing bud activity. The decline was probably due to the competition for growth factors necessary for rooting.

Third experiment

By comparing the leaf-bud cuttings with half and full blade, and terminal cuttings with 1, 2, 3 and 4 leaves, all of which were treated with IBA 2000 mg/l, was observed showed that increasing the leaf area, improves rooting. However, by comparing leaf-bud cuttings and one-leaf terminal cuttings, leaf-bud cuttings had a better rooting, which confirmed the results of the second experiment, indicating that the apical bud probably acted as a strong consumer and reduced rooting. The increase in the number of leaves improved rooting so that the highest rooting was observed in terminal cuttings with 3 and 4 leaves. This suggests that, although the apical bud is a strong source of auxin, it was not able to compensate for the lack of factors necessary for rooting. Here, the importance of other roles of the leaf becomes clear. The results of this experiment are consistent with reports by other researchers (Leakey *et al.*, 1982; Badji *et al.*, 1991; Tchoundjeu and Leakey, 1996; Tchoundjeu *et al.*, 2002; Atangana *et al.*, 2006; Opuni-Frimpong *et al.*, 2008). In an experiment by Leakey (Leakey, 2004), stem cuttings of the *Khaya senegalensis* showed that rooting was limited to leafy cuttings. The inability of rooting in leafless cuttings was associated with the rapid evacuation of carbohydrates in stem tissues, but their concentration in leafy cuttings increased (Leakey *et al.*, 1982). This shows that rooting depends on the formation and consumption of carbohydrates after the cuttings are separated from the mother plant (Leakey and Coutts, 1989). Van Overbeek *et al.* (1946) reported that rooting stimulation by the presence of leaves on Red hibiscus cutting could be replaced with sucrose and organic or inorganic nitrogen. Welander (1976) showed that *in vitro* rooting of Sugar Beet Hypocotyls was stimulated by an increase in sucrose and inorganic nitrogen in the presence of high concentrations of IAA, whereas low concentrations of auxin were ineffective. Gabryszewska (2011) studied *Syringa vulgaris* under *in vitro* conditions and showed that increasing the amount of sucrose in the medium causes spontaneous root formation on cultivated plantlets in the presence of low levels of nitrogen salts. The planting of Rosa 'Improved Blaze' shoots for the purpose of investigating the effect of sucrose and inorganic nitrogen on adventitious root formation showed that high concentrations of sucrose led to the production of higher and more elongated roots (Hyndman *et al.*, 1981). Therefore, the role of the leaf in helping rooting can occur by the presence of carbohydrates through photosynthesis and also by polyamines as a source of nitrogen.

In general, this study shows that the application of Put along with auxin improves rooting and root quality in *ficus* cuttings. Moreover, in terminal cuttings, due to the competition for nutritional and hormonal factors necessary for the simultaneous growth of buds and the occurrence of rooting, a certain amount of leaf area is necessary to provide these factors. The application of Put along with auxin can partly satisfy this requirement in cuttings with smaller leaf areas.

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Adv. Hort. Sci., 2019 33(1): 13-21 DOI: 10.13128/ahs-22679

AHS
Advances in Horticultural Science

Eco-physiological and biochemical characterization of *Rhus tripartita* (Ucria) Grande growing in Algerian Sahara under arid climate

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OPEN ACCESS

Key words: Ahaggar, aridity, biochemistry, ecophysiology, *Rhus tripartita*.

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Citation:
BENAISSA A., DJEBBAR R., BOUCELHA L., 2019 - *Eco-physiological and biochemical characterization of Rhus tripartita (Ucria) Grande growing in Algerian Sahara under arid climate*. - Adv. Hort. Sci., 33(1): 13-21

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 15 February 2018
Accepted for publication 7 September 2018

Abstract: *Rhus tripartita* (Ucria) Grande, is an Anacardiaceae autochthonous shrub of the Algerian Sahara. Its ecological, pastoral and therapeutics interests prompted us to carry out an eco-physiological and biochemical behavior in relation to aridity. Therefore, relative water content of shrub leaves were found on average 81.55% and the maximum of electrolyte leakage recorded was 14.29. The biochemical determination of proteins and sugars shows that leaves are a true source of protein (33.76 mg/g FM) and sugars (938.93 µg/g FM) while the proline assay gave a value of 824.40 µg/g. The quantitative study of flavonoids in the leaves gave a result of 36.53 mg/g. The analysis of photosynthetic pigments content showed respectively results of 28.1 µg/g, 31.24 µg/g, 56.47 µg/g and 11.23 µg/g for chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. The total antioxidant capacity was evaluated and gave result of 95.5 mg GAE/g. Therefore, *Rhus tripartita* was found to accumulate high proportions of primary and secondary metabolites which showed a good adaptation to its arid environment. In conclusion, the plant can be considered as a xeromorphic plant, that is, a desert-adapted plant that is not limited by the water availability.

1. Introduction

Rhus tripartita (Ucria) Grande is synonymous with *Searsia tripartita* (Ucria) Moffett, an Anacariaceae forage plant; it is traditionally used by the Tuaregs (local inhabitants) of Ahaggar (Algeria). This Saharo-Mediterranean shrub is widespread from North Africa to Egypt (Sahki and Sahki, 2004). Ferchichi (1999) has described the shrub as a very drought-resistant species characterized by abundant foliage throughout the year despite the soil's moisture status, and it can be planted successfully on poor and marginal lands. On the geomorphological level of the Algerian

Saharan environment, this plant belongs to the grouping of the Ahaggar mountainous massifs where it grows on shallow soils.

Plant growth is strongly influenced by many biotic and abiotic factors. Ecologists and physiologists have long been interested in the effects of environmental factors that lead to plant adaptation and distribution. Therefore, phyto-ecophysiological studies are important to understand the species response in extreme environments and especially their valuation in these areas. By staying in extreme environments, the Sahara is one of the driest and hottest deserts in the world (Dutil, 1971). The desert fact is explained by extreme values of climatic parameters which constitute the essential factors of vegetation's distribution and the grounds evolution (Daoud and Halitim, 1994). Moreover, aridity is a natural selection force that influences plant adaptive strategies to water stress (Sayed, 1998). Hence different adaptive strategies of plants to aridity have been developed through considerable survival mechanisms. These strategies are divided into three general categories: drought escape, drought avoidance and dehydration tolerance (Levitt, 1972; Turner, 1979). Drought escape is employed by plants under conditions where water limitation late in the growing season is likely, and it ensures that the plants can complete their life-cycle quickly during the brief period of favorable conditions. Drought avoidance is a strategy for avoiding lower water status during drought by maintaining relatively high tissue water content due to reduced evaporatory water loss and an efficient water uptake (Levitt, 1972). The third category, drought tolerance, is the ability of plants to withstand water deficit and maintain metabolism at low tissue water content (Valliyodan and Nguyen, 2008). In this strategy, osmotic adjustment, osmoprotection, antioxidation and scavenging defense system have been the most important physiochemical and biochemical bases responsible for drought tolerance. Cell tissue and water conservation, antioxidant defense, cell membrane stability, compatible solutes and plant growth regulators mainly contributes in above said physiochemical and biochemical mechanisms. The three strategies are not mutually exclusive, and the same plant may use more than one strategy in order to adapt to periods of drought stress (Nilsen and Orcutt, 1996).

Therefore, to adapt with these natural constraints, plants have had to produce primary metabolites such as proteins and soluble sugars. This includes photosynthetic mechanisms, osmoregulation and antioxidant enzymes (Liu *et al.*, 2011; Guo and Gan, 2012). Furthermore, water stress has a positive effect on the production and accumulation of secondary plant metabolites (Ncib *et al.*, 2018). Their biosynthesis is often stimulated in response to biotic or abiotic stress (Naczk and Shahidi, 2004).

The aim of this work is to study the adaptive physiological response of *Rhus tripartita* to its naturally arid ecosystem (Ahaggar, Algeria). For this purpose, we will content to discuss our results in relation to water deficit and oxidative stress.

2. Materials and Methods

Presentation of study area

Localisation. The native plants of *Rhus tripartita* (Fig. 1) are located in the Ilamane region (100 km away from Tamanrasset city) which is situated in the Ahaggar National and Cultural Park (22°49'59" north, 5°19'59" east). This park with an area of 4.500.000 ha, was created by the decree n. 87-231 of 03 November 1987. It is located in the mountainous center part of the volcanic Ahaggar in the extreme Algerian south (Fig. 1).

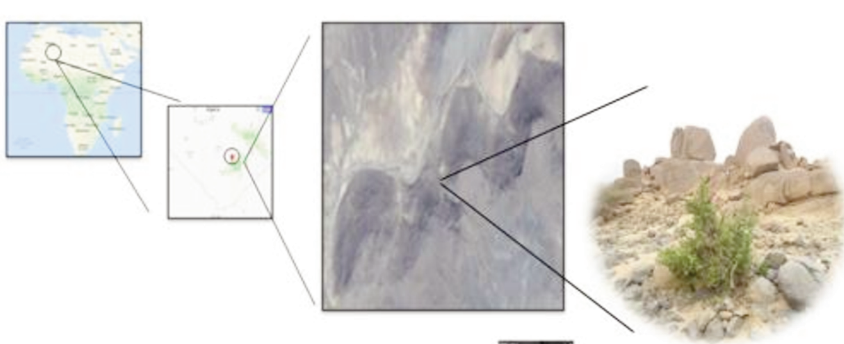

Fig. 1 - Location of *Rhus tripartita* growing in the rocky mountains of Ilamane in the Ahaggar National and Cultural Park.

Features edapho-climatic. Before looking more specifically at plant physiology that interests us, it is necessary to present the main characteristics of the Ahaggar. The bioclimate is arid, with very varied winter and summer temperatures and very low rainfall. The rains occur from May to September and usually increase in August. The annual rainfall varies between 180 mm and 250 mm. The thermal regime varies between the two very short winter and longer summer seasons. Dubief (1959) noted the maximum average on the ground, in July, at 52°C towards 2700 m of altitude (Assekrem) and estimates at 60°C this value at 1376 m (Tamanrasset). These high temperatures condition the biology of plants in this environment.

On the edaphic plane, soil encountered in this area is very little evolved because of a low rainfall. However, this park is marked by a geomorphological distinction (mountainous mass, wadis, hammadas, gueltates, plains and rocky ravines); this has resulted in a high heterogeneity in the soil composition (sandy, sandy-loamy, sandy-clayey, shallow and rocky). Therefore, in the Ilamane region, edaphic variability is a result of shallow skeletal soils that characterize this area.

Determination of physiological parameters

In order to study the *Rhus tripartita* physiology and biochemistry characteristics in relation to its arid environment, different measurements of primary and secondary metabolites are performed. All analyzes were performed on three replicates of six shrubs naturally growing in this area. The sampling was conducted during December 2017 (winter season). Therefore, we consider that 6 individuals are largely sufficient given the physiognomic homogeneity of the population.

Relative water content (RWC)

The RWC measurement is an old method (Slatyer, 1967) which is currently widely used to estimate the plant water status and in particular water deficit. A 12 mm leaf disc taken from the second leaf by means of a punch is weighed directly (weight of the fresh vegetable material, WFVM). It is then placed in the refrigerator in a test tube containing distilled water for 24 hours and weighed to obtain the turgor weight (TW). The fragments are finally placed in an oven at 80°C for 48 hours and then weighed to obtain the weight of dry vegetable matter (WDVM). The RWC is calculated according to the following equation:

$$RWC\ (\%) = (WFVM - WDVM) \times 100/(TW - WDVM)$$

Metabolites compositions

For the analysis of sugars, proteins and proline, 100 mg of fresh material from each repeat was used.

Total Hydro-soluble protein. Soluble proteins are assayed according to Bradford method (Bradford, 1976). This method is a colorimetric assay based on the color change of Coomassie blue after binding to aromatic amino acids (tryptophan, tyrosine and phenylalanine) and the hydrophobic residues of amino acids present in proteins.

Proline free foliar. The technique used for proline determination is that of Troll and Lindsley (1955) modified by Magné and Larher (1992). It is based on the ability of proline to react in acidic and hot conditions with ninhydrin (revealing aromatic amino acids including proline) to give a pink compound soluble in organic solvents such as toluene.

Ethanol soluble sugars. The soluble sugars assay method is based on the technique of McCready *et al.* (1950). In the presence of 91% sulfuric acid (H_2SO_4) and hot conditions, the oses produce furfural derivatives that react with the anthrone to give a blue-green compound.

Photosynthetic pigments. The assay was performed by colorimetric method; the pigments being naturally colored. To do this, read the OD of the samples using the spectrophotometer previously calibrated with 80% acetone (corresponding to OD= 0). The OD readings were carried out at wavelengths 647 and 663 nm for chlorophylls and 470 nm for carotenoids. The concentrations were determined according to Lichtenthaler (1987).

Antioxidant system

Total Anti-oxidant Capacity (CAT). The total antioxidant capacity of Rhus *tripartita* leaf was estimated using the method described by Prieto *et al.* (1999). The amount of 0.1 ml of methanolic extract (2 g of dry matter in 10 ml of methanol) was mixed with 1 ml of molybdate reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance was measured at 695 nm after incubation in boiling water bath about 90 min. The total antioxidant capacity was expressed as the milligramme gallic acid equivalent per gram of dry matter (mg GAE/g DM).

Lipid peroxidation. Lipid peroxidation is estimated according to the method of Cakmak and Horst (1991) which consists in grinding 100 mg of fresh plant material in 1 ml of 1% Trichloroacetic acid (TCA) followed by centrifugation at 12000 rpm/20 min. Then, 1 ml of Thiobarbituric acid (TBA) at 0.5% (prepared in 20% TCA) is added to 500 µl of extract and incubated with Marie-bath at 95°C/30 min. After cooling, the optical densities (OD) reading is carried out at 532 and 600 nm. The MDA measurement content is calculated using its molar extinction coefficient (ε= 155 mmol/l cm).

Electrolyte Leakage. Electrolyte Leakage (EL) was estimated by measuring the electrolytic conductivity according to modified method of Pike *et al.* (1998). This technique consists in placing ten foliar disks 12 mm in diameter in 20 ml of distilled water. A first measurement of the conductivity (EC) is carried out after stirring for 3 hours. Then, a second measurement of the conductivity (ET) is conducted, after Marie-bath heating (95°C) for one hour. Electrolyte leakage (%) is determined by the ratio (EC/ET).

Total flavonoid content. Total flavonoid content was estimated using the method reported by Dewanto *et al.* (2002). It consists to mix 250 µl of vegetable methanolic extract with 25 µl of 5% $NaNO_2$, added with 150 µl of $AlCl_3$ (2%). After 5 min, 0.5 ml of 1M NaOH is added to the solution. After 10 min of incubation, the absorbance was measured at 510 nm.

Statistical analysis

The data were subjected to statistical analysis using the Microsoft Excel 2010 program. All values of biochemical compounds and secondary metabolites are the mean ± SE (standard error) of three replicates of a single sample. Unidirectional analysis of variance (ANOVA) was used and differences between individual means were considered significant at P<0.05.

3. Results and Discussion

Aridity depends on several climatic factors (temperature, wind, radiations) and above all on evaporation, an essential factor to calculate the water deficit. Therefore, a rocky environment such as that of our study site, expresses accentuated drought effects because the soil scarcity. Moreover, relatively rapid soil drought after rains causes water stress even during the rainy season (Pimienta-Barrios *et al.*, 2001). To this end, we will discuss our results in relation to the influence of hydric and oxidative stress as the main parameters of aridity.

Therefore, plant eco-physiological study depends on several criteria, depending on environmental changes and their adaptation to them. Indeed, plant survival in arid environments depends on different coping mechanisms. In this work, we have analyzed the variation of physiological parameters of *Rhus tripartita* in relation to the arid climate of Ahaggar. These parameters are often measured to study the different plants responses to abiotic stress.

Relative water content

The low water availability can cause tissue dehydration, for this purpose, plant can control its hydric potential to cope with these high temperatures of arid zones. Therefore, the relative water content of the plant gave a high value ranging from 54.79% to 81.55% (Fig. 2). The highest content is recorded in shrub n. 5 with a significant difference compared to the same species shrubs. Poole and Miller (1975) reported that species of the same genus: *Rhus ovata, Rhus laurina* and *Rhus interguifolia;* showed high water potential in water-deficit conditions.

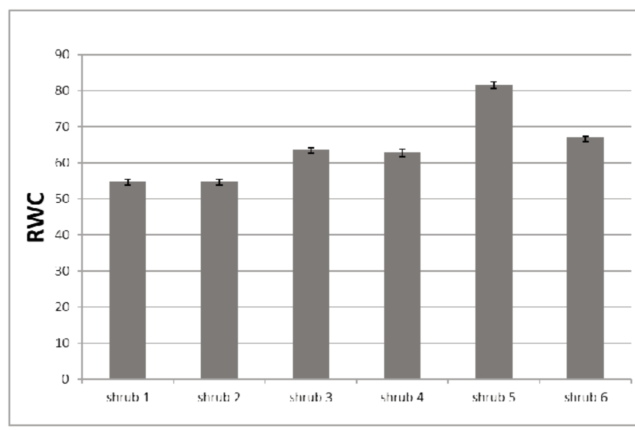

Fig. 2 - Relative water content (RWC%) of *Rhus tripartita* leaves according to climatic aridity conditions.

Metabolite content

In extreme temperatures, the plant can produce metabolites to protect itself from harmful and damaging effects. The total protein assay of *R. tripartita* showed levels that ranged from 20.03 mg/g in shrub n.3 to 33.76 mg/g in Shrub n. 1 (Fig. 3), which represent fairly high levels compared to *Periploca Angustifolia* Labill, a shrub growing in Tunisian arid areas with a rate of 5.204 mg/g DM (Dghim *et al.*, 2015). However, this amount is twice as high as that of the same species collected in Libya (10.1 mg/g DM) (Le Houérou, 1991) and four times higher than that found in other species of the same genus such as *Rhus lancea* (7.79 mg/g MS) (Aganga and Mosase,

2001) but it is one and a half times higher in other forage species: *Medicago sativa* (19.4 mg/g MS) (Le Houérou, 1991).

On the other hand, proline is an amino acid whose rate increases proportionally more rapidly than other amino acids in plants under water stress. It has been suggested as a study parameter for the selection of drought-resistant plants (Bates *et al.*, 1973). The proline level recorded in *R.tripartita* showed a significant difference (P<0.05) between the six shrubs of the same species with an interval of 212.45 µg/g - 851.28 µg/g (Fig. 3). Plants tolerant to stress, have relative stability or low accumulation of proline compared to sensitive plants (Lemzeri, 2006). According to Dix and Pearce (1981), the proline accumulation is not an adaptation reaction to stress, but rather a sign of metabolic disturbance. Alternatively, proline may confer a protective effect on the plant by induction of stress-protecting proteins (Vinocur and Altman, 2005).

However, osmotic stress can produce harmful effects in the plant's cell compartments. A wide range of metabolites can intervene to avoid these effects including a variety of sugars and alcoholic sugars such as mannitol and trehalose (Vinocur and Altman, 2005). The study of sugars compound in *Rhus tripartita* leaves was relatively high (938.93 µg/g) (Fig. 3). Several studies have investigated the soluble sugars accumulation in several stressful species (Garg *et al.*, 2002; Penna, 2003; Silva and Arrabac, 2004).

Ahaggar is a region known for its continuous sunshine all year round. In the presence of strong radiation, several plants use protective mechanisms to reduce the absorption of solar radiation (Harrison *et al.*, 2010). The study of *Rhus tripartita* photosynthetic pigments leaves showed results of 28.1 µg/g, 31.24 µg/g and 53.56 µg/g for chlorophyll *a*, *b* and total chlorophyll respectively (Table 1). Similar to those recorded in *Rhus typhina* under water stress conditions (Liping, 2007), these results indicate that dry conditions do not have significant effects on the degradation of photosynthetic pigments, which explains the dark green colour of the leaves. Nevertheless, photosynthesis of plants in arid zones that believe under permanent conditions of water deficiency; are subject to photoinhibition risk (Voronin *et al.*, 2003). Furthermore, the amount of photosynthetic pigments in the leaves of these plants has been shown to be relatively low (Valladares and Sanchez-Gomez, 2006) which contradicts the findings of this study. The water stress is considered one of the environmental factors limiting photosynthesis, therefore plant growth (Ozturk *et al.*, 2010).

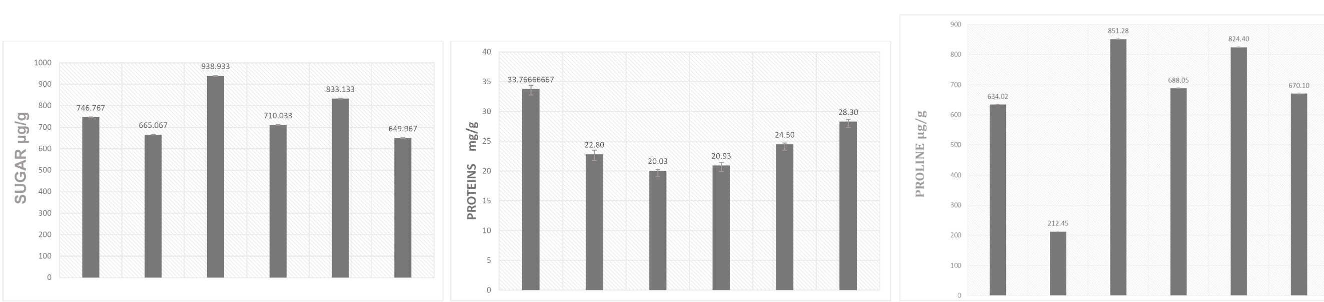

Fig. 3 - Soluble sugar content (µg/g), total proteins content (mg/g) and proline content (µg/g) in *Rhus tripartita* leaves according to climatic aridity conditions.

Table 1 - Content of photosynthetic pigments (µg/g) in the fresh leaves of *Rhus tripartitus* (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids)

Shrub	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll	Carotenoids
Shrub n° 1	17.367 ± 0.284	24.800 ± 0.527	41.300 ± 0.584	8.480 ± 0,006
Shrub n° 2	23.600 ± 0.156	29.967 ± 0.295	53.560 ± 0.383	8.022 ± 0.025
Shrub n° 3	24.133 ± 0.157	18.880 ± 0.271	43.067 ± 0.415	5.797 ± 0.017
Shrub n° 4	24.967 ± 0.151	31.243 ± 0.564	56.473 ± 0.378	11.237 ± 0.079
Shrub n° 5	24.033 ± 0.390	20.783 ± 0.533	44.577 ± 0.443	6.619 ± 0.089
Shrub n° 6	28.100 ± 0.340	21.203 ± 0.363	49.503 ± 0.257	6.841 ± 0.039

Antioxidant system

In this study, the oxidative stress effect is evaluated through the quantification of MDA and the electrolyte leakage since secondary aldehyde products from lipid peroxidation are generally considered to result from oxidative stress (Del Rio *et al.*, 2005). The results presented in figure n. 10 showed that the MDA content in the shrub is significantly higher (7.007 nmol/g) than other shrubs [5.165-5.673 nmol/g]. The electrolyte leakage was studied using conductivity measurement. It varies between 12.39 and 14.293 in the six shrubs of *Rhus tripartita* as shown in figure n. 4. Similary, in *Rhus typhina*, high levels of MDA have been recorded in water stress conditions (Liping, 2007). Therefore, MDA is a degradation product of lipid peroxidation reactions that are formed during the attack of polyunsaturated lipids by reactive oxygen species (ROS). However, this is the most widely used assay to characterize oxidative damage in plants (Shulaev and Oliver, 2006). Moreover, high temperatures can produce metabolic disturbances based on reactive oxygen species and antioxidant systems. However, the present work showed a variability in Total Anti-oxidant Capacity from one shrub to another and is within a range of 19 mg/g - 100 mg/g (Fig. 4). Therefore, it is known that TAC is mainly due to phenolic compound (Tlili *et al.*, 2014). On the other hand, the flavonoids constitute the main group of polyphenols, ubiquitous in plants, they are formed from aromatic amino acids (Hernandez *et al.*, 2009). They act as antioxidant molecules that ensure the binding of ROS produced during stress and thus neutralize their effects before the manifestation of oxidative damage at the cellular level (Lovdal *et al.*, 2010). The determination of total flavonoids contents in *Rhus tripartita* leaves gave result of 35.89 mg/g (Fig. 4). This level is relatively similar to the same species under water stress conditions (39.2 µg/mg) (Ncib *et al.*, 2018) but higher than in other species of the same family such as *Rhus punjabensis* (30.50 µg/mg) (Tabassum *et al.*, 2017). Several studies have demonstrated the richness of *Rhus tripartita* in flavonoids (Mahjoub *et al.*, 2007, 2010; Tlili *et al.*, 2014). Therefore, flavonoid is considered as a phytochemical adaptation to the biotic and abiotic environment (Dixon and Paiva, 1995). In the same perspective, the carotenoids are soluble antioxidant lipids that play a very important role in abiotic stress tolerance (Sieferman-Harms, 1987; Gill and Tuteja, 2010). The carotenoids content found in our shrub leaves is relatively low (11.63 µg/g) (Table 1) compared to another shrub of Tunisian arid zones: *Periploca angustifolia* Labill (Dghim *et al.*, 2015). It is known that carotenoids have a protective role against photooxidation (Ladygin *et al.*, 2008) which may explain the adaptive power of this shrub.

The relationship between aridity and the results found is certainly evident. It can be explained by the

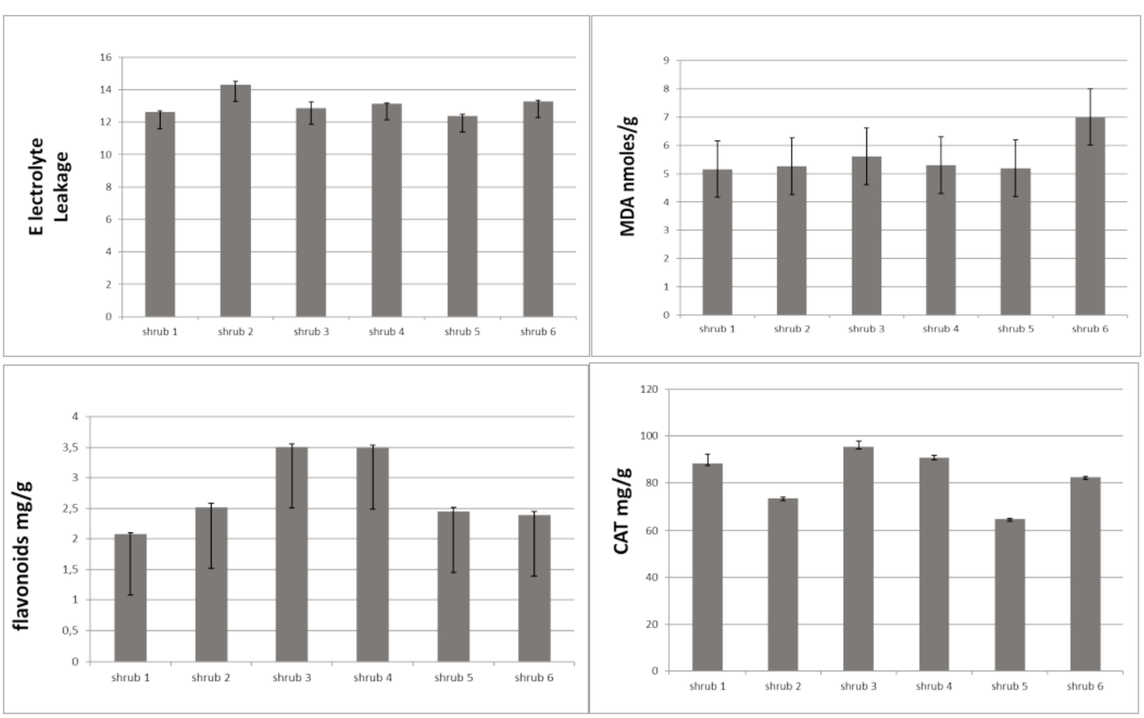

Fig. 4 - Malondialdehyde content (nmol/g), total antioxidant capacity (mg/g), electrolyte leakage and flavonoid content (mg/g), of *Rhus tripartita* leaves in relation to climatic aridity conditions.

fact that the plant increases in both primary and secondary metabolite production under drought conditions. It should be noted that a difference in the metabolites composition has been recorded among different individuals of the same species. That said, not all shrubs had the same orientation towards the sunshine and some were grouped with the species *Myrtis nivellei* and *Periploca laevigata*. These two parameters can explain the difference between the results of the six individuals.

4. Conclusions

In summary, we presented a simple preliminary work on the physiological information of *Rhus tripartita* in arid environments. Therefore, our results have shown that this shrub presents an interesting adaptation to its climatic and edaphic environment. The approaches developed in this study certainly contribute to the understanding of shrub ecophysiology and can be improved plant productivity in arid regions.

In conclusion, and according to classification of Jenks and Hasegawa (2005) of plants from desert and semi-arid zones, *Rhus tripartita* can be considered as a xeromorphic plant, a species adapted to the desert that is not limited by water availability.

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Adv. Hort. Sci., 2019 33(1): 23-31

DOI: 10.13128/ahs-23015

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Biochemical characterization of artichoke (*Cynara cardunculus* var. *scolymus* L.) spring genotypes from Marche and Abruzzo regions (Central Italy)

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Key words: antiradical activity, artichoke genotypes, capitula quality traits, total phenolic content.

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Citation:
GALIENI A., STAGNARI F., PISANTE M., PLATANI C., FICCADENTI N., 2019 - *Biochemical characterization of artichoke (Cynara cardunculus var. scolymus L.) spring genotypes from Marche and Abruzzo regions (Central Italy)*. - Adv. Hort. Sci., 33(1): 23-31

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 9 April 2018
Accepted for publication 12 December 2018

Abstract: Ten artichoke genotypes from Marche and Abruzzo Regions [Ascolano (As), Castorano (Cs), Clone Monsampolo, Jesino (Je), Mazzaferrata (Mz), Montelupone A, Montelupone B, Urbisaglia1 (Ub_1), Urbisaglia2 and Violetto Tardivo di Pesaro] were characterized for their quality traits and peculiar end-use attitudes, in comparison with the reference Romanesco Clone C3 (Cl_C3). Total polyphenols content (TPC), total flavonoid content (TFC) and antiradical activity were assessed in the receptacle and external bracts of both main and first order capitula. Cl_C3 showed high TPC and TFC values in the receptacle of the main flower heads (7.4 mg gallic acid equivalents, GAE, g^{-1} dry weight, DW, and 3.6 mg rutin equivalents, RUE, g^{-1} DW, respectively), confirming its attitude for fresh consumption. Je and Ub_1 showed great and stable (among main and first order capitula) head quality, highlighting their potential for breeding programs to enhance the content of functional compounds. Conversely, Mz and As could be appreciable for processing or pharmaceutical applications, being characterized by great TPC (external bracts, first order capitula: 2.8 and 2.7 mg GAE g^{-1} DW, respectively) and TFC (external bracts, first order capitula: 2.1 and 2.7 mg GAE g^{-1} DW, respectively) values in the waste parts. High correlations between TPC and TFC with antiradical activity were also observed. Our results suggest the possibility to promote the utilization in genetic breeding programs of the autochthonous artichoke populations, according to their peculiar characteristics, including also their biochemical composition.

1. Introduction

Globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori] belongs to the family of *Asteraceae* (*Compositae*) and it is an herbaceous perennial crop mainly cultivated in the Mediterranean Basin (about 65% of world production) followed by Americas and China (Sihem *et al.*, 2015, Lombardo *et al.*, 2017). In Italy it plays an important role in the agro-food chain with over 43.8 Kha and approximately 366 Kt of floral heads produced (FAO, 2016).

The Italian gene pool of globe artichoke includes hundreds of varieties and ecotypes, grouped into four main types i.e. "Catanesi", "Romaneschi", "Spinosi" and "Violetti". According to the harvesting period, they are classified as early or late distinct clonal varietal groups, with the former having a typical autumn-winter cycle in the southern Regions, while late including spring genotypes mainly grown in central Regions (Ciancolini *et al*., 2013 a, b). The varietal constitution of artichoke is restricted to clonal selection carried out within local populations propagated by agamic way which represent a patrimony of agrobiodiversity and a biological, cultural and economic heritage (Ficcadenti *et al*., 2013). However, several cases of homonymy and synonymy (different varieties are called with the same name in the first case, while the same variety comes call with different names in the second) as well as unsatisfactory, uniformity and identity of accessions occur (Ficcadenti *et al*., 2013). Traditional agricultural and food production must be safeguarded to avoid processes of globalization and homologation and, consequently, identification, collection, characterization and conservation of agrobiodiversity as well as development of genetic improvement strategies, particularly linked to nutritional and organoleptic traits, have been undertaken in these years (Mauromicale and Ierna, 2000; Ficcadenti *et al*., 2010; Ciancolini *et al*., 2012).

Recently, a renewed and growing interest for artichoke cultivation has been observed worldwide mainly due to its potential uses as functional food: its large immature inflorescences, called capitula or heads, represent a rich source of bioactive compounds - including polyphenols with a strong antiradical activity (Schütz *et al*., 2004) - inulin, fibres and minerals (Lattanzio *et al*., 2009; Lombardo *et al*., 2010; Pandino *et al*., 2011). Furthermore, the utilization of by-products of artichoke processing (i.e. external bracts) involves animal feedstuff (Megías *et al*., 2002) or extraction of functional molecules (Larossa *et al*., 2002). It emerges that the types and amount of bioactive substances and their activity (i.e. polyphenols content and the related antioxidant activity) could be used to characterize and select specific genotypes.

Nowadays, only few studies have investigated on polyphenols content, discriminating among the different head parts of artichoke (see for example Fratianni *et al*., 2007; Lombardo *et al*., 2010; Soumaya *et al*. 2013; Sihem *et al*., 2015); besides, the simultaneous determinations of total polyphenols and flavonoids content with radical scavenging capability, have not been considered at all. Consequently, in the present work we aimed at investigating such important biological properties in eleven spring accessions of artichoke collected in Central Italy (Marche and Abruzzo regions). The primary objectives were to obtain a preliminary: (i) genotype's characterization of the selected Central-Italy artichoke accessions from a biochemical point of view; (ii) evaluation of the suitability of the selected artichoke genotypes for fresh consumption or by-products production.

2. Materials and Methods

Plant material, management practices and head sampling

The study was carried out in 2014 at the experimental field of the Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics (CREA-OF), located in Monsampolo del Tronto (AP) (latitude 42°52'59.1" N, longitude 13°48'01.9" E), in the coastal area of the Marche Region (Central Italy) a typical area for globe artichoke cultivation.

Ten artichoke accessions from Marche and Abruzzo Regions, named as "Clone Monsampolo" (Cl_MSP), "Ascolano" (As), "Castorano" (Cs), "Jesino" (Je), "Mazzaferrata" (Mz), "Montelupone A" (ML_A), "Montelupone B" (ML_B), "Urbisaglia1" (Ub_1), "Urbisaglia2" (Ub_2) and "Violetto Tardivo di Pesaro" (VT_PS), were collected on the base of their peculiar sensory features and were compared with the reference genotype "Romanesco Clone C3" (Cl_C3), characterized by high market standards of the flower heads (purple with green shades, round shape, regular size and thick consistency). The selected globe artichoke genotypes differ for their biological and morphological profiles, as briefly synthetized in Table 1.

Plant material (shoots, named "carducci") was transplanted in August 2011 in rows spaced 1.00 m apart with row spacing of 1.20 m; each plot (artichoke genotype) consisted of thirty plants. The fertilization program, typical of the area, consisted in: 150 kg ha^{-1} of N, 80 kg ha^{-1} of phosphorus pentoxide (P_2O_5) and 100 kg ha^{-1} of potassium oxide (K_2O), respectively. The experimental field was kept weed-free by mechanical weed control and no pest control was needed.

Table 1 - Head characteristics of the eleven selected genotypes of globe artichoke

Genotype	Acronym	Colour of outer bracts	Colour of inner bracts	Bracts
"Clone C3"	Cl_C3	Green with Purple shades	Yellow	Spineless
"Clone Monsampolo"	Cl_MSP	Green	Yellowish-green	Spineless
"Ascolano"	As	Purple with green shades	Yellow-greenish with purple shades	Spineless
"Castorano"	Cs	Purple with light green shades	Yellow-purple	Spineless (but mucronate)
"Jesino"	Je	Purple with Green shades	Yellow purple	Spineless
"Mazzaferrata"	Mz	Purple with Green shades	Yellow-greenish	Spineless
"Montelupone A"	ML_A	Purple with Green shades	Yellow-purple	Spineless
"Montelupone B"	ML_B	Purple with Green shades	Yellow-purple	Spineless (but mucronate)
"Urbisaglia 1"	Ub_1	Purple	Yellow-purple	Spineless
"Urbisaglia 2"	Ub_2	Purple	Yellow-purple	Spineless
"Violetto tardivo PS"	VT_PS	Purple	Yellow-purple	Spine

At the marketing stage, six capitula per artichoke genotype were harvested, without floral stem, in two subsequently times (10th April and 10th May, considered as early and mid-spring), allowing to compare both main (first sampling data) and first order (second sampling data) capitula. Each flower head was separated into 'external bracts (~15 bracts)' (waste part) and 'receptacle' (edible fraction), freeze-dried, homogenized and stored at -20°C until biochemical characterization.

Chemical analysis

The extraction of polyphenols and flavonoids were carried out as described by Gouveia and Castilho (2012 a).

The Folin-Ciocalteu reagent method was used to evaluate the total polyphenols content (TPC) of the external bracts and receptacle following the method of Gouveia and Castilho (2011). Plant extracts were dissolved in methanol (10 mg mL^{-1}); aliquots of 50 µL were added to 1.25 mL of Folin-Ciocalteu (dilution, 1:10) and 1.0 mL of a 7.5% Na_2CO_3 solution. Solutions were maintained at room temperature for 30 min and the TPC was determined at 765 nm using a Beckman DU640B spectrophotometer (Beckman Coulter, Brea, California, USA). Gallic acid standard solutions were used to calibrate the method, so results were expressed as mg gallic acid equivalents (GAE) per g^{-1} dry weight (DW).

Total flavonoids content (TFC) was calculated following the procedure described by Gouveia and Castilho (2012 a) and estimated as rutin equivalents (RUE), i.e. expressed as mg RUE g^{-1} DW. Methanolic solutions (500 µL of sample solution) of the plant extracts (2.5 mg mL^{-1}) were mixed with 1.5 mL of methanol, 2.8 mL of water, 100 µL of potassium acetate (1 M) and 100 µL of aluminium chloride (10% in methanol). The absorbance of reaction mixture was read after 30 min at room temperature and at 415 nm using a Beckman DU640B spectrophotometer.

The radical scavenging activity of the extracts was determined using the stable radicals: (i) 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) - TEAC/ABTS assay (ABTS) (Re *et al*., 1999), modified as described by Gouveia and Castilho (2012 a); and (ii) 2,2-diphenyl-1-picrylhydrazyl - DPPH assay (Gouveia and Castilho, 2012 b). In each assay, Trolox was employed as reference standard and results were expressed as µmol Trolox equivalent (TE) g^{-1} DW.

Reagents and solvents were purchased from Sigma Chemicals Co. (St. Louis, MO). All reagents were of analytical grade.

Statistical analysis

In order to test (*F*-test) the effect of genotype on all the investigated variables, a one-way analysis of variance (ANOVA) was performed. The experiment was conducted following a complete randomized design and each sampled capitula represented a single repetition. When significant differences were detected, the means were compared based on the standard error of the difference (SED) between means, with significance being assigned using the least significant difference (LSD) value at the 5% ($p<0.05$) level of significance. Before the ANOVA, the data were analyzed to test for normality and homoschedasticity assumptions, through graphical methods.

To interpret and summarize the association between treatments (artichoke genotypes: Cl_C3, Cl_MSP, As, Cs, Je, Mz, ML_A, ML_B, Ub_1, Ub_2 and VT_PS) and variables (TPC, TFC, ABTS, DPPH) the principal component analysis (PCA) was applied. The PCA was performed separately for main and first order capitula; each principal component (PC) was calculated as a linear combination of the standardized original variables by using the eigenvectors of the correlation matrix. The results were visually explored in a two-dimensional PCA correlation biplot: standardized PC1 and PC2 scores were plotted as symbols, while the correlations between PCs and standardized variables (factor loadings) were plotted as vectors.

Statistical analyses were performed with the R software (R Core Team, 2017).

3. Results and Discussion

The TPC and TFC in the receptacle and external bracts of main and first order capitula of the eleven artichoke genotypes, are reported in Table 2. TPC ranged from 1.5 to 9.2 mg GAE g^{-1} DW, while TFC ranged from 1.8 to 4.1 mg RUE g^{-1} DW, matching with the literature data or, in some circumstances, resulting slightly higher (Lombardo *et al*., 2010; Pandino *et al*., 2011; Gouveia and Castilho, 2012 a; Pandino *et al*., 2012 a; Sihem *et al*., 2015; Dabbou *et al*., 2017; Marques *et al*., 2017; Petropoulos *et al*., 2017). Both traits were significantly (p<0.05) influenced by genotype: differences are related to both head part (receptacle or external bracts) and their location on plant architecture (main or first order capitula) (Table 2). Clear trends were observed: Je gave the highest TPC content (5.1 mg GAE g^{-1} DW, averaged over head parts and harvest time), followed by Ub_1, Ub_2 and VT_PS (5.0, 5.0 and 4.3 mg GAE g^{-1} DW on average, respectively), while Cl_MSP resulted as one of the worst genotypes in terms of polyphenols concentra-

Table 2 - Total polyphenols content [TPC, mg gallic acid equivalents (GAE) g^{-1} dry weight (DW)] and total flavonoids content [TFC, mg rutin equivalents (RUE) g^{-1} DW] in the receptacle and in the external bracts of different artichoke genotypes

Genotype§	Main capitula		First order capitula	
	TPC (mg GAE g^{-1} DW)	TFC (mg RUE g^{-1} DW)	TPC (mg GAE g^{-1} DW)	TFC (mg RUE g^{-1} DW)
Receptacle				
Cl_C3	7.4 ± 0.11	3.6 ± 0.23	3.8 ± 0.57	2.5 ± 0.38
Cl_MSP	3.6 ± 0.28	1.9 ± 0.16	3.1 ± 0.09	1.9 ± 0.12
As	5.7 ± 0.42	2.4 ± 0.21	3.9 ± 0.55	2.2 ± 0.19
Cs	6.3 ± 0.91	2.5 ± 0.30	2.6 ± 0.03	1.9 ± 0.08
Je	8.2 ± 1.09	3.3 ± 0.44	5.4 ± 0.47	3.0 ± 0.34
Mz	4.9 ± 0.73	2.8 ± 0.17	6.8 ± 1.35	3.5 ± 0.61
ML_A	5.8 ± 1.34	4.1 ± 1.01	2.1 ± 0.19	1.8 ± 0.19
ML_B	4.3 ± 0.22	2.3 ± 0.31	4.0 ± 0.64	3.1 ± 0.66
Ub_1	8.0 ± 0.58	3.4 ± 0.36	4.7 ± 0.66	2.9 ± 0.69
Ub_2	9.2 ± 1.55	3.9 ± 0.74	4.4 ± 0.15	2.6 ± 0.15
VT_PS	6.8 ± 0.45	3.5 ± 0.42	4.2 ± 0.45	3.2 ± 0.45
F-test	**	*	**	*
SED	1.2	0.7	0.9	0.6
External bracts				
Cl_C3	3.2 ± 0.12	2.6 ± 0.07	2.1 ± 0.06	2.3 ± 0.18
Cl_MSP	2.2 ± 0.15	2.0 ± 0.00	1.7 ± 0.11	2.0 ± 0.10
As	3.0 ± 0.19	2.4 ± 0.08	2.7 ± 0.42	2.7 ± 0.30
Cs	2.9 ± 0.28	2.1 ± 0.17	2.1 ± 0.04	2.6 ± 0.20
Je	3.9 ± 0.53	2.5 ± 0.18	3.0 ± 0.46	2.9 ± 0.56
Mz	2.4 ± 0.14	2.5 ± 0.08	2.8 ± 0.16	2.1 ± 0.04
ML_A	2.1 ± 0.25	2.4 ± 0.25	1.5 ± 0.08	2.3 ± 0.02
ML_B	2.1 ± 0.16	2.0 ± 0.07	2.2 ± 0.28	2.3 ± 0.28
Ub_1	4.8 ± 0.30	2.9 ± 0.17	2.4 ± 0.03	2.3 ± 0.22
Ub_2	4.1 ± 0.61	2.7 ± 0.25	2.2 ± 0.19	2.3 ± 0.02
VT_PS	4.0 ± 0.11	3.2 ± 0.05	2.2 ± 0.05	3.0 ± 0.21
F-test	**	**	**	NS
SED	0.4	0.2	0.3	

Data refer to both main and first order capitula (two different harvest times, at early and mid-spring 2014). Means ± standard errors of n=6 independent replicates are reported.
* p<0.05; ** p<0.01; *** p<0.001; NS = not significant.
SED, standard error of differences between means.
§ The list of the used acronomys is reported in Table 1.

tion in artichoke heads (on average 2.7 mg GAE g^{-1} DW) together with ML_A and ML_B (on average 2.9 and 3.2 mg GAE g^{-1} DW, respectively) (Table 2). These results were quite confirmed by TFC data (Table 2), indicating those genotypes' suitable for fresh consumption rather than food processing. Lower antioxidant compounds (i.e. polyphenols) is, indeed, considered a qualitative trait required by industry, thanks to the scarce propensity to enzymatic browning phenomena after cutting and storage operations (Lattanzio *et al*., 1994; Lombardo *et al*., 2010). The reference genotype (Cl_C3) confirmed its high value for fresh consumption, registering higher TPC and TFC, only in the combination early-spring harvest (main capitula)/receptacle (Table 2), mostly appreciated by consumers and with the highest commercial value.

As previously observed (Fratianni *et al*., 2007; Lombardo *et al*., 2010; Pandino *et al*., 2011; Pandino *et al*., 2012 b; Pandino *et al*., 2013 a; Sihem *et al*., 2015), polyphenols were not uniformly distributed in the different floral head parts (Fig. 1): regardless of the harvest time, higher TPC values were observed in the receptacle (5.2 mg GAE g^{-1} DW, averaged over genotypes) while lower in the external bracts (2.7 mg GAE g^{-1} DW, averaged over genotypes). Besides, no differences emerged in terms of TFC (2.5 *vs*. 2.8 mg GAE g^{-1} DW in external bracts and receptacle respectively, averaged over genotypes). The different amount of antioxidant compounds in the various head parts is of interest to identify genotypes rich in these molecules in the by-products (external bracts) and hence interesting for the industrial processes (i.e. animal feedstuff, fiber production, recovery of functional ingredients) (Femenia *et al*., 1998; Larossa *et al*., 2002; Megías *et al*., 2002; Lattanzio *et al*., 2009). Nonetheless, we observed some differences in terms of relative TPC among artichoke accessions, with particular regards in terms of relative TFC (Fig. 1): the genotypes Cl_MSP, As and Cs were characterized by the highest relative TFC in the waste products (Fig. 1), suggesting useful utilization for the by-products processes, with external bracts representing a potential innovative source for flavonoid extraction.

Lastly, TPC values lowered in both receptacle and external bracts shifting from main to first order capitula (Fig. 2A); this trend was confirmed by all the accessions with the exception of Mz (Fig. 2A), which gave the highest TPC values in the first order flower heads (Table 2), so maintaining a high content of functional compounds during the growing cycle. A similar behavior was recorded for some of the selected artichoke accessions in terms of TFC (see for example Cl_MSP, As and ML_B) (Fig. 2B). This was probably attributable to the environmental conditions recorded during the harvest season. Despite the solar radiation levels show the stronger effect on polyphenols accumulation in the artichoke' receptacle (Pandino *et al*., 2013 b), in our study the lower temperatures observed in April (-10% on average with respect to May - considering the mean air temperatures recorded during the first 10 days of each month) could have affected TPC and TFC, as previously observed in other crops (Klimov *et al.*, 2008; Hykkerud *et al*., 2018).

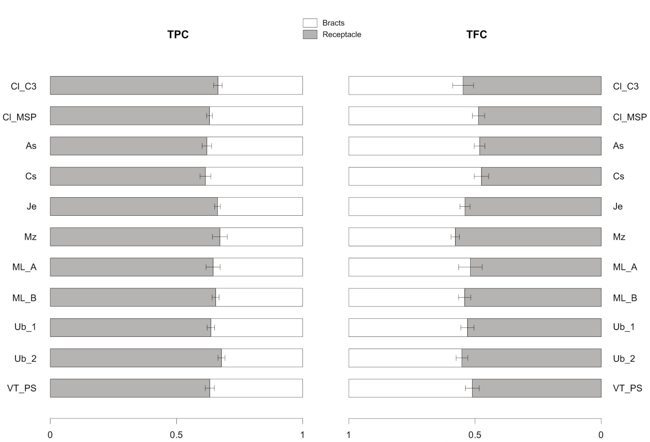

Fig. 1 - Relative proportion (as percentage) of total polyphenols content [TPC, gallic acid equivalents (GAE) g^{-1} dry weight (DW)] and total flavonoids content [TFC, mg rutin equivalents (RUE) g^{-1} DW] in the receptacle and external bracts averaged over both main and first order capitula of different globe artichoke genotypes (see Table 1 for the list of acronyms). Data represent means ± standard errors, n=12 independent replicates.

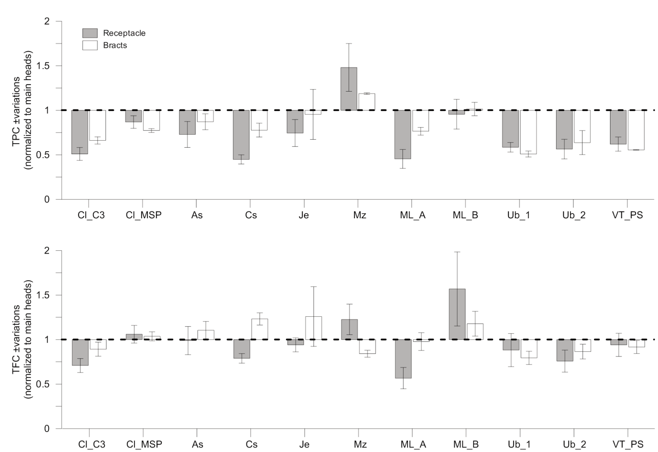

Fig. 2 - Variations normalized to main capitula values (dashed line) of (A) total polyphenols content [TPC, mg gallic acid equivalents (GAE) g^{-1} dry weight (DW)] and (B) total flavonoids content [TFC, mg rutin equivalents (RUE) g^{-1} DW] in the receptacle and external bracts of different globe artichoke genotypes (see Table 1 for the list of acronyms). Data represent means ± standard errors, n=6 independent replicates.

Radical scavenging activity (ABTS and DPPH assays) registered differences among thesis similar to those observed for TPC and TFC data (Table 3). ABTS values ranged from 21.2 to 146.8 µmol TE g^{-1} DW and DPPH ranged from 12.8 to 204.3 µmol TE g^{-1} DW, showing the same order of activity previously found in other antioxidant capacity assays on artichoke (Gouveia and Castilho, 2012 b; Rouphael *et al.*, 2017). The highest activity was concentrated in the receptacle (85.7 and 103.8 µmol TE g^{-1} DW for ABTS and DPPH, respectively *vs.* 41.9 and 38.3 µmol TE g^{-1} DW for ABTS and DPPH, respectively in the external bracts) regardless of genotype and harvesting time (Table 3) (Sihem *et al.*, 2015). Also for these biochemical traits, Je, Ub_1 and Ub_2 ranged at the first positions while Cl_MSP as the genotype with the lowest ABTS and DPPH values (Table 3). Moreover, we found significant (p<0.001) linear relationships between these variables, confirming previous results (Alghazeer *et al.*, 2012; Lombardo *et al.*, 2013). Follows the higher measured Pearson's correlation coefficients: TPC *vs.* ABTS, r=0.84; TPC *vs.* DPPH, r=0.91; TFC *vs.* ABTS, r=0.76; TFC *vs.* DPPH, r=0.81. Indeed, phenolic compounds are known to have the ability to block the chain reaction of reactive oxygen and nitrogen species through different pathways involving (i) direct reaction with free radicals, (ii) sequester metal ions able to spread the chain reaction, (iii) synergic action with other antioxidants (Khasawneh *et al.*, 2014).

The relationships between TPC, TFC, ABTS and DPPH, classified based on the analysed capitula parts

Table 3 - Radical scavenging activity (µmol trolox equivalents (TE) g^{-1} DW) obtained from two different assays: trolox equivalent antioxidant capacity with 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) in the receptacle and in the bracts of different artichoke genotypes

Genotype§	Main capitula		First order capitula	
	ABTS (µmol TE g^{-1} DW)	DPPH (µmol TE g^{-1} DW)	ABTS (µmol TE g^{-1} DW)	DPPH (µmol TE g^{-1} DW)
Receptacle				
Cl_C3	146.8 ± 6.91	165.5 ± 7.75	61.1 ± 14.29	61.3 ± 26.77
Cl_MSP	71.3 ± 8.85	47.3 ± 12.82	42.3 ± 7.39	31.7 ± 2.95
As	87.2 ± 14.10	109.9 ± 14.97	73.4 ± 7.23	78.2 ± 14.87
Cs	97.4 ± 28.90	127.2 ± 31.56	34.4 ± 5.83	33.5 ± 7.20
Je	119.5 ± 19.14	185.6 ± 34.58	76.0 ± 12.66	56.9 ± 11.52
Mz	86.6 ± 19.63	108.4 ± 28.80	95.0 ± 12.93	178.2 ± 42.51
ML_A	104.2 ± 32.54	144.8 ± 57.25	31.4 ± 3.08	26.2 ± 9.39
ML_B	68.2 ± 8.77	80.0 ± 12.90	82.3 ± 19.76	47.2 ± 12.23
Ub_1	140.3 ± 16.58	171.5 ± 19.28	67.5 ± 12.89	111.0 ± 26.09
Ub_2	128.2 ± 27.06	204.3 ± 49.60	95.2 ± 5.22	91.7 ± 9.76
VT_PS	107.4 ± 7.40	138.2 ± 12.15	70.2 ± 16.40	84.3 ± 22.80
F-test	NS	*	**	**
SED		42.4	16.7	28.5
External bracts				
Cl_C3	57.3 ± 5.56	42.9 ± 4.49	37.6 ± 8.53	18.2 ± 2.77
Cl_MSP	27.6 ± 0.77	12.8 ± 3.78	21.2 ± 3.39	16.1 ± 2.57
As	45.5 ± 4.93	37.0 ± 4.82	40.8 ± 3.63	42.6 ± 10.25
Cs	34.1 ± 8.87	38.1 ± 6.55	32.1 ± 2.04	24.2 ± 3.38
Je	64.7 ± 12.85	80.5 ± 13.40	54.4 ± 10.96	53.7 ± 16.83
Mz	27.5 ± 2.79	21.7 ± 5.90	48.1 ± 5.17	34.0 ± 8.95
ML_A	31.9 ± 6.81	36.6 ± 1.91	27.4 ± 3.67	17.9 ± 5.71
ML_B	27.5 ± 1.78	27.2 ± 5.04	31.5 ± 10.47	20.9 ± 7.52
Ub_1	80.9 ± 8.72	93.9 ± 10.09	35.8 ± 4.19	38.4 ± 2.52
Ub_2	58.8 ± 16.82	71.4 ±20.49	39.1 ± 6.21	19.1 ± 6.07
VT_PS	57.6 ± 2.45	59.4 ± 4.90	39.2 ± 6.46	36.7 ± 3.85
F-test	**	**	NS	**
SED	11.5	12.7		10.8

Data refer to both main and first order capitula (two different harvest times, at early and mid-spring 2014). Means ± standard errors of n=6 independent replicates are reported.

* p<0.05; ** p<0.01; *** p<0.001; NS = not significant.

SED, standard error of differences between means.

§ The list of the used acronomys is reported in Table 1.

(i.e. receptacle - TPC_Rec, TFC_Rec, ABTS_Rec and DPPH_Rec - and external bracts - TPC_Bra, TFC_Bra, ABTS_Bra and DPPH_Bra), and the eleven artichoke accessions, were summarized by PCA. The results, on the basis of harvest time, are graphically displayed in two correlation bi-plots (Figs. 3A and 3B, respectively); in Table 4 are reported the factor loadings, the eigenvalues and the percentage of the explained variance.

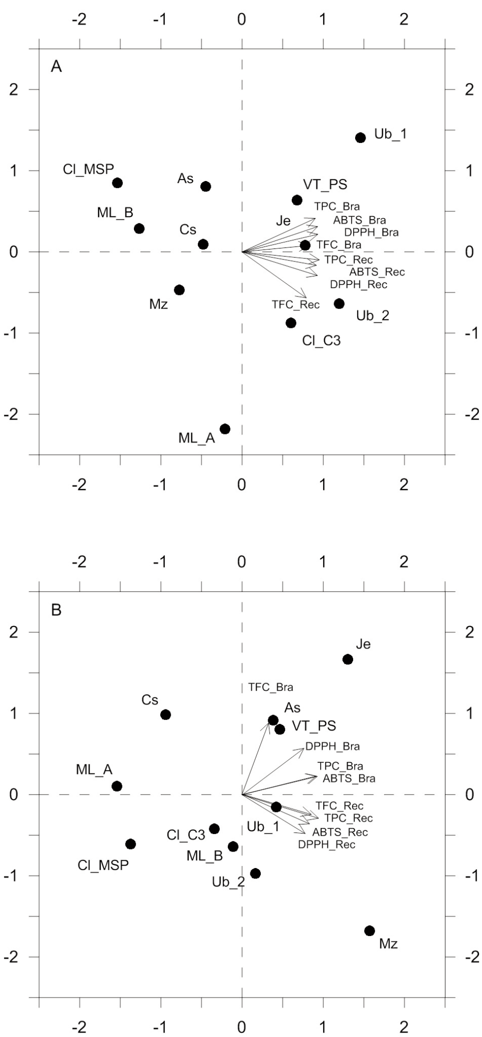

Fig. 3 - Two dimensional principal component analysis (PCA) correlation bi-plot [main capitula/first harvest time (A) and first order capitula/second harvest time (B)]: symbols show the standardized scores on PC1 (x-axis) and PC2 (y-axis) for the eleven artichoke genotypes (see Table 1 for the list of acronyms); vectors coordinates represent the correlations between standardized variables [total polyphenols content in receptacle and external bracts (TPC_Rec and TPC_Bra, respectively), total flavonoids content in receptacle and external bracts (TFC_Rec and TFC_Bra, respectively), radical scavenging activity obtained from two different assays in the receptacle and external bracts (ABTS_Rec and ABTS_Bra, respectively; DPPH_Rec and DPPH_Bra, respectively)] and PCs.

In early-spring harvesting time (i.e. referring to main capitula data), the first and second principal components explained 90.2% of the total data variability (80.7 and 9.5% for PC1 and PC2, respectively) (Table 4). The variables were grouped into two distinct clusters, separated by PC2: in the upper right quadrant, we found all the chemical data related to the external bracts samples (TPC_Bra, TFC_Bra, ABTS_Bra and DPPH_Bra) while in the bottom right section, those related to the receptacle ones (TPC_Rec, TFC_Rec, ABTS_Rec and DPPH_Rec); all the variables reached high PC1 scores (scores from 0.952 to 0.788 for TPC_Rec and TFC_Rec, respectively) (Table 4). Regarding genotypes, Cl_C3, Ub_2, Je, VT_PS and Ub_1 clustered separately on the right along PC1 and were positively associated with all the investigated variables; conversely, Cl_MSP exhibited the highest negative PC1 score (-1.533) followed by ML_B, Mz, Cs, As and ML_A (Fig. 3A).

With respect to the mid-spring harvesting time (i.e. referring to first order capitula data), the eigenvalues for PC1 and PC2 were 5.28 and 1.71, respectively, thus capturing 87.4% of the total data variability (Table 4). Again, variables were clearly separated by PC2 while genotypes by PC1 (Fig. 3B). In particular, all the variables reached high PC1 scores with the exception of TFC_Bra, which was mainly correlated with PC2 (scores: 0.328 and 0.886 for PC1 and PC2, respectively; Table 4). Je and Mz showed the higher PC1 scores (1.302 and 1.568, respectively) despite they performed very differently with respect to PC2 (scores: 1.668 and -1.679 for Je and Mz, respectively). As a consequence, Je was related to higher TPC, ABTS and DPPH values in the waste fractions (external bracts) while Mz in the edible parts (receptacle).

PCA proved to be a useful tool to summarize the biochemical characteristics of the different investi-

Table 4 - Principal component analysis (PCA): factor loadings, eigenvalues and percentage of the explained variance

Variables	Main capitula		First order capitula	
	PC1	PC2	PC1	PC2
TPC_core	0.952	-0.093	0.941	-0.288
TFC_core	0.788	-0.561	0.853	-0.242
DPPH_core	0.927	-0.286	0.777	-0.477
ABTS_core	0.916	-0.163	0.831	-0.356
TPC_bratee	0.903	0.418	0.926	0.228
TFC_bratee	0.823	0.084	0.328	0.886
DPPH_bratee	0.933	0.220	0.762	0.572
ABTS_bratee	0.930	0.315	0.912	0.226
Eigenvalue	6.455	0.761	5.284	1.711
Explained variance (%)	80.692	9.515	66.056	21.382

gated artichoke genotypes, and clear conclusions could be obtained, confirming the results in terms of single investigated biochemical parameters. In particular, the reference genotype Cl_C3 and the accessions Ub_1, Ub_2, Je and VT_PS confirmed higher TPC, TFC and, consequently, antiradical activity in the main capitula, highlighting their important attitude for fresh consumption. This greater head quality was maintained during all the growing season (i.e. as quality traits of the first order capitula) only for Ub_1, Je and VT_PS. Other genotypes, such as As and, principally, Mz, were clearly characterized by higher bioactive compounds in the first order flower heads and by smaller capitula. These accessions could represent a promising potential as germplasm for future breeding programs to select elite cultivars, characterized by: (i) higher and stable quality traits suitable for fresh consumption (i.e. Je); (ii) high concentrations of biochemical compounds, especially in the waste products, to be used for processing or pharmaceutical applications although further investigations on smaller and waste flower heads are needed.

4. Conclusions

In conclusion, our results confirm that the capitula of globe artichoke could be considered a functional food thanks to its relevant content of bioactive compounds accumulated in both receptacle and external bracts. The properties of the external bracts could be usefully exploited for other end-use purposes, although they are still edible fractions (Fratianni *et al*., 2007; Pandino *et al*., 2011). A great and appreciable variation among genotypes in terms of chemical composition and nutritional value exists. Such biodiversity of the accessions of Abruzzo and Marche Regions should be exploited and utilized, taking into account the peculiarity of each genotype (in terms of both yield and quality) as well as the actual end-use which can be reached.

Acknowledgements

We gratefully acknowledge Maria Assunta Dattoli for her technical assistance in the chemical analysis and Maria Silvia Sebastiani and Alessandro Giardini for their support in data collection and first elaboration.

This work was grant by RGV/FAO Project.

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Adv. Hort. Sci., 2019 33(1): 33-38 DOI: 10.13128/ahs-22953

AHS
Advances in Horticultural Science

Combination of alginate based edible coating-betel essential oil in extending the shelf life of rose apple cv. Dalhari (*Syzygium samarangense*)

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Key words: alginate, betel essential oil, edible coating, rose apple cv. Dalhari.

OPEN ACCESS

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Citation:
SETIAWAN C.K., UTAMA N.A., PRADANA B.A., 2019 - *Combination of alginate based edible coating-betel essential oil in extending the shelf life of rose apple cv. Dalhari (Syzygium samarangense)*. - Adv. Hort. Sci., 33(1): 33-38

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 1 January 2018
Accepted for publication 18 September 2018

Abstract: Rose apple cv. Dalhari is a local fruit grown in Berbah District, Special Region of Yogyakarta. This fruit perishable, easily loses its water content and is attacked by microbes. This research aimed to determine the best combination between alginate and betel essential oil to inhibit the growth of microbes and maintain quality of rose apple cv. Dalhari. Alginate and betel essential oil treatments were used at three concentration levels, alone and in combinations, respectively of 2%, 2.5%, 3% and 0%, 0.1%, 0.2%. The results showed that combination treatment of 2.5% alginate and 0.1% betel essential oil was the most effective to maintain the fruit quality. The sole addition of betel essential oil was not able to inhibit microbial growth. Furthermore, combination of alginate based edible coating and betel essential oil was able to maintain the quality of rose apple cv. Dalhari up to nine days.

1. Introduction

Rose apple cv. Dalhari is locally cultivated at Berbah District, Yogyakarta. Rose apple cv. Dalhari is one of the most famous fruits due to its large size, slightly fresh sour taste, and high water content. While, rose apple has thin skin makes low ability to maintain its water content and also is easily growth by microbes when it is kept at room temperature (Pertiwi *et al.*, 2012). Edible coating is an alternative technology to overcome the problem and its widely known as the ability to maintain the atmosphere condition around the fruit, such as control gas exchange and control water loss, maintain the fruit's texture, and also reduce the risk of microbial attack (Campos *et al.*, 2011).

Edible coating formulation can be derived from polysaccharides, lipids, proteins as long as the material are tasteless, odourless, and transparent. Polysaccharides include alginate that is widely used as primary ingredient for edible coating due to its ability in holding high oxygen and in resulting good quality of edible coating (Quirós-Sauceda *et al.*, 2014). Alginate is obtained from marine brown algae (*Phaeophyceae*) extraction. Recently, the use of edible coating in fruit preservation is combined with another active ingredient, such as antibrowning agent, nutrients, and antimicrobial agent to decrease microbial growth (Campos *et al.*, 2011; Guerreiro *et al.*, 2015; Atarés and Chiralt, 2016; Hamedi *et al.*, 2017).

Some essential oils have shown their effectiveness as food preservatives due to their antimicrobial activity and antioxidant content. Betel essential oil contains chavicol, eugenol, acetyl eugenol as the main compounds to prevent pathogen growth in different edible commodities (Prakash *et al.*, 2010; Basak and Guha, 2015). However there are still limited information available regarding the use of edible coating to maintain the freshness of rose apple cv. Dalhari. The only report from previous authors refers to effect of storage temperature of rose apple cv. Dalhari (Widoyo, 2013) , and none mentioned the use of edible coatings. This study aimed to determine the effect of betel essential oil combined with alginate-based edible coatings to prevent pathogen growth, and extend the shelf life of rose apple cv. Dalhari.

2. Materials and Methods

Rose apple Fruit cv. Dalhari was harvested in Berbah District, Special Region of Yogyakarta, Indonesia in the middle of April, 60 days after flowering. Harvested fruit has characteristic red colour skin and average weight 125 g/fruit from 243 fruits. The fruit was immediately brought to Postharvest Laboratory at the Universitas Muhammadiyah Yogyakarta. The fruits were selected based on defect and size, then stored in a refrigerator (14°C) until treatment. Food grade Sodium Alginate (AL) (IIS, Indonesia) was used for coating treatment, glycerol as a plasticizer, and betel essential oil (BE).

Preparation and application of edible coating

Edible coating was prepared with diluted alginate into aquadest solution and heated in 85°C. Solution was added with 1.5% glycerol and betel essential oil in different concentration. Alginate-betel essential oil solution were formulated into nine treatments: AL 2% (w/v) (A1S0); AL 2% (w/v)-BE 0.1% (v/v) (A1S1); AL 2% (w/v)-BE 0.2% (v/v) (A1S2); AL 2.5% (w/v) (A2S0); AL 2.5% (w/v)-BE 0.1% (v/v) (A2S1); AL 2.5% (w/v)-BE 0.2% (v/v) (A2S2); AL 3% (w/v) (A3S0); AL 3% (w/v)-BE 0.1% (v/v) (A3S1); AL 3% (w/v)-BE 0.2% (v/v) (A3S2).

Each treatment was performed in several steps as follow: the fruit was washed using sodium hypochlorite 200 µl L^{-1} and dried at room temperature followed by dipping in edible coating solution for 2 min. The excess of edible coating solution was dripped off for 30 s before being dipped in CaCl 2% solution for 1 min. Three treated fruits were packaged with polypropylene trays with perforated cover and stored at 14°C until analysis. Analysis was performed on days 0, 3, 6, 9, 12, and 15.

Fruit quality assessment

Twenty seven groups of rose apple (9 treatments x 3 replicates) were weighed during the storage time. The weight loss (WL) of rose apple was determined using the equation:

$$WL (\%)= [(W0-Wt)/W0] \times 100$$

where W0 is the initial weight and Wt is the sample weight at time t. Fruit firmness was measured using a Fruit Hardness Tester FHT200 (Extech Instruments, USA). Each fruit was tested in three different sides with a 6 mm diameter probe on equatorial position and recorded in N/mm^2. Total Acidity (TA) determination was performed with methods according to ISO 750-1998. Five grams of rose apple slices were homogenised and diluted to 100 mL with distilled water. The mixture was filtered and was added 3-5 drops of phenolphthalein (1% in 95% ethanol) to 10 mL solution. The solution was then stirred and titrated with 0.1 N NaOH. TA was determined as a percent of citric acid. Total Soluble Solid (TSS) was determined using a hand-refractometer (Atago, Japan). The tools determines the juice TSS content after being homogenised the flesh and it was expressed as a brix (%). Reducing Sugar (RS) were determined according to Nelson-Somogyi Technique (Nelson, 1944) and the result showed in percentage. The analysis was performed in 540 nm abs using Spectrophotometer UVmini-1240 (Shimadzu, Japan).

Antibacteria and antiyeast activity

Bacteria and yeast were isolated from decayed rose apple using paper discs and plate count. Suspension was obtained from diluting 1 gr samples on destilled water in seven-fold dilution series. A petri dish was prepared with poured 10 ml nutrient agar medium and allowed to solidify. The 0.1% inoculum suspension was poured and flattened on the medium. Sterilized filter paper disc dipped with 0.1% betel essential oil for 15 min and then placed on the surface of a medium. The petri incubated at room temperature for 48 h. After incubation time, measurement the inhibition zones were done with a ruler. In the other hand, 0.1% of betel essential oil was poured in medium before inoculated with 0.1 ml inoculum. After 48 h incubation at room temperature, the visible microbe was counted and the result was presented in log Colony Forming Unit (CFU).

After treatment was done, all samples were analyzed with microbial count for bacterial and yeasts based on Mola *et al.* (2017). One gram of homogenized sample was diluted using 9 ml sterilized water. Sample then diluted in seven-fold dilution series before plating. A 0.1 ml solution was poured on Plate Count Agar (PCA) surface and Potato Dextrose Agar (PDA) for enumerating total bacteria and molds. Microbiological analysis was performed in three replication and the results were expressed as CFU (Colony Forming Units) per grams fresh weight.

Sensory analysis

The sensory was analysed by 15 semi-trained panellists on the based of 5-point hedonic scales (1=very dislike; 2=dislike; 3=neither like or dislike; 4=like; 5= like very much) for the parameters of texture and appearance according to Guerreiro *et al.* (2015) with some modification. The panellists were recruited from staff and students of the department. They were trained in the initial test to be familiar with the fruit. All samples were analyzed on 0, 3, 6, 9, 12, 15 days after treatment.

Statistical analysis

The experiment was carried out in randomised experimental design. The experiment data were analysed using SAS statistical software package 9.4 for windows and Duncan multiple-range test ($P<0.05$) was performed for mean comparisons.

3. Results

Weight loss is one of the most important freshness indicators in fruit. As shown in figure 1, the weight loss increases in all fruit treatment during 15 days storage. However, a combination of alginate 2.5% and betel essential oil 0.1% can significantly ($P<0.05$) prevent the fruit weight loss, while the treatment of alginate 2% has shown the highest weight loss. Weight loss of rose apple has no relation to its firmness. It can be shown in figure 2. Although it shows that the value of firmness is not constant, overall the trend tends to show the similar pattern

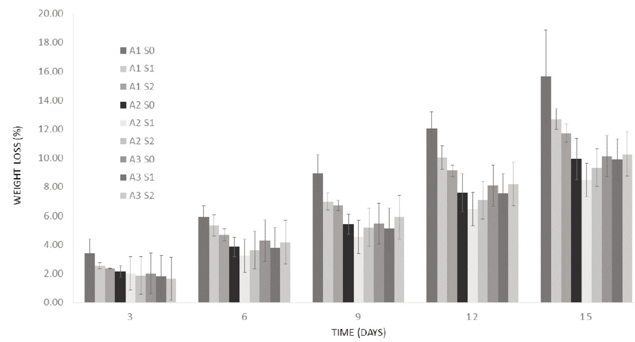

Fig. 1 - Effect of alginate based edible coating-betel essential oil on weight loss of rose-apple cv. Dalhari.

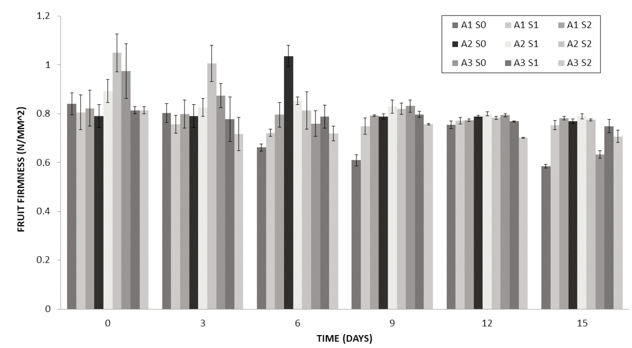

Fig. 2 - Effect of alginate based edible coating-betel essential oil on fruit firmness of rose-apple cv. Dalhari.

during 15 days of storage. Alginate can maintain the fruit firmness' decline even there is no significant effect between all treatment ($P>0.05$). A similar trend was also shown in pineapples (Azarakhsh *et al.*, 2012), and apple (Rojas-Graü *et al.*, 2007 a) (Oms-Oliu *et al.*, 2008).

The TA, TSS and RS content show a regular decreasing during 15 days of storage time. The TA decreases after three days storage time in all treatment. However, as can be seen in figure 3A, the fruit treated with 3% alginate shows slower TA reduction. The value of TSS slowly decreases on all treatments during 15 days storage (Fig. 3B) despite no significant effect ($P>0.05$) is observed. Rose apple fruit being non-climacteric fruit, tends to maintain similar TSS content during storage. Concerning RS content, there is no significant effect ($P>0.05$) in all treatments. RS value decreased after three days storage as showed in figure 3C.

The effect of the edible coating containing a different concentration of betel essential oil on microbial growth is shown in figure 4A and 4B. As

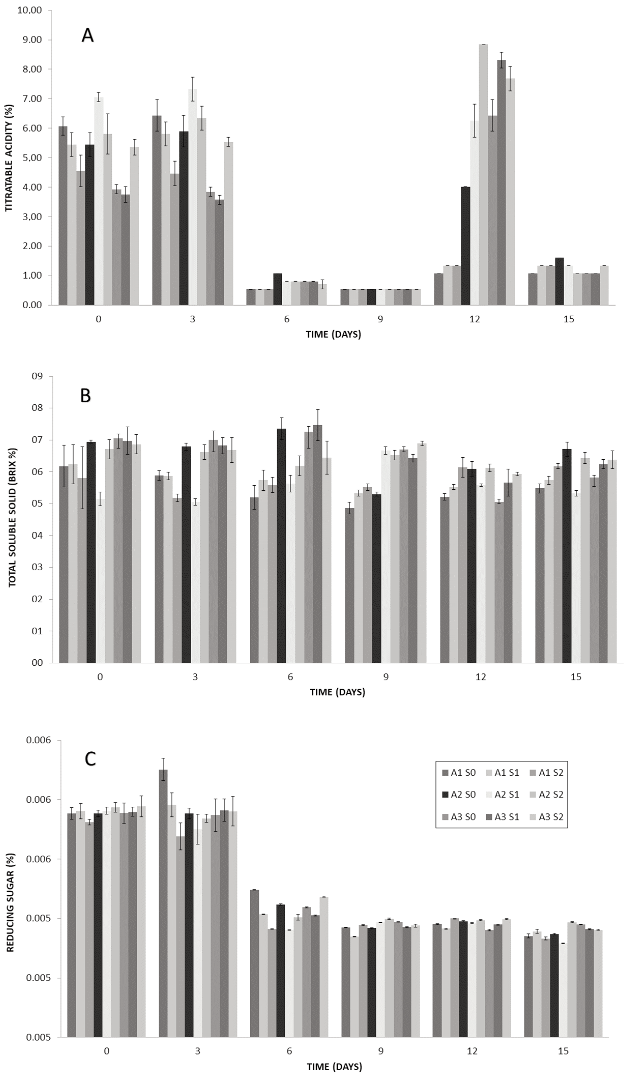

Fig. 3 - Effect of alginate based edible coating-betel essential oil on TA (A), TSS (B) and RS (C) of rose-apple cv. Dalhari.

shown in the figure, edible coating can inhibit both bacteria and yeast in rose apple cv. Dalhari. The increase in the population of both bacteria and yeast is observed during 15 days of storage time. The addition of betel essential oil can inhibit the yeast growth until nine days, while bacteria can be shown in no essential oil addition treatment after six days storage (Fig. 4A).

The sensory quality evaluation is not significantly different (P>0.05) during 15 days of storage time based on both treatments. The result indicates that different changes happen in the treatment during storage time although panellists did not apprehend those differences. The result also showed that rose apple is not suitable for consumption at 9 d after storage since most panellists give a score under minimum acceptable value in overall score (Fig. 5).

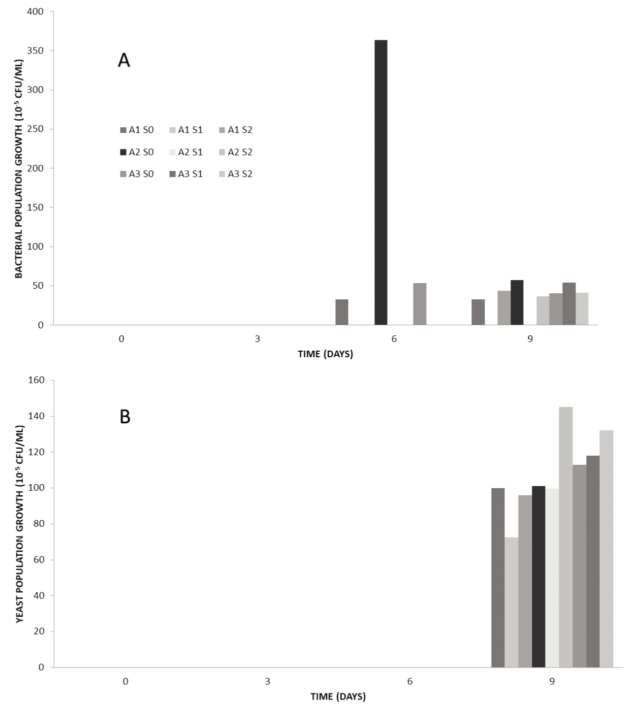

Fig. 4 - Effect of alginate based edible coating-betel essential oil on bacteria (A) and yeast (B) population of rose-apple.

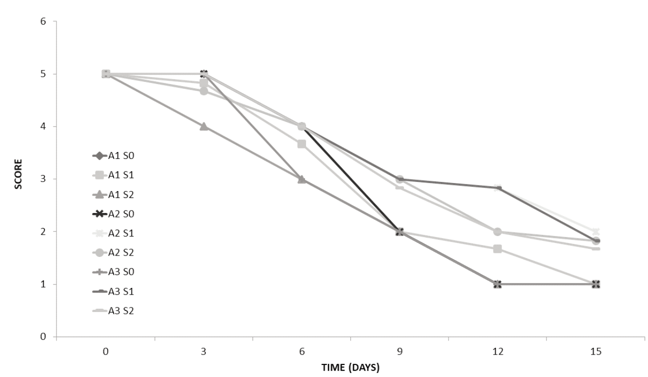

Fig. 5 - Effect of alginate based edible coating-betel essential oil on panelist preferences of rose-apple cv. Dalhari.

4. Discussion and Conclusions

Reducing effect from essential oil to weight loss can be described with conjunction of betel essential oil with polysaccharides based edible coating. This relation make the edible coating more stable and permeable. Similar result was described in the experiment using lemongrass (Azarakhsh *et al.*, 2014), cinnamon and palmarosa (Raybaudi-Massilia *et al.*, 2008), oregano and vanillin (Rojas-Graü *et al.*, 2007 a). The use of alginate as edible coating is expected can decrease the weight loss. In the present study, the results show that increasing alginate concentration determine an increase in weight loss. In a previous study, this phenomenon has been reported and weight loss was explained as coming from edible coating itself (Guerreiro *et al.*, 2015).

Addition of betel essential oil does not affect the firmness (Fig. 3). In a previous study, the use of lemongrass essential oil on alginate based edible coating decreases the fruit firmness due to its lower pH that triggers pectic acid hydrolysis (Rojas-Graü *et al.*, 2007 a). Fresh cut pineapple which are treated with alginate-lemongrass essential oil edible coating shows a decrease in firmness value during storage (Azarakhsh *et al.*, 2014).

In TA observation, the result show that high concentration of alginate can decrease oxygen permeability which led reduction on the respiratory process (Campos *et al.*, 2011). TA changes are related to organic acid production and an acidity reduction maybe represent as a metabolic changes result in fruit or caused by the use of organic acid as respiratory substance (Selcuk and Erkan, 2015). TSS content is a critical attribute which has contribution to quality and consumer acceptability. Our result demonstrate that alginate based edible coating shows ability to maintain the TSS content of rose apple. This findings are in line with the previous study in sweet cherry (Díaz-Mula *et al.*, 2012), guava (Nair *et al.*, 2018), cantaloupe (Zhang *et al.*, 2015), blueberry (Mannozzi *et al.*, 2017) and Ber fruit (Ramana Rao *et al.*, 2016).

RS value is an essential indicator for determining respiratory process in the fruit. Betel essential oil addition does not affect the RS value. However, many studies showed alginate can maintain RS value which is reported in carambola (Gol *et al.*, 2015), cherry (Díaz-Mula *et al.*, 2012)

Fresh fruit has high nutrient and high water content which allow microbial to multiply (Rojas-Graü *et al.*, 2007 a). Betel essential oil can inhibit the microbiological growth in food preservation (Prakash *et al.*, 2010). In a previous study, the result showed that there is no fungi that can grow in media which have been added with betel essential oil more than 0.6% (v/v) (Basak and Guha, 2015). However, our previous study in preferences (unpublished data) on alginate-betel essential oil edible coating showed that most panellists accepted to consume rose apple with the addition of essential oil less than 0.2%.

Addition betel essential oil shows proper appreciation up to 9 d in appearance value. This result is related to low microbial spoilage compared to no addition of essential oil. Similarly, changes are shown in rose apple cv. Dalhari texture preference. The panellist show acceptable score up to 9 d. This overall result is consistent with in previous study where it is no differences between fruit with low concentration essential oil addition with control in sensory test (Raybaudi-Massilia *et al.*, 2008; Azarakhsh *et al.*, 2014; Bustos *et al.*, 2016; Guerreiro *et al.*, 2016).

In conclusion, edible coating treatment with alginate 2.5% and betel essential oil 0.1 % maintained the quality of rose apple cv. Dalhari by reducing weight loss, fruit firmness, microbial growth and appearance. In a study of microbial growth inhibition using paper disc test, betel essential oil has shown to inhibit both yeast and bacteria. However, in this experiment, a combination of alginate-betel essential oil has not shown the different effect to microbial growth on rose apple cv. Dalhari. The main limitation of this study is the lack of information in the fruit. Future research needs to be done for more findings and better understanding.

Acknowledgements

The authors thank to the Universitas Muhammadiyah Yogyakarta for financially supporting this research. The research grant was awarded under the Dosen Muda research schemes. Special thanks go to Indira Prabasari, PhD and Puthut Ardianto, M.Pd for proofreading the manuscript.

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Adv. Hort. Sci., 2019 33(1): 39-48 DOI: 10.13128/ahs-23606

AHS
Advances in Horticultural Science

Reflective materials and management practices on the physicochemical and biochemical quality of Merlot grapes

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Citation:
PORTO A.H., WAGNER JUNIOR A., BANDEİRA CABRAL V., CİTADİN I., ZANELA J., NUNES I.B., , CONCEIÇÃO P.C., 2019 - *Reflective materials and management practices on the physicochemical and biochemical quality of Merlot grapes*. - Adv. Hort. Sci., 33(1): 39-48

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 10 July 2018
Accepted for publication 4 October 2018

Key words: fruit growing, irradiation, vine, *Vitis sp.*

Abstract: The aim of this study was to evaluate the use of reflective materials on the soil surface and management practices on the physicochemical and biochemical quality of the Merlot grapes (*Vitis vinifera* L.). The experimental design was of randomized blocks, in factorial 3 x 2 ("material type" x "management practice"), with 4 replications, using 4 plants by plot. The "management practice" factor was divided into two levels, with and without its realization. For "material type", two reflective films were tested in the soil surface, it being white raffia plastics of polypropylene (reflective film 1), metallic raffia plastic (reflective film 2) and without the use of any reflective material. When they reach the harvesting point in the 2009/2010 and 2010/2011 production cycles, the physicochemical and biochemical evaluations of fruit quality were submitted; physiological characteristics of the plant and microbiological properties of soil were also evaluated. The use of reflective material on the soil surface improved the quality of the grape in some aspects, especially determining the reduction of the berry dropping and, consequently, an increase in the productivity. Furthermore, it provided greater microbial activity in the soil, which may be important for grapevine. The cultural practices had no influence on the physicochemical and biochemical quality of the vine fruits, providing only a greater number of berries per bunch.

1. Introduction

Grape is one of the most economically important fruits in the world, with Brazil in 2014 occupying 78,765 hectares, while production was 1,454,183 tons (FAO, 2014; Camargo *et al.*, 2011). Approximately 57% of this production was used for winemaking (Mello, 2011), with the rest consumed *in natura* or as dried fruits (Conde *et al.,* 2007).

Viticulture for winemaking is mainly concentrated between the 30th and 50th parallel of North latitude and between 30th and 45th of South latitude. The main climates that allow the cultive of vines are those of temperate, Mediterranean and arid areas, in different levels (Tonietto and Mandelli, 2003).

However, the state of Paraná, Brazil, presents potential for the cultivation of grapes destined for the *in natura* consumption and for the elaboration of juices or wines. In particular, in the Southwest region of Paraná there are numerous vine orchards in small rural properties even though the grapes produced in this region had sporadically shown inferior quality to what is considered as good for wine and/or juice, especially when related to soluble solids content.

Fruit quality is linked directly to genetic characteristics, edaphoclimatic factors and cultural practices. The management adopted in the vine orchard usually aims to promote the penetration of solar rays inside the canopy, allowing the leaves located in the lower part of the plant to increase the photosynthetic rate and increase the availability of the photo assimilates for the fruits (Mota *et al*., 2009). The solar radiation is one of the main factors to obtain fruits with high quality, with regard to size, firmness, sugar content, coloration, anthocyanins, starch content and acidity (Procton and Lougheed, 1976; Erez and Flore, 1986; Jackson, 1989). Thus, the increase in the efficiency of its use is fundamental for obtaining more attractive fruits.

Among the management techniques performed in the pre-harvesting of the grape, which can improve the penetration of light and aeration inside the canopy, favouring the obtaining of quality fruits, are green pruning, defoliation, thinning, removal extra shoots (suckering) in the grape tree leaf (Mendonça *et al*., 2016).

However, many winegrowers are resistant to these practices, preferring the adoption of management techniques that allow them to obtain fruits with quality, without the need to increase the demand for labour and the costs in the property.

One of the alternatives that can be adopted is the use of reflective materials in the soil, since they allow to increase the total light absorbed and the distribution of the same inside the canopy, improving the quality of the fruits (Chavarria and Santos, 2009). In 'Fuji' apple the use of reflective materials in the soil was able to increase fruit quality as the red intensity of the epidermis and increase the size of fruits from the interior of the canopy (Andris and Crisosto, 1996).

The use of reflective plastics, under the canopy of plants, is a very used management technique in some countries of America, Europe and Asia. The main objective is to reflect the sunlight to the inside of the crown, to intensify the coloration of the fruit epidermis, to improve the flavour, as well as to antecipate maturation (Trevisan *et al.,* 2006; Meinhold *et al.,* 2011). This additional light is beneficial for photosynthesis and anthocyanin production (Layne *et al.,* 2002).

The principle of the use of these materials is the ability to reflect the solar radiation to the interior of the canopy and it can provide a 40% increase in the photosynthetically active radiation reflected when the soil is completely covered and, around 24%, when partially covered (Green *et al*., 1995).

The present study aimed to evaluate the use of reflective materials and management practices on plant physiology and physicochemical and biochemical quality of Merlot grape (*Vitis vinifera* L.).

2. Materials and Methods

The experiment was conducted in a commercial vineyard in the Dois Vizinhos city - PR, Brazil, in Santa Lucia community (25° 51' 08" S, 53° 06' 15" W, 594 m of altitude), for two productive cycles (2009/2010 and 2010/2011), indicated as cycles 1 and cycles 2 in the following text.

The studied vineyard was planted in 2004 with Merlot (*Vitis vinifera* L.) grafted 1103 rootstock Paulsen (*V. berlandieri* x *V. rupestris*). The spacings between rows are 3.0 m and between plants are 2.0 m in an espalier system, totalling 1.666 plants per hectare.

The experimental design was of randomized blocks, in multifactorial 3 x 2 ("material type" x "management practice"), with 4 replications, considering each 4 plants as a plot, the two central ones being useful, disregarding those near the border.

The "management practice" factor was divided into two levels, with and without its realization. The treatments with the management practices consisted of the adoption of the shoot topping, withdrawal of tertiary branches, disbudding and defoliation. In the treatments without the management practices, only the disbudding was done.

For "material type", two reflective films were tested in the soil: polypropylene white raffia plastic (reflective film 1) and metallic raffia plastic (reflective film 2), both placed below the projection of the crown of the plants, in the lines and between the lines. The films were placed in the vineyard 30 days after plants broke their dormancy; as third level, none film was used.

Upon reaching the harvesting point, in both cycles, the fruits were harvested and taken to the Laboratory of Plant Physiology, (Universidade Tecnológica Federal do Paraná - Campus Dois Vizinhos) for physicochemical and biochemical evaluations.

The evaluated variables were rot incidence (bunch and berry), bunch and berries weight, number of berries and bunches, berry drop, total soluble solids content (°Brix) (SS) titratable total acidity (TTA) (g tartaric acid 100 mL^{-1}), SS/TTA *ratio*, pH and production per plant (kg).

The incidence of rot was determined visually, considering fruits with damage when they presented lesions and typical characteristics of pathogen attack. The berry drop was determined by the percentage of berries detached from the general total of the bunch. The SS contents were determined by refractometry. The titratable acidity was determined by titrating 10 mL of juice in 90 mL of water diluted with 0.1 N NaOH solution to pH 8.1; the results being expressed in g of tartaric acid 100 mL^{-1}.

Analysis of biochemical variables of productive cycle 1 fruits included total proteins, total phenols, total and reducers sugars, phenylalanine ammonia-lyase enzyme (PAL), flavonoids and anthocyanins, constituting a total of four samples per treatment.

For total protein dosage, the pulp samples from the fruits of each treatment were macerated in a mortar with 10 mL of 0.2 M phosphate buffer (pH 7.5). Then the material was centrifuged (14.000 *g* for 10 min at 4°C) and the supernatant collected. For the quantification of the total protein content in the samples, the Bradford test (1976) was used and the spectrophotometer, model UV-SP2000-Spectrum at 630 nm, with bovine serum albumin as standard.

The quantification of the total phenolic compounds of the pulp of the fruits of each treatment was carried out in two stages; the first one, following the method adapted from Bieleski and Turner (1966) and the second one according to Jennings (1991). The total soluble sugar concentrations of fruit pulp from each treatment were determined by the phenol-sulfuric method as described by Dubois *et al.* (1956). The activity of PAL was evaluated based on the difference in absorbance resulting from the conversion of phenylalanine to trans-cinnamic acid (Hyodo *et al*., 1978).

Were evaluated the efficiency of water use (%), CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1}), water conductance (mol H_2O m^{-2} s^{-1}), intracellular CO_2 concentration (µmol CO_2 mol^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}) and foliar temperature (°C) in the productive cycle 1 and productive cycle 2. These physiological analyses of the plants were performed on three different dates (8th, 15th and 21st of January, 2010) for the productive cycle 1 and a date (14th of December, 2010) during the productive cycle 2. The gas exchange readings always started at 9:30 A.M. using an open gas measurement system equipped with a LI-6400XT infrared gas analyser (IRGA - LI-COR, Lincoln, Nebraska - USA) and an artificial source of red and blue light, performed on the fully developed and healthy middle third leaves of two plants per plot. The microclimatic conditions in the sample chamber were maintained constant during the readings, being 1100 µmol m^{-2} s^{-1} PAR (photosynthetically active radiation) and ambient CO_2 concentration (average of 383 µmol CO_2 mol^{-1}).

Microbiological analyses of the soil were also performed according to the type of coverage adopted, by means of the quantification of the respiratory activity of the same, according to method proposed by Öhlinger (1993). The analyses were carried out in the laboratory after the collection of the same, in the period of seven days after the harvest of each productive cycle to the depth of 10 cm.

Maximum, average and minimum temperatures of the plant body were obtained after reflection of the solar radiation for the productive cycle 1, captured by infrared images (thermographic) by a Therma CAM SC500 camera, always installed in the direction of the region of the plant, at 1 m of distance from the projection of the plant, discarding those of the border of each treatment. The temperature was obtained using the Therma CAM 200 Professional software based on the readings of 15th and 22nd of January, 2010 starting at 9:00 A.M.

The results of all variables were submitted to analysis of variance and the means were compared by the Tukey's test (α = 0.05), except for the soil microbiological analyses where the Duncan's test (α = 0.05) was applied. The data expressed in percentage was transformed by *sine arc* $\sqrt{x/100}$ and when expressed in numbers by $\sqrt{x+1}$, according to the Lilliefors' normality test. All analyses were performed by the statistical software SANEST (Zonta and Machado, 1984).

3. Results and Discussion

In the course of the experiment, excessive rainfall occurred during the two productive cycles (Figs. 1A and 1B). In addition to the occurrence of hail in productive cycle 1, which impaired the performance of phytosanitary treatments, creating a favourable environment for disease incidence, it being observed among these, a greater incidence of mildew (*Plasmopara viticola*), close to 25% of bunches or berries.

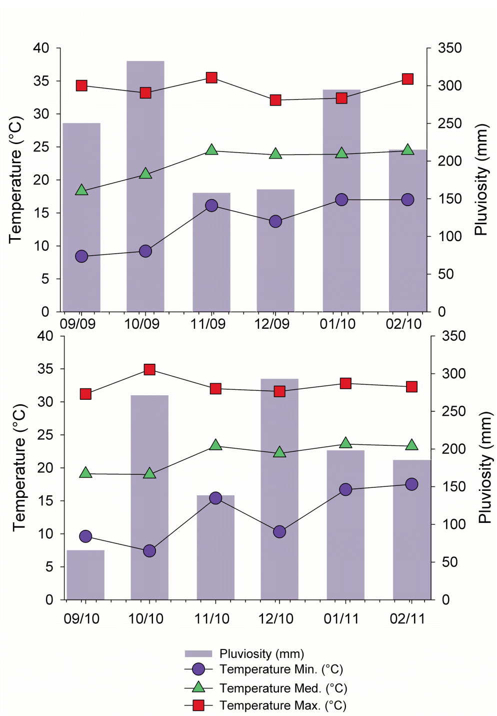

Fig. 1 - Data obtained from INMET's meteorological station, from productive cycle 1 (2009/2010) (A) and productive cycle 2 (2010/2011), located at Universidade Tecnológica Federal do Paraná - campus Dois Vizinhos - PR.

There were no statistically significant differences for the multifactorial interactions between the type of material and the management practices in the physicochemical variables (Table 1) during productive cycles 1 and 2 and biochemical (Table 2) of productive cycle 1, except for the SS/ATT *ratio* of the productive cycle 2. When factors were analyzed separately, significant results were observed, except for the total weight of berries and bunch, berry drop, yield per plant and total sugars concentration for the factor "material type" of production cycle 1 (Table 3) and number of berries per bunch for the factor "management practice" of cycle 1 (Table 4).

For berry drop of the grapes, in the productive cycle 1, it was verified that the use of the reflective film 2 (metallized raffia plastic) presented the lowest values in comparison to the reflective film 1 (white raffia plastic) and without film (Table 1). However, in production cycle 2 these values were lower in relation to cycle 1. The highest values of berry drop productive cycle 1 obtained in the reflective film 1 and without film, may be related to the greater attack of fungal diseases in the berries, because the cycle had an average temperature around 25°C and a high incidence of rainfall (280 mm) near the harvest season

Table 1 - Berry drop (%), bunch weight (g), weight of total and individual berries (g), SS (°Brix), ATT (g of tartaric acid 100 mL^{-1}), pH, SS/TTA ratio, number of berries per bunch, number of bunches, rot berries and bunches (%), production per plant (Kg) of Merlot grapes submitted or not to management practices with or without the use of reflexive films on the soil, in productive cycle 1 of (2009/2010) and productive cycle 2 of (2010/2011)

Treatment (z)	Berry drop	Bunch weight	Total weight of berries	Berry weight (g)	SS	ATT	pH	SS/ATT	Berries No. per bunch	Bunches No.	Berries with mildew	Bunch with mildew	Production (Kg)
						Productive cycle 1 (2009/2010)							
T1	12.71 NS	132.4 NS	125.70 NS	1.73 NS	16.64 NS	5.57 NS	3.44 NS	2.99 NS	72.76 NS	25.67 NS	25.24 NS	24.02 NS	3539.04 NS
T2	15.29	162.65	155.17	2.43	15.74	4.71	3.47	3.34	63.62	27.69	20.44	25.57	4222.51
T3	4.59	155.96	148.5	1.98	15.89	4.84	3.53	3.28	74.87	26.71	21.96	24.92	4535.66
T4	16.76	117.29	111	1.98	16.64	5.34	3.42	3.12	56.06	23.91	25.24	22.5	2831.5
T5	11.36	148.45	139.96	2.22	15.74	4.77	3.43	3.3	62.95	30.06	20.45	27.48	4503.96
T6	4.68	157.17	150.16	2.48	15.89	4.51	3.45	3.52	60.65	29.12	21.96	25.66	4554.81
CV (%)	18.77	14.06	14.29	18.33	7.98	8.29	2.21	16.52	8.12	8.79	4.54	5.1	24.37
						Productive cycle 2 (2010/2011)							
T1	1.59 NS	250.58 NS	237.82 NS	1.75 NS	60.25 NS	4.04 NS	3.67 NS	4.14**	137.2 NS	60.25 NS	4.47 NS	15.61 NS	15321.1 NS
T2	4.8	259.4	241.74	1.76	62.63	3.77	3.36	4.44	139.75	62.62	6.25	15.98	16389.38
T3	5.42	283.37	268.07	1.74	70.25	4.58	3.06	3.72	152.97	70.25	4.13	14.13	19997.24
T4	3.72	245.29	233.95	1.78	59.63	4.4	3.37	3.86	131.97	59.62	6.04	11.26	14907.88
T5	3.63	265.81	253.84	1.75	60.88	4.08	3.2	3.93	145.06	60.88	4.85	17.11	16278.66
T6	3.78	246.34	238.1	1.62	70.3	4.43	3.35	4.48	147.22	69.38	8.06	18.99	17504.40
CV (%)	27.72	18.25	17.92	6.6	25.57	10.93	2.99	11.79	16.16	25.57	49.36	26.24	30.64

NS= non-significant by the F test. ** Significant at 5% of probability.T1= with management practices and no reflexive film; T2= with management practices with reflexive polypropylene white raffia plastic (film 1); T3= with management practices with reflexive metallic raffia plastic (film 2); T4= without management practices and no reflexive film; T5= without management practices with reflexive polypropylene white raffia plastic (film 1) and (T6) without management practices with reflexive f metallic raffia plastic (film 2).

(Fig. 1), favouring the development of pathogens. Although they did not present statistically significant difference in both cycles, it is assumed that the use of metallized raffia plastic as a reflective film beneath the Merlot vines had provided greater heat in the area of the crown projection as compared to the use of white raffia plastic without film creating an environment with low levels of moisture, unfavourable to the attack of diseases. The same was visualized in productive cycle 2 whose incidence of fungal diseases attack was reduced, resulting in lower values of berry drop.

This hypothesis can be verified by the analysis of plant surface temperatures (minimum, average and maximum) after reflection of the solar radiation, captured by infrared (thermographic). In fact, we found divergent results for the type of cover and management practice, and the use of reflective film allowed an increase of up to 3°C in the average and maximum temperatures, in relation to the non-use of reflective film (Tables 3 and 4, respectively).

The same significant effect was not obtained for the maximum, average and minimum temperatures of the plants during the second evaluation (Table 2).

Table 2 - Flavonoids (mg/100 g), phenols (mg/g), total and reducing sugar (mg/g), anthocyanins (mg/100 g), PAL (UAbs/min/mg prot) and proteins (mg/g), minimum, average and maximum surface temperatures of the second evaluation, intracellular CO_2 concentration (µmol CO_2 mol^{-1}) of the first and third evaluation, of Merlot vine submitted or not to management practices and the use or not of reflexive films on the soil, in productive cycle 1 (2009/2010)

Treatment (z)	Flavonoids (mg/100 g)	Phenols (mg/g)	Total sugar (mg/g)	Antho-cyanins (mg/100 g)	PAL (UAbs/min/mg prot)	Proteins (mg/g)	Surface temperature in 2nd evaluation			Intracellular CO_2 concentration	
							Min (°C)	Med (°C)	Max (°C)	1st evaluation	3rd evaluation
T1	159.12 NS	1.78 NS	26.92 NS	128.08 NS	0.09 NS	0.92 NS	26.33 NS	30.23 NS	37.90 NS	255.55 NS	242.07 NS
T2	153.31	1.01	28.02	119.43	0.02	1.02	25.2	29.98	38.77	251.23	250.12
T3	110.41	1.32	30.76	86.01	0.04	0.79	24.85	31.38	41.65	246.61	241.07
T4	121.79	1.56	26.82	94.87	0.04	0.45	25.92	29.6	35.75	253.19	235.12
T5	151.74	1.63	27.76	118.22	0.03	0.74	26.83	31.13	39.73	255.55	244.82
T6	137.52	1.27	28.16	107.13	0.39	0.54	23.05	31.1	38.6	246.94	242.71
CV (%)	31.83	33.38	5.67	30.82	282.43	71.27	8.13	4.76	7.99	3.1	3.99

NS= non-significant by the F test. ** Significant at 5% of probability. (z) T1= with management practices and no reflexive film; T2= with management practices with reflexive polypropylene white raffia plastic (film 1); T3= with management practices with reflexive metallic raffia plastic (film 2); T4= without management practices and no reflexive film; T5= without management practices with reflexive polypropylene white raffia plastic (film 1) and (T6) without management practices with reflexive f metallic raffia plastic (film 2).

Table 3 - Berry drop (%), total weight of bunches and berries (g), production per plant (Kg) and total sugar (mg/g), minimal, medium and maximum surface temperatures during the first evaluation, of Merlot vines submitted or not to management practices with the use or not of reflexive films on the soil, in productive cycle 1 (2009/2010)

Type of material (z)	Berry drop	Total weight of berries	Bunch weight	Production per plant	Total sugar	Surface temperatures in first evaluation		
						Min (°C)	Med (°C)	Max (°C)
Reflexive film 1	13.26 a*	147.57 a	155.55 a	4519.81 a	27.92 ab	20.43 ns	29.84 ab	39.98 ab
Reflexive film 2	4.63 b	149.33 a	156.57 a	4388.66 ab	29.46 a	18.81	30.45 a	41.55 a
No film	14.68 a	118.35 b	124.84 b	3185.27 b	26.87 b	18.26	29.09 b	38.20 b
CV (%)	24.62	14.29	14.06	24.37	5.67	14.35	2.75	5.83

NS= non-significant by the F test. *Means followed by the same lowercase letter in the column don't differ significantly by the level of 5% by Tukey's test. (z) reflexive polypropylene white raffia plastic (Reflexive film 1); reflexive metallic raffia plastic (Reflexive film 2).

Table 4 - Minimum, medium and maximum surface temperatures during the first evaluation, number of berries per bunch in Merlot vines submitted or not to management practices, in productive cycle 1 (2009/2010)

Management practice	Surface temperature in first evaluation			N° of berries per bunch
	Min (°C)	Med (°C)	Max (°C)	
With management	20.75 a *	29.79 a	38.63 b	70.33 a
Without management	17.58 b	29.79 a	41.8 a	59.86 b
CV (%)	14.35	2.75	5.83	5.67

* Means followed by the same lowercase letter in the column do not differ significantly at 5% level of significance by Tukey's test.

Regarding the bunch weight, total berries weight, and production per plant, the highest values were obtained when the reflective films 1 and 2 (white raffia plastic and metallized raffia plastic, respectively) were used, and when observed in the productive cycle 2 these values were even more expressive, but without statistical difference for the effect of the treatments (Table 1). Observing the productive cycles 1 and 2, we noticed the alternation of productivity, which may be related to the lower pluviosity in the final period of development of the fruits (Fig. 1) and lower incidence of fungal diseases, providing best sanitary conditions of the vines resulting in increased production.

It is believed that the use of reflective films in productive cycle 1 may have provided an increase in the use of the photosynthetically active radiation by the plant, since when it reaches the films, it is reflected, mainly in the regions below the canopy, usually more shaded, increasing the production of photo assimilates, which allowed a greater weight of bunch and berries and, consequently, greater production. This fact can also be observed for productive cycle 2, however, with no statistical difference.

The influence of the radiation on the weight of the berries was also verified by Cargnello (1992), Kliewer and Lider (1968), and Todic *et al.* (2007), who verified that the weight of berries in plants exposed directly to the radiation was superior to those kept under shading conditions. This fact was also verified by Morrison (1988), reporting that shading of the leaves undermined the weight of the berries. During fruit development, shading affects the photosynthetic rate, which consequently limits carbohydrate sources for their development (Garriz *et al*., 1998).

In part, this hypothesis can be verified by the results obtained regarding the intracellular CO_2 concentration during the second evaluation in productive cycle 1, since there was a significant effect for the interaction between the factors tested (Table 5), demonstrating that when the management practice was performed, there was a lower value with the use of reflective film 2 (metallized raffia plastic), followed by the use of reflective film 1 (white raffia plastic) and during productive cycle 2, the results remained favourable for the use of some kind of material, however, with no statistical difference.

It is assumed that these lower values in the intracellular CO_2 concentration with the use of the reflective films are due to the greater photosynthetic activity by the plant, quickly converting all CO_2 absorbed into photo assimilates, as previously attributed to the results regarding bunch weight, total of berries weight and production per plant. The same effect of the films was obtained for the contents of total sugars, with a higher mean obtained in fruits, where the plants had reflective films 1 and 2, which is also related to the greater use of the photosynthetically active radiation.

Table 5 - Intracellular CO_2 concentration (µmol CO_2 mol^{-1}) during the second evaluation in Merlot vines submitted or not to management practices with or without reflexive films on the soil, in productive cycle 1 (2009/2010)

Material type [z]	Intracellular CO_2	
	With management	Without management
Reflexive film 1	266.35 abA*	259.50 aA
Reflexive film 2	261.19 bA	261.53 aA
No film	274.13 aA	254.73 aB
CV (%)	2.03	

* Means followed by the same lowercase letter in the column don't differ significantly by the level of 5% by Tukey's test.

[z] Reflexive polypropylene white raffia plastic (Reflexive film 1); reflexive metallic raffia plastic (Reflexive film 2).

In grapevine, Kliewer (1980) described that the fruits located inside the canopy, therefore, with lower illumination, presented less accumulation of sugar. This fact also occurred in the fruits of those plants that did not have reflective films below the canopy, which demonstrates that the use of these is important for the greater use of photosynthetically active radiation.

When submitted to the management practices the SS/ATT ratio of the fruits obtained higher results significantly using the reflective film 1 and without the use of reflective films. However, when they observed the non-use of management practices, the use of reflective material did not present statistical difference (Table 6).

The same phenomenon can be also observed concerning, the management practice. With its realiza-

Table 6 - SS/ATT ratio in Merlot vines submitted or not to management practices with or without reflexive films on the soil, in productive cycle 2 (2010/2011)

Material type [z]	SS/ATT ratio	
	With management	Without managment
Reflexive film 1	4.44 aA	3.93 aA
Reflexive film 2	3.72 bA	4.48 aA
No film	4.14 aA	3.86 aA
CV (%)	11.8	

* Means followed by the same lowercase letter in the column don't differ significantly by the level of 5% by Tukey's test.

[z] Reflexive polypropylene white raffia plastic (Reflexive film 1); reflexive metallic raffia plastic (Reflexive film 2).

tion, it is believed that there was increase in the photosynthetic rate of the plant, as results in the intracellular CO_2 concentration (Tables 5, 7, and 8), since practices like disbudding and defoliation allow higher incidence of light to enter the plants. In part, the greater light penetration can be proven by obtaining the increase in the minimum temperature of the plants during the first evaluation, when the management practice is carried out (Table 4), which is advantageous for greater metabolic activity of the plant, consequently providing increase in energy production and photo assimilates.

However, when there was no management practice the plants showed a higher maximum temperature in comparison with those in which it was carried out (Table 4). This demonstrates that the accomplishment of the management practice besides increasing the minimum temperature of the plant, provides lower maximum temperature, favouring the photosynthetic activity, since very high temperatures can provide stomatic closure, reducing the entrance of CO_2, which diminishes the photosynthetic activity in addition to reducing the absorption of water and nutrients. On the other hand, the average surface temperature of the plant presented equal means with or without the management practice (Table 4).

As for the other physiological variables (water use efficiency, CO_2 assimilation rate, water conductance, transpiration rate and leaf temperature), there was no significant interaction or for the factors individually during the production cycle 1 in the three evaluations carried out (Table 7), also verified for intracellular CO_2 concentration in the first and third evaluation (Table 2), and in the water use efficiency, CO_2 assimi-

Table 7 - Water use efficiency (%), CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1}), water conductance (mol H_2O m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), leaf temperature (°C), intracellular CO_2 concentration (µmol CO_2 mol^{-1}) of Merlot vines submitted or not to management practices with or without reflexive films on the soil, during the first, second and third evaluations, in productive cycle 1 (2009/2010)

Treatment (z)	Water use efficiency (%)			CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1})			Water conductance (mol H_2O m^{-2} s^{-1})			Transpiration rate (mmol H_2O m^{-2} s^{-1})			Leaf temperature (°C)		
	1ª Ev.	2ª Ev.	3ª Ev.	1ª Ev.	2ª Ev.	3ª Ev.	1ª Ev.	2ª Ev.	3ª Ev.	1ª Ev.	2ª Ev.	3ª Ev.	1ª Ev.	2ª Ev.	3ª Ev.
T1	0.26 NS	0.25 NS	0.38 NS	12.12 NS	10.55 NS	10.9 NS	0.23 NS	0.24 NS	0.18 NS	4.76 NS	4.76 NS	2.94 NS	32.59 NS	31.58 NS	28.3 NS
T2	0.27	0.27	0.38	12.57	11.93	11.8	0.26	0.25	0.23	4.69	4.69	3.41	31.82	31.16	28.38
T3	0.31	0.3	0.37	11.92	12.53	10.69	0.22	0.23	0.2	4.57	4.57	3.27	32.45	31.13	28.58
T4	0.28	0.3	0.39	12.44	11.52	10.98	0.23	0.22	0.18	4.5	4.5	2.96	31.84	31.21	28.6
T5	0.24	0.27	0.39	12.96	11.05	11.5	0.26	0.21	0.2	5.34	5.34	3.23	32.94	31.82	28.49
T6	0.26	0.3	0.37	12.9	12.62	11.78	0.23	0.25	0.17	4.34	4.34	2.8	31.78	30.93	28.02
CV (%)	15.03	9.78	10.72	7.22	9.69	10.72	10.85	15.89	20.67	11.69	13.37	13.43	2.99	2.07	1.41

NS= non-significant by the F test. (z) (T1) with management practices and no reflexive film; (T2) with management practices with reflexive polypropylene white raffia plastic (film 1); (T3) with management practices with reflexive metallic raffia plastic (film 2); (T4) without management practices and no reflexive film; (T5) without management practices with reflexive polypropylene white raffia plastic (film 1) and (T6) without management practices with reflexive f metallic raffia plastic (film 2).

Table 8 - Intracellular CO_2 concentration (µmol CO_2 mol^{-1}), water use efficiency (%), CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1}), water conductance (mol H_2O m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), leaf temperature (°C) of Merlot vines submitted or not to management practices with or without reflexive films on the soil, in productive cycle 2 (2010/2011)

Treatment (z)	Intracellular CO_2 concentration	Water efficiency (%)	CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1})	Water conductance (mol H_2O m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Leaf temperature (°C)
T1	261.31 NS	1.43 NS	14.67 NS	0.23 NS	3.29 **	24.44 NS
T2	263.7	1.46	14.36	0.23	3.2	24.51
T3	263.09	1.46	14.09	0.22	3.36	24.32
T4	260.05	1.41	15.02	0.23	3.23	24.37
T5	263.43	1.44	14.71	0.23	3.67	24.5
T6	261.82	1.44	14.44	0.22	3.04	24.26
CV (%)	2.78	7.84	7.85	8.74	7.48	2.85

NS= non-significant by the F test. (z) (T1) with management practices and no reflexive film; (T2) with management practices with reflexive polypropylene white raffia plastic (film 1); (T3) with management practices with reflexive metallic raffia plastic (film 2); (T4) without management practices and no reflexive film; (T5) without management practices with reflexive polypropylene white raffia plastic (film 1) and (T6) without management practices with reflexive f metallic raffia plastic (film 2).

lation rate, water conductance for productive cycle 2. The transpiration rate of productive cycle 2 showed significant interaction indicating that the use of reflective film 2 in conjunction with the management practice were superior to the other treatments, which possibly may have contributed to the quantity and quality of the fruits in the cycle productive 2 (Table 9).

Table 9 - Transpiration rate (mmol H_2O m^{-2} s^{-1}) of Merlot vines submitted or not to management practices with or without reflexive films on the soil, in productive cycle 2 (2010/2011)

Material type [(z)]	Transpiration rate (mmol H_2O m^{-2} s^{-1})	
	With practice	Without practice
Reflexive film 1	3.20 b A	3.67 a A
Reflexive film 2	3.36 a A	3.04 a AB
No film	3.29 a A	3.23 a B
CV (%)	7.48	

* Means followed by the same lowercase letter in the column don't differ significantly by the level of 5% by Tukey's test.

(z) Reflexive polypropylene white raffia plastic (Reflexive film 1); reflexive metallic raffia plastic (Reflexive film 2).

It was verified that the adoption of management practices in spite of not influencing most of the variables analysed in the present study, provided a greater number of berries when carried out on the Merlot vines in productive cycle 1 (Table 4), demonstrating the importance of its execution for the winegrower.

It is believed that the lower number of berries in the plants without management practices can be due to the attack of fungal diseases. Champagnol (1984) and Fregoni (1998) reported in their studies that mildew, after effective fruiting, may be one of the causes for the lower number of berries per bunch. Indeed, the practices of shoot topping, suckering, withdrawal of tertiary branches and defoliation may have improved the penetration of light and air in the area of projection of the plants, not allowing the formation of favourable microclimate for fungal diseases.

Regarding the microbiological analysis of the soil, a statistical difference was observed for the type of reflexive film used in productive cycle 1, with the highest microbial activity in the soil when white raffia plastic and metallized raffia plastic were used. The latter did not differ statistically from the treatment without reflexive film on the soil (Table 10). For production cycle 2, although the values obtained were higher, which makes it favourable for productivity, the same did not present statistical differences.

It is assumed that the use of the white or metallic raffia plastic films to reflect the solar radiation allowed lower temperature in the soil, as well as reduced water loss, favouring the full metabolic activity of soil microorganisms, given the maintenance of conditions optimum temperature and humidity, decreasing problems with water stress for the plant. Hungria *et al.* (1997) mentioned that the reduction of soil microbiota impairs the temporary fixation of nutrients, increasing their losses and resulting in soil impoverishment.

Table 10 - Microbiological respiratory activity of the soil according to the material type addopted for Merlot vines, in productive cycle 1 (2009/2010) and productive cycle 2 (2010/2011)

Material type [(z)]	Microbiological resiratory activity (mg of CO_2 kg of soil^{-1})
Productive cycle of 2009/2010	
Reflexive film 1[(z)]	45.65 a
Reflexive film 2	43.45 ab
No film	35.75 b
CV (%)	11
Productive cycle of 2010/2011	
Reflexive film 1	100.10 NS
Reflexive film 2	102.3
No film	82.13
CV (%)	30.87

* Means followed by the same lowercase letter in the column don't differ significantly by the level of 5% by Tukey's test.

(z) Reflexive polypropylene white raffia plastic (Reflexive film 1); reflexive metallic raffia plastic (Reflexive film 2).

In general, soils with high microbiological activity indicate low human interference with the environment and are, in principle, desirable for crops, since they contribute to a faster decomposition and re-synthesis of organic matter, to nutrient cycling, to specific biochemical transformations (nitrification, denitrification, oxidation and sulphur reduction) for biological nitrogen fixation (Silveira and Freitas, 2007).

Thus, the use of reflective material on the soil presents additional benefits to the crop and the environment, and according to the peculiarities of the area, the effects of the application of the technology on the soil microbial population can become as important or more than the benefits obtained directly on the quality of the fruits. Attention must be paid to determining the most appropriate moment for the installation of the reflective film in the soil, since the influence of the radiation on the quality of the fruit varies according to the phenological stage of the crop (Weston and Barth, 1997; Kader, 2002; Meinhold *et al.,* 2011).

4. Conclusions

The use of reflective films on the soil improved the quality of Merlot grapes in some aspects as reducing berry drop, consequently increasing productivity.

Management practices here tested didn't have influence on the physicochemical and biochemical characteristics of the fruits, resulting only in more berries per bunch.

The use of reflective films on the soil provided higher microbiological activity in the soil, which may be important for grapevines.

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Adv. Hort. Sci., 2019 33(1): 49-56 DOI: 10.13128/ahs-22863

AHS
Advances in Horticultural Science

Ameliorate the cadmium toxicity in *Solanum tuberosum* L. plants with selenium and silicon application

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Citation:
DORNELES A.O.S., SOARES PEREIRA A., POSSEBOM G., PELIGRINOTTI TAROUCO C., ROSSATO L.V., ALMERI TABALDI L., 2019 - *Ameliorate the cadmium toxicity in Solanum tuberosum L. plants with selenium and silicon application*. - Adv. Hort. Sci., 33(1):49-56

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 9 March 2018
Accepted for publication 4 October 2018

Key words: elements beneficial, potato, toxic metals, toxicity.

Abstract: The present study aimed to prove the efficiency of Se or Si as relievers of the Cd toxicity in potato plants. *Solanum tuberosum* plants, Asterix genotype, from *in vitro* propagation were placed in pots with sand and irrigated with complete nutrient solution for 60 days under six treatments: T1: Control (nutrient solution); T2: 2.5 µM Se; T3: 2.5 mM Si; T4: 50 µM Cd; T5: 50 µM Cd + 2.5 µM Se; T6: 50µM Cd + 2.5 mM Si. The treatments were arranged in completely randomized design, with four replicates for each treatment and six plants per replicate. The plants were collected at 30 and 60 days after application of the treatments. Cadmium was highly toxic in all parameters (dry and fresh weight, plant height, leaf number, leaf area, root and photosynthetic parameters), in both assessments. However, Se and Si were effective in mitigating Cd toxicity in all parameters, although Si has been shown to be more efficient than Se in dry weight and plant height parameters. Thus, from data obtained in this study, it is clear that the beneficial elements tested have power to ameliorate Cd toxicity.

1. Introduction

Plants differ in their ability to absorb, accumulate and tolerate heavy metals, including cadmium (Cd), and toxic levels of heavy metals affect a variety of plant processes (Gupta *et al.,* 2013). Cadmium is one of most toxic heavy metals, having a high mobility in environment (Tang *et al.*, 2015), being absorbed by roots and transported to shoot of many plant species (Shi *et al.,* 2005). Although Cd has no known biological function in plants (Pence *et al*., 2000; Pereira *et al*., 2016), it can be easily absorbed and transported by xylem (Lux *et al.,* 2011), since it has an electronic configuration and state of zinc-like valence (Nan *et al.,* 2002). This heavy metal is toxic even at low concentrations, inducing stress responses in plants from 5-10 µg g^{-1} soil (While and Brown, 2010). Several studies have reported significant reductions in biomass accumulation in plants exposed to Cd (Farooq *et al.,* 2013; Said *et al.,* 2014; Vaculík *et al.,* 2015), and this inhibition in biomass production can occur in short time of exposure to this heavy metal (Han *et al.,* 2015). In contrast to others toxic heavy metals, Cd present in soil is easily absorbed by plant roots, particularly in acidic soils (Guimarães *et al.,* 2008). The plant development stage and time of exposure to heavy metal affect the absorption and distribution of Cd in different parts of plant (Gonçalves *et al.,* 2009 a).

Thus, it is necessary to develop strategies that result in lesser absorption of these toxic elements present in soil by plants, optimizing the use of natural resources and production of safe food, especially when it comes to a plant used in food such as potatoes. Potato (*Solanum tuberosum* L.) is one of main foods for mankind, consumed by more than one billion people worldwide, due to its composition, gastronomic and technological versatility, as well as the low market price of tubers (Coelho *et al.,* 1999; Dorneles *et al.,* 2016). According to Birch *et al.* (2012), potatoes are third most important crop in world, behind only rice and wheat. The potato is susceptible to Cd, and this sensitivity can be accentuated by level of Cd in soil, time of exposure and cultivar (Gonçalves *et al.,* 2009 b).

One of options sought to solve this problem with Cd in plant growth is use of beneficial elements, which when used in low concentrations can alleviate the damaging Cd effects. In this sense, selenium (Se) and silicon (Si) are recognized as beneficial elements for growth of some plants, and can increase the tolerance of plants to abiotic stresses. These elements are recognized as being capable of mitigating metal toxicity in plants (Wu *et al.,* 2017). However, it is necessary to evaluate the potential use of these mitigating elements in the presence of Cd, since some studies show that these may not be effective to reduce the toxicity of this element in some species (Khattab, 2004; Liu *et al.,* 2013). However, Si has been shown to be effective in alleviating Al toxicity in potato plants (Dorneles *et al.,* 2016) and Cd in species such as peanuts (Shi *et al.,* 2010), Chinese cabbage (Song *et al.,* 2009), rice (Tripathi *et al.,* 2013), maize (Lukacová *et al.,* 2013), wheat (Khan *et al.,* 2015) and sunflower (Said *et al.,* 2014). Selenium, however, has stress-alleviating properties that are more focused on biochemical mechanisms activation (Kumar *et al.,* 2012; Feng *et al.,* 2013; Tamaoki and Maruyama-Nakashita, 2017). However, it already has great potential in use to relieve stresses by metals (He *et al.,* 2004; Pezzarossa *et al.,* 2012). However, this element, to date, has not been tested as potential to relieve the stress caused by Cd toxicity in potato plants. Thus, the aim of this work was to test the possibility of using Se or Si as reliever of Cd toxicity in potato plants.

2. Materials and Methods

Plants of *Solanum tuberosum* L., Asterix genotype, were used for the experiment, which were propagated *in vitro* from nodal segments for 25 days in MS culture medium (Murashige and Skoog, 1962).

After this period, plants were transferred to plastic vessels containing 1 plant each and 5 kg of sand, being watered daily with complete nutritive solution. This solution had the following composition (mg L^{-1}): 85.31 N; 7.54 P; 11.54 S; 97.64 Ca; 23.68 Mg; 104.75 K; 176.76 Cl; 0.27 de B; 0.05 Mo; 0.01 Ni; 0.13 Zn; 0.03 Cu; 0.11 Mn and 2.68 Fe ($FeSO_4$/Na-EDTA). After five-day of acclimation period, treatments were applied, which consisted of following combinations: Treatment 1: Complete nutritional solution and absence of Cd, Se and Si; Treatment 2: Complete nutritional solution + 2.5 µM Se; Treatment 3: Complete nutritional solution + 2.5 mM Si; Treatment 4: Complete nutritional solution + 50 µM Cd; Treatment 5: Complete nutritional solution + 50 µM Cd + 2.5 µM Se; Treatment 6: Complete nutritional solution + 50 µM Cd + 2.5 mM Si. These solutions were applied daily maintaining 80% of the vessel capacity, which was monitored by daily determining the weight of the vessel. The treatments were arranged in a completely randomized design, with four replicates for each treatment and six plants per replicate. The pH of the solutions was adjusted daily (4.5±0.1).

Two collects were performed, the first collect being at 30 days of exposure to treatments and the second at 60 days. In both collections, the following evaluations were carried out:

- Photosynthetic parameters were evaluated in fourth fully expanded leaf of four plants per replicate: the photosynthetic rate (A - µmol CO_2 m^{-2} s^{-1}), the stomatal conductance of water vapors (Gs - mol H_2O m^{-2} s^{-1}), internal CO_2 concentration (Ci - µmol

m^{-2}), water use efficiency (WUE - mol CO_2 mol H_2O^{-1}), and the transpiration rate (Tr mmol - mmol H_2O m^{-2} s^{-1}) obtained by the ratio between the amount of CO_2 fixed by photosynthesis and the internal CO_2 concentration. The evaluations were carried out in period between 8 and 11h with use of IRGA portable meter, brand LI-COR, model LI-6400XT.

- Growth parameters: fresh and dry biomass, height, leaf area, number of leaves, and morphological parameters of the root system (length, diameter, volume and number of root branches), according to methodology described by Dorneles *et al.* (2016).

For statistical analysis of data, it was verified the normality of error distribution through Anderson-Darling test and homogeneity of error variances through the Bartlett test (Estatcamp, 2012) for all variables of experiment. The averages were submitted to analysis of variance and compared by the Scott-Knott test, with 5% significance, using Sisvar (Ferreira, 2011). The graphic program used was SigmaPlot 12.5.

3. Results

For experimental conditions tested, cadmium (Cd) presented great toxicity to potato plants both at 30 and 60 days of growth. This is evidenced by averages of fresh weight accumulation obtained by plants exposed to Cd, which did not increase after 30 days of cultivation, remaining unchanged up to 60 days (Fig. 1) both being statistically smaller than in the control treatment. Besides, in general, when applied in isolation, Si and Se did not promote greater accumulation of fresh and dry weight in comparison to control plants (Figs. 1, 2). However, both elements tested proved to be effective in mitigating Cd toxicity from first collect, inducing a higher accumulation of fresh weight in both tissues, compared to the treatment where only Cd was applied. The enhancing effects of Se and Si are more evident in shoot, where plants exposed to Cd combined with Se or Si maintained some growth in second collect (Fig. 1 A). The roots of potato plants (Fig. 1 B) also show a significant improvement in presence of both beneficial elements. This probably contributed to growth of shoot, considering that roots are the first tissue to come into contact with Cd.

For dry weight, potato plants showed similar behavior to that observed for fresh weight in both tissues (Fig. 2). Cadmium showed to be toxic to both tissues for dry weight, inhibiting this accumulation

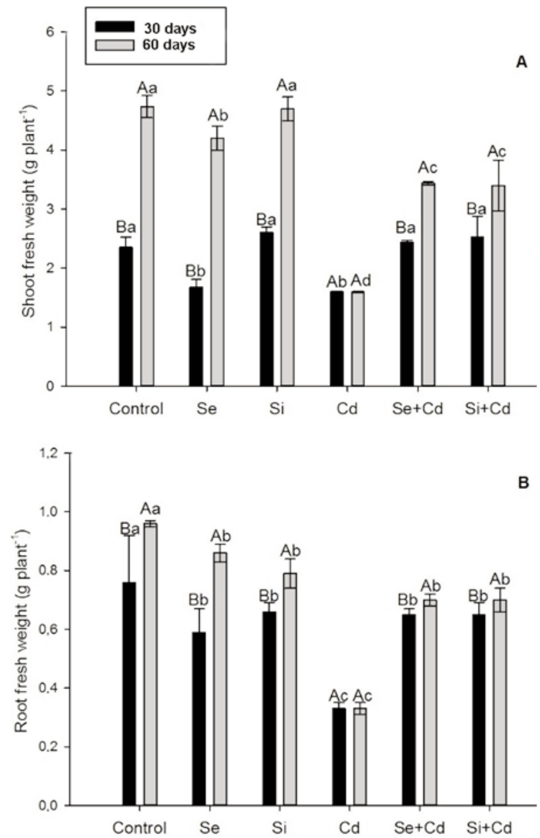

Fig. 1 - Effect of selenium (2.5 µM) or silicon (2.5 mM) on shoot (A) and roots fresh weight (B) of potato plants grown in presence of cadmium (50 µM) at 30 and 60 days after application of the treatments. Different lowercase letters indicate significant differences between treatments in same collect. Different uppercase letters indicate significant differences between collects for same treatment.

until last collect. However, Se and Si induced biomass accumulation similar to control plants, even when applied together with Cd. Silicon induced greater accumulation of shoot dry weight when compared to Se (Fig. 2 A). In roots, Cd prevented the dry weight increase, while plants treated with Se and Si presented increase in root dry weight in second collect (Fig. 2 B), that is, Se and Si alleviated the toxic effects of cadmium.

In addition to affecting the production of fresh and dry biomass, Cd inhibited the production and expansion of leaves, as well as the growth in height of potato plants. However, plants that received Si and Se together with Cd obtained higher means even in comparison to control plants (Figs. 3 A, B). In addition, the number of leaves and height of plants exposed to Cd were higher when applied Si, in comparison to plants treated with Se as amendment. Both Se and Si were effective in reducing the toxicity of cadmium from first collect, inducing higher height, number of leaves and leaf area than plants with only

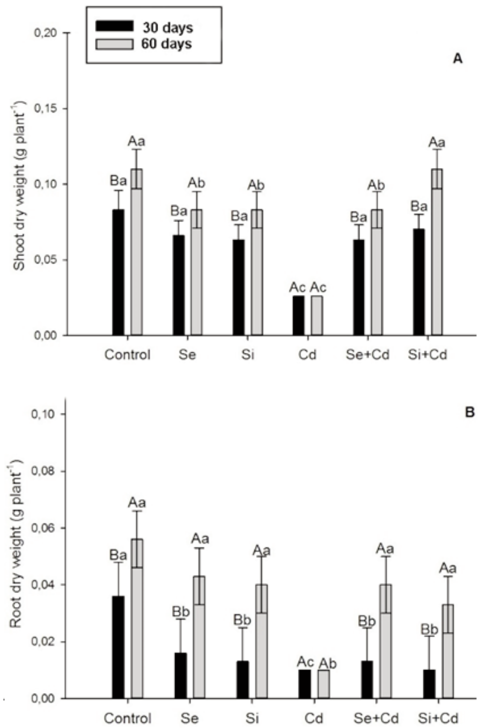

Fig. 2 - Effect of selenium (2.5 µM) or silicon (2.5 mM) on shoot (A) and roots dry weight (B) of potato plants grown in cadmium presence (50 µM) at 30 and 60 days after application of the treatments. Different lowercase letters indicate significant differences between treatments in same collect. Different uppercase letters indicate significant differences between collects for same treatment.

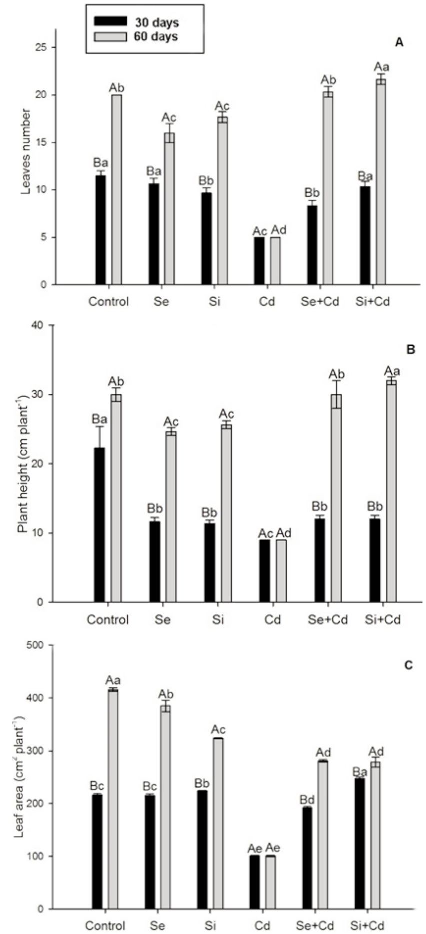

Fig. 3 - Effect of selenium (2.5 µM) or silicon (2.5 mM) on leaves number (A), plants height (B) and leaf area (C) of potato plants grown in cadmium presence (50 µM) at 30 and 60 days after application of the treatments. Different lowercase letters indicate significant differences between treatments in same collect. Different upper case letters indicate significant differences between collects for same treatment.

Cd in nutrient solution.

There was no statistical difference between treatments for root length and root diameter at 30 days, but plants treated with Cd presented root length 45% lower than the control (Table 1). At 60 days, plants exposed to Cd showed root length 16% and root

Table 1 - Length, diameter, volume and number of branch roots of *Solanum tuberosum* plants grown in presence of selenium (2.5 µM), silicon (2.5 mM) and cadmium (50 µM) at 30 and 60 days after application of the treatments

Collect	Treatments	Root length (cm)	Root diameter (mm)	Root volume (cm^3)	Branch number
30 days	Control	798±0.24 Aa	0.35±0.02 Ba	1.64±0.49 Aa	715±68 Aa
	Se	776±18.3 Aa	0.38±0.04 Ba	0.91±0.25 Ab	394±18 Bb
	Si	743±9.24 Aa	0.35±0.02 Ba	0.84±0.19 Ab	401±20 Bb
	Cd	438±3.76 Ba	0.34±0.02 Aa	0.37±0.07 Ac	201±13 Ad
	Se + Cd	650±4.62 Aa	0.36±0.00 Ba	0.65±0.05 Ab	323±35 Bc
	Si + Cd	711±3.43 Aa	0.41±0.03 Aa	0.83±0.08 Ab	318±38 Bc
60 days	Control	998±0.57 Aa	0.45±0.02 Aa	1.83±0.21 Aa	790±48 Aa
	Se	884±21.6 Aa	0.45±0.01 Aa	0.85±0.03 Ab	794±58 Aa
	Si	862±25.2 Aa	0.45±0.02 Aa	0.76±0.08 Ab	790±62 Aa
	Cd	839±10.11 Ab	0.34±0.02 Ab	0.34±0.02 Ac	253±24 Ac
	Se + Cd	795±5.50 Aa	0.46±0.00 Aa	0.75±0.04 Ab	423±21 Ab
	Si + Cd	791±6.11 Aa	0.44±0.02 Aa	0.85±0.07 Ab	452±22 Ab

Different lowercase letters indicate significant differences between treatments in same collect. Different upper case letters indicate significant differences between collects for same treatment.

diameter 25% lower, compared to the control. On the other hand, all treatments presented lower root volume compared to control, but this effect was more significant in the treatment containing only Cd in the growth medium, both at 30 and 60 days of cultivation. This same behavior was observed for the number of branches at 30 days. At 60 days, branch numbers was lower in the treatments containing Cd, Se + Cd and Si + Cd, and this effect was more significant in the treatment containing only Cd in the growth medium. Cadmium showed to be highly toxic, reducing volume of roots and ramifications to less than half of values presented by control plants.

However, Si and Se presented an amendment effect on all root variables, mainly for total length and diameter, both at 30 and 60 days of cultivation. In these variables, plants exposed to Cd treated with both Si and Se were statistically the same as control plants.

Plants exposed to Cd had photosynthetic rate (A) reduced by 37% compared to control plants at first collect, and 42% at second collect (Table 2). Besides, Cd caused significant reductions in stomatal conductance (GS), internal CO_2 concentration (Ci) and transpiratory rate (Trmmol) in both collects. The water use efficiency (WUE) in both collects was higher only for the treatment with Se + Cd. However, in both collects Si and Se were effective in mitigation of Cd toxicity for all variables except for WUE (Table 2).

4. Discussion and Conclusions

In the present study, Cd promoted a significant reduction in biomass production in potato plants (Fig. 1). The reduction in biomass accumulation caused by Cd may be due, in part, to its effect on the inhibition of nutrient uptake by roots (Cao *et al.,* 2014; Li *et al.,* 2016).

The effects of Cd on absorption of nutrients may be due to damage caused by this element in the roots. Since root tissues are first to come into contact with the Cd present in solution, they are also the most affected. Several studies have already reported negative effects of Cd on cellular level of root tissues (Benavides *et al.,* 2005; Lux *et al.,* 2011; Martinka *et al.*, 2014).

The data presented in this work show the significant reduction in root parameters caused by Cd. This behavior may be due to effect of Cd on degradation of membranes and nucleotides of root cells (Han *et al.,* 2015). This degradation of membranes and organelles can be explained by the increase in concentration of reactive oxygen species (ROS) induced by Cd (Chou *et al.,* 2012; Farooq *et al.,* 2013; Said *et al.,* 2014; Han *et al.,* 2015). It is possible that this effect of Cd on increase of ROS, reported in these studies, may inhibit cell division of roots (Said *et al.,* 2014) In addition, the Cd has affinity for phosphates and some amino acids components of enzymes and

Table 2 - Effect of selenium (2.5 µM) or silicon (2.5 mM) on photosynthetic rate (A-µmol CO_2 m^{-2} s^{-1}), stomatal conductance (GS - mol H_2O m^{-2} s^{-1}), internal CO_2 concentration (Ci - µmol m^{-2} s^{-1}), transpiration rate (Trmmol - H_2O mmol m^{-2} s^{-1}),and water use efficiency (WUE - CO_2 mol H_2O mol^{-1}) of *Solanum tuberosum* plants grown in cadmium presence (50 µM) at 30 and 60 days after application of the treatments

Collect	Treatment	A	GS	Ci	Trmmol	WUE
30 days	Control	9.47±0.13 Ba	0.20±0.07 Aa	288±0.48 Aa	9.47±0.13 Ba	192±4.59 Bb
	Se	8.39±0.32 Bb	0.16±0.04 Aa	295±11.8 Ba	8.39±0.32 Bb	178±11.8 Bb
	Si	8.92±0.31 Bb	0.04±0.00 Ac	272±2.44 Bb	8.92±0.31 Bb	200±20.3 Bb
	Cd	5.92±0.27 Bc	0.10±0.00 Ab	257±8.54 Ac	5.92±0.27 Bc	228±8.63 Ab
	Se + Cd	8.73±0.07 Bb	0.06±0.03 Ac	297±5.57 Aa	8.73±0.07 Bb	308±3.05 Aa
	Si + Cd	8.26±0.03 Bb	0.10±0.00 Ab	255±9.29 Ac	8.26±0.03 Bb	236±12.6 Ab
60 days	Control	12.5±0.14 Aa	0.20±0.03 Aa	289±0.24 Aa	12.5±0.14 Aa	205±0.25 Ab
	Se	10.3±0.32 Ab	0.14±0.01 Aa	268±14.6 Ab	10.3±0.32 Ab	215±0.24 Ab
	Si	10.1±0.06 Ab	0.06±0.00 Ab	298±5.53 Aa	10.1±0.06 Ab	236±0.02 Ab
	Cd	7.41±0.31 Ad	0.04±0.00 Bc	269±8.13 Ab	7.41±0.31 Ad	220±0.26 Ab
	Se + Cd	9.08±0.72 Ac	0.08±0.00 Ab	257±8.54 Bc	9.08±0.72 Ac	298±0.14 Aa
	Si + Cd	8.98±0.61 Ac	0.10±0.00 Ab	255±9.29 Ac	8.98±0.61 Ac	245±0.22 Ab

Different lowercase letters indicate significant differences between treatments in same collect. Different upper case letters indicate significant differences between collects for same treatment.

proteins, which leads, in addition to damage to membranes, genetic damage and can disrupt oxidative phosphorylation in exposed tissues (Hasanuzzaman and Fujita, 2012; Nahar *et al.,* 2016).

The present study also shows the effect of Cd under photosynthetic parameters, in which there was a significant reduction in plants exposed to this heavy metal. This reduction in photosynthetic parameters may be a consequence of the degradation of chlorophylls caused by Cd (López-Millán *et al.,* 2009). Furthermore, Cd causes disorder in arrangement of grana and thylakoids, which limits the efficiency of most photosynthetic parameters (Han *et al.,* 2015; Bayçu *et al.,* 2016).

The potato plants used in present study, treated with Si or Se as amendments of Cd toxicity, showed higher biomass production than plants exposed to only Cd. In addition, Se and Si significantly reduced the damage caused by Cd in photosynthetic parameters. These results of biomass and photosynthetic rate are related, since the beneficial effects of Si and Si on the Cd toxicity in photosynthetic parameters are expressed, to a greater or lesser degree, in higher production of biomass by plants.

Each element used as amendment in this work (Se or Si) act in different ways in the plant. Silicon have been proven to be effective in easing stress by Cd in different species such as cucumber (Feng *et al.,* 2010), corn (Malčovská *et al.,* 2014; Vaculík *et al.,* 2015) and cotton (Farooq *et al.,* 2016). This effect of Si may be due to its deposition on cell wall which increases its plasticity and elasticity (Vaculík *et al.,* 2009). The Si deposited on cell wall of the plants may increase Cd retention in apoplast (Lukačová *et al.,* 2013; Vaculík *et al.,* 2015), which may reduce the availability and translocation of Cd. Due to these cell wall modulations, Si can inhibit Cd uptake (Liu *et al.,* 2013). These effects of Si can explain the more evident amelioration of Cd in shoot than in roots for dry weight in potato plants (Fig. 2). In addition, silicon has photoprotective properties when deposited on leaves (Tripathi *et al.,* 2017), which may explain the mitigation of Cd toxicity in gas exchange parameters.

There is a chance that Se also has the property of forming complexes with toxic or heavy metals, but were only found complexes of Se-mercury (Hg) in plants (Said *et al*., 2014). However, there are reports that selenium reduces Cd accumulation in tissues (*Lactuca sativa* L.) (He *et al.,* 2004). While Si is recognized for increasing the enzymatic antioxidants activity (Debona *et al.,* 2017), Se has the property of inducing resistance by activating routes of synthesis of hormones linked to stress response and antioxidant activities (Freeman *et al.,* 2010; Feng *et al.,* 2013; Tamaoki and Maruyama-Nakashita, 2017). Thus, the mechanism of mitigation of Se may be more directly related to antioxidant system. Many studies have reported this effect of Se under plant antioxidant enzymes, as well as its effect on direct removal of ROS (Cartes *et al.,* 2010; Zembala *et al.,* 2010). In present study, the Cd toxicity was reduced in most of evaluated parameters, mainly under photosynthetic parameters. The possible effect of Se under enzyme activity may help to explain its most evident effect under these parameters. Selenium was more effective in reducing the effects of Cd on photosynthetic parameters than for other parameters evaluated.

For potato plants under experimental conditions used in this study, Si and Se elements proved to be effective in mitigating Cd toxicity. Thus, these elements have potential for use in fertilizers applied to contaminated soils. Future biochemical, histological and molecular analyzes may complement the elucidation of action mechanisms these elements.

Acknowledgements

The authors thank the Coordenação e Aperfeiçoamento de Pessoal de Nível Superior, Conselho Nacional de Desenvolvimento Científico e Tecnológico, and Fundação de Amparo à Pesquisa de Estado do Rio Grande do Sul for the research fellowships.

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DOI: 10.13128/ahs-23328

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Effects of pre-harvest applications of different source of calcium on the cell wall fractions and stem bending disorder of Gerbera (*Gerbera jamesonii* L.) cultivar flowers

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Key words: catalase, cellulose, lignin, membrane stability index, superoxide dismutase.

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Citation:
AGHDAM M., HASSANPOUR ASIL M., GHASEMNEZHAD M., MOUSAVI MIRKALAEI S.A.A., 2019 - *Effects of pre-harvest applications of different source of calcium on the cell wall fractions and stem bending disorder of Gerbera (Gerbera jamesonii L.) cultivar flowers* - Adv. Hort. Sci., 33(1): 57-65

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 9 March 2018
Accepted for publication 4 October 2018

Abstract: Gerbera flower belongs to the composite family and is one of the top five cut flowers in the world in terms of production and consumption, which has a great economic value in the international flowering industry. This study was designed to evaluate whether calcium pre-harvest application, provided through 0, 0.5, 1 and 1.5% of calcium chloride ($CaCl_2$) and calcium nitrate ($CaNO_3$), could extend the day of stem bending of gerbera cut flower. In the present study, we used two gerbera cultivars 'Intense' and 'Rosaline' as resistant and sensitive to stem bending, respectively. For evaluation of associated traits with stem bending, the produced flowers were kept in a vase solution containing 200 mg/L hydroxyquinoline with temperature conditions of 20°C. The results showed that day of stem bending of flowers extended 9.62 and 10.37 days by application of 1% $CaCl_2$ for 'Rosaline' and 'Intense' respectively. All treatments were effective in the increasing relative water content of flower due to increase water uptake. The results also revealed that the calcium pretreatment delayed flowers senescence and increased antioxidant enzyme activity. Application of calcium resulted in an increase in membrane stability index in the cut flowers of both cultivars, providing evidence for delay of senescence in calcium-treated cut flowers. Also, results showed that calcium application significantly increased lignin, cellulose and hemicellulose content of both cultivars. The maximum and the minimum lignin and cellulose content were observed in resistant and sensitive cultivars, respectively. In general, pre-harvest application of calcium (especially 1% $CaCl_2$) with increasing of antioxidant enzyme activity and stem lignification led to decreasing of stem bending disorder in both cultivars.

1. Introduction

Gerbera is one of the most important ornamental plants in the world used widely as cut flower or potted flower (Çelikel and Reid, 2002; Perik *et al.*, 2014). Gerbera, followed by rose, chrysanthemum, tulip, and lily, occupies fifth position in top ten cut flower in world in terms of its production and consumption (Nair *et al.*, 2006; Perik *et al.*, 2014). The longevity or postharvest life of gerbera flowers depends on the extent of stem bending and the lack of stem strength (Naing *et al.*, 2017; Nazarideljou and Azizi, 2015). In this regard, although flowers of some gerbera cultivars are apparently not wilted or faded, their stems have drooped due to their inappropriate stem strength; and based on flower quality and grading, flowers with stem bending more than 30° are not worth maintaining (Çelikel and Reid, 2002; Perik *et al.*, 2014). The lack of mechanical protection and cell wall lignification (especially secondary cell wall) as well as lowering stem strength are of more likely reasons for emerging stem bending in gerbera (Perik *et al.*, 2012). In addition to its remarkable effect on stem hardening and growing gerbera uprightly, lignin plays an utmost role in promoting water flow inside xylem vessels (Vanholme *et al.*, 2010). Also, there is a negative relationship between the rate of stem lignification and the extent of stem bending (Perik *et al.*, 2012). Accordingly, increasing stem lignification directly influences stem strength and thereby reducing stem bending of gerbera cut flowers (Nazarideljou and Azizi, 2015).

In past years, many attempts have been made to increase postharvest life and quality of cut flowers through different techniques. In cut flowers industry, inappropriate condition and inadequate nutrition often lead to a reduction in the quality and quantity of produced gerbera flowers; and regarding these issues can economically improve gerbera flowers production (Khangoli, 2001). Calcium is an essential element affecting on plant growth and development; and its accumulation in plants facilities pectin polymers' linkage so as to improve mechanical strength of stem in line with reducing stem bending and extending flowers vase life (Gerasopoulus and Chebli, 1999; Helper, 2005; Li *et al.*, 2012). The role of intra and intercellular calcium on cell metabolism change is attributed to its effect on the membrane structure and function and cell walls (Ferguson and Drobak, 1988). The results of previous studies showed that calcium, due to its role in the synthesis of pectin located in cell wall of xylem vessels, facilitates water translocation in stem and inhibits stem bending (Van Ieperen and Van Gelder, 2006). Also, applying calcium as pre or postharvest treatments caused to increase the vase life of many cut flowers (Gerasopoulos and Chebli, 1999; Hepler, 2005; Li *et al.*, 2012; Geshnizjany *et al.*, 2014). In spite of existing such documents, the effects of calcium on reducing stem bending of gerbera cut flowers are in needs of more investigations. Therefore, in present research, the effect of different calcium concentrations in forms of chloride and calcium nitrate on stem bending and other related parameters in two gerbera cultivars were scrutinized.

2. Materials and Methods

This research was conducted using a factorial experiment in completely randomized design (CRD) with four replicates and fourteen plots in each replicate and five pots in each plot (totally 280 pots) in local commercial greenhouse (Persian Gulf, Tehran, Iran).

Based on the sensitivity to stem bending, two cultivars of gerbera, namely 'Intense' as a tolerant cultivar and 'Rosaline' as a sensitive cultivar to stem bending (Nazarideljou and Azizi, 2015), were used in this experiment. Gerbera seedlings were cultivated in pots with 18 cm diameter filled with coco peat and perlite (1:1) maintained in greenhouse with 20-25/15-18°C (day/night), 70±5% relative humidity and 14 h with a light intensity of 40 μmol^{-2} m^{-2} s^{-1} (Naing *et al.*, 2017). Before receiving the treatments, the seedlings were feeded based on a nutritional formula used by most commercial greenhouses in Iran (Table 1).

Table 1 - Nutrient elements used in gerbera plant fertilization during excremental period

Elements	Content (mmol/L)
Macro-elements	
Nitrogen	10
Phosphorous	1.8
Potassium	5.5
Magnesium	2
Sulphate	3
Micro-elements	
Iron	40
Magnesium	5
Zinc	5
Copper	1
Molybdenum	1
Boron	30

After planting seedlings in pots and undergoing their perfect establishment, experimental seedlings received calcium chloride and calcium nitrate treatments at three levels (0.5%, 1%, and 1.5%) in form of foliage spray (Mehran *et al.*, 2007) while controls received distilled water. The flowers were harvested when two to three rows of ray floret got color but the stamens still did not open. After preparing of flowers stem in the length of 40 cm, they were placed in the Erlenmeyer containing 500 cc distilled water and hydroxiqinolin sulphate (HQS) in concentration 200 mg/L. Then, they were transferred into the ambient temperature and a light with intensity of 28 μmol^{-2} m^{-2} s^{-1} and 60% relative humidity (Gerasopoulos and Chebli, 1999). The treatments were measured on 9th of experiment.

Stem bending was determined by assessing the accompanying change in the position of the flower head as described previously (Perik *et al*., 2012). Ten excised petals per each flower were weighed (fresh weight, FW) and placed in distilled water in the dark for 6 hours to allow them to reach full turgidity and, hence, to determine their turgid weight (TW). These samples were then dried at 70°C for 24 h and their dry weight (DW) was recorded. Finally, relative water content was calculated using the FW-DW/TW-DW (Abdolmaleki *et al.*, 2015).

Membrane stability index (MSI) was recorded using an electrical conductivity meter based on Ezhilmathi *et al.* (2007). Petal samples were rinsed and immersed in 10 ml of distilled water. The samples were incubated at room temperature for 60 min with shaking (150 rpm). The electrical conductivity of the solution (EC1) was read after shaking using a conductivity meter. Samples were incubated in 95°C water bath for 20 min the second reading (EC2) was taken after the solutions had cooled to room temperature. MSI was calculated as [1-(EC1/EC2)] ×100.

Petal samples (0.2 g) was homogenized with 2 ml of 50mM phosphate buffer (pH=7) containing 1 mM EDTA, 1mM phenylmethylsulfonyl fluoride (PMSF) and 2% polyvinylpyrrolidone (PVP) (w/v) in ice water-bath and then centrifuged at 12000 *g* for 20 min at 4°C. The supernatant was collected and used for enzyme assay with a UV-Visible spectrophotometer (Cary 100, Varian, USA).

The activities of enzymes were determined according to standard methods previously reported for CAT (EC1.11.1.6) (Aebi, 1984) and SOD (EC 1.15.1.1) (Giannopolitis and Ries, 1977). Enzyme activities were expressed as enzyme units.mg^{-1} protein. Protein content was determined according to the method of Bradford (1976).

The cell wall materials of the proximal end of flower stem were fractioned following the method of Li *et al.* (2012). Briefly, the stems were ground into fine powder in liquid nitrogen and extracted with 95% alcohol, then washed twice with boiling alcohol and methyl alcohol: chloroform (1:1, *v/v*), respectively. Finally, the cell wall residues were dried over night at 50°C and used for analysis. Hemi-cellulose content was determined by the phenol colorimetric assay (Dubois *et al.*, 1956) and the cellulose content was measured by the anthrone colorimetric assay (Updegraff, 1969). Lignin content was determined following the method of Müse *et al.* (1997). All measurements were reported as μg/mg DW. Stem calcium concentration was determined by atomic absorption spectrophotometer (Abdolmaleki *et al*., 2015).

The data were analyzed using SAS software and the comparing means was carried out using Duncan multiple range test at 5% probability and the drawing figures were carried out using excel 2013 software.

3. Results and Discussion

Stem bending

The results of variance analysis showed that the main effects of treatment and cultivar (p<0.01) and their interaction effects (p<0.05) were significant on stem bending (Table 2). All calcium treatments, except 0.5% calcium nitrate in 'Intense', significantly increased the days until onset of stem bending. Also, calcium chloride had better effect on stem bending in 'Rosaline'.

Stem bending is the most important factor affect-

Table 2 - Analysis of variance (ANOVA) for cultivar, calcium treatment and their interactions effects on evaluated dependent variables in gerbera cut flower

Dependent variable	Cultivar (C)	Treatment (T)	C × T
Stem bending	**	**	*
Relative water content	NS	**	NS
Membrane stability index	**	**	*
Catalase activity	**	**	**
Superoxid dusmutase activity	*	**	*
Lignin content	**	**	*
Cellulose content	**	**	**
Hemicellulose content	NS	**	*
Calcium content	**	**	NS

** and * represent significance at the 1 and 5% probability levels, respectively, and ns represents non-significance at p<0.05.

ing the quality of cut flowers, flower vase life, and flower loss during harvesting in gerbera. Genetic, nutrition, storage temperature, and the lack of water balance in xylem vessels are of the most important factors facilitate stem bending (Van Meeteren, 1978; Perik *et al.*, 2012; 2014). Reduction in rigidity of the stems carrying flower is associated with the reduction in the formation of lignin compounds. In this regard, it has been stated that the higher-vase life cultivars of *chrysanthemum* contain more lignin compared to lower-vase life ones (Lv *et al.*, 2011). It has been described that tolerance to stem bending is contributed to the presence of a sufficient amount of sclerenchyma and lignin in the stems of flowers (Perik *et al*., 2012). So, each factor being able to increase the stem rigidity also reduce stem bending. Calcium ions maintain cell wall rigidity as they bind to pectin molecules, thereby increasing cell wall stiffness. The positive effects of calcium chloride on the reduction of stem bending in gerbera were proved by Gerasopoulos and Chebli (1999). Also, Perik *et al.* (2014) reported that calcium treatments decreased stem bending in gerbera cultivars likely due to enhancing stem rigidity. It seems that calcium inhibits vascular blockage and escalates stem strength in line with reduction in stem bending and consequently increasing flower vase life (Fig. 1).

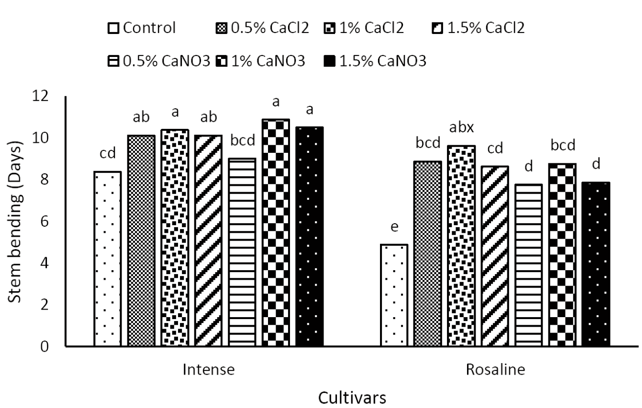

Fig. 1 - The effects of calcium treatments on day of stem bending of two gerbera cultivars. Bars represent standard error of four replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

Relative water content

The results showed that just treatment had a significant effect on relative water content (RWC). The effects of cultivar and its interaction with treatment were not statistically significant (Table 2). Compared to controls, all applied calcium treatments effectively increased RWC and among all applied treatments, 1% calcium chloride had the better effect on RWC while 1.5% calcium nitrate gained the lowest effects on RWC (Fig. 2).

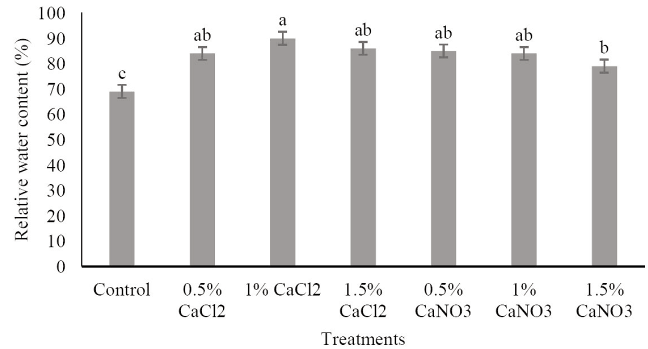

Fig. 2 - The main effects of calcium treatments on petals relative water content of two gerbera cultivars. Bars represent standard error of four replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

The results of this research are in agreements with those obtained by Cortes *et al.* (2011) who reported that calcium treatments increased the relative fresh weight of cut flowers. In gerbera, applying calcium treatments extended flowers vase life by enhancing water uptake by stem and maintaining flower water content (Geshnizjany *et al.*, 2014). Also, it has been stated that the flower fresh weight and solution absorption decreased whereas flower wilting increased, these mainly occur due to water loss by different organs of flowers (Borochov and Woodson, 1989; Urban *et al.*, 2002). In other words, when the stem vessels are blocked, flower spent the absorbed water through transpiration and subsequently reduced RWC (Urban *et al.*, 2002; Van Meeteren, 1978). In results, upholding a proper water uptake by cut flowers is of crucial issues in order for extending the vase life of flowers (Urban *et al.*, 2002). Whenever the rate of transpiration exceeds more than that of water uptake, water deficiency occurs and leads to flower wilting and reduction in flower weight (Van Meeteren, 1978). In current research, the significant effect of calcium treatments on increasing the petal RWC of cut flowers was observed, it can be resulted from a reduction in stem blockage owing to increasing lignin biosynthesis.

Membrane stability index

The results of variance analysis revealed that the main effects of cultivar and treatment as well as their interaction cultivar × treatment on MSI was significant (Table 2). Also the mean comparison of the interaction effect revealed that calcium treatments used in both cultivars significantly increased MSI. It was found a significant different among calcium treatments of both forms on MSI. The highest rate of MSI was observed in 'Intense' cultivar by 1% calcium chloride which increased as much as 13% to controls, but the highest rate of MSI was found in 'Rosaline' cultivar by applying 1% calcium nitrate which increased as much as 15% to controls (Fig. 3).

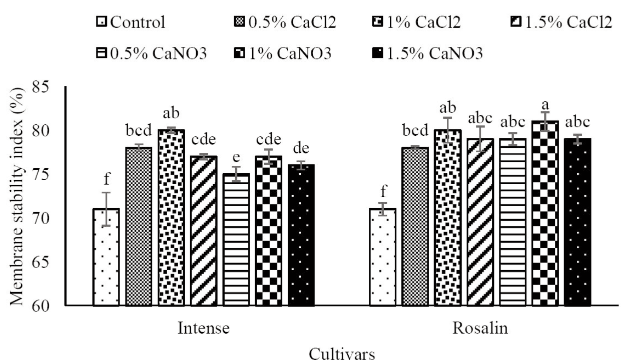

Fig. 3 - The effects of calcium treatments on petals membrane stability index of two gerbera cultivars. Bars represent standard error of four replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

In general, the senescence in plants is an oxidative and controlled process including biological, physiological, hormonal, and structural changes destroying macro molecules such as protein, nucleic acids, and lipids (Iqbal *et al.*, 2017). The lack of balance between generating reactive oxygen species (ROS) and cleaning ROS by plant defensive systems paves the way for inducing oxidative stresses which inflect damage on different parts of cells and destroy them. The destruction of cell membrane is one of the processes which intensifies in the presence of different ROS, resulted in increasing cell electrolyte leakage (Abdolmaleki *et al.*, 2015). In our research, pretreatment of different sources of calcium increased MSI in petals of gerbera cut flowers. calcium is supposed to alleviate the adversary effect of ethylene on initiating cell senescence through inhibiting cell membrane destruction and consequently to extend the vase life of cut flowers (De Capdeville *et al.*, 2005). Also, the structural and functional roles of calcium on maintaining cell membrane and cell walls have been proved (Ferguson and Drobak, 1988). In other words, according to the results of our experiment, calcium treatments were found to enhance antioxidant enzyme activities resulted in decreasing oxidative stress and preserving cell membrane structure. Our results are in agreement with those of Abdolmaleki *et al.* (2015) who reported that applying calcium preharvest treatment retarded damage to cell membrane and hence increased vase life of rose cut flowers. It appears that calcium treatments directly increase cell wall stability and indirectly influence enzyme processes in order to increase the content of MSI in gerbera cut flowers.

CAT and SOD activity

The results showed that the interaction effects of cultivar × treatment was significant in terms of catalase (CAT) and superoxide dismutase (SOD) (Table 2). Compared to controls, all applied treatments significantly increased CAT in both cultivars and the effects of the treatments in both cultivars were the same (Fig. 4A). Also, calcium nitrate and calcium chloride significantly improved SOD activities on both cultivars. The highest SOD activity was observed in 'Intense' cultivar by using 1.5% calcium nitrate, whereas in 'Rosaline' cultivar it was found by applying 1.5% calcium chloride (Fig. 4B).

The process of senescence in cut flowers is usually accompanied with a modulation in the antioxidant enzymes' activities (Borochov and Woodson, 1989).

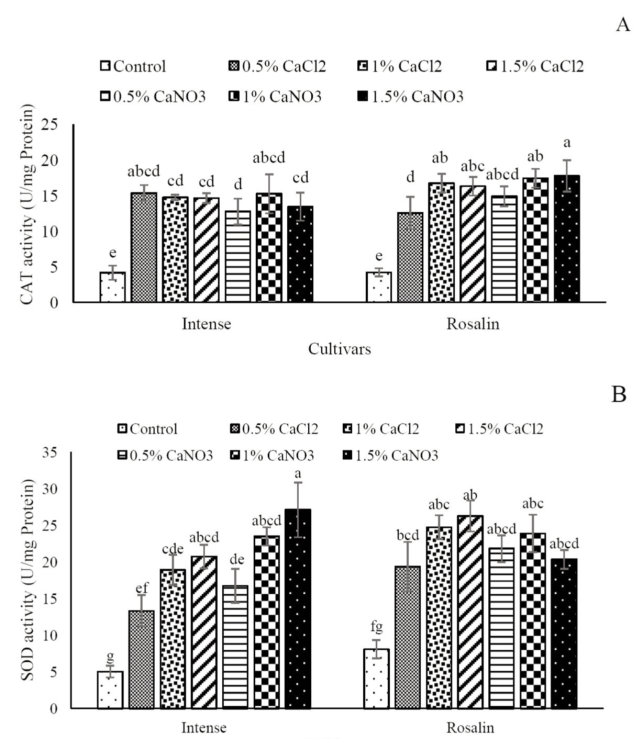

Fig. 4 - The effects of calcium treatments on catalase (CAT) (A) and superoxide dismutase (SOD) (B) activities of gerbera petals. Bars represent standard error of four replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

The results of different studies showed that increasing antioxidant enzyme activity extended flower longevity because these antioxidants retarded the process of senescence (Ezhilmathi *et al.*, 2007). It has been demonstrated that calcium treatments, through changing in antioxidants activities, delayed flower senescence and maintained chlorophyll and protein contents in gladiola flower during storage (Sairam *et al.*, 2011). In this respect, Zhao *et al.* (2006) showed that calcium chloride enhanced the activities of CAT and SOD in the cut flowers of *Rosa hybrida* and *Dendrobium phalaenopsis*. In current research, both forms of calcium increased CAT and SOD activities in gerbera flowers (Fig. 4). It is obvious that improving antioxidant activities prevents cell membrane from breakdown and maintains membrane integrity. Calcium may serve as a secondary messenger in a pathway to stimulate the synthesis of antioxidant enzymes (Jiang and Zhang, 2003). With respect to what mentioned above, calcium may indirectly stimulate the antioxidant activities and consequently reduce the oxidative stresses through lessening the peroxidation activity and maintaining the membrane integrity, or it may directly keep the membrane integrity and cell wall and delay the process of senescence in cut flowers.

Lignin, cellulose, and hemicellulose

The results showed that the interaction effect of cultivar × treatment was significant on lignin ($p<0.05$), cellulose ($p<0.01$), and hemicellulose ($p<0.05$) (Table 2). Comparing to 'Rosaline', more lignin and cellulose were observed in 'Intense', but the rate of hemicellulose in both cultivars was not significantly different (Fig. 5). Application of both forms of calcium at all their concentrations significantly improved the rates of lignin and hemicellulose in both cultivars (Fig. 5A and 5B). The highest rate of lignin was found in 'Intense' by 0.5% calcium nitrate while it was obtained in 'Rosaline' by 1.5% calcium chloride (Fig. 5A). On the other way, all calcium treatments increased cellulose in 'Intense' in comparison to control, but just 1% and 1.5% calcium chloride and 0.5% of calcium nitrate increased cellulose in 'Rosaline' as comparing to controls (Fig. 5B).

One of probable factors on providing stem bending in cut flowers is contributed to the lack of sufficient lignin formation in cell wall, especially in the secondary wall besides its effect on stem rigidity, causes to take up water continuously by flowers (Vanholme *et al.*, 2010). Peroxidase (POD) and phenylalanine ammonia lyase (PAL) are of key

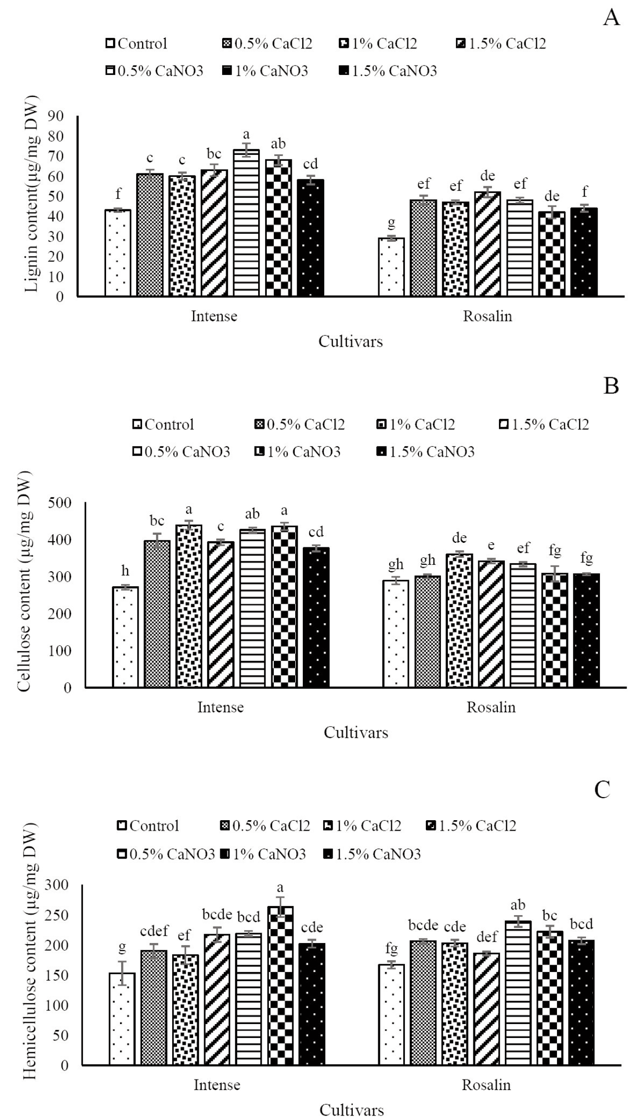

Fig. 5 - The effects of calcium treatments on lignin (A), cellulose (B), and hemicellulose (C) of gerbera petals. Bars represent standard error of four replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

enzymes involving in the pathway of lignin biosynthesis; their roles in stem strength and delaying stem bending in gerbera flowers have been proved (Nazarideljou and Azizi, 2015). It has been reported that the cut flowers prepared from the long-lasting cultivars contain more POD and PAL compared to short -lasting cultivars (Lv *et al.*, 2011; Nazarideljou and Azizi, 2015). In the present research, 'Intense' showed a higher tolerance to stem bending than 'Rosaline' because of containing more lignin and cellulose. The results of this research also revealed that calcium treatments significantly increased lignin, cellulose, and hemicellulose contents in gerbera. Perik *et al.* (2014), by investigating the effects of different treatments on stem rigidity, figured out that using calcium chloride lessened stem bending in gerbera flowers through increasing stem strength resulted from lignin formation. In this regard, Li *et al.* (2012) reported that applying calcium chloride at pre-harvest stage significantly enhanced the rate of pectin, lignin, cellulose, and hemicellulose in the cut flowers of herbaceous peony. Also, it has been stated that using some materials such as ethylene and salicylic acid, through affecting on the some enzymes like PAL involving in lignin biosynthesis, had a direct influence on stem bending of flower (Ferrante *et al.*, 2007). Also, Naing *et al.* (2017) reported that applying sodium nitroprusside augmented the gene expression of lignin biosynthesis; and likewise PAL extended flower vase life and diminished stem bending of gerbera flowers.

Calcium content

The interaction effect of cultivar × treatment was not significant in terms of calcium, but the main effects of treatment and cultivar were significant (Table 2). The rate of calcium in 'Intense' was observed higher than of that in 'Rosaline' (Fig. 6A). In other words, all calcium treatments significantly increased the amount of calcium in comparison to controls. There was a significant difference among all treatments and 0.5% calcium nitrate and calcium chloride had the lowest effect on calcium content of the stem cut flower (Fig. 6B).

Calcium is an essential element effecting on the plants' growth and development processes in a way that its accumulation in them facilitates creating the connections among pectin polymers; and accordingly it escalates the mechanical strength of stem and lignin production. Eventually, they lead to reducing stem bending and increasing flower longevity (Gerasopoulos and Chebli, 1999; Li *et al.*, 2012). The internal and inter cellular roles of calcium are contributed to its part in plants leading to the changes on cell metabolism as well as its effect on the structure and function of cell wall and membrane (Ferguson and Drobak, 1988). The results showed that application of calcium, due to its role in synthesis of the pectin located in cell wall of xylem vessels, improved water translocation in stem and prevented cut flowers from stem bending (van Ieperen and van Gelder, 2006). In reality, a reduction in the resistance towards water conductivity in plants is one of calcium duties for extending vase life of cut flowers (Cortes *et al.*, 2011). Calcium improves the flower longevity and this may delay many issues such as physiological phenomena related to senescence, reduction in water uptake, and losing more water from flower through transpiration, and thereby reducing flower fresh weight and stem bending (Gerasopoulos and Chebli, 1999; De Capdeville *et al.*, 2005; Sosanan, 2007). In current research, applying calcium increased calcium concentration in stem of cut flowers. In a similar way, using different forms of calcium at growth stage of herbaceous peony enhanced the internal calcium, lignin formation, and consequently the rigidity of the stems carrying flowers (Li *et al.*, 2012). Nikbakht *et al.* (2008) reported that calcium accumulation in the stem of gerbera delayed stem bending. Therefore, it seems that an increase in the content of calcium inside stems is associated with the reduction of stem bending and extending flower longevity.

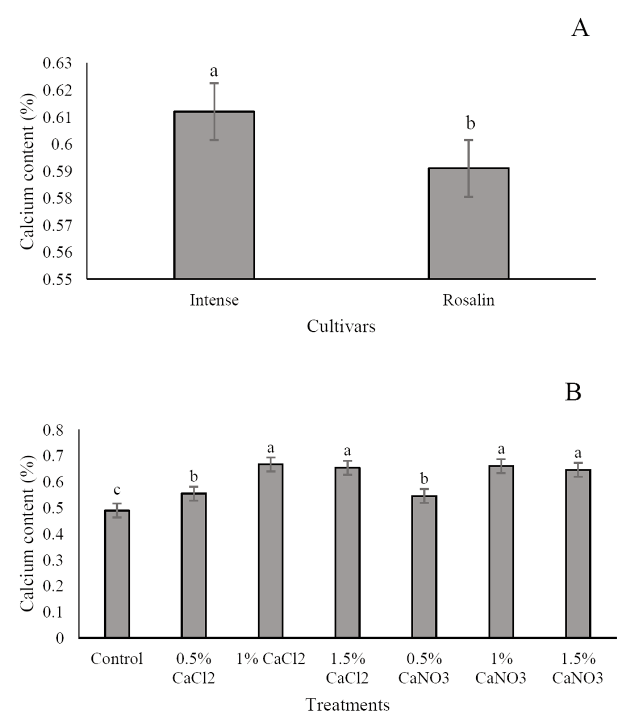

Fig. 6 - The main effects of cultivar (A) and calcium (B) treatments on calcium content of two gerbera flower stem. Bars represent standard error of four replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at $P<0.05$.

4. Conclusions

In general, the results showed that applying calcium in different forms of nitrate and chloride reduced the stem bending in gerbera cultivars. Both forms of calcium had a positive effect on gerbera cultivars, but mostly calcium chloride had better effect on stem bending in comparison to calcium nitrate; and its 1% concentration compared to other its concentrations gained the best results. The positive effect of calcium on stem bending is ascribed to its role on increasing the synthesis of lignin, cellulose, and hemicellulose. The results of this research also showed that the antioxidant enzymes activities were increased while applying calcium treatments led to delaying the senescence processes in flowers.

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Adv. Hort. Sci., 2019 33(1): 67-75 DOI: 10.13128/ahs-23043

AHS
Advances in Horticultural Science

Influence of post-véraison water deficit on berries yield and quality of three table grape cultivars

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Key words: antioxidant activity, anthocyanin, drought, total phenol, water requirement.

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Citation:
ASHORI M., GHASEMNEZHAD M., BIGLOUEI M.H., 2019 - *Influence of post-véraison water deficit on berries yield and quality of three table grape cultivars*. - Adv. Hort. Sci., 33(1): 67-75

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 10 April 2018
Accepted for publication 17 October 2018

Abstract: In order to investigate the effect of regulated deficient irrigation (RDI) on some quantitative and qualitative characteristics of the three commercial grapevine cultivars (i.e. Keshmeshi, Sahebi and Sharabi), an experiment was set in split plot arranged in an RCBD design with three replications. In this experiment, irrigation treatments including 100 (as control), 80%, 60% and 40% crop evapotranspiration (ETc) were implemented in a period between onset of berries color change (véraison) to harvest of fruits. At the end of experimental period, some traits such as berry weight, berry length, berry diameter and cluster weight as well as some fruit quality traits such as total soluble solids (TSS), titratable acidity (TA), flavonoids, anthocyanin, total phenol, and total antioxidant capacity were measured. The results of this experiment revealed that effect of RDI at different levels on yield, berry and cluster weight, berry length and diameter, TSS, TA, anthocyanin, phenol, and flavonoid was significant. Also, there was no significant difference between control and 80% ETc in terms of yield, berry diameter, TSS, and TA; and these treatment enhanced total phenol, anthocyanin and antioxidant activity in the three cultivars. In both years of experiment, RDI remarkably enhanced anthocyanin, flavonoids, antioxidant activity and phenolic concentration. Overall, the results indicated that 80% ETc might be sufficient to gain adequate yield in 'Keshmeshi', 'Sahebi' and 'Sharabi' without undermining the quality of fruit.

1. Introduction

Water deficiency is one of limiting factors influencing grape production throughout the world (Rabiei *et al.*, 2003). In order to improve water use efficiency (WUE), it is necessary to recognize factors restricting WUE and then introduce appropriate approaches for mitigating the harmful effects of such factors (McCarthy *et al.*, 2002). In this respect, balancing between vegetative and reproductive phases in plants in favor of hindering excess vegetative growth is one of these approaches being used to improve not only WUE but also fruit quality of grapevine, because excess growth of shoots may have adverse impact on fruit quality (Chalmers *et al.*, 1981). Reduction in vegetative growth in favor of improving WUE can be implemented by many approaches. One of such approaches is to constrict the amount of water required for plant growth through reducing the number of irrigation or decline in amount of water in each irrigation (Chalmers *et al.*, 1981; Goodwin and Jerie, 1992). For achieving this purpose, regulated deficit irrigation (RDI) was introduced (Santesteban and Mirandam Royo, 2011). As more than 82% of Iran territory is located in arid and semi-arid zone with drought climate and extreme temperature changes in the world (Amiri and Eslamian, 2010), this approach may pave the way for planting crop in a vast majority of abandoned farmland deprived of adequate water distribution, although employing this approach may reduce crop production because of reduction in water use (Chalmers *et al.*, 1981). Under drought condition, RDI abridges vegetative growth in favor of enhancing reproductive growth and this could improve yield (Santesteban and Mirandam Royo, 2011).

At critical stages of plant growth and development, change in water status certainly influences fruit quality by affecting vegetative growth and fruit development (Van Leeuwen *et al*., 2004; Ezzhaouani *et al*., 2007). Therefore, RDI improves cluster quality through reduction in berry drop (Ojeda *et al.*, 2002). Under a mild drought stress, Acevedo-Opazo *et al.* (2010) proposed an appropriate threshold for managing RDI schedule to keep quality of grape fruits. The positive RDI effect on phenolic compounds, TSS, and anthocyanins has previously been documented (Ojeda *et al.*, 2002; Van Leeuwen *et al*., 2004). The highest yield (48 Kg a tree) with high fruit quality was observed in seedless grape cultivars experiencing RDI under drought condition (Faci *et al*., 2014). According to the results obtained by Zabihi and Azarpajouh (2004) and Dolatibaneh and Norjo (2012), RDI probably decrease photosynthetic capability in leaves of grape; and as a result, it reduces (fruit) yield. In other research, it was shown that RDI increased berry quality and increase water use efficiency in grape trees Pinillos *et al.* (2016). Rabiei *et al*. (2003) pointed out that using RDI at the end of growth season significantly increased phenolic compounds in Merlot cultivar. With respect to mentioned issues, the current experiment was designed to investigate effects of different RDI treatments on yield and fruit quality of the three grape cultivars (i.e. Keshmeshi, Sahebi and Sharabi) during the onset of véraison to harvest under climate of Khorramabad province, Iran.

2. Materials and Methods

Vineyard site and experimental design

This experiment was conducted during the 2015 and 2016 growing seasons at semiarid climate in Lorestan province, Iran (47°E, 51°N, 1400 M). Own-rooted *Vitis vinifera* L. cvs. Keshmeshi, Sahebi and Sharabi were planted in 2007 by spacing of 3×2 m in N-S oriented row. Vines were trained to spur-pruned bilateral cordon with 6, 4 and 4 nodes per vine in 'Keshmeshi', 'Sahebi' and 'Sharabi', respectively according to flowering type. The vineyard was drip-irrigated using pressure-compensated emitters (flow rate 4 Lh^{-1}). The vines were fertigated from June with a nutrient compound containing 150 kg ha^{-1} N, 25 kg ha^{-1} K, 6 L ha^{-1} S and 4 kg ha^{-1} Fe chelate.

Three RDI strategies were compared with the full irrigation practice (Control). Specifically, the control received full irrigation over fruit growth and development (ETc 100), whereas RDI treatments were: 80 % of ETc, 60% of ETc and 40% of ETc. RDI treatments were applied at the onset of berries color change (véraison) to harvest time. Daily metrological data were obtained from a database of Khoramabad's meteorology station located closely to the experimental vineyard and water requirement for each vine was calculated according to CROPWAT 8.0 software. Furthermore, irrigation scheduling was based on soil water holding capacity, daily crop water requirement, effective depth of root, percentage of wetted soil surface according to defined treatments (100%, 80%, 60% and 40% ETc) using the following relations.

$$In = \frac{FC - PWP}{100} . \rho_b . Dr . Pw . F$$

$$I_i = \frac{In}{K(ETc)}$$

where *In*= Net irrigation water content (mm), FC= Field capacity (%), PWP= Permanent wilting point (%), *pb*= Soil bulk density ($gr.cm^{-3}$), *Dr*= effective depth of root (120 Cm) *I*= Irrigation interval (day), *K*= Operation Coefficient of RDI treatments, ETc= Plant water requirement.

All vines were equally irrigated based on their water requirement before starting RDI treatment. In this study, the drip irrigation system was equipped with two droplets adjusted manually for 4 Lh^{-1} per vine. In order to access required pressure, a floating pump was used and the amount of water allocated to each irrigating turn was measured by water counter with 0.1 L accuracy. Directing water into each plot containing three grapevines was performed through stopcock water equipped in drip irrigation.

For each irrigation treatment, three replications were established according to a randomized block design. Each replication consisted of five lines of 60 vines each, and the experimental measurements were performed on 20 homogeneous vines from the 3 central lines chosen at the beginning of the study according to their trunk cross sectional area (TCSA).

Sampling and analysis

The berries were harvesting from 10 vines per each treatment and three samples for each vine, based on minimum maturity index of sugar content and berry color change. The mean total yield, clusters weight, number of berry per cluster and berry weight was measured from 10 vines for each treatment and three samples for vines. Soluble solid content (SSC) was measured by a digital refractometer (Model Eurromex RD). Titratable acidity (TA) was measured with a digital titrator in presence of 0.1 NaOH and pH was determined by a digital pH meter (Model Sclto TT).

Total phenolic was determined by using the Folin-Ciocalteau method (Brand-Williams *et al.*, 1995) with slight modification in a UV-Vis spectrophotometer (T80+, PG instruments Ltd). Briefly, 0.5 g of extract was mixed with 3 ml of methanol 85%, and then centrifuged for 10 min by 10000 per min. Then, 300 µl of diluted extract was mixed with 1.5 ml of diluted Folin-Ciocalteau reagent (1:10 in distilled water). After vortexing, 1.2 ml of 7.5% sodium carbonate was added and allowed to stand for 90 min at room temperature. The absorption at 760 nm was measured and results were expressed as gallic acid equivalents (GAE) per 100 g fresh weight.

Total flavonoids content were determined spectrophotometerically according to the Zarrouk *et al.* (2012). Catechin was used as a standard. The flavonoids content were expressed as mg catechin equivalents (RE) per 100 g FW.

Total antioxidant capacity (TAC) was measured using the 2, 2 diphenyl-1-pic-rylhydrazyl (DPPH) radical scavenging method described by Brand-Williams *et al.* (1995) with slight modification. The amount of 75 µl of fruit extract was mixed with 2925 µL 0.1 mol L^{-1} DPPH in methanol. After incubating at room temperature for 30 min in the dark, the absorbance of the mixture was measured at 517 nm using UV-Vis spectrophotometer. For each sample, three separate determinations were recorded. Antioxidant activity was expressed as the percentage decline in absorbance in comparison with the control, corresponding to the percentage of DPPH scavenged (% DPPHsc), which was calculated as:

$$\%DPPHsc = [(Acontrol-Asample)/Acontrol] \times 100$$

Data analysis

The results obtained for each irrigation treatment were compared using ANOVA and when significant differences were found, Duncan's test was used to identify differences in mean values. Data from each year were analyzed separately; all analyses were performed with SAS 17.0.

3. Results and Discussion

Fruit yield

The results of ANOVA showed that simple effect of RDI on total yield at first year was significant ($p<0.01$). Compared to controls, 80% ETc did not show a significant effect on yield at first year, whereas applying 40% and 60% ETc reduced fruit yield by 33% and 48%, respectively. Also, it was found that Sahebi in comparison to Sharabi gained higher fruit yield at first year (Table 1). The results of this experiment showed that the interaction effect of Cultivar × RDI had a significant effect on yield in second year of experiment (Fig. 1). In 'Keshmeshi' and 'Sharabi', using 40% ETc significantly resulted in a yield reduction, while 40% and 60% ETc decreased fruit yield in Sahebi. Santesteban and Mirandam Royo (2011) and Di Vaio *et al.* (2001) showed that RDI brought about a reduction in grape yield, although they proposed RDI as a useful approach to enhance quality and quantity of fruits in the grapes grown especially in subtropical climate. On the contrary, Dolatibane and Norjo (2012) and Zabihi and Azarpajouh (2004) stated that RDI could reduce grape yield. The negative effect of RDI on fruit yield is associated with its harmful effect on photosynthesis activities and consequently supplying required assimilates for berry's formation

Table 1 - The effects of regulated deficit irrigation (RDI) on fruit yield and quality of grape cultivars

Season	Treatment	Levels	Yield (kg vine^{-1})	Berry weight (g)	SSC (%)	TA (%)	pH
2015	RDI	Control	23.70 a	1.11 a	17.55 a	0.68 a	3.51 a
		80% ETc	21.75 a	1.08 a	17.18 a	0.69 a	3.40 a
		60% ETc	15.90 b	0.89 ab	17.24 a	0.72 a	3.48 a
		40% ETc	12.24 d	0.72 b	17.12 a	0.66 a	3.48 a
		Significance	**	**	NS	NS	NS
	Cultivar	Keshmeshi	19.47 ab	0.60 b	19.84 a	0.78 a	3.35 b
		Sahebi	22.77 a	1.28 a	14.15 b	0.6 2b	3.59 a
		Sharabi	15.20 b	0.98 a	17.83 a	0.67 b	3.46 ab
		Significance	**	**	**	**	**
2016	RDI	Control	24.40 a	0.96 a	20.25 a	0.66 a	3.50 a
		80% ETc	23.56 ab	0.92 a	21.31 a	0.64 a	3.47 a
		60% ETc	21.82 b	0.78 b	20.33 a	0.68 a	3.45 a
		40% ETc	18.71 c	0.62 c	21.67 a	0.67 a	3.42 a
		Significance	**	**	NS	NS	NS
	Cultivar	Keshmeshi	17.31 b	0.57 b	24.50 a	0.75 a	3.37 b
		Sahebi	29.02 a	0.99 a	17.95 c	0.59 b	3.59 a
		Sharabi	20.04 b	0.90 a	20.22 b	0.65 ab	3.41 b
		Significance	**	**	**	**	**

NS= not significant, ** significant at p<0.01, * significant at p<0.05.

(Conesa *et al.*, 2016). Also, a remarkable increase in transpiration rate of berries under severe drought stress can be accounted for losing berries weight and lowering grape yield. Conesa *et al.* (2016) reported that RDI had not a significant effect on grape yield and RDI-experiencing plants had the same yield as controls. In this study, we also didn't find any significant differences between 80% RDI and control during two consecutive growing seasons.

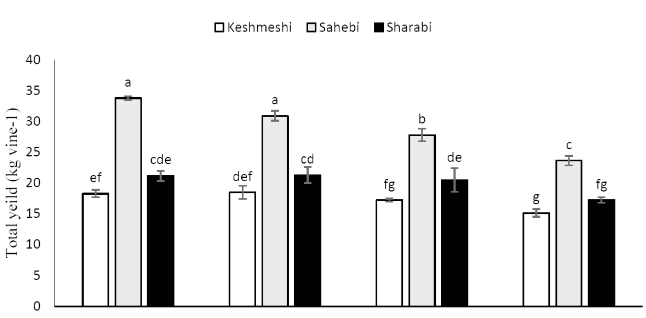

Fig. 1 - Effect of regulated deficit irrigation (RDI) on fruit yield of three grape cultivars in the second year. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

Berry weight and cluster weight

The results of ANOVA showed that berry weight was significantly affected by simple effects of cultivar and RDI (p<0.01). The interactional effect of Cultivar × RDI had a significant effect on cluster weight in both years of experiment (p<0.01). In general, the weight of grape berry and cluster was significantly decreased as the percentage of RDI was gradually decreased (from 100% to 40% ETc) in both experimental years. Comparing to controls, although applying 40% and 60% ETc reduced cluster weight of 'Keshmeshi' at first year, all RDI treatments did not render a reduction in cluster weight of Keshmeshi in second year of experiment. In 'Sahebi', cluster weight was diminished by applying 40% and 60% ETc at first year, whereas by just 40% ETc in second year of experiment. In this regard, all RDI treatments reduced berry and cluster weight of Sharabi while 80% ETc did not significantly reduce cluster weight in Sharabi only in second year (Fig. 2). The results of this research revealed that effect of RDI on berry and cluster weight is strongly correlated to cultivars. According to Coombe (1992), change in berry's water status under drought is directly related with change in berry weight. Rabiei *et al*. (2003) figured out that RDI may be responsible for a reduction in berry weight in Merlot cultivar. In this regard, Dolatibaneh and Norjo (2012) stated that the highest berry weight was obtained at full irrigation; and, in spite of finding non-significant difference between 50% and 75% ETc treatments, they concluded that berry weight is often depressed under RDI performance. As compared to controls, the cell size of fruit experienced different RDI regimes reduced due to reduction in vegetative growth, leaf area, and photosynthesis products, and this probably led to reduction in berry and cluster weight (Ezzhaouani *et al.*, 2007). In current research, the grape cultivars had different responses to RDI especially at 40% and 60% ETc regimes. Regarding these results, different responses to RDI treatments suggests the sensitivity of different cultivars to drought stresses.

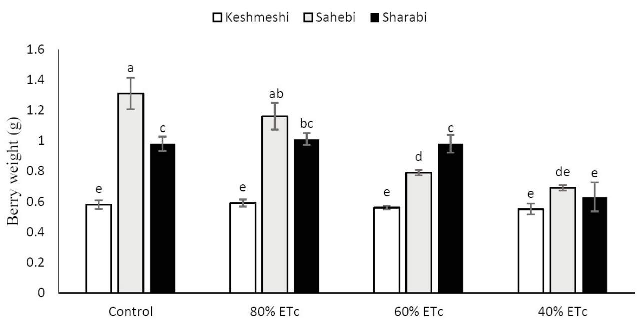

Fig. 2 - Effect of regulated deficit irrigation (RDI) on berry weight of three grape cultivars in the second year. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

Berry length and diameter

The data presented in figure 3 showed a significant interaction effect of cultivar × RDI on traits of berry length and diameter. In both years of experiment, 40% ETc reduced berry length and diameter, whereas 80% ETc did not have a significant effect on both of them. Accordingly, the cultivar response to RDI was found different (Fig. 3). Under RDI performance, the cell size was lower than that of at full irrigation. Due to reduction in the vegetative growth and leaf area of RDI-subjected plants, the size of berry and photosynthetic products were reduced (Deluc *et al*., 2009). Zabihi and Azarpajouh (2004) reported that water deficiency resulted in reduction in the size of berry, which is in agreement with our findings.

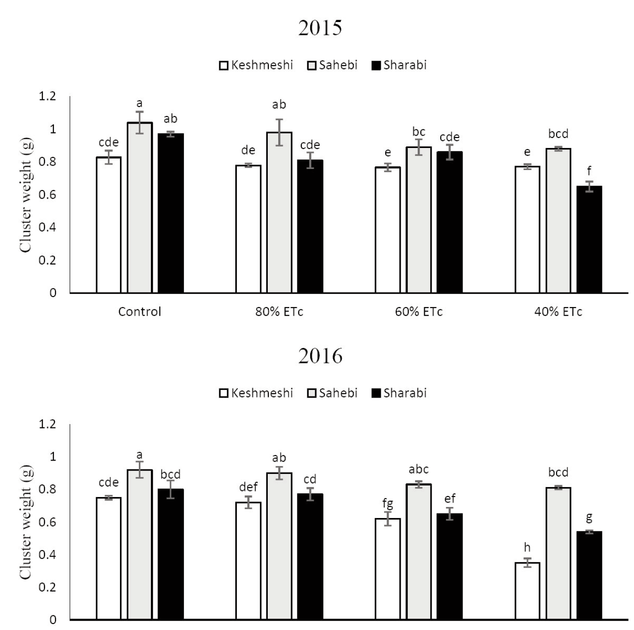

Fig. 3 - Effect of regulated deficit irrigation (RDI) on rachis weight of three grape cultivars in two growing seasons. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

Total soluble solid

The results of this research revealed that only simple effect of cultivar on TSS was significant in both years of experiment, and the highest and lowest TSS were found in 'Keshmeshi' and 'Sahebi', respectively (Table 1). Contrary to Dolatibaneh and Norjo (2012) who stated that RDI had a positive effect on TSS of grape fruit, Lanari *et al.* (2014) figured out a non-significant effect of RDI of TSS in grape fruit. According to previous research, mild drought may stimulate ABA production in favor of increasing some sugar production through different ways in order to cope with stressful condition. In this respect, some researchers pointed out that water deficiency could reduce photosynthesis activities and consequently constrict sugar production, but whenever water deficiency is exacerbated even fruits may not ripe properly and this probably affects TSS status in fruits (Dolatibaneh and Norjo, 2012).

Titratabe acidity and pH

The results of this experiment demonstrated that only cultivar treatment had a significant effect on TA at first year of experiment, and additionally Sahebi and Sharabi obtained the lower TA as compared to Keshmeshi cultivar. Contrary to the results obtained at first year of experiment (Table 1), an interaction effect of cultivar × RDI had a significant effect on TA in second year of experiment. Although, RDI did not have effect on TA in 'Keshmeshi', RDI at 60% ETc significantly increased TA in Sahebi cultivar. In addition, applying 40% and 60% ETc caused a reduction in TA of Sharabi cultivar (Fig. 4).

In both years of experiment, pH was only affected by cultivar; and highest pH was observed in Sahebi as compared to the two other cultivars. Dolatibaneh and Norjo (2012) pointed out that RDI could decline the rate of organic acid of grape fruit. During cluster ripping, coinciding with rising environmental temperature, drought stress is able to decrease acidity of berry and accordingly paves the way for minimizing the rate of TA in grape berries. Lanari *et al*. (2014) reported that RDI did not have a significant effect on

TA, whereas findings of Zabihi and Azarpajouh (2004) showed water availability increased organic acids in grape berries.

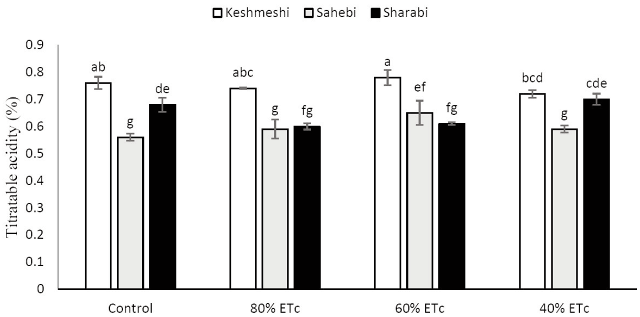

Fig. 4 - Effect of regulated deficit irrigation (RDI) on titratable acidity percentage of three grape cultivars in second year. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

Total phenol

The results of this experiment revealed that the interaction effect of cultivar × RDI had a significant impact on total phenol (Fig. 5). Comparing to controls, applying only 80% ETc increased total phenol in 'Keshmeshi' while applying all RDI treatments in Sahebi enhanced total phenol in both years of experiment. In Sahebi, the highest amount of phenol was found by employing 40% (at first year) and 80% (in second year) ETc treatments. The results also revealed that using 40% and 60% ETc (at first year) as well as 60% and 80% ETC (in second year) had significantly affected total phenol in Sharabi.

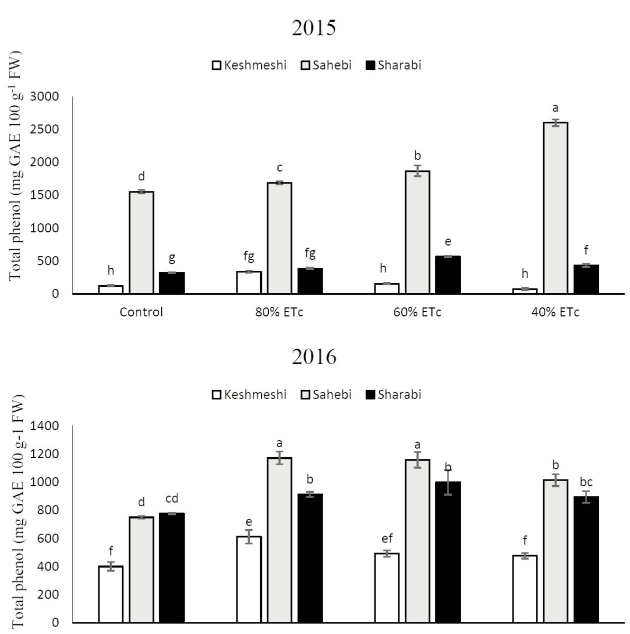

Fig. 5 - Effect of regulated deficit irrigation (RDI) on total phenol of three grape cultivars during two seasons. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

Rabiei *et al*. (2003) reported that applying RDI at the end of growth season significantly increased phenol content of Merlot comparing to controls, which is in agreement with our findings. A prolonged drought stress was found to significantly decrease total phenol and other phenolic compounds such as frolic acid, coumaric acid, and caffeic acid (Krol *et al*., 2014). By investigating effects of different RDI regimes on phenolic compounds in Shirazi cultivar, Ojeda *et al.* (2002) demonstrated that RDI, dependent on type of phenolic compounds, may change the amount of phenol in grape berries. In this regard, some factors including type of cultivar, climate, soil, media culture, maturely period, and yield are effective on the amount of phenols and other biocompounds in grapes (Deluc *et al*., 2009; Bindon *et al*., 2011; Zarrouk *et al*., 2012). In our research, RDI treatments had different impacts on phenol; and the results of this research, dependent on type of cultivar, suggest that an appropriate RDI can increase total phenol in grape berries.

Flavonoids

The results of this research revealed that flavonoid in berries was influenced by RDI treatments. In general, applying 60% and 80% ETc significantly increased flavonoid in all the three cultivars. As compared to other cultivars, 'Keshmeshi' was lowly affected by RDI, in a way that 60% ETc at first year and 80% ETc in second year of experiment significantly enhanced flavonoid content in berries. In contrast, applying 80% and 60% ETc at first year and 40%, 60%, and 80% ETc in second year significantly increased flavonoid content in Sahebi. As compared to the second year of experiment, the rate of flavonoid in Sharabi was significantly elevated by all RDI treatments at first year of experiment relative to controls (Fig. 6). In grape berries, flavonoids are the abundant secondary metabolites influencing wine's physical features (especially color and astringency) (Brillante *et al*., 2017). The biosynthesis of such secondary metabolites is highly affected by environmental stresses (Kuhn *et al.*, 2013). The previous research has demonstrated that regulating vineyard irrigation is efficiently able to increase these metabolites (Kennedy *et al*., 2002). Similarly, findings of Zarrouk *et al*. (2012) revealed that RDI significantly increased flavonoid compounds in grape's Aragonez cultivar in the two subsequent years, which are in consistent with ours. Deluc *et al.* (2009) reported that effect of RDI on flavonoid content of Chardonnay and Cabernet Sauvignon cultivars was different, in a way that its content in former cultivar increased while in latter one decreased. In current research, drought stress at 60% and 80% ETc raised flavonoid content in the all grape cultivars and this manifests the positive effect of RDI on increasing grape quality.

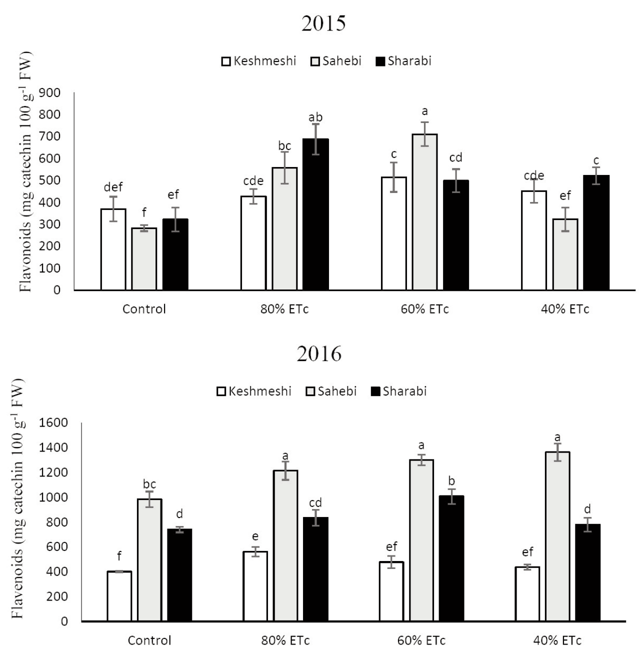

Fig. 6 - Effect of regulated deficit irrigation (RDI) on flavonoid content of three grape cultivars on both seasons. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

Total antioxidant capacity

The results of ANOVA revealed that interactional effect of cultivar × RDI had a significant effect on total antioxidant capacity (TAC) in both years of experiment (Table 1). As compared to full irrigation, applying 60% and 80% ETc at first year of experiment significantly increased TAC in 'Keshmeshi' and 'Sahebi'. In second year of experiment, all RDI treatments interestingly enhanced TAC in Sharabi relative to controls. Moreover, TAC was increased by using 60% and 80% ETc in Keshmeshi, as well as 60% and 80% ETc in Sahebi, and just 80% ETc in Sharabi (Fig. 7). In a similar way, Soukhtesaraee *et al.* (2017) figured out that drought stress significantly increased TAC in Chefta and Yaghoti cultivars, but did not affect Bidanehsefid. Tangolar *et al*. (2015) showed that RDI increased TAC at different growth stages of Razaki cultivar. Comparing to full irrigation, RDI depending upon some factors such as time of applying RDI and type of cultivar diminished TAC in leaf and root of Kiszmisz cultivar (Krol *et al.*, 2014). Plant materials possessing antioxidant properties surely contain different phenolic compounds. The antioxidant properties of these materials, due to their redox ability and chemical structures are able to neutralize free radicals through forming a complex with metal ions (Krol *et al*., 2014). In many previous research, the strong correlation between TAC and phenolic content were reported (Tangolar *et al*., 2015). In our research, a positive relationship was found between phenol and TAC.

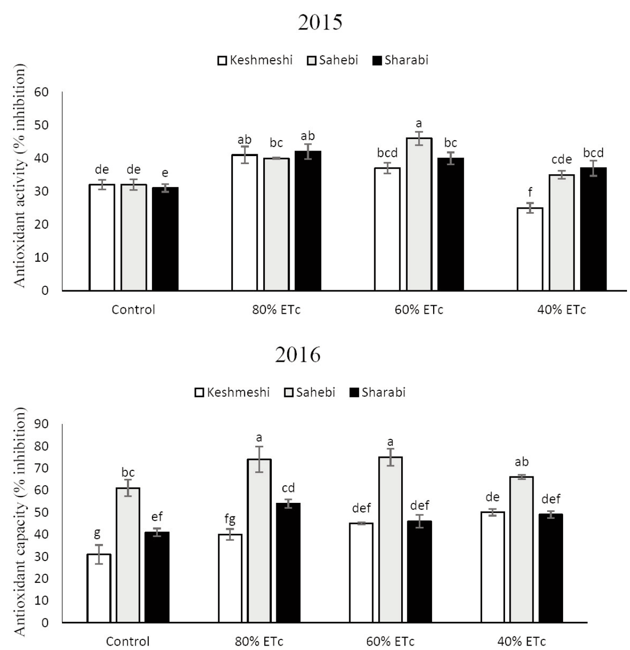

Fig. 7 - Effect of regulated deficit irrigation (RDI) on antioxidant capacity of three grape cultivars on two seasons. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

Anthocyanin

The results of this research showed that the interaction effect of cultivar × RDI had a significant effect on anthocyanin content in both years of experiment (Table 1). Comparing to controls, all the three RDI treatments increased anthocyanin content in Keshmeshi cultivar. In other words, applying 40%, 60%, and 80% ETc at first year and 60% and 80% ETc in second year increased anthocyanin contents in Sahebi (Fig. 8). Anthocyanins are a class of phenolic compounds responsible for generating black and red colors in grape epidermis. These compounds possess powerful antioxidant property and also play a significant role in physiological process. According to previous study, water shortage can strongly influence anthocyanin accumulation in grape cultivars (Ozden *et al.*, 2010; Kyraleou *et al.*, 2016). Also, Bindon *et al.* (2011) reported that an increase in time of irrigation could remarkably hinder anthocyanin biosynthesis. So far, the effect of drought stress on reduction in canopy's density in favor of entrance more light radiation into tree canopy has been reported. Through this, the light quantitatively required to anthocyanin biosynthesis is supplied (Tangolar *et al.*, 2015). Previous research showed that RDI, through influencing physiological and hormonal responses, changed the pathway of anthocyanin biosynthesis (Shellie, 2011; Nelson *et al.*, 2015). In present research, it was found that RDI significantly increased anthocyanin accumulation of Iranian grape cultivars in both years of experiment, although the cultivar responses towards RDI were various.

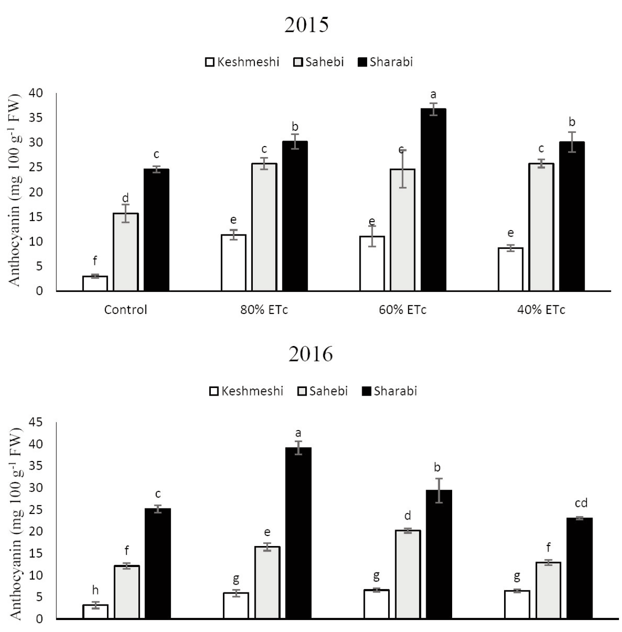

Fig. 8 - Effect of regulated deficit irrigation (RDI) on anthocyanin content of three grape cultivars on two seasons. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

4. Conclusions

Overall, the results of this research revealed the positive effect of RDI (especially 80% ETc) on yield and some fruit qualities in Keshmeshi, Sahebi, and Sharabi cultivars. In this regard, RDI significantly increased not only yield but also some fruits' qualitative features like anthocyanin, phenol, flavonoid, and total antioxidant capacity in comparison to controls. Therefore, 80% ETc can be advised to maintain and increase fruit qualities of Keshmeshi, Sahebi, and Sharabi cultivars under regional conditions.

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 DOI: 10.13128/ahs-23298

AHS
Advances in Horticultural Science

Apple seed stocks affected scion tree vigor and performance based on maternal self(in)compatibility

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Key words: breeding, dwarfness, genetic purity, inductive effect, *Malus × domestica* Borkh., seed rootstock.

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Citation:
HAJNAJARI H., 2019 - *Apple seed stocks affected scion tree vigor and performance based on maternal self(in)compatibility*. - Adv. Hort. Sci., 33(1): 77-85

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 28 May 2018
Accepted for publication 12 December 2018

Abstract: In a breeding program to increase uniformity of apple saplings size, shape and vigor the genetic improvement of seed rootstocks was considered as a key point. The Half-Sib seeds of two selected native crab parents, self-compatible "Morabbaei" and self-incompatible "Azayesh", both dwarf, were considered as the main sources of variances into the six grafted scions. The inductive effects of the two seed masses were assessed on vegetative traits of 6 and 7-year-old scion trees. According to the results, shoot length, shoot thickness and internode length as main components of tree vigor showed significant differences at 1% level in all the six scions. The shoot length mean of the grafted scions on "Azayesh" seed progeny was higher than "Morabbaei" seed stock. The grafted scions on self-compatible "Morabbaei" seed stock gave place to the shoots with higher thickness than the same grafted on "Azayesh" seed source. Combinations of "Red Delicious"-"Morabbaei" and "Braeburn"-"Azayesh" demonstrated max and min values of internodes length during two consecutive years. Both rootstock and scion trunk diameters on self-incompatible "Azayesh" seeds resulted higher than seed progeny of self-compatible "Morabbaei". Shoot number was not affected by rootstock type, whereas the effect of rootstock × scion, scion and year resulted significant.

1. Introduction

Malus genus is characterized by great phenotypic and genetic diversity. High heterozygosity is reinforced by dominant self-incompatibility besides inbreeding depression (Korban and Skirvin, 1984). Among 6000 documented apple cultivars, a large part of commercial production still relies on few cultivars including "Golden Delicious", "Granny Smith", "Fuji", "Gala" and Delicious group (O'Rourke, 2003). Rootstocks can influence vigor, habit and cropping of the scion cultivar as well as tolerance to unfavorable climatic or edaphic conditions (Webster, 2005). Rootstock performance is highly correlated with the genetic potential of rootstock to provide anchorage, adaptaion to pedo-climatic conditions and resist to abiotic stress efficiently (Fazio, 2014). Trees with limited height are requested due to facilitate orchard management. Smaller trees are also easier to target with sprays, reducing undesirable spray drift and increasing efficiency of usage (Jobir, 2016). Good performance, dwarfness, precociousness, scion compatibility, free from suckers and burrknots are some of the attributes of an ideal rootstock (Wertheim and Webster, 2005). Clonal rootstocks are being developed rapidly while seed rootstocks have gradually decreased. Still today, many nurseries in Canada, Belarus and Finland use apple seed rootstocks. “Antonovka” was selected as a seed source due to the freeze resistance and its cytoplasmic heritability (Żurawicz and Lewandowski, 2014), the system for which some traits are transmitted to the next generation by maternal parent (Lorenzetti and Ceccarelli, 1980). Seed rootstocks improvement is applied for dwarfing effect, and tolerance to dry soil conditions (Brown and Maloney, 2005). In Iran seed rootstocks are used because of the alkaline and dry soils and also due to bad topography and exaggerated fragmentations. In absence of a specialized sector for pure seed production, the mixture of recycled seeds of processing industry characterized by high genetic diversity are used in saplings production (Hajnajari and Tarrahi, 2009; Hajnajari, 2010). Increasing genetic purity of seed rootstocks will enhance scion tree size and uniformity by taking advantage of their neglected aspects (Hajnajari and Mizani, 2015). Similarly, almond trees are raised on wild peach and bitter almond rootstock (Sharma *et al.*, 2004). “Mazzard” seedlings followed by “Mahaleb” are the most widely used rootstocks for cherry tree production (Demirsoy *et al.*, 2017). Each open pollinated seed has its specific hormonal status due to the different genetic provenience different from the rest which will cause a gradient of tree growth habits and vigor within the same scion cultivar. While tree vigor is provoked by hybrid heterosis (Lorenzetti *et al.*, 2011), the mandatory inbreeding ruling the self-compatible cultivars as maternal seed source may cause vigor decline of scion trees. It was suggested that the inbred F1 seed stocks could weaken the vigor of scion. Vigorous trees with higher canopy size need increased planting distance (Kosina, 2010). Selection of proper parents as seed source was the unique pass to way out from the emblematic situation (Hajnajari, 2018). Cultivar evaluation program led to selection of few native dwarf crabs “Morabbaei”, “Zinati” and “Azayesh” as seed source parents (Hajnajari, 2010). Contemporaneous field screening program for determination of self-compatibility level of the 92 cultivars followed by fluorescent microscope studies showed complete self-compatibility of “Morabbaei”, “Zinati” and “Azayesh” as self-incompatible (Forughikia *et al.*, 2014; Hajnajari and Moradi, 2014). Using such maternal parents that profit high level of self-compatibility accompanied with dwarfness can minimize seed genetic variability leading to production of the F1 seed progeny marked by high genetic purity. Both traits of dwarfism and low vigor within the seedlings can be used for production of uniform saplings. The same parents were used for production of clonal rootstocks resistant to the crown rot disease (Hajnajari *et al.*, 2012). The effects of two seed masses, as F1 progeny of “Azayesh” (self-incompatible) and “Morabbaei” (self-compatible) on growth characteristics of six apple scion cultivars were investigated. Practically, the two open pollinated seed masses derived from two different maternal parents are assumed as two family’s genetically, diverse within the lots and among them, however two various sister masses belonging to different families. Nevertheless, both parents as crab apples carry jointly dwarfness trait and are native to Iranian habitat. Here is shown that the seed rootstocks bred by self- compatible parent incur more morphological uniformity and control of tree vigor in scion trees compared with the rival sister seeds produced by heterozygote parent “Azayesh” which afford more vigorous and less homogenous tree architecture due to the need of self-incompatible parent for foreign pollen.

2. Materials and Methods

The mature apple trees, 6 and 7-year-old (“Golden Delicious”, “Red Delicious”, “Granny Smith”, “Braeburn”, “Gala” and native “Golab-e Kohanz”) pruned in spindle form, grafted on two selected seed masses were studied to understand the effects of seed sources on vegetative characteristics of the scion cultivars. Genetically, the two seed lots were identified as F1 Half-Sib progenies derived from two different maternal parents including the self-compatible cultivar “Morabbaei” and the other self-incompatible “Azayesh”, revealed as homozygote and heterozygote, correspondingly. The two seed lots were obtained from the maternal parents exposed equally to open pollination, so forming two genetically diverse seed masses as two families considered as the main treatments. The investigated traits included annual diametric growth of scion trunk, graft point and rootstock. For each tree, 4 annual shoots were selected and labelled, and internodes number, internodes length, shoot length, shoot thickness and lenticels number were assessed. Also, shoot numbers per tree and crotch angle were studied. The entire comparative inter and intra varietal measurements were achieved to distinguish the influences of the two seed rootstocks as the main treatments. Interaction of scion per rootstock was recorded to study also the role of scion tree vigor against dwarfing effect of the seed source. Six trees, 3 × 3 m, were assigned for each combination of "Scion cv-Seed progeny" within six replications, large enough to explain seed source inductive effects. Growth traits of grafted scions were measured in two successive vegetative seasons (2014 and 2015). The experimental orchard was established in Meshkin-Abad Station under Temperate and Cold Fruit Research Center located in Karaj, Alborz Province (Iran). The experiment was set as according to a Completely Randomized Blocks Design (RCBD) with six replications for statistical analysis. ANOVA was performed by combining the analysis of the collected data regarding all growth traits in exam, both seed rootstocks and cultivars. All statistical analyses were carried out using the general linear model (GLM) procedure of the SAS version 9.0. The Duncan' multiple range test ($P\leq0.01$) was used to evaluate differences between treatments.

3. Results

Shoot length and thickness

Shoot length and shoot thickness were affected significantly by rootstock, scion and year interactions (Table 1). All variables are measured at annual shoots.

Shoot length

In 2014, "Red Delicious"-"Azayesh" combinations showed the longest shot lenght, while "Golden Delicious" on "Morabbaei" had the lowest value, in 2015. Comparing the treatments, no significant differences were observed for effects of the two seed progenies "Azayesh" and "Morabbaei" on the single cultivars, at the end of the first growing season. In the second year, significant differences were observed by different sources of seed rootstocks for grafted cultivars (Table 2). In 2014, it was found that shoot length value for all scions of "Golab-e Kohanz", "Gala", "Granny Smith" and "Braeburn" combined with "Morabbaei" seed rootstocks showed lower values than "Azayesh" F1 progeny; albeit "Braeburn" demonstrated significant difference, "Golden Delicious" and "Red Delicious" showed no significant differences on the 2 seed sources for shoot length. These results indicate that the maternal self-incompatible "Azayesh" gave place to more vigorous seed progeny. Whereas in 2015, the entire scions had lowered values of Shoot length showing significant difference affected by seed source (Table 2). Considering the prevailing traits of dwarfness associated with homozygosis carried by "Morabbaei" it becomes clear how these are transmitted to the relative F1 seed progeny whom could control the vigor of the combined scion trees. Though both parents may produce seeds which reinforced vigor control of scion trees but the "Morabbaei" seed progeny showed the capability both to induce dwarfism and uniformity in size and shape into the scion cultivars (Table 2). Parallel researches of artificial self-pollination followed by florescent microscopy studies put in evidence how the pollen tubes of "Morabbaei" could penetrate to its own ovary confirming the total self-compatibility of selected maternal parent (Forughikia, *et al.*, 2014). Future investigations are

Table 1 - Analysis of variance regarding the effects of year, rootstock and scion on vegetative traits

Source	DF	Mean Square									
		Shoot length	Shoot thickness	Internode number	Internode length	Rootstock diameter	Graft diameter	Trunk diameter	Shoot number	Branch angle	Lenticels number
Year (Y)	1	44376.9 **	3.92 **	7.65 **	1.14 **	1912.11 **	4460.61 **	0.22 **	0.25 *	67.79 NS	0.007 NS
Block (year)	10	4982.37 **	0.04 **	0.17 **	0.07 **	223.66 NS	108.48 NS	0.01 NS	0.03 NS	420.71 **	0.07 *
Rootstock (R)	1	39402.37 **	0.67 **	0.04 NS	0.56 **	1005.01 *	911.83 *	0.03 NS	0.03 NS	3679.76 **	1.29 **
Scion (S)	5	10396.58 **	0.063 **	0.24 **	0.12 **	28.27 NS	111.47 NS	0.02 NS	0.14 *	833.27 **	0.24 **
R × S	5	6614.24 **	0.067 **	0.06 NS	0.23 **	409.78 *	143.15 NS	0.02 NS	0.17 **	1749.75 **	0.76 **
Y × R	1	17580.61 **	0.003 NS	0.05 NS	0.56 **	56.99 NS	31.91 NS	0.03 NS	0.006 NS	667.52 *	0.10 NS
Y × S	5	7960.64 **	0.06 **	0.12 **	0.08 **	82.02 NS	79.06 NS	0.02 NS	0.10 NS	440.02 *	0.20 **
Y × R× S	5	11061.31 **	0.07 **	0.12 **	0.06 **	52.05 NS	46.51 NS	0.01 NS	0.01 NS	668.91 **	0.37 **
CV (%)		25.62	12.16	16.52	10.74	24.007	23.11	7.63	22.32	16.80	14.83

**= Significant differences at 1% level, *= Significant differences at 5% level, NS= Not significant.

required to elucidate the mechanisms and biochemical pathways by which a self-compatible cultivar can accept insider pollen leading to fertilization, within a self-incompatible species.

Shoot thickness

Combination of “Red Delicious”-”Morabbaei”, in the first year, showed the highest shoot thickness, while both combinations of “Golden Delicious” and “Red Delicious” on “Azayesh”, in the second year, showed the lowest rates. It could be inferred that low vigor of “Morabbaei” F1 progeny inhibited Shoot length growth rising inversely shoot thickness. In 2014, Converse growth rhythm was registered in “Golden Delicious” and “Braeburn” on heterozygote seeds of “Azayesh” inducing significant differences, while “Golden Delicious”, “Red Delicious” and “Gala” were affected significantly by “Azayesh” F1 progeny for the measured variables in 2015. Moreover, in 2014 “Morabbaei” seeds induced significant differences between “Golden Delicious” and “Granny Smith”. Similar differences were observed among “Golden Delicious”, “Red Delicious” and “Gala”, in 2015 (Table 2). Such differences in successive years within the combinations are attributed to the normal annual growth.

Internode number

Year and scion main effects and year × scion and year × rootstock × scion interaction significantly affected internode number (Table 1). The highest number of internode was found in 2014 in combination of “Golden Delicious”-“Morabbaei” confirming the role of homozygosity on lowering vegetative growth, changes in metabolic pathways and relative hormonal modifications at intra-cellular level. However, the lowest expression of the genes for internode number was found in “Gala”-“Azayesh” combination, in 2015. As a reason, the latter low vigor of “Gala” occurred in “On” year could influence such result. In 2014, there were no significant differences among the cultivars for internode number on “Azayesh” seeds, while in the second year significant differences were observed caused by annual growth. Contrarily, “Morabbaei” seed stocks induced significant differences in “Gala”, “Golden Delicious” and “Granny Smith”, in 2014, which confirms relatively lower vigor of “Gala”, whilst in 2015 significant differences was observed among the grafted cultivars (Table 2). In 2014, there were no significant differences between rootstocks for each scion combination, but in 2015, “Golden Delicious” and “Red

Table 2 - The interaction effects of year × scion × rootstock on some vegetative traits of the apple scion cultivars

Year	Seed stock	Scion	Shoot length mm	Shoot thickness mm	Internodes number	Internodes length mm	Rootstock diameter mm	Graft diameter mm	Trunk diameter mm	Shoot number	Crotch angle	Lenticels number
2014	Azayesh	'Golden Delicious'	179.24 b-g	5.70 ef	21.06 ab	14.66 b-e	45 a	43 a	30.2 a	7.75 a	61.67 cd	16.25 ij
		'Red Delicious'	208.22 a	6.43 de	19.26 a-d	17.20 bc	43.6 a	47.2 a	38.5 a	10.2 a	68.33 bcd	24.23 cde
		'Granny Smith'	177.39 b-h	6.87 bcd	20.17 abc	17.19 bc	57.5 a	58.25 a	52.25 a	17.66 a	73.75 a-d	33.12 a
		'Braeburn'	175.25 b-h	5.10 ghi	18.08 b-e	15.54 b-e	49.4 a	56 a	40.5 a	11.16 a	83.33 a-d	14.53 j
		'Golab-e Kohanz'	168.75 b-h	6.80 cd	19.40 a-d	14.66 cde	47.33 a	51.66 a	50.83 a	8.5 a	87.50 ab	10.22 k
		'Gala'	178.45 b-g	6.86 bcd	18.16 b-e	17.83 b	62.83 a	60.83 a	47.66 a	10.5 a	85.00 abc	18.16 e-i
	Morabbaei	'Golden Delicious'	193.63 abc	6.18 de	22.86 a	16.83 bcd	47 a	43.66 a	35.83 a	14.5 a	82.17 a-d	21.21 c-f
		'Red Delicious'	195.06 ab	8.00 a	19.46 a-d	20.33 a	56 a	41 a	51 a	17.16 a	95.00 a	21.94 c-g
		'Granny Smith'	174.61 b-h	6.36 de	20.20 abc	13.73 b-e	40.25 a	49.6 a	41.8 a	13 a	90.00 ab	21.40 c-h
		'Braeburn'	159.70 e-i	7.63 ab	19.58 a-d	15.75 b-e	43.4 a	49.6 a	43.4 a	9.33 a	60.00 d	23.80 bcd
		'Golab-e Kohanz'	165.74 c-h	7.41 abc	19.56 a-d	15.30 b-e	44.6 a	54.5 a	43.16 a	6.33 a	82.50 a-d	29.27 ab
		'Gala'	171.44 b-h	7.86 ab	15.57 d-g	15.03 b-e	41.2 a	44.5 a	36 a	8.00 a	73.33 a-d	15.50 hij
2015	Azayesh	'Golden Delicious'	176.64 b-h	3.94 k	13.39 fgh	13.24 ef	69.33 a	70.33 a	58.66 a	6.33 a	68.33 bcd	14.18 ij
		'Red Delicious'	149.59 hi	4.03 k	9.52 hi	11.36 fg	51 a	62 a	52.33 a	10.16 a	70.00 bcd	22.65 c-g
		'Granny Smith'	164.23 d-h	4.47 h-k	14.17 efg	14.89 b-e	63.2 a	70.2 a	58.8 a	10.66 a	69.00 bcd	19.34 c-h
		'Braeburn'	181.71 a-f	4.31 jk	11.32 ghi	9.97 g	62.16 a	69.16 a	61 a	9.4 a	59.00 d	16.30 hij
		'Golab-e Kohanz'	187.42 a-e	4.30 ijk	14.11 efg	10.23 g	60.33 a	68.66 a	58 a	7.5 a	76.67 a-d	18.22 d-i
		'Gala'	185.61 a-e	4.63 g-j	8.25i	10.36 g	68.33 a	68.5 a	57.5 a	9.16 a	83.33 a-d	17.28 ghi
	Morabbaei	'Golden Delicious'	143.23i	6.10 de	9.11i	14.67 cde	53 a	58.33 a	45.83 a	10.00 a	90.00 ab	21.08 c-g
		'Red Delicious'	191.06 a-d	4.33 h-k	16.10 c-f	21.24 a	60 a	62 a	53 a	17.16 a	95.00 a	22.56 bc
		'Granny Smith'	116.40 j	4.63 g-j	14.70 efg	13.79 ef	44.6 a	57.5 a	44.66 a	7.33 a	85.00 abc	17.26 hij
		'Braeburn'	151.71 ghi	4.68 g-j	11.31 ghi	16.79 bc	51.66 a	64 a	50 a	6.66 a	76.67 a-d	23.19 bc
		'Golab-e Kohanz'	156.16 f-i	4.97 gh	14.43 efg	15.61 b-e	58.25 a	65.75 a	53 a	7.83 a	73.33 a-d	19.94 c-h
		'Gala'	150.88 ghi	5.24 fg	11.82 f-i	11.50 fg	44 a	57.66 a	44 a	9.33 a	83.33 a-d	17.50 f-i

In each column, means with similar letters are not significantly different at the 1% probably level using Duncan multiple range test.

Delicious" showed significant differences on "Morabbaei" and "Azayesh" seed sources (Table 2).

Internode length

Internode length was affected by year, scion cultivar and rootstock main effects and their interactions (Table 1). The longest internode was found in "Red Delicious" on "Morabbaei" rootstocks in both years and the lowest value was registered in combination of "Braeburn"-"Azayesh", in 2015 (Table 2). In the first year, among all the scions combined with "Azayesh" rootstocks, there was significant difference between "Golab-e Kohanz" and "Gala" and among the grafted cultivars on "Morabbaei" sole "Red Delicious" showed significant difference. "Golab-e Kohanz" is the most vigorous Iranian cultivar early and "Gala" with moderate tree vigor. Tree vigor, denoted as potential of vegetative growth, is measured based on height and spread of adult trees self-rooted, or relative to reference cultivars on the same rootstock in apple descriptors (Watkins and Smith, 1997). "Golden Delicious" and "Red Delicious" were used as reference cultivars to assess rootstocks efficiency for tree vigor, the trait that encompasses other components as shoot length, shoot thickness, internode number, internode length, shoot number, and crotch angel by which the tree overall size, shape and architecture will be reflected. Fazio (2014), assumed that rootstock performance is highly correlated with the genetic potential to impart positive architectural properties to the scion like vigor control. Considering the ruling genetic purity carried by "Morabbaei" seed rootstocks, it imparted uniformity in the size of internode length in all the scion cultivars. In the second year, "Golden Delicious" and "Granny Smith" showed significant differences against other scions grafted on "Azayash", while "Red Delicious" and "Gala" had significant difference relative to the other scions on "Morabbaei" (Table 2). Such differences are attributed to the different genetic vigor of scion materials.

Rootstock, graft point and scion diameter

According to the Table 1, the effect of year was significant for rootstock, graft union and scion trunk diameters. Overall, rootstock diameter, graft and trunk were higher in the second year. Rootstock and graft point were affected equally by rootstock factor. Interaction of rootstock × scion was significant for rootstock (Table 1). Interaction effect of scion × rootstock on rootstock demonstrated that combination of "Granny Smith" on "Azayesh" and "Morabbaei" rootstocks endured modifications on the trait, for example 60.66 mm to 42.66 mm, respectively. There was only significant difference between "Red Delicious" (47.30 mm) and "Gala" (65.58 mm) for rootstock limiting factor to "Azayesh" rootstock. Similar comparison within seed lots of "Morabbaei" revealed significant difference in combination with "Granny Smith" (42.66 mm) and "Gala" (60.22 mm). Surprisingly, both seed rootstocks influenced "Gala" increasing rootstock to the highest values of 65.58 mm and 60.22 mm (Table 3). Moreover, significant difference was observed between the combinations of "Granny Smith"-"Azayesh" and "Granny Smith"-"Morabbaei" for the effect of rootstock type on each scion for rootstock, while no difference was observed between seed rootstocks for other combinations (Table 3). This result is assumed positive for uniform apple tree saplings production on the improved seed rootstock.

Shoot number

Shoot number was not affected by rootstock type, while the effects of year, scion and rootstock × scion were significant (Table 1). In 2014, more lateral branches were grown than 2015. Among scions-stocks combinations, "Morabbaei" seed stock showed both max. and min. shoot number, on "Red Delicious" (17.16) and on "Golab-e Kohanz" (7.08), respectively (Table 3). No significant difference was found among the six scion cultivars for shoot number on "Azayesh", whilst "Morabbaei" rootstock induced significant differences of shoot number on "Golden Delicious" and "Red Delicious".

Crotch angle

In this study crotch angle was affected by year,

Table 3 - The interaction effect of scion × rootstock on rootstock diameter and shoot number

Rootstock	Scion	Rootstock diameter (mm)	Shoot number
'Azayesh'	'Golden Delicious'	55.43 abc	7.14 c
	'Red Delicious'	47.30 bc	10.18 bc
	'Granny Smith'	60.66 ab	13.00 bc
	'Braeburn'	56.36 abc	10.36 bc
	'Golab-e Kohanz'	53.83 abc	8.00 bc
	'Gala'	65.58 a	9.83 bc
'Morabbaei'	'Golden Delicious'	50.27 abc	12.25 ab
	'Red Delicious'	58.00 abc	17.16 a
	'Granny Smith'	42.66 c	10.16 bc
	'Braeburn'	46.50 bc	8.00 bc
	'Golab-e Kohanz'	50.67 abc	7.08 c
	'Gala'	60.22 ab	9.25 bc

In each column, means with similar letters are not significantly different at the 1% probably level using Duncan multiple range test.

rootstock and scion interaction (Table 1). The combination of “Red Delicious”-“Morabbaei” showed the widest value of crotch angle probably as a result of heavy cropping, while light bearing trees in the combination of “Braeburn”-“Azayesh” had the lowest crotch angle rates, in both years. In general, the scion cultivars grafted on “Morabbaei” had wider crotch angle than on “Azayash” progeny (Table 2). Except partial genetic role of the scions regarding growth habit, this effect might be result of higher tree vigor control by self-compatible parent “Morabbaei” rootstocks and subsequent decrement of woody parts in favor of flower bud initiation and fruit set (Wertheim and Webster, 2005). Clearly, bearing shoots under higher fruit weight were bent downward increasing crotch angle.

Lenticels number

Lenticels number was affected by year × rootstock × scion interaction (Table 1). The max and min values of lenticels number were found in “Granny Smith” and “Golab-e Kohanz” both on Half-Sib seed stocks of “Azayesh”, in the first year. It can be deduced that the increased leaf lenticels number of the “Golab-e Kohanz” as the most vigorous scion is a normal physiological trend appeared trough interaction of the grafting with as much vigorous seed stock “Azayesh” in the year. More vegetative growth induced by vigorous seeds demands higher respiration rate for which a higher lenticels number equilibrate the defined biological status. In both years, significant difference was observed among the fruit scions on “Azayesh” and “Morabbaei”. This confirms significant effect of rootstock seed masses for lenticels number. In 2014, comparisons of rootstocks for each cultivar showed that “Golden Delicious”, “Granny Smith”, “Braeburn” and “Golab-e Kohanz” were affected by seed stocks for lenticels number. Also in 2015, “Golden Delicious” and “Braeburn” were affected for this trait (Table 2). The comparison between two seed sources put in evidence of high dominant uniformity of lenticels number trait for scions grafted on the seed stocks and originated from self-compatible “Morabbaei”.

4. Discussion and Conclusions

According to the results, shoot length, shoot thickness and internode length as 3 main components of tree vigor showed significant difference in all the six cultivars grafted on two seed masses. Dominant homozygosity carried out by self-compatible “Morabbaei” and relative progeny overcame heterosis effects which induced by “Azayesh” seeds and tree vigor interaction. Our results regarding control of tree vigor “Morabbaei” seed stocks demonstrated that it could reduce shoot length; infact this trait decreased in all the cultivars except for “Golden Delicious”, in 2014. This cultivar showed the same opposite rhythm for all growth traits like crotch angle, internode thickness, internode number, roostock, and scion diameter on “Morabbaei” seed stock. Also, other four scion cvs. decreased shoot length value and were affected by dwarfing seeds in the range of 13 to 3 mm for “Red Delicious” and “Golab-e Kohanz”, 7 and 3 mm for “Gala” and “Granny Smith”, respectively. These results confirm the reciprocal influences of two genomes, scion and seed rootstock within the same genus, each one specialized differently for which were selected in the selection procedure. There is no clear reason for reaction of “Golden Delicious” for measured trait when compared with five scion cultivars grafted on the same seed pure line progeny. This may not be related to the distant geographical origin of two genomes, Iran-USA. With studying scion tree vigor was suggested that J-TE-E as an acceptable rootstock for “Rubin” among 14 rootstocks (Kosina, 2010). The scion cultivars on “Morabbaei” seeds showed higher shoot thickness than the same scions on “Azayesh” seed progeny. Increased shoot thickness as the diametric growth of annual shoots on “Morabbaei” seeds indicated accumulation of carbohydrates for productive functions. However the final canopy size is determined genetically by scion-rootstock interaction. Higher dwarfing efficiency raised in “Morabbaei” than “Azayesh” progeny is in agreement with Tworkoski and Miller (2007 a), who indicated that Malling rootstocks have different size-controlling effects on different apple scions. The lowest interned number (8.25) was found in “Gala”-“Azayesh” combination, in 2014, while the highest (22.86) was found in combination of “Golden Delicious”-“Azayesh”, in 2015. The biennial comparisons evidenced that the higher mean of internode number was induced by “Morabbaei” in both years, 22.86 and 9.11, related to “Azayesh” equal to 21.06 and 8.25. These finding confirmed stronger dwarfing effects of the self-compatible parent. Dwarfing mechanism acted through an increase of internode length and contrarily shortening internode length. Highest value of interned length was found in “Red

Delicious"-"Morabbaei" combination for 2014 and 2015 and the lowest in "Braeburn" on "Azayesh" in 2015 (Table 2). Others reported that wild peach rootstocks influenced shoot growth and leaf area for the scions only during the first year (Sharma *et al.*, 2004). In general, in the first year, the internode number and internode length were higher than the second year. It can be attributed also to the difference between orchard management during two years. Interesting results were obtained in 2015 comparing effects of two seed stocks on major part of the scions. Dwarfing property of "Morabbaei" seed stocks overcame in the next season which caused shoot length shortening in appropriate growth rhythm and shoot thickness increasing and internode number and decreasing rootstock and scion diameter. A part of less expected behavior of "Red Delicious" on "Morabbeai" stock which shoot length and internode length was both increased, but other traits like shoot thickness, internode numbe , and scion diameter followed normal dwarfing trend. All the other growth traits assessed in the rest of four scion cultivars were highly affected by dwarfing effect of "Morabbeai" seed progeny, without exception (Table 2). Weibel *et al. (*2003) indicated that differences in peach shoot length were related primarily to the internode length rather than the node number, whereas Seleznyova *et al.* (2003) attributed the differences in apple branch size to the reduction in neoformed internode length and node number. Mean of internode length depended on node number. It means that shorter internodes are caused by lower node number (Costes and Garcia-Villanueva, 2007). Measured diametric values of graft union and scions on self-incompatible "Azayesh" seed stocks showed higher than those on self-compatible "Morabbaei" F1 seed progeny. Scion-Rootstock discontinuity may lead to Burrknot formation and high diametric difference between rootstock and trunk that may be a site where xylem may be affected by modified cell diameters or cell disorganization between different scions and rootstocks (Atkinson *et al.*, 2003; Tworkoski and Fazio, 2011). Such morphological differences may in turn affect hydraulic conductivity, root hormones production, or nutrient transport and explain the differences in growth. Tworkoski and Fazio (2015) reported that the scion cultivars "Gala", "SM.9", and "SM.27" reduced rootstock of "G.41" and "G.11" compared with "Fuji" scion. In the second year, the most of fruit scions on "Morabbaei" seed had smaller diameter than "Azayesh" without significant difference, except for "Red Delicious" (Table 2). This implies that rootstocks may affect scion. So, shoot number was not affected by rootstock type, whereas the effect of rootstock×scion, scion and year resulted significant. "Red Delicious" and vigorous "Golab-e Kohanz" on "Morabbaei" seed rootstock had the highest and lowest BrN, respectively (Table 3). "Morabbaei" F1 seeds induced more new shoots than "Azayesh" seed stocks. Generally, the mandatory cut back of leader in Spindle pruning method activates the latent buds, forming new lateral shoots due to removal of apical dominance. This practice however was performed equally on the leader of all combinations. Thus, it may be concluded that while, on one side, homozygosis affects canopy size and decreases shoot length, on the other side, the reserved carbohydrates might be used in activation of latent buds in absence of apical dominance. Obviously, winter pruning obliges crossing out of adventitious shoots for flower initiation purposes. Across all scions, the dwarfing M.9, and seedling rootstock induced the lowest and the highest tree height and scion diameter, respectively. Significant interactions indicated that effects of size-controlling rootstock on components of shoot growth vary with apple tree growth habit (Tworkoski and Miller, 2007 a). In this study all the six scions carried the spread habit, for which crotch angle was investigated. Meantime, it might be attributed to the hormonal factor due to the genetic factor derived from seed stocks, scions and relative interaction. Seedling rootstock might affect growth habits by increasing the Auxin/Cytokinin ratio. Changing in hormonal ratio might be a factor regulating the development of growth habit in apple scions, and rootstock might modify the hormone concentrations in shoot tips (Tworkoski and Miller, 2007 b). As prerequisite, crotch angle is mentioned as a stable trait and used in cultivar differentiation and cultivar/progeny evaluation (Watkins and Smith, 1997), and less affected by external factors which is agreed with our results of crotch angle among five scions on two diffrent stocks, except for "Red Delicious". In general, the grafted scions on "Morabbaei" seeds showed wider crotch angle than "Azayash" progeny, probably for higher fruit weight as a result of higher set. The significant differences of crotch angle between "Golden Delicious" and "Granny Smith" on "Azayesh" seed rootstocks, in the first year, can be attributed to the stability of this trait, whereas lack of differences in the second year would be justified by pruning effect. It is however very important that the single cultivars on "Azayesh" didn't show significant differences in terms of crotch angle with the same cultivar on "Morabbaei", except for "Red Delicious" (Table 2). However, endogenous growth regulators mainly auxins and cytokinins play a major role in the control of lateral bud development (Sachs and Thimann, 1967). There are numerous reports of growth-regulating chemicals affecting branch crotch angle (Verner, 1938; Williams and Billingsley, 1970; Elfving and Forshey, 1977). Warner (1991) reported that rootstock affects primary scaffold branch crotch angle of apple trees, which is consistent with our results. Also lenticels number of annual shoots unit, defined as stable trait like crotch angle, was affected by rootstocks and year, and interaction of rootstock × scion × year. Comparisons of rootstocks for each cultivar, in 2014, showed that "Golden Delicious", "Granny Smith", "Braeburn" and "Golab-e Kohanz" were affected by seed stocks for lenticels number, and in 2015, "Golden Delicious" and "Braeburn" were affected for this trait (Table 2). However, the use of lenticels number may be successfully applied for cultivar/hybrid evaluations when more cultivars are grafted on similar rootstocks.

The self-compatible parents are able to supply seeds characterization with high genetic purity. Furthermore, the improved seed rootstocks seem to be the preferred solution to combat genetic variability, suitable for heavy and alkaline soils and inappropriate slopes. Conventional breeding remains the main method to release new clonal or seed rootstock (Wertheim and Webster, 2005). Seed rootstock improvement program was started to impede use of a mixture of seeds with unknown origin and with high genetic variability and evolved into adequate genetic purity, which is the basic element for standard apple tree production.

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Adv. Hort. Sci., 2019 33(1): 87-95 DOI: 10.13128/ahs-23794

AHS
Advances in Horticultural Science

Comparison of salinity effects on grafted and non-grafted eggplants in terms of ion accumulation, MDA content and antioxidative enzyme activities

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Citation:
TALHOUNI M., SÖNMEZ K., KIRAN S., BEYAZ R., YILDIZ M., KUŞVURAN Ş., ELLIALTIOĞLU Ş.Ş., 2019 - *Comparison of salinity effects on grafted and non-grafted effplants in terms of ion accumulation, MDA content and antioxidative enzyme activities*. - Adv. Hort. Sci., 33(1): 87-95

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 9 April 2018
Accepted for publication 12 December 2018

Key words: APX, CAT, eggplant, lipid peroxidation, NaCl, Na^+, Cl^-, K^+, Ca^{++}, scion/rootstock combination, SOD.

Abstract: Grafting onto resistant/tolerant rootstocks is known to alleviate the negative effects of abiotic stress factors like salinity by enhancing their enzymatic antioxidant defense system and having more efficient nutrient uptake. This study was carried out under greenhouse conditions, different rootstock/scion eggplant combinations were grown under two salinity treatments 1.8-2 dS/m (control) and 6-7 dS/m (stress) with seven eggplant genotypes as rootstocks (commercial and Turkish genotypes). Two genotypes were used as the scion. Leaf MDA and ions (Na^+, Cl^-, K^+ and Ca^{++}) content, antioxidant enzymes activity were evaluated as indicators for plant tolerance level. It was found that the rootstock-grafted plants were more efficient in preventing Na^+ ions to be transferred to the plants upper parts and had higher SOD, CAT, and APX activity levels compared to the self- and non-grafted plants which resulted in better tolerance and growth in these plants.

1. Introduction

Eggplant (*Solanum melongena* L.) is an important vegetable crop worldwide, its production reaches about 48.5 million ton while in Turkey eggplant production reaches 800-900 thousand tons (TUIK, 2015). One of the most stress factors that affect eggplant production is salinity. Salinity is a major environmental factor limiting plant growth and productivity, especially in the arid and semiarid regions (Parida and Das, 2005). Conflicting literature exists on eggplant tolerance to soil salinity and this difference could be related to the varieties or cultivars used and to the different environmental conditions in those studies (Ünlükara *et al*., 2010).

Overcoming salt stress problems would have a positive impact on agriculture production. Attempts have been made to improve salt tolerance of crops by traditional breeding programs but with limited success due to the complexity of the trait (Flowers, 2004). Even the use of genetic transformation of plants to raise their tolerance, despite its success in some cases (Rus *et al.,* 2001); the complexity of the trait and lack of public acceptance, limiting its wide spread and use (Munns*,* 2002). One way of avoiding or reducing losses in production caused by salinity would be to use the tolerant rootstocks. In relation to salt tolerance, many studies have been conducted to determine the response of grafted plants to salinity. According to these studies, the improvement of salt tolerance by grafting is related to the capability of rootstocks to reduce toxicity of Na^+ and/or Cl^- through exclusion and/or reduction of absorption of Cl^- by the roots, and the replacement or substitution of total K^+ by total Na^+ in the foliage (Estañ *et al*., 2005; Martinez-Rodriguez *et al.,* 2008). It is supposed that useful rootstocks should be able to reduce the uptake and transport of saline ions to the shoot, which will slow or prevent the accumulation of the toxic salt ions in the leaves (Usanmaz and Abak, 2018). Salt stress causes a range of adverse effects in plants, mainly ionic disorders, osmotic stress and nutritional imbalance. A common feature of these effects is the overproduction of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress (Ashraf and Foolad, 2007). Salt stress causes stomatal closure, which reduces the CO_2/O_2 ratio inside leaf tissues and inhibits CO_2 fixation (Hernández *et al.,* 2000). Plants antioxidant enzymes such (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; guaicol peroxidase, GOPX and glutathione-S- transferase, GST) work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging of ROS (Scandalios, 1997; Dixit *et al*., 2001; Shalata *et al*., 2001).

Superoxide dismutase (SOD) reacts with the superoxide radical at almost diffusion-limited rates to produce H_2O_2 (Scandalios, 1993). H_2O_2 is scavenged by peroxidases, especially ascorbate peroxidase (APX), and catalase (CAT).

In the present study, we exposed non-grafted, self-grafted and rootstock-grafted eggplants to conditions of salt stresses to investigate whether grafted plants could improve tolerance to salinity by alleviating the expression of antioxidant enzymes.

Using local genotypes in breeding programs is of vital importance to find new rootstocks with the ability to alleviate the effects of salinity and reduce its effect on plant growth and productivity.

In the present study, two eggplant genotypes were grafted onto seven rootstocks to compare the ability of the different rootstock genotypes in increasing eggplant tolerance as it is related to the ability of the rootstock to 1) control the transport of Na^+ and Cl^-, 2) to maintain better K^+ and Ca^{++} uptake, 3) to increase the enzymatic defense mechanism scavenging the ROS induced by oxidative stress resulting in less leaf malodialdehyde (MDA) content. Another goal was to evaluate the potential of the Turkish genotypes Burdur and Mardin as rootstocks under salinity conditions in comparison to the commercial genotypes.

2. Materials and Methods

The field part of the experiment was carried out between August-November 2014, in a 300 m^2 plastic house belongs to the private sector (Genta General Agricultural Products Marketing Co.) in Antalya-Turkey while laboratory works and analysis were carried out in Ankara University Faculty of Agriculture, Departments of Horticulture and Agronomy laboratories.

Plant material

Two eggplant (*Solanum melongena* L.) genotypes, Naomi F_1 cv., a commercial cultivar, and Artvin, a salt sensitive breeding line, were used as scions. And for rootstocks five commercial genotypes were used, AGR703 (*Solanum aethiopicum*), Köksal F_1, Yula F_1 and Vista (*S. incanum* x *S. melongena* hybrids), and Hawk (*S. torvum*), these genotypes are the most common used rootstocks in grafting eggplants due to their tolerance. In addition two local salt-tolerant genotypes Burdur and Mardin (*S. melongena* L.) were used as rootstocks, their tolerance to salinity were confirmed in previous studies screening for Turkish tolerant genotypes (Yaşar, 2003).

Grafting and salt treatment

Eggplant seeds were sown in germination trays filled with 2:1 peat:perlite. After sowing trays were kept under controlled conditions of temperature (25°C) and humidity (80%). When the seedlings reached 2-4 true leaves stage, grafting was carried out. The tube-grafting was used in this study because it is the most widely used grafting method in *Solanaceae* family (Rivard *et al.,* 2009). Then grafted seedlings were kept under controlled conditions of humidity (90%) for four days, then seedlings were transferred to the greenhouse under shading before planting for acclimatization. Ten days after grafting seedlings were placed in the plastic house and were ready for transplantation in 8 L plastic pots filled with 3:1 perlite:vermiculite.

In one of our earlier studies we examined these genotypes tolerance under salinity conditions at seedling stage in a hydroponic experiment (Talhouni, 2016), in this study we wanted to assess these same genotypes at flowering and fruit set stage. When plants reached the flowering and fruit set stage salinity treatment began. 6-7 dSm^{-1} water EC was used as the stress treatment by solving NaCl into the nutrition solution; according to the volume of barrels used in fertigation about 8 kg of iodine-free sodium chloride were required , while for the control the EC level was kept at 1.8-2 dSm^{-1} (no NaCl was added).

Leaf ion concentrations

After 60 days from salinity stress application, samples were taken from the control and the salinity treated plants for the different analysis. For the leaf-Na^+, Cl^-, K^+ and Ca^{++} concentration measurements, leaves were dried at 65°C for 48 hours, grounded, dissolved in 1% (v/v) HCl. For the analysis of Na^+, K^+ and Ca^{++} contents, atomic absorption spectrophotometer (Varian Spectra AA 220 FS) was used (Kuşvuran, 2012). While for Cl^-, titration procedure was followed as described by Taleisnik *et al.* (1997) using Buchler - Cotlove chloridometer.

Enzyme extractions and assays

Fresh leaf samples were submersed for 5 min in liquid nitrogen. The frozen leaves were kept at -80°C for further analyses. Enzymes were extracted from 0.5 g leaf tissue using a mortar and pestle with 5 ml extraction buffer containing 50 mM potassium phosphate buffer, pH 7.6 and 0.1 mM Na-EDTA. The homogenate was centrifuged at 15,000 *g* for 15 min and the supernatant fraction was used to assay for the various enzymes. All steps in the preparation of enzyme extracts were performed at 4°C.

APX activity was determined by measuring the consumption of ascorbate by following absorbance at 290 nm. One unit of APX activity was defined as the amount of enzyme required to consume 1 µmole ascorbate min^{-1} (Cakmak and Marschner, 1992).

SOD was assayed according to Cakmak and Marschner (1992), by monitoring the superoxide radical-induced nitro blue tetrazolium (NBT) reduction at 560 nm. One unit of SOD activity was defined as the amount of enzyme which causes 50% inhibition of the photochemical reduction of NBT.

Catalase (CAT) activity was measured as the decline in absorbance at 240 nm due to the decomposition decline of extinction of H_2O_2. The reaction was started by adding H_2O_2.

Lipid peroxide content

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) determined by the thiobarbituric acid (TBA) reaction. Frozen samples were homogenized in a pre-chilled mortar with two volumes of ice-cold 0.1% (w/v) tricloroacetic acid (TCA) and centrifuged for 15 min at 15000 x *g*. Assay mixture containing 1 ml aliquot of the supernatant and 2 ml of 0.5% (w/v) thiobarbituric acid in 20% (w/v) tricloroacetic acid (TCA) was heated to 95°C for 30 min and then rapidly cooled in an ice-bath. After centrifugation (10000 x *g* for 10 min at 4°C), the supernatant absorbance (532 nm) was read and values corresponding to non-specific absorption (600 nm) were subtracted. The MDA content was calculated according to the molar extinction coefficient of MDA (155 mM^{-1} cm^{-1}).

Statistical analysis

Randomized complete block design with three replicates was used. Each replicate included 108 pots (18 rootstock/scion combination * 2 salinity level * 3 plants of each combination) with one plant/pot. Data were subjected to Duncan's multiple range tests using the SAS program (P≤0.01)(Version 6.12, SAS Institute Inc., Cary, USA).

3. Results

Na^+ concentrations

In general, the concentrations of Na^+ in the leaves increased significantly due to increased NaCl concentration (Table 1) with significant differences between grafting combinations, and a significant 'salinity x scion/rootstock combination' interaction at P≤0.01.

After 60 days of salinity stress application, the combinations that showed the least concentrations of Na^+ in their leaves were; (rootstock/scion) Köksal/Artvin, Vista/Naomi, Köksal/Naomi, AGR703/Naomi and AGR703/Artvin (9.75, 9.65, 9.92 10.02 and 10.46 µg/mg FW respectively) with increase rates of 3511, 1035, 4623, 1721, 3506% respectively (Fig. 1) which indicated that these rootstock genotypes could limit Na^+ to the leaves more successfully. No significant effects of grafting per se were noticed, no differences were observed between non-grafted and self-grafted combinations.

Cl^- concentration

As in leaf Na^+ concentration, leaf Cl^- concentration also increased significantly under salinity treatment in all combinations, with a significant differences and a significant 'salinity x scion/rootstock combination' interaction at P≤0.01 (Fig. 1). The highest Cl^- concentration was observed in Artvin, Naomi, (rootstock/scion) Artvin/Artvin, Naomi/Naomi, Mardin/Artvin, Mardin/Naomi (11.83, 10.81, 9.54, 9.06, 8.83 8.12 µg/mg FW) combinations, while the lowest was observed Köksal/Artvin, AGR703/Artvin, AGR703/Naomi and Burdur/Artvin (5.06, 5.06, 5.27, 5.49 µg/mg FW respectively) and there were no significant differences between non- and self-grafted combinations (Table 1).

K^+ concentrations

The amounts of K ion measured in leaf samples taken from plants treated with EC 6-7 dS / m NaCl gave lower values in some combinations than control plants (Table 1), the highest decrease in leaf K^+ content was observed in non-grafted Artvin (-9.82%).followed by (rootstock/scion) Artvin/Artvin with decrease rate of (-6.25%) (Fig. 1). The highest values were obtained from (rootstock/scion); Köksal/Artvin, Köksal/Naomi, Mardin/Naomi, AGR703/Artvin, Yula/Naomi (4.86, 4.64, 4.36, 4.30, 4.27 µg/mg FW, respectively) (Table 1). Among these combinations, Köksal/Artvin, AGR703/Artvin and Yula/Naomi had the highest K^+ ions increase rate (47.27%, 22.16%, 20.96%) (Fig. 1). Combinations that gave the lowest K^+ ion amount measurements were (rootstock/scion) Hawk/Artvin, Naomi, Burdur/Naomi, Naomi/Naomi,

Table 1 - Leaf ions concentration (µg/mg FW); in the different grafting combinations under control and salinity treatments

Grafting combination	Na^+		K^+		Ca^{++}		Cl^-	
	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity
Köksal/Artvin	0.27±0.00 a	9.75±0.25 a	3.30±0.15 ab	4.86±0.33 b	0.50±0.03 f	0.48±0.03 e	0.06±0.02 a	5.06±0.64 a
AGR703/Artvin	0.29±0.03 a	10.46±0.53 a-c	3.52±0.34 a-c	4.30±0.26 ab	0.49±0.02 f	0.41±0.04 de	0.06±0.02 a	5.06±0.90 a
Vista/Artvin	0.51±0.03 ab	12.14±0.86 b-e	3.89±0.11 a-e	3.93±0.53 a	0.46±0.02 b-f	0.41±0.03 de	0.12±0.04 a-d	6.30±1.10 a-c
Yula/Artvin	0.24±0.02 a	11.08±0.58 a-e	3.74±0.18 a-e	3.89±0.12 a	0.43±0.01 a-c	0.35±0.03 b-d	0.19±0.02 c-f	6.63±0.75 a-d
Burdur/Artvin	0.49±0.01 b	10.91±0.53 a-d	3.57±0.12 a-c	3.97±0.24 a	0.49±0.02 ef	0.39±0.05 c-e	0.09±0.03 ab	5.49±0.13 ab
Mardin/Artvin	0.61±0.09 b-d	12.36±0.29 c-e	3.70±0.23 a-d	4.22±0.09 ab	0.44±0.01 a-d	0.35±0.06 b-d	0.14±0.03 a-e	8.83±1.08 c-f
Hawk/Artvin	0.26±0.05 a	10.89±0.18 a-d	4.05±0.28 c-e	3.84±0.31 a	0.46±0.02 c-f	0.41±0.03 de	0.11±0.02 a-c	6.45±1.50 a-d
Artvin/Artvin	0.83±0.08 e-g	12.36±0.70 c-e	4.32±0.05 de	4.05±0.15 ab	0.41±0.01 a	0.35±0.02 b-d	0.19±0.02 c-f	9.54±1.21 e-g
Artvin	0.88±0.04 fg	12.77±0.46 de	4.38±0.28 e	3.95±0.13 a	0.40±0.01 a	0.29±0.02 ab	0.22±0.02 ef	11.83±0.62 g
Köksal/Naomi	0.21±0.02 a	9.92±0.37 ab	4.11±0.22 c-e	4.64±0.23 ab	0.49±0.02 d-f	0.31±0.01 a-c	0.09±0.04 ab	6.06±0.38 ab
AGR703/Naomi	0.55±0.12 ab	10.02±1.26 ab	3.87±0.06 a-e	3.96±0.28 a	0.48±0.01 d-f	0.36±0.02 b-d	0.10±0.04 a-c	5.27±0.55 a
Vista/Naomi	0.85±0.05 fg	9.65±0.71 a	3.67±0.15 a-d	4.15±0.08 ab	0.44±0.02 a-e	0.36±0.02 b-d	0.15±0.03 b-f	6.13±0.19 ab
Yula/Naomi	0.73±0.04 d-f	10.93±0.97 a-d	3.53±0.10 a-c	4.27±0.29 ab	0.42±0.01 a-c	0.25±0.02 a	0.18±0.05 b-f	7.51±0.54 a-e
Burdur/Naomi	0.49±0.05 b	11.14±1.16 a-e	3.50±0.36 a-c	3.88±0.23 a	0.48±0.01 d-f	0.40±0.01 c-e	0.13±0.01 a-d	6.37±1.01 a-c
Mardin/Naomi	0.68±0.04 c-e	12.07±0.13 b-e	3.95±0.32 b-e	4.36±0.24 ab	0.43±0.01 a-c	0.34±0.03 a-d	0.14±0.03 a-e	8.12±0.63 b-e
Hawk/Naomi	0.80±0.10 e-g	11.14±1.20 a-e	3.80±0.11 a-e	4.09±0.27 ab	0.41±0.01 ab	0.35±0.01 b-d	0.20±0.02 d-f	7.29±0.24 a-e
Naomi/Naomi	0.94±0.04 g	12.34±0.84 c-e	3.33±0.20 ab	3.96±0.36 a	0.40±0.01 a	0.34±0.01 a-d	0.22±0.02 ef	9.06±1.06 d-f
Naomi	0.52±0.06 ab	13.30±0.73 e	3.27±0.29 a	3.85±0.30 a	0.40±0.02 a	0.28±0.03 ab	0.23±0.03 f	10.81±1.41 fg
CV (%)	*43.07*	*9.78*	*8.81*	*6.83*	*8.01*	*15.22*	*38.38*	*27.17*
Treatment	**		**		**		**	
Combination	**		**		**		**	
Combination x treatment	**		**		**		**	

AGR703/Naomi, Artvin (3.84, 3.85, 3.88, 3.96, 3.96, 3.96 85 µg/mg FW). No significant differences were obtained for self-grafted combinations.

Ca^{++} concentrations

EC 6-7 dSm^{-1} NaCl treatment led to a decrease in leaf Ca^{++} concentration in all combinations (Fig. 1) with significant differences between treatments and combination ($P \leq 0.01$) and with a significant 'salinity x scion/rootstock combination' interaction. The highest leaf Ca^{++} concentrations were obtained in (rootstock/scion) AGR703/Artvin, Köksal/Artvin, Burdur/Naomi, Hawk/Artvin and Vista/Artvin (0.48, 0.42, 0.41, 0.41 µg/mg FW, respectively). No significant effects were observed in self-grafted combinations.

Antioxidant enzyme activities

Salt treatments increased superoxide dismutase (SOD) activities in all of the plants (Table 2). However,

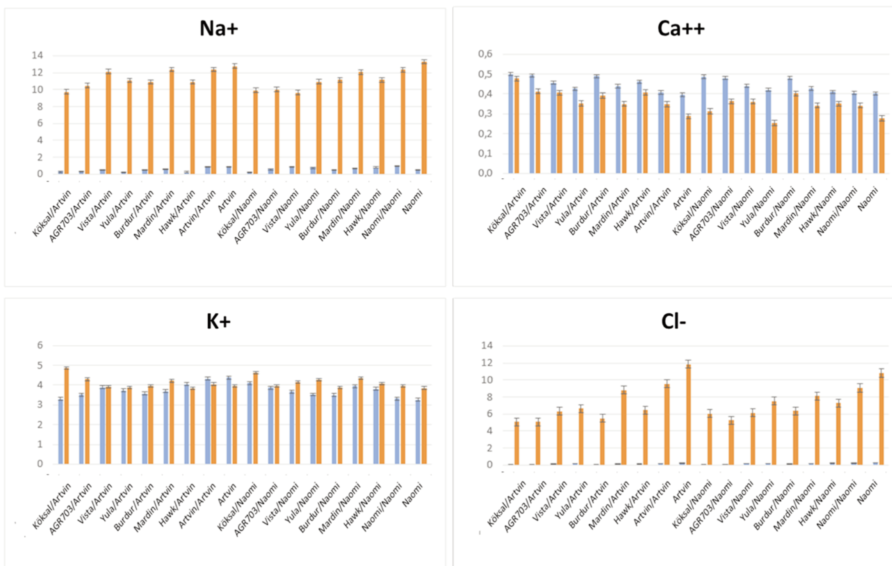

Fig. 1 - Leaf ions concentration (µg/mg FW); in the different grafting combinations under control (blue) and salinity treatments (red).

Table 2 - SOD, CAT, and APX enzymes activities (µmol/g FW) in the different grafting combinations under control and salinity treatments

Eggplant	SOD		CAT		APX		MDA	
	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity
Köksal/Artvin	217.03±8.00 a	635.46±27.84 gh	139.90±13.74 ab	593.91±28.44 h	2042.77±12.67 h	5387.96±560.98 e	5.12±0.29 a-d	10.44±0.54 a
AGR703/Artvin	207.06±6.28 a	645.64±38.29 h	129.82±10.00 ab	576.91±27.73 gh	2020.91±97.38 h	4820.66±545.40 de	5.02±0.13 ad	10.23±1.24 a
Vista/Artvin	215.92±11.75 a	464.88±44.39 c-f	113.03±11.99 a	484.17±25.96 c-f	1758.36±28.48 e-h	3829.29±568.57 a-d	4.86±0.27 ab	12.83±0.26 ab
Yula/Artvin	193.54±6.29 a	414.49±26.02 b-d	103.86±6.46 a	409.13±1.88 bc	1765.71±141.18 e-h	3307.96±550.14 a-c	5.12±0.26 a-d	10.14±0.61 a
Burdur/Artvin	208.97±10.32 a	508.88±55.72 d-f	131.44±4.26 ab	548.67±19.02 e-h	1882.51±52.64 f-h	3839.81±57.28 a-d	5.19±0.10 a-d	11.16±0.44 a
Mardin/Artvin	195.91±10.91 a	470.99±16.48 c-f	138.76±14.25 ab	434.69±6.55 b-d	1395.16±235.41 b-d	3105.27±93.76 ab	5.49±0.25 a-e	11.50±0.21 a
Hawk/Artvin	203.44±10.29 a	436.05±50.00 b-e	118.68±15.42 a	464.12±25.38 c-e	1926.37±199.83 gh	4303.30±32.77 b-e	4.80±0.31 a	11.95±0.98 ab
Artvin/Artvin	186.29±10.04 a	341.33±58.60 ab	123.87±6.38 ab	350.16±52.43 ab	1134.59±102.54 ab	2731.28±37.49 a	6.32±0.13 fg	12.54±0.15 ab
Artvin	189.95±12.89 a	295.15±16.97 a	113.49±7.29 a	374.84±27.07 b	903.43±37.94 a	2614.61±37.06 a	6.34±0.33 g	14.81±1.23 b
Köksal/Naomi	190.92±25.52 a	553.92±32.30 f-h	116.33±14.37 a	553.17±30.84 f-h	1968.59±112.34 g-h	4427.68±48.03 c-e	4.95±0.18 a-c	10.93±1.20 a
AGR703/Naomi	213.81±4.16 a	491.39±39.05 d-f	165.69±17.37 b	503.21±14.17 d-g	1924.53±144.55 gh	4326.69±614.68 b-e	4.87±0.23 ab	10.54±1.04 a
Vista/Naomi	194.09±17.87 a	457.84±29.92 c-f	124.91±15.83 ab	405.95±29.79 bc	1547.82±154.87 c-f	3072.32±48.96 ab	5.59±0.25 b-f	11.18±0.16 a
Yula/Naomi	200.41±10.91 a	427.66±11.33 b-e	99.34±6.30 a	401.18±19.92 bc	1499.31±168.98 c-e	3663.61±635.13 a-d	5.26±0.28 a-d	11.06±0.93 a
Burdur/Naomi	196.48±16.49 a	534.86±35.10 e-g	117.08±28.01 a	503.48±27.99 d-g	1813.06±32.67 e-h	3789.02±90.92 a-d	5.09±0.39 a-d	10.36±0.85 a
Mardin/Naomi	191.44±20.27 a	378.05±10.08 a-c	104.70±15.90 a	367.67±14.99 ab	1608.48±112.47 d-g	3448.81±529.02 a-c	5.71±0.19 d-g	10.86±0.74 a
Hawk/Naomi	193.35±9.53 a	421.65±43.89 b-d	140.49±24.51 ab	360.07±7.01 ab	1695.49±80.45 d-h	4471.40±613.60 c-e	4.80±0.12 a	10.89±0.92 a
Naomi/Naomi	201.11±19.08 a	272.74±22.75 a	125.68±6.97 ab	366.67±43.39 ab	1205.04±58.49 a-c	2693.56±51.33 a	6.05±0.16 e-g	14.66±2.05 b
Naomi	199.68±10.99 a	271.57±36.99 a	123.98±8.26 ab	289.02±39.88 a	1033.83±54.71 ab	2782.04±593.00 a	5.66±0.18 c-g	14.91±1.65 b
CV (%)	*4.65*	*24.53*	*12.87*	*19.94*	*21.9*	*21.7*	*9.37*	*13.58*
Treatment (T)	**		**		**		**	
Combination (C)	**		**		**		**	
C x T	**		**		**		**	

in the rootstock-grafted plants, SOD activity increased faster and with higher rates than in the non- and self-grafted plants. Köksal/Artvin and AGR703/Artvin had the highest SOD activity level (645.64 and 635.46 Umol/min/mg FW respectively). Followed by Köksal/Naomi, Burdur/Naomi, and Burdur/Artvin combinations (553.92, 534.8, and 508.88 Umol/min/mg FW respectively). The highest increase rate in SOD activity was obtained for AGR703/Artvin combination (211.8%) while Naomi/Naomi and Naomi combinations had the lowest increase with rates of 35.62 and 36% respectively (Fig. 2). Naomi and self-grafted plants had the lowest values of the enzyme activity with no significant differences between non- and self-grafted combinations.

Catalase (CAT) activity increased under salt treatment in all combinations compared to the control plants (Table 2), (rootstock/scion) Köksal/Artvin and AGR703/Artvin had the highest CAT activity (593.91 and 576.91 µmol/min/mg FW respectively). Followed by Köksal/Naomi, Burdur/Artvin, AGR703/Naomi and Burdur/Naomi. On the other hand Artvin and Naomi (non-grafted) and the self-grafted plants had the lowest CAT activity. Burdur/Naomi had the highest increase rate in CAT activity (330%) while non-grafted Naomi had the lowest rate (133%) (Fig. 2). No significant differences were observed between non- and self-grafted combinations.

Under NaCl-salinity conditions, ascorbate peroxidase (APX) activity was increased in all plants (Fig. 2). However, AGR703/Artvin and Köksal/Artvin had the highest APX activity levels indicating their better tolerance level (4820.66 and 5387.96 µmol/min/mg FW respectively) as shown in Table 2, while the lowest APX enzyme activity was found in non-grafted plants Naomi and Artvin indicating their poor tolerance. No significant differences were obtained between non- and self-grafted combinations.

Malondialdehyde (MDA)

Salinity resulted in a significant increase on the MDA content compared to the controls due to oxidative stress induced peroxidation (Fig. 2). With regard to the MDA, significant differences were found among grafting combinations and a significant 'salinity x rootstock/scion combination' interaction at P≤0.01 (Table 2). According to the results Naomi, Naomi/Naomi and Artvin were found to be more sensitive with MDA content increase rate of 163.4, 142.3, 133.6% respectively (Fig. 2). No significant differences were observed between non- and self-grafted combinations.

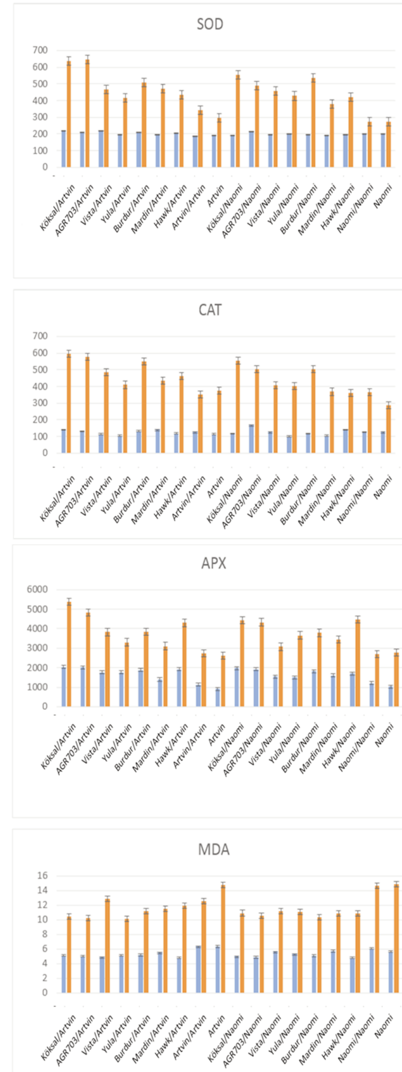

Fig. 2 - SOD, CAT and APX enzymes activities (µmol/g FW) in the different grafting combinations under control (blue) and salinity treatments (red).

4. Discussion and Conclusions

As a result of high salinity level, Na^+ and Cl^- ions can be accumulated in toxic levels in plant tissues depends on the plant species. Even though these two ions are suitable for osmotic adjustment, excess concentrations will be toxic enough to prevent plant growth. In the study, combinations Vista/Artvin, Köksal/Artvin, Vista/Naomi, and Köksal/Naomi had less Na^+ accumulation in their tissues which indicates that these combinations were able to keep Na^+ ions away from their leaves. Concerning leaf Cl^-, Vista/Artvin combination had the least concentration. Grafted plants tend to hold Na^+ and Cl^- ions in their root tissues preventing them from being translocated to the shoots and leaves in high concentrations (Levitt, 1980; Estañ *et al*., 2005). Most vegetables like, cucumbers, melons, tomatoes and eggplant are injured by excess Na^+ ions (Tester and Davanport, 2003). In Giuffrida *et al.* (2009), increased NaCl level led to Na^+ concentration increase in tomato leaves and fruits. Kuşvuran *et al.* (2007), under salinity conditions Na^+ and Cl^- were accumulated in higher rates in the salinity-sensitive melon plants compared to the salinity-tolerant ones.

K^+ decrease rate was different between the different combinations. Akinci and Lösel (2012), different eggplant genotypes showed different tolerant level to salinity. Pala cv. showed better tolerance to salinity compared to Kemer and Aydın Siyahı cultivars with better K^+/Na^+ ratio. Yaşar *et al.* (2006), in tissue culture study on eggplant, there was an increase in Na^+ and Cl^- tissues concentrations with decrease in K^+ and Ca^{++} due to salinity. However there were significant differences between different genotypes, the salinity-tolerant MK and BB showed higher K^+ and Ca^{++} concentrations compared to the salinity sensitive GR and AH genotypes. Consequently MK and BB had higher K^+ and Ca^{++} uptake decreased under stress treatment (Savvas and Lenz, 2000). In a similar study on pepper, the same results were obtained (Aktaş *et al.,* 2002).

Combinations with the highest leaf Ca^{++} concentrations under salinity were Vista/Naomi, AGR703/Naomi and Burdur/Artvin. And maybe for this reason Burdur genotype can be considered a potential rootstock for increasing eggplant tolerance against salinity. The decrease in Ca^{++} uptake due to NaCl salinity was observed by many authors, and in contrary to K^+, the decrease was not due to the competition between Na^+ and K^+ at the absorption site on the root surface, it was always found because of the decline in the transpiration rate under stress conditions (Maggio *et al.,* 2007). In Gao *et al.* (2005), under stress conditions of low temperatures (5°C) grafted eggplants maintained higher leaf Ca^{++} concentrations compared to the non-grafted plants which gave the grafted plants higher tolerance under such stress conditions.

Plant adaptation to salinity may depend on different mechanisms, including the capacity to maintain high levels of antioxidants and/or through the induction of antioxidant enzymes (SOD, CAT, GR, and APX, etc.) (Sevengör, 2010). In the present study, rootstock-grafted plants had higher activity of antioxidant enzymes under salinity conditions, which was translated to lower MDA content in their leaves which means these combinations, were less affected by the ROS-induced lipid peroxidation and they were more tolerant to salinity than the non- and self-grafted plants.

MDA content always found higher in salinity-sensitive plants compared to salinity-tolerant ones (Yaşar, 2003; Kuşvuran *et al*., 2015) and a significant relation between MDA content and antioxidant enzymes activity is first proven by Shalata and Tal (1998). Meloni *et al.* (2001) in cotton, Yaşar (2003) in eggplant, Doğan (2004) in tomato, and Sevengör (2010) in pumpkin, all found that MDA content was low in plants with high antioxidant enzymes activity under salinity stress conditions.

In this study, antioxidant enzymes activity showed a higher increase in rootstock-grafted plants compared to the non- and self-grafted plants, this increase was significantly different between grafting combinations. In another study where cucumber was grafted onto salinity-tolerant rootstock, H_2O_2 level was found to be low, whereas SOD, CAT, POD enzymes activity level were found higher. Öztekin and Tuzel (2011), CAT activity level differed according to the rootstock genotypes, but always was higher in the grafted plants compared to the non-grafted plants.

All results indicated that grafting per se had no significant role in alleviating negative effects of salinity as there were no significant differences between non- and self-grafted combinations in all parameters measured in this study.

In general, local genotypes (landraces) are adapted to prevailing environmental conditions like salinity. In this work, the local Turkish genotype Burdur showed a good potential to compete commercial genotypes. On the other hand, Mardin was way behind and did not show enough potential in this study.

Acknowledgements

This research was supported by the Coordination Unit of the Scientific Research Projects of the University of Ankara (Project no: 15H0447001).

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Adv. Hort. Sci., 2019 33(1): 97-104 DOI: 10.13128/ahs-22618

AHS
Advances in Horticultural Science

Morphological traits and yield of Ajowan affected by different irrigation intervals and growth regulators

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Key words: *Carum copticum*, field, grain yield, growth regulator, stem diameter, water stress.

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Citation:
GHASSEMI S., ZEHTAB-SALMASI S., GHASSEMI-GOLEZANI K., ALIZADEH-SALTEH S., 2019 - *Morphological traits and yield of Ajowan affected by different irrigation intervals and growth regulators*. - Adv. Hort. Sci., 33(1): 97-104

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 6 February 2018
Accepted for publication 23 November 2018

Abstract: Two field experiments were carried out to evaluate the effect of salicylic acid and abscisic acid applications on some morphological traits and yield of ajowan (*Carum copticum* L.) under different watering conditions. Irrigation intervals (irrigation after 70, 100, 130 and 160 mm evaporation from class A pan) were located in main plots and exogenous applications of water (control), salicylic acid (0 and 1 mM) and abscisic acid (0 and 50 micro-molar) were allocated to sub plots. Water stress negatively affected the morphological traits of the Ajowan. Stem diameter, branches per plant, leaves number per plant, biological yield and grain yield per plant of Ajowan were considerably improved by application of salicylic acid and abscisic acid. Salicylic acid treatment was more effective than abscisic acid treatment, in this study, salicylic acid treatment increased the stem diameter and leaves number per plant more than abscisic acid and caused a yield enhancement in Ajowan plants. In general, foliar spray of salicylic acid and abscisic acid could alter morphological traits and yield of Ajowan.

1. Introduction

Medicinal plants have been used for centuries as remedies for diseases and human health, because they contain secondary metabolites of medicinal value. Ajowan (*Carum copticum* L.) is an annual herbaceous plant belonging to the *Umbelliferae* family, which grows in the east of India, Iran, and Egypt, with white flowers and small, brownish seeds. Its fruit has been widely consumed as a food flavoring agent and spice. During the past centuries in the Iranian traditional medicine, several therapeutic effects including anti-vomiting, antiasthma and anti-spasm, is postulated for ajowan fruits (Boskabady *et al.*, 2005).

Water deficit, defined here as an unbalance between soil water availability and evaporative demand which can naturally occur in the field and causes a decrease in carbon assimilation, tissue expansion and actually cell number. Each of these macroscopic processes involves a large number of genes, enzymes, hormones and metabolites (Skirycz and Inze, 2010). Abiotic stresses such as drought cause metabolic changes ranging from synthesis of limited quantities of specialized metabolites to large shifts in primary metabolite composition as well as many other physiological responses. During drought there is a need for osmoticum to accumulate inside the plant cell to retain water and maintain positive turgor pressure (Verslues and Juenger, 2011). It is generally accepted that, at whole plant level, adaptation to water depletion starts with a change in stomatal conductance, leading to reduced net CO_2 assimilation and impaired photosynthesis, which result in shoot growth termination. The result of Nassiri *et al.* (2014) showed that seed yield, water use efficiency and harvest index of ajowan reduced in water stress treatment. Also, Fresh and dry weight of *Ocimum sp.* significantly reduced in water stress conditions (Khalid, 2006). Adaptation to water stress can be highly controlled by plant growth regulators (Popko *et al*., 2010).

Salicylic acid is a phenolic compound capable of enhancing plant growth and yield in some plants (Arfan *et al*., 2007). Salicylic acid can regulate various stress responses and development such as abiotic stress responses, flowering, senescence, thermogenesis and resistance to pathogens (Vicente and Plasencia, 2011). Among abiotic stresses, this growth regulator has been reported to counter low temperature (Tasgin *et al*., 2003), water stress (Abbaspour and Ehsanpour, 2016), salinity stress (El Tayeb, 2005) and high temperature (He *et al*., 2005). Salicylic acid plays diverse physiological roles in most of plants, which include nutrient uptake, stomatal movements, enzyme activities, thermogenesis, ethylene biosynthesis, photosynthesis, flower induction and plant growth (Hayat and Ahmad, 2007). Applications of 1 or 2 mM SA significantly reduced chilling injury and fruit decay of apricot fruit as well as membrane electrolyte leakage and ascorbic acid content. Fruits treated with SA resulted in high total polyphenolic content, antioxidant capacity and carotenoids content (Ezzat *et al*., 2017). Also, application of SA significantly enhanced activity of phenylalanine ammonialyase (PAL) and content of hydrogen peroxide in apricot fruit. Treated fruits showed significantly lower activity of catalase and ascorbate peroxidase but higher activity of superoxide dismutase and peroxidase than those in control fruits (Wang *et al*., 2015). Exogenous application of salicylic acid or its derivates affects diverse plant processes. Since, this growth regulator is heavily involved in crosstalk with other plant hormones; its effect on some of these processes may be indirect (Pieterse *et al*., 2009).

Abscisic acid plays critical roles in numerous biological processes, such as gene transcription, seed dormancy and stomatal closure (Cutler *et al*., 2010). This growth regulator appears to be a major player in mediating the adaptation of the plant to water stress (Pal *et al*., 2011). Abscisic acid enhances drought tolerance in wheat and many of the other plant species (Travaglia *et al*., 2010). That plays an important role in the regulation of abiotic stress resistance in plants and orchestrates complicated signalling pathways involved in the response to reduced water availability as well as in multiple developmental processes (Kim *et al*., 2010).

Based on the aforementioned studies and other researchers, it is acceptable that exogenous application of salicylic acid and abscisic acid can improve abiotic stress tolerance in plants. Thus, this study was designed to investigate the effect of salicylic acid and abscisic acid applications on some morphological traits of Ajowan plants under water stress.

2. Materials and Methods

At the Research Farm of the Faculty of Agriculture, University of Tabriz, Tabriz, Iran, during the growing seasons of 2014 and 2015, two field experiments were carried out to evaluate the effect of salicylic acid and abscisic acid applications on some morphological traits and yield of *Carum copticum* L. under different irrigation treatments. Irrigation intervals [I_1, I_2, I_3, I_4: irrigation after 70, 100, 130 and 160 mm evaporation from class A pan (a cylinder with a diameter of 120.7 cm that has a depth of 25 cm) as normal irrigation and mild, moderate and severe water deficit, respectively] were assigned to main plots and foliar applications of water (control), salicylic acid (0 and 1 milli-molar) and abscisic acid (0 and 50 micro-molar) were allocated to subplots. Average maximum and minimum temperatures and rainfall during the experiment in 2014 and 2015 are shown in Table 1.

Each plot had 6 rows of 4 m length, spaced 25 cm apart. Seeds of this plant were treated with benomyl (Benlat wp 50%; Shanghai Bosman Industrial Co. Ltd, China) at a rate of 2 g kg^{-1} before sowing. Then sown by hand on April 2014 and 2015 at a depth of about 1 cm in a sandy-loam soil. All plots were regularly irrigated after sowing until seedling establishment, and thereafter irrigations were carried out according to the treatments. During plant growth and development, weeds were controlled by hand as required. Salicylic acid and abscisic acid were sprayed on plants at vegetative (once) and reproductive (once) stages.

Table 1 - Averages of maximum and minimum temperatures and rainfall during the work in Tarbiz, Iran, 2014-2015

Month	Temperature (°C)		Rainfall (mm)	
	2014	2015	2014	2015
April	23.7	12.6	50.2	43.2
May	29.5	30.45	10.7	1.5
June	37.1	38.9	18	0.9
July	38.9	40.2	1.3	0
August	34.75	31.1	0	26.4
September	20.65	23.45	84.2	24.1

Leaf number per plant was measured by hand at grain filling stage. Also, at maturity stage, other traits such as plant height (by meter), stem diameter (by digital caliper) and branches per plant (count by hand) were determined. At maturity, 10 plants of the middle part of each plot were harvested and grain yield per plant were determined. Then above ground biomass was oven-dried at 80°C for 48 hours and weighed and subsequently plant biomass was calculated. Measurements for all morphological traits were normally distributed and confirmed through the Kolmogorov-Smirnov test. Randomness was confirmed using the Run Test, and the descriptive statistics were calculated. MSTATC software used to the data analyzed and the means of traits were compared using Duncan multiple range tests at *P≤0.05*.

3. Results and Discussions

Combined analyses of variance showed significant effects of irrigation and growth regulators on stem diameter, branches per plant and leaf number per plant, biomass and grain yield per plant. Plant height was affected by irrigation treatments and plant biomass was significantly influenced by year. The interaction of year × irrigation, year × growth regulator and year × irrigation × growth regulator for plant biomass were significant. Grain yield per plant significantly affected by interaction of irrigation × growth regulator (Table 2).

Plant height of Ajowan decreased with increasing water stress. There were no significant differences between hormonal treated and control plants (Fig. 1). Stem diameter decreased with increasing irriga-

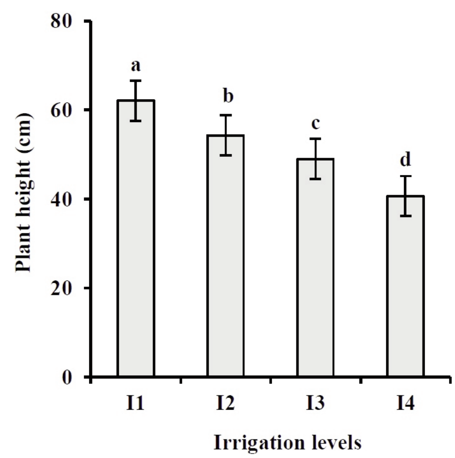

Fig. 1 - Means of plant height of ajowan for different irrigation levels. Different letters in each column indicate significant difference at p≤0.05 (Duncan test). I1, I2, I3, I4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

Table 2 - Combined analysis of variance of morphological traits of Ajowan under different irrigation and growth regulators in Tarbiz, Iran, 2014-2015

Source	df	Mean square					
		Plant height	Stem diameter	Branches per plant	Leaf number per plant	Plant biomass	Grain yield per plant
Year (Y)	1	0.056 NS	0.001 NS	0.68 NS	74.01 NS	65.532 **	10.178 ns
Repeat	4	213.63	0.001	7.94	233.44	24.849	5.580
Irrigation (I)	3	1465.38 **	0.169 **	47.90 **	5662.23 **	709.482 **	294.030 **
Y×I	3	0.05 NS	0.001 NS	1.71 NS	24.82 NS	15.325 *	2.631 ns
Error	12	48.69	0.004	1.35	190.24	3.320	2.420
Growth regulator (G)	2	0.72 NS	0.05 **	6.12 *	1762.16 **	80.355 **	18.847 **
Y×G	2	0.05 NS	0.001 NS	0.10 NS	73.39 ns	5.137 **	0.296 ns
I×G	6	72.50 NS	0.001 NS	2.40 NS	90.44 ns	16.430 **	3.944 **
Y×I×G	6	0.05 NS	0.001 NS	1.07 NS	24.93 ns	2.724 *	0.316 ns
Error	32	54.43	0.002	1.25	67.27	0.834	0.498
CV (%)		14.32	9.86	10.44	16.81	8.53	12.03

NS,*,**= No significant and significant at P≤0.05 and P≤0.01, respectively.

tion intervals. Hormonal application, especially treatment with salicylic acid significantly increased this trait (Fig. 2A and B). With increasing water deficit branches per plant of ajowan decreased. However, difference between I_2 and I_3 was not significant. Salicylic acid treatment increased this trait but, there was no difference between salicylic acid and abscisic acid (Fig. 2C and D). Similarly, leaf number per plant decreased as water deficit increased. Salicylic acid treatment significantly increased this trait. However, there was no significant differences between abscisic acid treated and control plants (Fig. 2E and 2F).

In both years plant biomass of Ajowan decreased with decreasing water supply. Salicylic acid application enhanced this trait under I_1 and I_2 in 2014 and under I_1 in 2015, but abscisic acid treatment only increased plant biomass under I_1 in both years. Salicylic acid was advantage that of abscisic acid on this trait (Fig. 3).

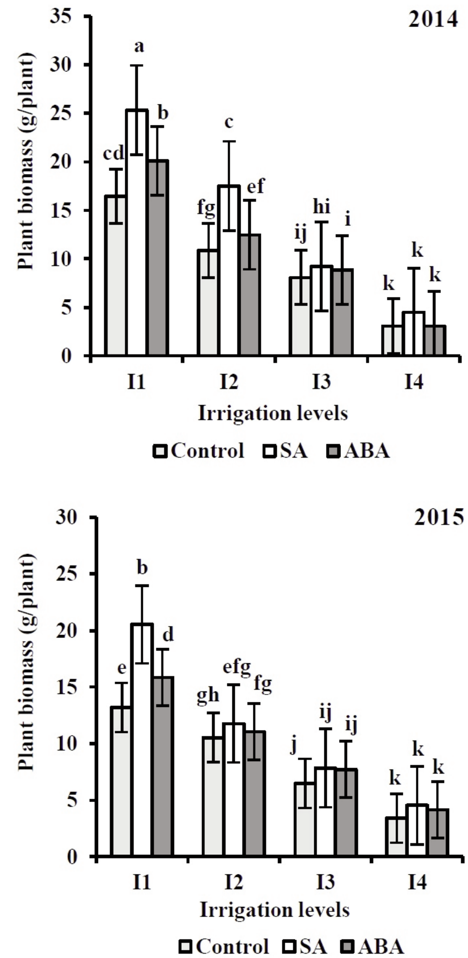

Fig. 3 - Means of plant biomass of Ajowan for year × irrigation × growth regulator. Different letters in each column indicate significant difference at p ≤ 0.05 (Duncan test). I1, I2, I3, I4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively. SA= Salicylic acid - ABA= Abscisic acid.

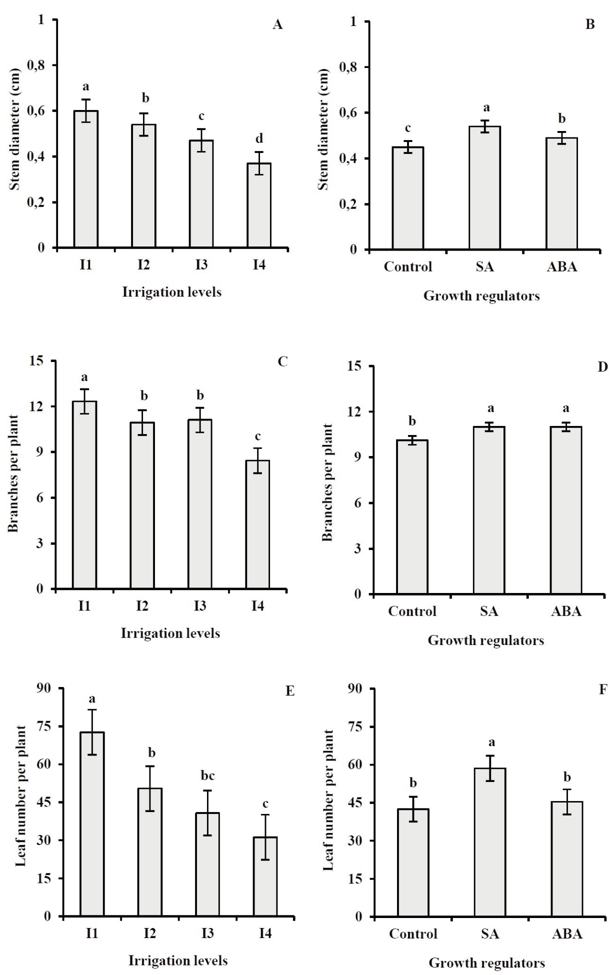

Fig. 2 - Means of stem diameter (A and B), branches per plant (C and D) and leaf number per plant (E and F) of ajowan for different irrigation and growth regulators. Different letters in each column indicate significant difference at p ≤ 0.05 (Duncan test). I1, I2, I3, I4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively. SA= Salicylic acid; ABA= Abscisic acid.

With increasing water deficit, grain yield of salicylic acid treated plants significantly decreased, compared with control plants. Exogenous application of salicylic acid under I_1 and I_2 and also abscisic acid application under I_2 treatment improved grain yield of ajowan plants. However, under I_3 and I_4 irrigation treatments there was no significant difference between hormonal treated and non-treated plants (Fig. 4).

Reduction in plant height due to water stress (Fig. 1) is related with the competition of plants for nutrients and water availability (Ghassemi-Golezani *et al.*, 2010). Water stress during vegetative stages has the greatest impact on biomass and plant height (Ghassemi-Golezani *et al.*, 2008). It has been confirmed by many researchers that water stress lead to growth reduction, which was reflected in leaf area, plant height, dry mass, and other growth functions (Fischer *et al*., 1980; Kriedemann *et al*., 1981). The impact of water stress on plant growth can be explained as a method of adaptation to the conditions of water shortage to limit the rate of transpiration (Lu and Neumann, 1998), in order to maintain the water supply in the soil around plant roots to increases the chance of survival of the plant (Passioura, 2002). The mechanism, by which plant height is reduced under water stress, is through the reduction of cell elongation, which leads to the reduction of cell size and therefore the reduction of plant height (Schuppler *et al*., 1998).

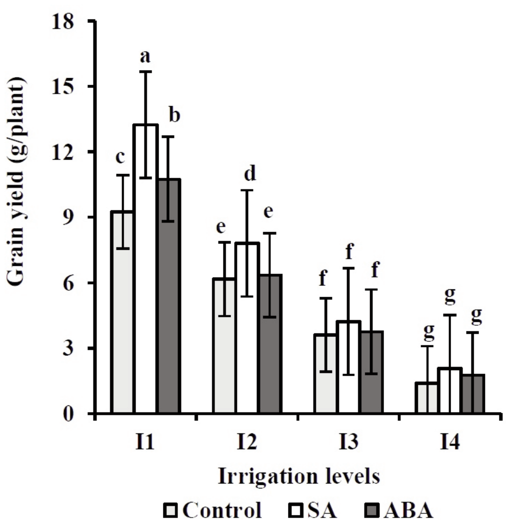

Fig. 4 - Mean grain yield of ajowan for interaction of irrigation × growth regulator. Different letters indicate significant difference at $p \leq 0.05$ (Duncan test). I_1, I_2, I_3, I_4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively. SA= Salicylic acid, ABA= Abscisic acid.

A reduction in leaf turgor and photosynthesis under water stress condition suppresses cell expansion and growth, leading to the diminution of stem diameter (Fig. 2A) (Anjum *et al*., 2011). The decrease in growth parameters under drought stress could be considered as an avoidance mechanism which minimizes water losses (Rodriguez *et al*., 2005). Stem diameter fluctuates daily because of transpiration-induced tension changes in the stem sap (Irvine and Grace, 1997; Perämäki *et al*., 2001). Salicylic acid application promotes cell division and enlargement (Hayat *et al*., 2005). It has been reported that salicylic acid significantly enhances the average growth speed of tomato stems (Stevens and Senaratna, 2006), which is in line with the results of the present study. Salicylic acid was reported to increase cytokinins in corn and these hormones increased the stem diameter (Shakirova *et al*., 2003). Foliar spray of abscisic acid is favouring vegetative growth of plants as shown for ajowan (Fig. 2B) and soybean (Travaglia *et al*., 2009). Exogenous application of abscisic acid was able to increase plant adaptive response to various environmental conditions (Abraham *et al*., 2008).

Number of branches and leaves per plant decreased under drought condition (Fig. 2C and 2E). This might be related with the suppression of cell expansion and cell growth due to the low turgor pressure. Reduced number of leaves by moisture stress is in line with the finding of Stolf-Moreira *et al*. (2010) who found that water stress affected number of leaves for soybean. This is because water stress leads to decreased rate of leaf initiation and reduction in leaf area of already formed leaves. This can be related with lower photosynthetic activity in the affected leaves. The overall effect is a decrease in the rate of new leaf initiation and increase in leaf shedding thereby resulting to reduction in number of green leaves per plant (Yunusa *et al*., 2014).

Salicylic acid stimulatory effect on growth estimated characteristics could be related with the effect of this growth regulator on the endogenous phytohormones such as growth promoters i.e. cytokinins, gibberellins and auxins (Waffaa *et al*., 1996; Shehata *et al*., 2000). Application of these growth regulators increased number of branches per plant (Fig. 2D) and leaves (Fig. 2F), which could lead to increment of number of flowers and subsequently seed yield. In agreement with these results Fathy *et al*. (2003) on eggplant and Gharib (2007) on basil and marjoram they mentioned that salicylic acid increased plant height, number of branches and leaves per plant and dry mass as well, respectively. Abscisic acid by induction of genes that encode enzymes and other proteins involved in cellular dehydration tolerance, plays a critical role in regulating plant water status through guard cells and growth as well as (Luan, 2002; Zhu, 2002) which might be the main reason for increased branches per plant. Treatment with abscisic acid in *Sesamum indicum* L. increased number of branches to a large extent (Abraham *et al*., 2008).

Plant biomass was reduced under water stress (Fig. 3) due to leaf senescence and decline in the cell enlargement resulting from reduced turgor pressure (Shao *et al*., 2008). Severe water stress may result in arrest of photosynthesis and disturbance of metabolism (Liang *et al*., 2006). Water stress inhibits cell enlargement and that reduces various biochemical and physiological processes (Shao *et al*., 2007).

Drought stress leading to stomata closure and reduction in photosynthesis rate and leaf growth (Ozturk, 1999), which ultimately decreases plant biomass. This reduction in plant biomass resulted in decreasing the plant height (Fig. 1), stem diameter, branches per plant and leaf number per plant (Fig. 2) and consequently grain yield per plant (Fig. 4).

Salicylic acid improved the production of plant biomass (Fig. 3) by increasing the stem diameter, branches per plant and leaf number per plant (Fig. 2). Salicylic acid influences a wide variety of plant processes, including stomatal regulation, chlorophyll content and photosynthesis (Yildirim *et al*., 2008). Also, by increasing ribulose 1,5-bisphosphate (Rubp) content under drought condition, protecting the photosynthetic machinery from reactive oxygen species produced during drought stress (Shehata *et al*., 2001) and maintaining LAI and photosynthetic activity under stress (Bayat and Sepehri, 2012) increased plant biomass. Abscisic acid plays a critical role in regulating plant water status through guard cells and growth as well as by induction of genes that encode enzymes and other proteins involved in cellular dehydration tolerance (Zhu, 2002), which might be resulted in increasing dry mass under drought stress (Fig. 3).

Reduction of grain yield under water stress (Fig. 4) has been attributed to reduced plant height (Fig. 1), stem diameter, branches per plant, leaf number per plant (Fig. 2) and plant biomass (Fig. 3). Water stress severely limits growth and yield of plants by reducing chlorophyll content of leaves, photochemical efficiency of photosystem II (Ghassemi-Golezani and Lotfi, 2012), photosynthesis (Munns *et al*., 2006) and ground green cover (Ghassemi-Golezani and Ghassemi, 2013). Water stress during vegetative stages largely reduces plant height and biomass, while during reproductive stages it has the greatest negative impact on grain yield (Ghassemi-Golezani *et al*., 2008).

Grain yield was improved by growth regulators (Fig. 4). The increase in yield might be due to increased sink size mainly number and mass of grains. This could be related to increased photosynthetic efficiency by stabilization of chlorophyll and higher production and translocation of organic material from source to sink. Salicylic acid stimulates physiological processes that were reflected on improving vegetative growth (Fig. 2, 3) followed by active translocation of the photosynthesis products from source to sink. Abscisic acid is also able to activate metabolism of carbohydrates temporally stored in the plant stem (Yang *et al*., 2003; Travaglia *et al*., 2010).

4. Conclusions

Water stress negatively affected the morphological traits and yield of the Ajowan. Stem diameter, branches per plant, leaf number per plant, individual plant biomass and grain yield were considerably improved by application of salicylic acid and abscisic acid. Salicylic acid treatment had more positive effects on plants, compared with abscisic acid treatment. Foliar spray of abscisic acid and especially salicylic acid could, therefore, alter morphology and yield of Ajowan.

Acknowledgements

We appreciate the support of this work by the University of Tabriz.

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Adv. Hort. Sci., 2019 33(1): 105-111 DOI: 10.13128/ahs-22596

AHS
Advances in Horticultural Science

Propagation of *Rosa hybrida* L. cv. Dolce Vita by stenting and stem cutting methods in response to different concentrations of IBA

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Key words: Indole-3-butyric acid (IBA), propagation, rooting, rootstock, stenting.

Citation:
POURGHORBAN M., KHAGHANI S., AZADI P., MIRZAKHANI A., CHANGIZI M., 2019 - *Propagation of Rosa hybrida L. cv. Dolce Vita by stenting and stem cutting methods in response to different concentrations of IBA*. - Adv. Hort. Sci., 33(1): 105-111

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 4 February 2018
Accepted for publication 30 November 2018

Abstract: This study was conducted to investigate the effect of different concentrations of Indole-3-butyric acid (IBA) in propagation of *Rosa hybrida* L. cultivar Dolce Vita by stenting (cutting and grafting) and stem cutting methods in greenhouse conditions. Different concentrations of IBA (0, 1500, 3000 and 4500 mg/L) were considered on *Rosa hybrida* L. cv. Dolce Vita grafted onto *Rosa hybrida* L. 'Natal Briar' rootstock. Then, the stentings and stem cuttings were cultured in a cocopeat + perlite (in 1:1 ratio) medium under mist system. The research was conducted as a completely randomized design with three replications. The results suggested that all IBA treatments significantly increase rooting percentage compared with the control plants, and the highest specifications of roots and shoots were observed in 1500 mg/L IBA in both methods of propagation. The results approved the superiority of stem cutting in rooting. However, the higher content of chlorophyll a, b and total were obtained in stenting method.

1. Introduction

Rose flower (*Rosa hybrida* L.) is one of the world's most popular flowers among ornamental plants (Castilon *et al.*, 2006), and cut rose flower industry is the most important aspect of rose culture industry in the world (Bleeksma and Van Doorn, 2003) with a turnover of 735 million Euros in 2015 (Azadi *et al.*, 2016). Rose plants are propagated by seed and asexual methods such as stem cutting, grafting, budding, cutting-grafting (stenting), cutting-budding, root grafting and tissue culture (Salehi and Khosh-

Khui, 1997 a, b; Dole and Wilkins, 2005; Azadi *et al.*, 2007, 2013). Vegetative propagation is the sole method for maintaining desirable characters in a superior cultivar particularly when it is heterozygous and polyploid. The easiest and most common method of growing roses is the use of stem cuttings (Anderson and Woods, 1999). The success of rooting in stem cuttings depends on species and cultivar, growth season, the condition of cutting wood, type of cuttings like hardwood cuttings, semi hard wood cuttings, softwood and herbal cuttings, and many other factors (Hartmann *et al.*, 2002). However, stenting method is an efficient technique for quick propagation of plants. This method is an effective technique of rose propagation (Van de Pol and Breukelaar, 1982), in which cutting and grafting is performed simultaneously and the scion is grafted onto a non-rooted rootstock. Formation of the union and adventitious roots on the rootstock occurs simultaneously (Nazari *et al.*, 2009; Karimi, 2011; Babaie *et al.,* 2014). Cutting and grafting is also a valuable technique in propagation of several horticultural species including roses (Koepke and Dhingra, 2013), conifers, rhododendrons, and also a number of citrus fruits, apples, plums and pears (Hartmann *et al.*, 2002), therefore the use of rootstocks in propagation is a common practice. Plant propagation by stenting has been practiced in several nurseries as a substitute for budding, since it has advantages such as better yield and quality, and resistance to crown gall disease (Park and Jeong, 2010). Efficiency and flower productivity in grafted plants is higher than those in plants growing on their own roots and this is due to the use of rootstock (Cabrera, 2002). Because of different climatic and soil conditions in different areas of the world, different rootstocks are recommended to be the main subject of attention of compatibility between rootstock and scion (Niu and Rodriguez, 2008). 'Natal Briar' is a rootstock of unknown lineage, probably coming from South Africa, and is becoming the desirable rootstock for hydroponic production systems in Netherlands, USA and Colombia (Cabrera, 2002). 'Natal Briar' is the rootstock for grafted roses, which represents 60-70% of the world's cultivation since it is easy to root and gives well stem length and head size (Otiende *et al.*, 2015). Exogenous auxin is shown to have a necessary role in root formation in cuttings and has an effect on the speed and the percentage of rooting of cuttings. Plants produce natural auxin in their fresh shoots and leaves, however, the synthetic auxin must be used for successful rooting to prevent cuttings death (Stefanic *et al.*, 2006; Kasim and Rayya, 2009), and has been reported in many species, e.g. *Hibiscus rosa sinensis* (Kumar and Singh, 2012). For accelerating the formation of adventitious roots, auxin is widely used on the stem cuttings (Galavi *et al.*, 2013), and it increases the speed and percentage of rooting (Kasim and Rayya, 2009). Photosynthetic pigments in plants comprise chlorophylls a and b and these pigments play an important role in light absorption during photosynthesis (Lobato *et al.*, 2009). Carotenoids are a group of natural tetraterpenoid pigments distributed widely in plants, algae, fungi, and bacteria. Many flowers, fruits, and roots owe their vivid orange, yellow, and red colors to carotenoids. Carotenoids play necessary roles in photosynthesis and photoprotection (Domonkos *et al.*, 2013; Niyogi and Truong, 2013; Hashimoto *et al.*, 2016). This research was carried out to evaluate different concentrations of IBA in propagation of *Rosa hybrida L.* cultivar 'Dolce Vita' using stem cutting and stenting.

2. Materials and Methods

Greenhouse conditions

This research was carried out in February 2017 in a greenhouse with plastic cover located in Markazi Province, Arak, Iran. The greenhouse was equipped with mist system (Fig. 1 a) as well as hot water system for adjusting temperature and humidity. During the study cycle, the average temperature and relative humidity of day were maintained at 20±5°C and 85±5%, respectively. At night, the mist system was automatically switched off and temperature was maintained at 10-15°C and relative humidity of 65±5%.

Plant materials and propagation (stenting and stem cutting)

Plant materials were prepared from a Rose commercial greenhouse (located in Markazi Province, Arak, Iran). The scions consisted of a single-node and one leaf (including 2 leaflets) were collected from *Rosa hybrida L*. cv. Dolce Vita as soon as their mother plants entered to faded flower stage. The scions were grafted onto a 4.0 cm length internode taken from the semi-hard wood cuttings of the rootstock *Rosa hybrida L*. 'Natal Briar'. In this method, scions and rootstocks with a suitable flat cut could be grafted together with the maximum overlap of the cambium layer (Fig. 1 b). The scions were selected based on thickness of the stem rootstock. Scions then were grafted using splice grafting method. Scions and rootstocks with an appropriate smooth cut could be grafted together with the maximum overlap of the cambium layer. Plastic tape was used for wrapping the graft union. The end bud in the rootstock was removed for better rooting and avoiding sucker production (Fig. 1 c). Stem cuttings with at least 3 nodes and of 5-8 mm diameter and 15-20 cm length were selected from the middle portion of the vigorously growing shoots of *Rosa hybrida L.* cv. Dolce Vita as soon as their mother plants entered to faded flower stage. The end node of the stem cuttings were removed for better rooting. The distal end of rootstocks and stem cuttings were treated with different concentrations of IBA (1500, 3000 and 4500 mg/L) by quick dip method, and control plants were treated with distilled water. The stentings and stem cuttings were rooted in a mixture of cocopeat and perlite medium (in 1:1 ratio) under mist system. To prevent fungal infection, the beds were disinfected with fungicides 0.2% ‘Captan’ solution every two weeks. The grafted plants and stem cuttings were grown for 60 days under the mentioned greenhouse conditions. At the end of the study, the plants were removed from the culture medium and some of their morphological and biochemical traits including healing percentage, rooting, root number, longest root, fresh weight of roots, dry weight of roots, shoot percentage, leaf number, shoot number, longest shoot, content of chlorophyll (a, b and total) and carotenoid were measured.

Chlorophyll (a, b and total) and carotenoid contents measurement procedure

To measure the chlorophyll content, 0.2 g of fresh leaves were ground to a fine pulp by adding 10 ml of 80% acetone. The supernatant was transferred to a 25 ml volumetric flask. The washings were collected in the volumetric flask and the volume was made up to 25ml with 80% acetone. The solution was centrifuged (5000 rpm) for five minutes. The absorbance of the solution was read at 663, 645 and 470 nm against the solvent (80% acetone) blank (Lichtenthaler, 1987).

Chl a (mg/ml) tissue = [12.7(A663) - 2.69 (A645)] V / 1000 W

Chl b (mg/ml) tissue = [22.9(A645) - 4.68 (A663)] V / 1000 W

Total Chl (mg/ml) tissue = [20.2(A645) + 8.02(A663)] V / 1000 W

Car (mg/ml) tissue = [(1000 A470 - 1.8 Chl a - 85.02 Chl b)/198] V / 1000 W

where Chl= Chlorophyll, Chl a= Chlorophyll a, Chl b= Chlorophyll b, Car= Carotenoid, A= Absorbance at specific wavelengths, V= Final volume of chlorophyll extract in 80 % acetone (25 ml), and W= Fresh weight of tissue extracted (0.2 g).

Data collection and statistical analysis

Sixthy days after planting, the stentings and stem cuttings were taken out from the media and some traits such as rooting percentage, shoot number, root number, root length (cm), leaf number, shoot length (cm), contents of leaf chlorophyll (a, b and total) and carotenoid (mg/g), root fresh and dry weight (g), and healing (graft-take) percentage were recorded for each stenting and stem cutting. After calculating fresh weight of roots, they were wrapped in paper envelopes and dried in oven at 60°C for 24 hours to calculate their dry weight. Content of leaf chlorophyll and carotenoid were determined using spectrophotometric method (Saini *et al.*, 2001). The experiment was conducted as a completely randomized design with three replications, and each replication consisted of 16 samles. The data were analyzed with SAS software and means were compared through Duncan test ($p<0.05$).

3. Results

The influence of treatments on morphological parameters and graft healing of stentings are shown

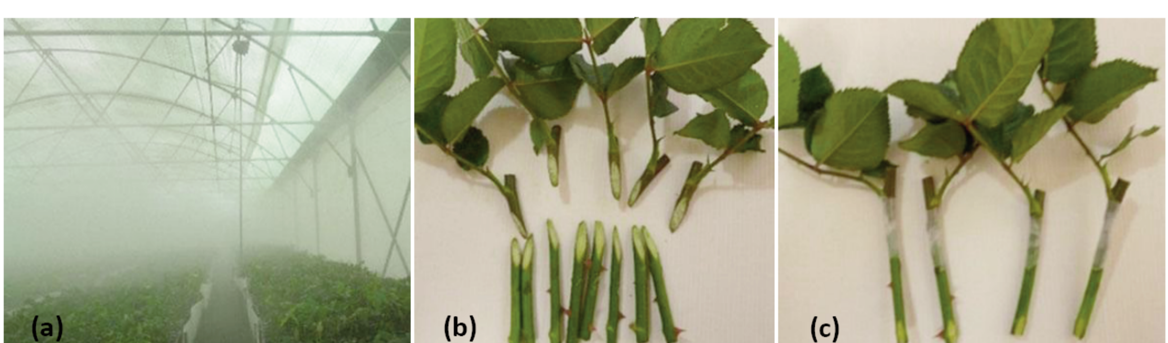

Fig. 1 - Greenhouse space and the stages of preparation of stentings. Mist system in the greenhouse (a), Flat cut in Scions and rootstocks (b), Connection scions and rootstocks by plastic tape, and remove end bud (c).

in Table 1. The presented data clearly revealed that different concentrations of IBA significantly affected various traits of roots and shoots. Maximum rooting percentage, root number, average root length, and fresh and dry weight of roots were recorded in 1500 mg/L of IBA. The highest grafting success, number of leaves, number of shoots per stenting, and average shoot length were observed in 1500 mg/L of IBA concentration (Table 1). An example of rooted stentings is shown in figure 2 (a-d). The influence of treatments on morphological parameters in stem cuttings is also shown in Table 2. Maximum rooting percentage, root number, and fresh and dry weight of roots in stem cuttings were recorded in 3000 mg/L of IBA. An example of rooted stem cuttings is shown in figure 3 (a-d). Different concentrations of IBA had significant effect on rooting percentage, root number, and fresh and dry weight of the roots. The highest rooting percentage, root number, fresh and dry weight of roots was obtained in stem cuttings treated with 3000 mg/L of IBA. However, there was no significant difference between treatments (1500 and 3000 mg/L) in most of the traits of stem cuttings (Table 2). Results showed that contents of leaf Chlorophyll (a, b and total) and carotenoid in stentings and stem cuttings were affected by different concentrations of IBA ($p<0.01$). An example of rooted stentings is shown in figure 4 (a-d).

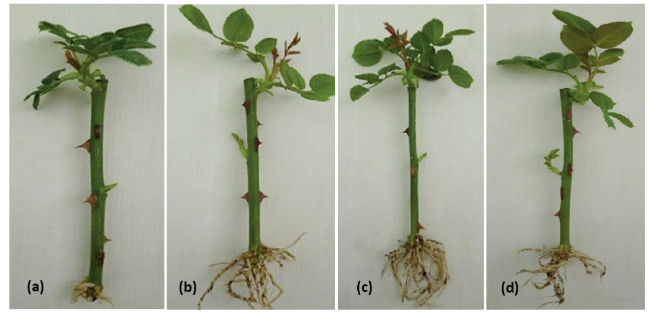

Fig. 3 - Rooting level at different concentrations IBA in Rosa hybrida L. cv. Dolce Vita stem cuttings. Control plant (a), Concentration of 1500 mg/L IBA (b), Concentration of 3000 mg/L IBA (c) and Concentration of 4500 mg/L IBA (d).

Table 1 - The effect of IBA treatments on growth parameters and graft healing of *Rosa hybrida* L. cv. Dolce Vita stentings

IBA (mg/L)	Healing (%)	Rooting (%)	Root number	Longest root (cm)	Roots fresh weight (g)	Roots dry weight (g)	Shoot (%)	Leaf number	Shoot number	Longest shoot (cm)
0	37.50 b	35.41 b	1.96 b	0.63 c	0.25 c	0.02 c	37.5 b	1.25 b	0.44 b	2.22 b
1500 mg/L	66.66 a	81.25 a	9.60 a	3.00 a	0.95 a	0.12 a	66.66 a	2.50 a	0.70 a	5.32 a
3000 mg/L	43.75 b	50.00 b	4.54 b	1.93 b	0.60 b	0.07 b	43.75 b	1.96 ab	0.46 ab	3.51 b
4500 mg/L	37.50 b	41.66 b	3.73 b	1.71 b	0.51 b	0.06 b	37.50 b	1.59 b	0.37 b	2.80 b

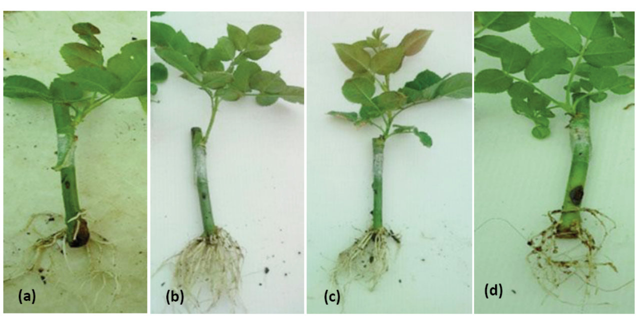

Fig. 2 - Rooting level at different concentrations IBA in *Rosa hybrida* L. cv. Dolce Vita stentings. Control plant (a), Concentration of 1500 mg/L IBA (b), Concentration of 3000 mg/L IBA (c) and Concentration of 4500 mg/L IBA (d).

Healing percentage in stentings

The presented data clearly revealed that maximum grafting success (66.66%) was in 1500 mg/L of IBA, and the minimum healing percentage was observed in control and 4500 mg/L of IBA (Table 1).

Table 2 - The effect of IBA treatments on growth parameters of *Rosa hybrida* L. cv. Dolce Vita stem cuttings

IBA (mg/l)	Rooting (%)	Root number	Longest root (cm)	Roots fresh weight (g)	Roots dry weight (g)	Shoot (%)	Leaf number	Shoot number	Longest shoot (cm)
0	83.33 b	13.95 d	5.06 b	1.23 d	0.12 c	93.75 a	4.8 a	1.69 a	7.87 b
1500 mg/L	95.83 a	22.94 b	8.21 a	1.76 b	0.16 b	95.83 a	4.83 a	1.61 ab	11.20 a
3000 mg/L	97.91 a	26.49 a	7.73 a	2.01 a	0.22 a	97.91 a	4.29 b	1.41 ab	10.46 a
4500 mg/L	85.41 b	20.72 c	5.80 b	1.55 b	0.15 bc	85.41 a	3.39 c	1.35 b	8.73 b

Root indices and fresh and dry weight of roots

The highest percentage of rooting were observed in stentings (81.25%) and stem cuttings (97.91%) treated with 1500 and 3000 mg/L of IBA, respectively, and the minimum rooting percentages (35.41% and 83.33%) in both propagation methods were

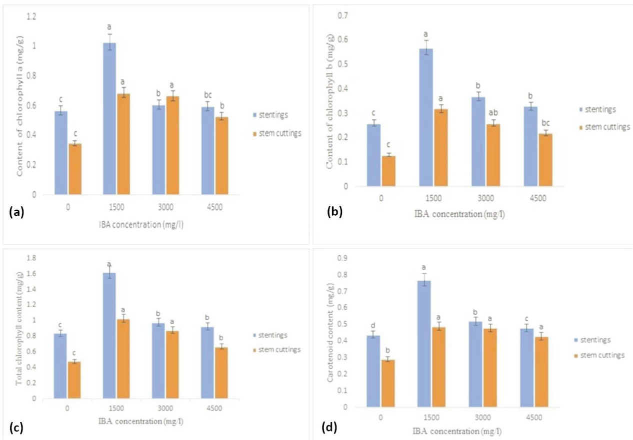

Fig. 4 - Comparison content of chlorophyll and carotenoid (mg/ml) in *Rosa hybrida* L. cv. Dolce Vita stentings and stem cuttings treated with different concentrations of IBA (mg/L). Content of Chlorophyll a (a), content of Chlorophyll b (b), total chlorophyll content (c) and carotenoid content (d).

recorded in control plants (Table 1, 2). Based on this study, different concentrations of IBA affected root number in stentings and stem cuttings. The maximum root number in stentings (9.60) and stem cuttings (26.49) were observed in 1500 mg/L and 3000 mg/L of IBA, respectively (Table 1 and 2). Results showed the greatest average root length in stentings (3 cm) and stem cuttings (8.21 cm) on 1500 mg/L of IBA, and the smallest rooting length in stentings (0.63 cm) and stem cuttings (5.06 cm) were observed in control plants (Table 1 and 2). The greatest root fresh weights were found in 1500 and 3000 mg/L of IBA respectively in stentings (0.95 g) and stem cuttings (2.01 g), and the lightest root fresh weights in both propagation methods were observed in control plants. The greatest dry weight of the root in stentings (0.12 g) and stem cuttings (0.22 g) were also observed at 1500 and 3000 mg/L of IBA, respectively, and the lightest dry weight of the root in both propagation methods were recorded in the control plants (Table 1 and 2).

Number of leaves, number and length of shoots

In stentings, highest leaf number (2.50), shoot number (0.70) and shoot length (5.32 cm) were observed in 1500 mg/L of IBA. The lowest leaf number (1.25) and shoot length (2.22 cm) were recorded in control plants, while the lowest shoot number (0.37) was observed in 4500 mg/L of IBA (Table 1). In stem cuttings, highest leaf number (4.83) and shoot length (11.20 cm) were observed in 1500 mg/L of IBA, while highest shoot number (1.69) was observed in control plants (Table 2).

Contents of leaf Chlorophyll (a, b and total) and carotenoid

In this study, contents of chlorophyll a, b and total and contents of carotenoid were measured in stentings and stem cuttings. In 0.2 g of fresh leaves, the highest content of chlorophyll a (1.03 and 0.69 mg/g), chlorophyll b (0.57 and 0.32 mg/g), total chlorophyll (1.62 and 1.03 mg/g) and carotenoid (0.77 and 0.49 mg/g) were observed in the 1500 mg/L of IBA in stentings and stem cuttings, respectively (Fig. 4 a-d).

4. Discussion and Conclusions

Physiologically, stenting is more complicated than cutting propagation since formation of the graft union must occur simultaneously with rooting and there are interactions between photosynthesis, root formation, and bud development (van de Pol *et al.*, 1986). Karimi (2011) also reported that in stenting, the graft union must be formed before root initiation. Therefore, after leaf formation on the scion, carbohydrates and natural hormones are produced and transmitted from the leaves to the rootstock for growing. Another study reported that rootstock plays an important role in the entire process of root growth, and propagation through bench grafting will bring success. In propagation of *Chinese hibiscus* through stenting showed that IBA treatments significantly increased rooting percentage (Izadi and Zarei, 2014). The effect of different concentrations of auxin on rooting of *Stewartia pseudocamellia*, reported that rooting percentages in cuttings treated with rooting hormones (71.9% to 93.6%) are higher than rooting percentages(53%) in the control plants (Nair *et al.*, 2008). For root formation on stem cuttings, natural or synthetic auxins are essential. Auxin increases the formation of adventitious roots in many species through facilitating carbohydrates and nitrogen materials transfer to the cutting base and motivating primordial root. The high level of auxin may have a negative effect on root length. Auxin leads to transfer of leaf carbohydrate and nitrogen to the roots and therefore causes an increase in the root dry weight (Hartmann *et al.*, 2002). Al-Salem and Karam (2001) reported that auxin concentration had a significant effect on rooting regardless of its chemical structure or type. The maximum rooting percentage, number of root, length, and fresh and dry weight were obtained by basal cuttings treatment with 24 mM of IBA. The maximum dry weight of roots may be ascribed to increased roots length and number of roots (Ingle and Venugopal, 2009). Auxins promote adventitious root formation and formed roots enhance the uptake of water and mineral nutrient and production of hormones (cytokinins) which are required for shoot growth and development (Otiende *et al.*, 2015). Growth amount of shoots and roots are interdependent (Tonutti and Giulivo, 1990). The probable cause for increase in shoot length may be the better utilization of carbohydrates, nitrogen and other nutrients which has been assisted by growth regulators (Chandramouli, 2001). Izadi and Zarei (2014) reported that the highest leaf number was observed in stentings with higher root number. Buds and leaves are considered significant factors to improve root induction (Hartmann *et al.*, 2002). Leaf chlorophyll content was affected by the interaction between cultivars and propagation methods and was significant in all grafted cultivars; as a result, leaf chlorophyll content and quality index were higher in grafted plants compared to those propagated by cuttings. This might be the effect of rootstock (Nazari *et al.*, 2009). In conclusion, the study demonstrated that the application of IBA plays an important role in success of *Rosa hybrida L.* cv. Dolce Vita propagation through stenting and stem cutting methods. IBA had significant effect on rooting compared to control plants, and the highest rate of rooting percentage was observed in 1500 mg/L IBA in both methods of propagation. Superiority of stem cutting in the case of percentage of rooting was approved. However, the higher content of chlorophyll a, b, total and carotenoids were obtained in stenting which probably will affect the quality and flower size at reproductive stage. It is needed further study on the growth stages of the plant until to the flower harvesting in both cuttings and stenting method.

Acknowledgements

Authors are grateful to Mr. Feizian, Dr. Edrisi, Mr. Bahrami, and Ms. Heidari for their assistance during this experiment. The study was done in Greenhouse of Mr. Feizian.

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Advances in Horticultural Science

A new device to improve the mechanical winter pruning in olive trees hedgerows

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OPEN ACCESS

Key words: branches, fruit bearing, leaves, *Olea europaea*, shoots, superintensive plantations.

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Citation:
OTTANELLI A., MARONE E., FIORINO P., 2019 - *A new device to improve the mechanical winter pruning in olive trees hedgerows*. - Adv. Hort. Sci., 33(1): 113-122

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 30 November 2018
Accepted for publication 9 March 2019

Abstract: The economic success of superintensive olive plantations is mainly due to the full mechanization of the harvesting and pruning. While the advantage of straddling machines is undoubted, winter mechanical pruning determines falls in productions. This is due to the indiscriminate suppression of both fertile leafy shoots destined to fruiting and growth, and exhausted parts of the plant. To reduce this damage, an innovative device has been developed, applied to a pruning machine, able to selectively cut the "aged" parts of vegetation. The selection is achieved by an air's fluid dynamic action obtained throughout defined and directional air jets able to push the young and flexible shoots upwards and downwards; in this way they are saved by the cut, regenerating at least one year in advance the new fruiting hedge. Tests were carried out on the cultivar Arbequina, Tosca and Sikitita, in three superintensive olive groves located in the province of Grosseto, Rome and Latina, assessing the amount of leaves, shoots and branches, as well as fruits present at harvest, preserved from the pruning thanks to the action of the air flow, respectively for the East and West side of the rows. To get a profile of the biomass distribution along the cross section of the tree canopy, in the Grosseto farm a trial was also carried out to better assess leaves, shoots and branches distribution in the canopy. The statistical data analyses immediately evidenced two different populations due to the selective pruning. The work highlighted the remarkable effectiveness of the air jet in safeguarding the flexible and leafy vegetation and allowed to quadruple leaf surface and production.

1. Introduction

The improved knowledge and agronomic techniques during the last decades have allowed the development of olive cultivation beyond the original distribution area (Mediterranean basin), in new zones where olive growing represents one of the most promising crops (Marone and

Fiorino, 2012).

Since the beginning of the 1990s, the introduction of a canopy management and training system defined as "superintensive" contributed to the spread of olive cultivation (Tous *et al.*, 1997; Tous *et al.*, 2010; Rallo *et al.*, 2013), as the high number of trees per hectare (over 1600), with particularly short distances between the plants on the row; the close canopies form, such as in the vine, a single hedge; the system allows the complete mechanization of the harvesting, carried out with straddling machines that detach, intercept and storage the fruits at once, working continuously; the system also allows the mechanical pruning (Vivaldi *et al.*, 2015), performed by circular saws fitted on adjustable bars, by eliminating the vegetation along horizontal (topping) or vertical (hedging) planes. The continuous harvesting yards, based on the use of straddle machines, allow strong scale economies and guarantee the greatest efficiency with respect to other systems like shakers and vibrating combs, which can work in traditional or intensive farming systems (Fiorino *et al.*, 2010).

The mechanical pruning using circular saws fitted on bars, borrowed from viticulture, can be applied to both intensive (individual canopies) and superintensive (hedge) plantations. The comparison between manual and mechanical pruning highlight a greater operative speed of the second (Giametta and Zimbalatti, 1997), partially compromised by the decreasing of productivity (Ferguson *et al.*, 2002; Peça *et al.*, 2002). In fact, the mechanical pruning simultaneously eliminates, together with the exhausted, also the young vegetation, influencing the canopy vegetative-productive ratios, as well as the distribution of the natural resources and of the light (Cherbiy-Hoffmann *et al.*, 2012). In intensive plantations, it needs several years to evaluate the influence of regular winter pruning on plant productivity (Dias *et al.*, 2012) and, according to Albarracín *et al.* (2017), depending on the intensity of the intervention, it can take up to two growth seasons to reconstitute a fruiting canopy and three to bring the plants back to full production.

While in traditional and intensive plantations the mechanical pruning can be considered an economical and convenient alternative to the manual one, it is the only technique available to manage the canopy of adult plants grown in superintensive plantations. In this case its role changes, from tool to preserve the vegetative-productive plants equilibrium, to tool for constraining the plant dimensions within defined limits of height and width, required to perform the harvesting by straddling machines (Vivaldi *et al.*, 2015); as a consequence it is largely applied in these plantations, despite the negative consequences on the productive vegetation, due to the particular growth model of the olive tree. In fact, in the olive tree, the fruiting takes place on the vegetation of the previous year, simultaneously with the elongation of the shoots apex that will take fruits the following year; over the time, these shoot elongations progressively move away from the central leader of the tree and, as the competition between the different sinks (apical shoot growth and fruits energy demand), progressively become weak and less productive. At the same time, from the branch that originated them, some latent buds can sprout, fated to reiterate the cycle, by substituting the fertile vegetation that has already produced fruits (Fiorino and Marone, 2010).

These vegetative structures coexist on the surface of the plants, or along a "wall" in the case of hedgerows, where the renewal vegetation is dragged downwards by the weight of the sprouts, being the wood of olive branches flexible, and due to the weight of olives, which also curve the young branches. The "aged" and exhausted parts are periodically eliminated by the winter pruning (Fiorino and Marone, 2010).

An innovative device has been designed and built, inserted on a bar equipped with counter-rotating circular saws, able to delivering defined and directional air jets that push the shoots upwards and downwards, bending them. In this way the young flexible shoots inserted on the two or three year-old branches escape the action of the pruning machine blades, recovering their natural position after the course of the tool, while the short shoots inserted on the rigid vegetation that produced fruits, depleted and to remove, are cut.

The present study would to test the efficiency of a new selective blower device, with the goals: a) to save leaves on the green wall, improving its efficiency in light interception, and b) to save a greater number of fertile buds since the first year after cutting and at least a part of the fruits resulting from the subsequent flowering.

Moreover, the study would determine the quantity of the biomass produced in olive hedgerows as by-product of the mechanical pruning, characterized by a remarkable presence of leaves, to verify the possibility of alternative uses, in addition to those already tested for manual pruning residues that, mixed with olive pomace, contribute to the production of pellets (Barbanera *et al.*, 2016) and, as already demonstrated for pomace, to identify a possible use as supplements/components of livestock feed (González *et al.*, 2012; Castellani *et al.*, 2017; Taticchi *et al.*, 2017) or as a natural source of antioxidant compounds (Talhaoui *et al.*, 2015).

2. Materials and Methods

Plant material

Three different pruning trials were carried out in three different localities in high density plantations, on rows of olive trees trained with a central leader, allowing their natural lateral branching.

1) Year 2014. The first trial was performed in the "Castello di Torrimpietra" farm, located in Torrimpietra (Rome) (41° 53 '43'' North Latitude, 12° 14' 8'' East Longitude, 44 m asl), on trees of the cv. Arbequina and Tosca, 8 years old, with planting density of 3.8 x 1.6 m (2630 m of rows per hectare). The plants were branched about 60 cm from the soil; the thickness of the canopy walls at the time of the pruning was about 120 cm, the height of 2.20-2.40 cm. The useful height of the fruiting wall was 180 cm. The soil is sandy (over 60% sand), pH = 7.6.

2) Year 2015. The second trial was performed in the "Casale San Giorgio" farm, located near Latina (41° 37' 7'' North Latitude, 12° 34' 50'' Est Longitude, 64 m asl), on trees of the cv. Arbequina and Sikitita, 5 years old, with planting density of 3.8 x 1.6 m (2630 m of rows per hectare). The plants were branched about 60 cm from the soil; the thickness of the canopy walls at the time of the pruning was 120 cm, the height of 2.00-2.20 cm. The useful height of the fruiting wall was 160 cm. The soil is clayey (36% clay), with a high percentage of sand (40%), pH = 6.7.

3) Year 2018. The third trial was performed in the "Tombolo" farm located in Grosseto (42° 44' 3'' North Latitude, 10° 59' 10'' Est Longitude, 8 m asl), on trees of the cv. Arbequina, 10 years old, with planting density of 4.0 x 1.6 m (2500 m of rows per hectare). The plants were branched about 60 cm from the soil; the thickness of the canopy walls at the time of the pruning was 140-160 cm, the height of 2.00-2.20 cm. The useful height of the fruiting wall was 160 cm. The soil is sandy-silty (over 60% sand), pH = 7.2.

The rows in the three farms are oriented North-South. All the plants were subjected to fertigation, soil management by grassing between rows and weed control on the rows, and fight against *Spilocaea oleagina* and *Bactrocera* (*Dacus*) *oleae*. In all trials the mechanical pruning was set to leave a maximum thickness of the canopy of the hedge of 80 cm (40 cm on the East side and 40 cm on the West side).

To verify the efficiency of the air-jet apparatus, samplings were carried out to quantify leaves, shoots (up to 1 year old) and branches (over 1 year old) saved when cut as pushed by the air of the blower device towards the inside of the canopy and then come back to its original position.

During the pruning, the machine proceeded along each row keeping constant the driving speed and the cutting depth of the blades, let the blower device operating for a defined number of spans (15-20 m each, depending on the plantation), alternating with spans where it remained off, and for each row on both sides (East and West orientation of the plants, respectively). The surveys were made at the center of the spans.

To perform the surveys on the vegetation, a square frame (1.0 x 1.0 m) was used, placed in correspondence to the central axis of a plant, at a height of 1.0 m from the soil, and positioned at the theoretical cutting blades surface (Fig. 1); all the plant material (leaves, shoots, branches) found outside this frame after the cutting was removed.

Table 1 shows the list of the number of plants chosen for each locality, from which the different samples of vegetal material were taken, used to carry out the experimental surveys, which concerned, for the East side and the West side, respectively: leaves number and surface, shoots length, branches length. In the third test, surveys were also made on the number of fruits present at harvest on pruned plants with and without the presence of the air flow, respectively, to evaluate the difference in the production ability of the plants determined by the two types of techniques, for the two sides of the rows (East and West).

Fig. 1 - Sampling units after the pruning in the Casale San Giorgio farm (2015): A) Air flow Off; B) Air flow On.

Table 1 - List of the sampling units related to different cultivar and locality

Codex	Samples provenience	East air On	East air Off	West air On	West air Off	Total
1	cv. Arbequina, Torrimpietra, Roma (2014)	6	6	6	6	24
2	cv. Tosca, Torrimpietra, Roma (2014)	9	9	9	9	36
3	cv. Arbequina, Casale San Giorgio, Latina (2015)	12	12	12	12	48
4	cv. Sikitita, Casale San Giorgio, Latina (2015)	4	4	4	4	16
5	cv. Arbequina, Grosseto, (2018)	12	12	12	12	48
	Total	43	43	43	43	172

In the Tombolo farm, the day before the winter pruning (March 2018), on 6 plants of the cv. Arbequina, the amount of leaves, shoots and branches on unpruned trees was determined, separately for the East and the West side, to obtain a profile of the canopy biomass distribution along the transversal section of the hedgerow. At this aim, the vegetal material present in the selected sampling units (1 m^2) was collected, keeping separated the outer 15 cm of the canopy (outer layer), corresponding to a "light" pruning, the next approximately 25 cm (intermediate layer), corresponding to a "severe" pruning, and the remaining 40 cm up to the permanent structures (inner layer) of the hedge.

For all the samples the leaves number and the leaf surface, the leaves dry weight, the shoots total length, the branches total length have been determined.

The leaf surface was measured by collecting 50-100 leaves per sampling unit; for the trials before the pruning of 2018 in the Tombolo farm the leaf area was instead determined through a sampling of at least 100 leaves completely developed per sampling unit (total 600 leaves), chosen in the different sections (outer, intermediate and inner) in which the canopy has been subdivided. The leaf area was determined by scanning and analyzing the image (UTHSCSA Image Tool IT Version 2.03), and the dry weight was determined for the same samples.

All trials and surveys were carried out in the second half of March, after the winter cold and shortly before the vegetative restart.

The selective pruning device

The pruning machine (BMV-FL480S), designed for use in intensive olive groves, is equipped with a removable device able to perform selective cutting, unlike commonly adopted solutions, that cut all the shoots and branches which are in a predetermined position. This is achieved by the air's fluid dynamic action.

Characteristics: the pruning machine used for the tests consists of a 240 cm cutting bar fitted on a frame that allows the adjustment in lateral height and inclination, composed of six blade disks with a diameter of 500 mm, a fluid dynamic flow generator and four air flow conveyors arranged in front of the cutting bar (Fig. 2). In all the trials a vehicle of more than 80 HP was used.

Fig. 2 - A) Cutting bar equipped with the selective pruning device; B) The pruning machine working along a row.

Statistical analyses

The experimental design was chosen to guarantee the uniformity of sampling units, deriving from the same starting population for each locality and cultivar. Average and standard deviations of raw data related to the four treatment (East air On, East air Off, West air On, and West air Off) for each locality and cultivar were compared; since in this experiment Authors were interested in the evaluation of the effectiveness in the performances of a pruning machine (i.e. kg of production or cm of elongation tree^{-1}, or ha^{-1}), only raw data were submitted to the parametric test, as logarithmic transformation is most suitable to express magnitude discrepancy; for each data set, tests were carried out to evaluate the normality of the distributions, and Levene's test were performed to evaluate homoscedasticity at 95% confidence level. Since in the most of cases the data distribution indicates some significant nonnormality (the standardized skewness and/or kurtosis is outside the range of -2 to +2), and since significant difference amongst the standard deviations at the 95.0% confidence level were evidenced, neither analyses of variance nor non-parametric tests to compare the medians instead of the means could be performed; as a consequence, average values and s.d. were only reported; Box and Whiskers plot were built up to compare the medians, as the effect of the applied air flow to the pruning machine is so high to generate two populations different at all. This aspect is very important to consider, as the standard deviation can play a meaningful role also when it results to high to allow the application of the common descriptive statistical tests (Marone *et al.*, 2017). The same approach was applied to evaluate the differences in the fruit bearing parameters measured in olive trees of cv. Arbequina (Tombolo farm, 2018), and the same conclusions were drawn.

One-way analyses of variance (ANOVA) were performed to compare vegetative growth parameters in olive trees of cv Arbequina (Tombolo farm, 2018) measured in the sampling units (1 unit = 1 m^2) before carrying out the pruning trials, evaluating the side effect (Est/West); separation of means was performed by the Fisher's LSD test ($p = 0.05$). Levene's test to check the variance, and Mood's median χ-square test to evaluate the medians samples differences, if necessary, were also applied.

All the computations were performed on the raw data by Statgraphics Centurion XV v. 15.0.04. A multivariate partial least squares-discriminant analysis (PLS-DA, supervised method), based on venetian blinds cross validation procedures, was applied on the data obtained from all the samples of the cv. Arbequina, after applying the two alternative pruning methods, coming from the sampling units collected in the three different zones, to highlight the possibility of differentiating the sampling submitted to the pruning with the air flow On, based on the chosen canopy vegetative parameters. For a description of the method, see Colzi *et al.* (2017). Data were preprocessed by a logarithmic transformation (log+1) to express magnitude discrepancy. The analysis was performed by PLS-Toolbox v. 8.0.2 (Eigenvector Research Inc., West Eaglerock Drive, Wenatchee, WA) for MATLAB® R2015b (Mathworks Inc., Natick, MA, USA).

A Factor Analysis (FA) was performed on (log+1) data obtained from the sampling units of cv. Arbequina (farm of Tombolo, 2018), considering as factors both vegetative and productive parameters (leaves number and areas, shoots and branches length, fruits number). Computations were performed by XLSTAT 2014.5.03.

3. Results

Vegetative growth and fruiting parameters

Table 2 shows the data related to the canopy parameters measured on the (residual) vegetation outside the cutting surface: leaves number (n.) and surface (cm^2), shoots total length (cm) and branches total length (cm). For each locality and cultivar, the average and standard deviation values of the sampling units (1 m^2), obtained with and without air flow, are separately indicated for the two sides of the row (East/West).

All the cultivars under study belong to the class of vigor suitable for their use in superintensive plantations. The data shows the great difference between the values of all the "On" tests parameters compared to those of the "Off" tests. In particular, the number of leaves (and axillary buds) results in many combinations more than quadrupled in the "On" tests (Table 2, Fig. 3), and the same proportion is found in the leaf surface present after the cut which, in the West On test of the cv. Tosca overcome the coefficient of 1.12 m^2 of leaf area per m^2 of cutting area. It can be also seen that, in general, the west side of the row is richer in leaves, compared to the east side, except the cv Arbequina in Casale San Giorgio (2015) and Tombolo farms (2018), which show a behavior that tends to be opposite.

Vegetation up to 1 year old shows the same

Table 2 - Differences in vegetative growth parameters in olive trees measured as residual vegetation in the survey units (1 m2) due to action of the selective device and the side (East/West) effect (average ± S.D.)

Treatment	Leaves (number)	Leaves (cm^2)	Shoots (cm)	Branches (cm)
1 East Off	99.0±65.2	471.6±350.4	273.8±201.2	21.0±35.3
East On	406.5±75.3	2060.7±359.6	1040.5±361.4	125.0±75.7
West Off	179.4±100.8	795.4±434.2	368.3±226.5	27.8±20.9
West On	714.2±322.4	3322.3±1480.7	1134.0±428.5	118.2±56.8
2 East Off	660.6±211.6	2542.5±926.3	782.2±220.1	66.3±40.2
East On	2358.1±564.7	8823.2±2098.2	2926.4±840.2	133.8±48.9
West Off	855.7±255.9	3287.1±1124.6	855.4±233.6	78.2±33.4
West On	2980.3±665.3	11160.5±2527.3	3479.2±917.9	142.5±47.7
3 East Off	115.7±65.1	542.7±305.3	270.2±83.3	5.2±9.0
East On	928.9±379.8	4356.5±1781.5	1453.9±552.6	57.08±35.9
West Off	57.1±22.4	268.0±105.0	196.9±67.0	10.2±8.9
West On	596.0±232.4	2795.2±1090.2	1124.1±418.3	69.3±42.6
4 East Off	15.0±10.0	46.8±31.0	200.0±92.5	9.2±14.8
East On	79.5±36.1	247.2±112.4	911.7±282.4	65.5±72.8
West Off	22.3±10.9	69.5±33.9	256.5± 12.1	8.7±17.5
West On	103.3±21.4	321.3±66.4	1342.0±434.9	80.7±34.6
5 East Off	35.6±15.2	167.2±71.3	99.0±34.3	2.4±4.5
East On	285.7±68.6	1335.6±321.9	572.9±223.9	48.3±37.5
West Off	35.0±14.0	164.1±65.5	84.0±19.5	0.0±0.0
West On	572.7±223.9	2681.4±1050.1	943.9±295.2	29.2±12.8

Samples 1, 3 and 5 refer to the same cv. Arbequina. Sample 2 to the cv. Tosca, sample 4 to the cv. Sikitita.

trend. The amount of branches is generally negligible, except for the cv. Tosca, which gives length values from 66 (East Off) to 142 cm (West On).

Figure 4 shows the score plot and the summary statistics of the PLS-DA model, that compare the data related to all the samples of the cv. Arbequina, coming from the sampling units collected in the 3 localities under test, after applying the two alternative pruning methods, based on the chosen vegetative parameters of the trees canopy. The model statistic indicators and the score plot clearly confirm the presence after pruning performed with and without air flow of two completely different populations. As the model is representative of three distinct agronomic situations (Castello di Torrimpietra, Casale San Giorgio and Tombolo farms) and of the two sides (East/West) of the rows, this output confirms the validity of the air jet system in safeguarding a significant part of the most important vegetation useful for the fruit production and the subsequent growth of the fruiting shoots, subtracting it from the indiscriminate mechanical cutting inevitable using the current pruning machines without blower device.

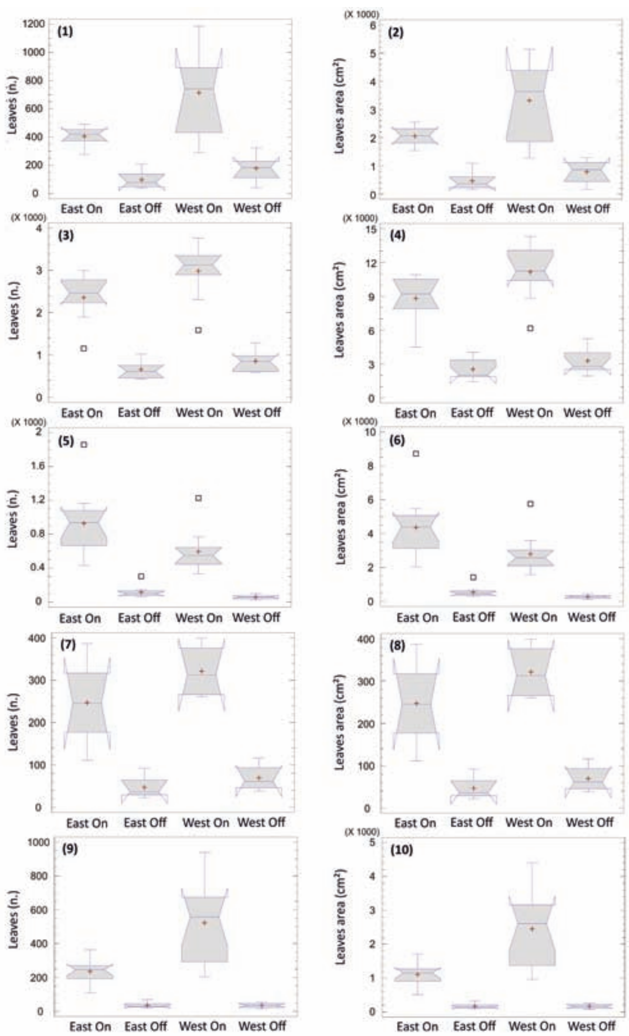
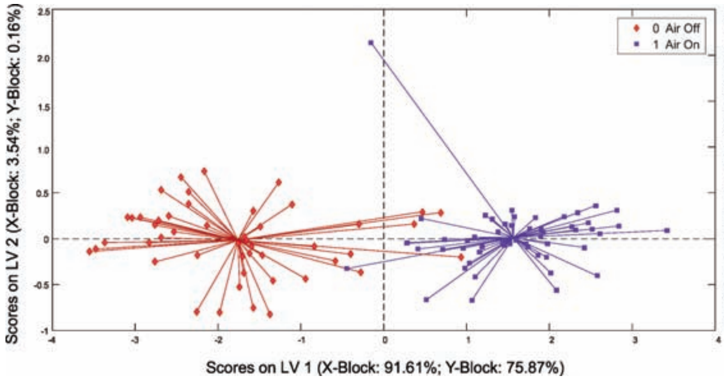


Fig. 3 - Box-and-Whisker plots related to the leaves number and area as influenced by the flow (On/Off) and side (Est/West). 1-2: 'Arbequina', 2014; 3-4: 'Tosca', 2014; 5-6: 'Arbequina', 2015; 7-8: 'Sikitita', 2015, 9-10: 'Arbequina', 2018. Median notches, and mean (+) ± S.D. Notches added to the plot show the estimation error associated with each median; if they do not overlap, highlight which medians are significantly different from which others at 95% confidence level. Mean markers (+) indicate the location of samples mean. Outliers are indicated ( ) if present.

In the Tombolo farm, the number of fruits per m^2 of fruiting area (sampling unit) present on the plants of the cv. Arbequina was also determined. The numerical differences between Air On and Air Off units related to the two orientations (East and West), respectively, highlight the enormous advantage obtained using the selective blower device, exceeding 100 fruits (with an increase of 107 fruits for the East and 116 for the West) (see table in Fig. 5). Calculating an average increase of only 100 fruits per m^2, multiplied by an useful area of about 8000 m^2 ha^{-1}, there would be a productive advantage already on the first yield after the pruning of 800000 fruits; multiplying this value by the average weight of 1 fruit of cv. Arbequina in the area (about 2.0 g), gives over 1600 kg of increased product in average per hectare, compared to the production of 284 kg that would have been obtained without the support of the device, with an average increase of 412% in olive production, and a recovery of over 1.7 t of product already in the pruning year.

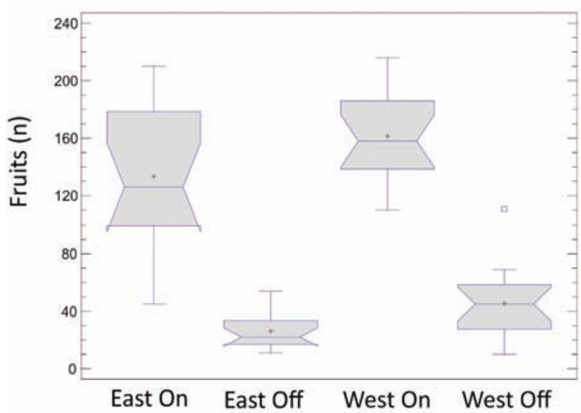
In the figure 6 a biplot from Factor Analysis is shown, simultaneously representing the relationships amongst the vegetative and productive parameters measured on the cv. Arbequina (Tombolo farm, 2018).

The first axis explains the 97.83% of the total variability in the data. The four populations deriving from the treatments and the orientation are well separated in the four plots of the diagram. The two clusters that represent the trials with the application of the blower device (on the right in the figure) are significantly related to the vegetative-productive parameters of the leaves. It is interesting to note how the parameters related to leaves, shoots and fruits are also influenced by orientation to the West, as shown by the direction and length of the arrows in the graph.



Statistics	AIR ON	AIR OFF
LVs	4.000	4.000
SE (Cal)	0.917	0.981
SP (Cal)	0.981	0.917
SE (CV)	0.917	0.963
SP (CV)	0.963	0.917
SE (P)	0.917	1.000
SP (P)	1.000	0.917
Class. error (Cal)	0.050	0.050
Class. error (CV)	0.060	0.060
Class. error (P)	0.041	0.041
RMSEC	0.239	0.239
RMSECV	0.263	0.263
RMSEP	0.298	0.298
Wilcoxon (Cv=Self P)	0.000	0.000
Sign Test (Cv=Self P)	0.000	0.000
Rand-t-Test (Cv=Self P)	0.005	0.005

Fig. 4 - Score plot of PLS-DA model and summary statistics for the cv. Arbequina samples in the three different zones after the pruning, based on the measured vegetative growth parameters: 0) Air Off (Red), 1) Air On (Blue).



	Fruits (n.)
East On	133.4 ± 47.9
East Off	26.1 ± 12.5
West On	161.4 ± 34.3
West Off	45.5 ± 24.4

Fig. 5 - Differences in fruit number in olive trees of cv. Arbequina (Tombolo farm, 2018). In the table: average ± S.D.; in the Box and Whisker plot: median notches, and average (+) ± S.D.

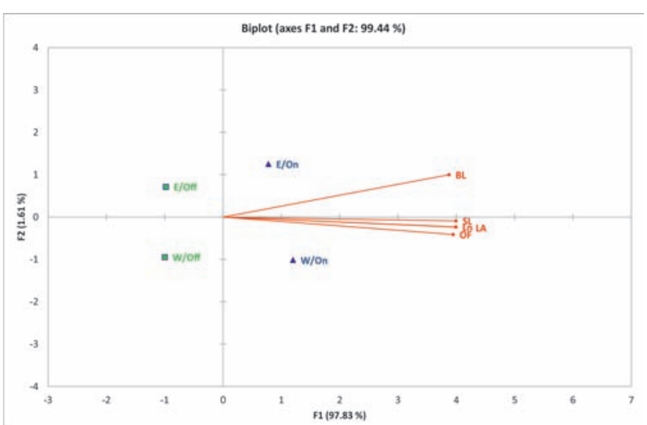


Fig. 6 - Biplot from Factor Analysis. Relationships among leaves number (LN), leaves area (LA), shoots length (SL), branches length (BL), fruits number (FN), and the four different treatment: East air On (E/On), East air Off (E/Off), West air On (W/On), West air Off (W/Off); cv. Arbequina, Tombolo farm, 2018.

Canopy distribution of biomasses in hedgerows trained olive trees

Table 3 compares the vegetative parameters (leaves number and area, shoots and branches length) per m^2 of the three sections in which the canopy has been transversely divided. An initial evaluation seems to indicate that, for the particular area of Tombolo farm, the East/West orientation plays an important role in determining the vegetative parameters taken into consideration; in fact, in the West section, regardless of the depth of the evaluation, the leaves number and surface (cm^2), and the shoots length (cm) is greater. Branches cannot be taken into consideration, since they are absent from the outer part, and almost absent from the intermediate section. This advantage determined by the orientation, whose causes are to better investigate, seems to be present also in the plot of Castello di Torrimpietra farm for both the cultivar (Arbequina and Tosca) and in the plot of Casale San Giorgio farm limited to the cv. Sikitita.

Table 3 - Partition of components (leaves number and surface, shoots and branches lenght) of the three canopy transversal sections in olive trees of cv Arbequina (Tombolo farm, 2018) measured in the sampling units (1 unit = 1 m^2), side (Est/West) (average ± S.D.). Average leaves dry weight for the three sections.

Taking into account the East/West averaged values, it results that a "light" pruning (taking away the

Table 3 - Partition of components (leaves number and surface, shoots and branches length) of the three canopy transversal sections in olive trees of cv. Arbequina (Tombolo farm, 2018) measured in the sampling units (1 unit = 1 m^2), side (Est/West) (average ± S.D.)

Section	Parameter	East	West	Average leaves dry weight (g)
Outer section (15 cm)	Leaves (no.)	689.1 ± 66.2 a	815.2 ± 86.9 b	67.69 (33.4%)
	Leaves (cm^2)	3232.0 ± 310.4 a	3823.3 ± 407.5 b	
	Shoots (cm)	1234.7 ± 30.6 a	1121.5 ± 111.3 b	
	Branches (cm)	0.0 ± 0.0	0.0 ± 0.0	
Intermediate section (about 25 cm)	Leaves (no.)	680.4 ± 39.7 a	1243.4 ± 88.2 b	86.57 (42.7%)
	Leaves (cm^2)	3191.3 ± 186.2 a	5831.5 ± 413.5 b	
	Shoots (cm)	2132.7 ± 510.7 a	2979.7 ± 232.3 b	
	Branches (cm)	214.0 ± 64.1 a	240.3 ± 82.0 a	
Inner section (40 cm from the main tree axis)	Leaves (n.)	367.8 ± 145.1 a	708.4 ± 206.6 b	48.43 (23.9%)
	Leaves (cm^2)	1725.1 ± 680.6 a	3322.6 ± 968.9 b	
	Shoots (cm)	2016.2 ± 423.8 a	1817.4 ± 904.2 a	
	Branches (cm)	580.2 ± 234.3*	709.2 ± 71.3*	

Average leaves dry weight for the three sections

*There is more than a 3 to 1 difference between the smallest standard deviation and the largest. Since the Levene's test P-value is less than 0.05 (0.0004) there is a statistically significant difference amongst the standard deviations at the 95.0% confidence level. Since the Mood's median χ-square test is greater than or equal to 0.05 (0.102467), the medians of the samples are not significantly different at the 95.0% confidence level.

first 15 cm of the vegetation), removes about 1/3 of the leaves thus reducing 1/3 the leaf surface of the plant, and a "severe" pruning (at a depth of about 40 cm), would remove more than ¾ of the present leaves, leaving the final permanent structure with a highly reduced leaf apparatus. Even considering the shoots length (cm) it is possible to evaluate the meaningful effect of the intervention: a "light" pruning would remove about 1/5 of the present shoots, while a "severe" pruning would leave only 35% of the shoots, thus eliminating most of the vegetation potentially productive.

Figure 7 shows an example of partition between the different components of the canopy for each of the three considered sections.

It is also interesting to consider the amount of leaves biomass that, by the pruning, can be made available for other uses: a "light" pruning, limited to a 15 cm layer, could produce, in this typology of rows, 541 kg of leaves (dry weight), and further 692 kg (dry weight) would also be removed from the intermediate section, for a total of 1.2 t of dry weight leaves to be considered an additional resource and not longer as a residual.

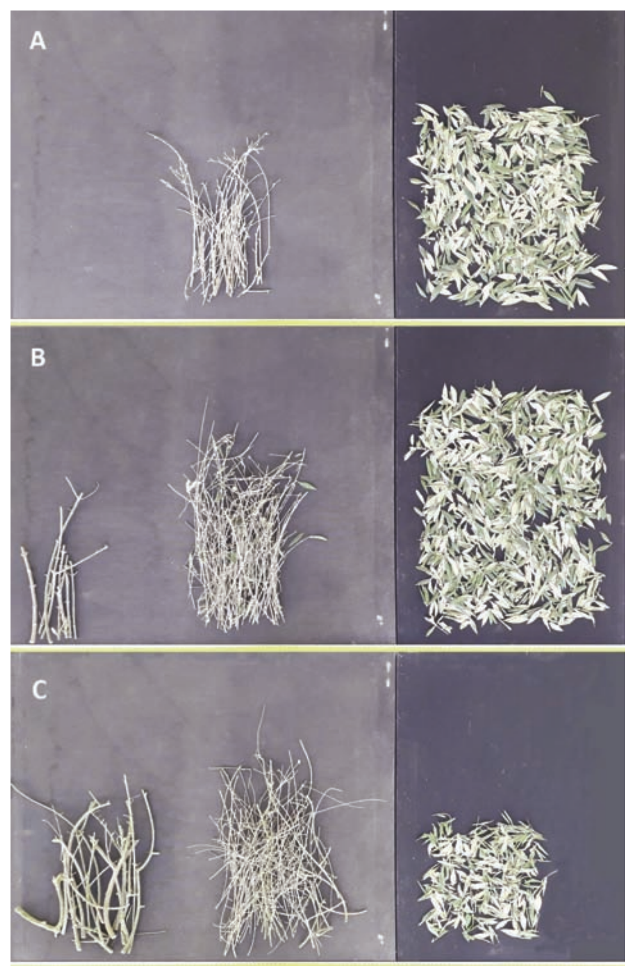


Fig. 7 - Example of partition of components (leaves, shoots, and branches) of the canopy transversal sections in a olive tree of cv. Arbequina (Tombolo farm, 2018) measured in a sampling units (1 unit = 1 m^2), side West. A) Outer section (15 cm); B) Intermediate section (25 cm); C) Inner section (40 cm from the main tree axis).

4. Discussion and Conclusions

Mechanical winter pruning on adult hedges in superintensive plantations, unlike manual pruning, is a useful tool to control the size of the vegetative structure, thus decreasing its traditional role of balancer between vegetative growth and production (Ferguson *et al.*, 2002). In fact, by manual winter pruning, the aged, shaded and exhausted parts of the plant are selectively eliminated, to leave light and space for the parts destined to growth and to produce the following years (Peça *et al.*, 2002). Mechanical winter pruning, on the other hand, proceeds by vertical sections, indiscriminately eliminating the part exceeding the size limits imposed by the use of straddling machines; this leads, as immediate result, as pointed out by several Authors (Vivaldi *et al.*, 2015; Albarracín *et al.,* 2017), a sharp decline in productivity, a long unproductive period, due to the need to regenerate a fruiting canopy and, therefore, an economic damage that decreases the benefits offered by the mechanization of the harvesting (Peça *et al.*, 2002; Albarracín *et al.,* 2017).

In this work the authors tried for the first time to overcome the problem by verifying the effectiveness of a tool that combines the action of circular saws with a jet of air which push the shoots away from the cutting area, allowing to reduce the removal of plants young leafy parts. The main effect of the air flow is evidenced by the presence of two population (air On and air Off), totally different and distinct. The new air-jet system, compared to traditional pruning machines, preserves over 400% of the leaf surface and relative shoots length in the canopy zones where it has been applied, determining a proportional increase in production, already economically interesting the first year after the pruning.

The difference in olive produced resulted higher than 1 t ha^{-1} using the air jet, demonstrating the true economic advantage of the new device, able to prevent an almost total fall in production in the winter pruning year. This improves the use of agronomic practices, keeping in mind that in superintensive olive groves, fertigation, soil and pests management are always to be applied, without exceptions (Vivaldi *et al.*, 2015). At the same time, the preservation of a larger leaf surface on the vegetation of the year, also guarantees a more suitable use of the solar radiation (Cherbiy-Hoffmann *et al.*, 2012), which allows both the activation and development of new growth points, and the growth of new potentially productive vegetative structures on the shoots elongations, able to guarantee an adequate continuity of the production (Fiorino and Marone, 2010).

Further research will be needed to improve the efficiency of this tool, and better understand the mechanisms and the evolution of the different buds present in the different parts of the canopy, which regulate the distribution of resources, also verifying the possible causes determining the growth differences due to the orientation East/West of the row canopy.

It is also important to determine the quality of the removed biomass which, in the case of "light" pruning, is exclusively composed of leaves and shoots, and could constitute a considerable amount of vegetal material to be used as a supplement/component of the livestock feed ration, or become an important source for the growing demand for phenolic antioxidants of nutraceutical interest (Talhaoui *et al.*, 2015; Castellani *et al.*, 2017).

Acknowledgements

The Authors are grateful to: Dr. Ing. Stefano Borio, BMV di Borio Valerio & C. S.N.C., Alba (CN), Italy, for his invaluable technical support and to having provided the pruning machine equipped with the new selective device; the farms: Azienda Casale San Giorgio, Latina, Azienda Castello di Torrimpietra, Roma, Azienda Tombolo, Grosseto, to have allowed and hosted the trials, and the Olive Consultant Alessandro Mersi, for its helpful collaboration in the organization of the tests.

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Adv. Hort. Sci., 2019 33(1): 123-131 DOI: 10.13128/ahs-22952

Mini review

AHS
Advances in Horticultural Science

Spiralling whitefly and its management practices in the South Pacific. A review

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Key words: abundance, *Aleurodicus dispersus* Russell, management, South Pacific, spiralling whitefly.

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Citation:
CHAND R.R., JOKHAN A.D., KELERA R., 2019 - *Spiralling whitefly and its management practices in the South Pacific. A review*. - Adv. Hort. Sci., 33(1): 123-131

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 31 March 2018
Accepted for publication 7 September 2018

Abstract: A few species of whiteflies are considered a serious insect pest of vegetation and ornamental plants across many countries. The Spiralling whiteflies, *Aleurodicus dispersus* Russell has been recorded on many different plant species across countries. These whiteflies feed exclusively on leaves and have the ability to spread plant diseases. A great deal of research has been done on whiteflies in relation to which control methods would be most effective in pest management. The management of Spiralling whitefly in the South Pacific is heavily reliant on biological control (using the parasitic wasps, predators and entomopathogenic fungi). Other control methods include physical, botanical, and chemical to keep the whitefly population at lower levels. In this paper, an overview of Spiralling whiteflies and its management practices in the South Pacific will be reviewed.

1. Introduction

Spiralling whiteflies, *Aleurodicus dispersus* Russell, 1965 (*Hemiptera: Aleyrodidae*), are polyphagous pest of agricultural and horticultural crops in glasshouses and fields worldwide (Oliveira *et al.*, 2001; Mani and Krishnamoorthy, 2002; Stansly and Natwick, 2010). It is a native to the Caribbean region and Central America (Waterhouse and Norris, 1989). It was first noticed as a pest in Hawaii in 1978 and since then has spread to the Pacific islands and other continents (Kumashiro *et al.*, 1983; Waterhouse and Norris, 1989).

Whitefly adults and larvae feed on leaves, stems and fruits by inserting stylets into the plant. Some specific plants that are usually attacked include cassava, pepper, papaya, mango, eggplant, citrus, guava, banana, coconut, breadfruit, tropical almond, sea grape, paper bark and rose (Russell, 1965; Jayma *et al.*, 1993; Neuenschwander, 1994; Reddy, 2015). When the stylets are in the phloem, the whiteflies ingest large quantities of sap that contains a lot of sugar. They excrete the excess liquids and sugar which is called honeydew. The honeydew is deposited on leaves and fruits, fostering the growth of black sooty mould fungi and falling of premature leaves. In addition, these moulds influence the rate of photosynthesis and transpiration as it hinders the light penetration, vapour movement and exchange, leading plants to exhibit yellowish specks on leaves, to wilt or die off (McAuslane *et al.*, 2004; Al-Shareef, 2011; Reddy, 2015).

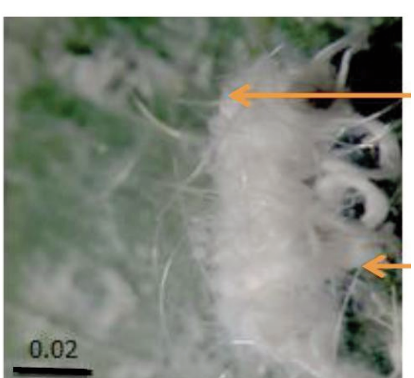
Spiralling whiteflies have caused detrimental effects in the production of crops and ornamental plants. It is one of the most common pest that has the ability to spread diseases and influence the global food production (ECPA, 2015). Over 300 plant species from approximately 77 families have been recorded as hosts of *A. Dispersus* worldwide (Lambkin, 1999). The spread of the Spiralling whitefly continues to increase rapidly due to the general ineffectiveness of chemical control and other pest control measures (Mani and Krishnamoorthy, 2002). Alternative measures for controlling Spiralling whiteflies include physical, cultural and biological control. These approaches have been used in the South Pacific but there is no data available (except biological control) that supports their practice and effectiveness in terms of whitefly management (Waterhouse and Norris, 1987). Hence, the present review builds information on studies carried in the South Pacific on *A. dispersus* Russell alternative control methods, which would be valuable in terms of effective management of the pest.

2. Origin, biology and whitefly-plant interactions

Aleurodicus dispersus is a tropical pest to a variety of horticultural crops, ornamental plants and shade trees (Department of Agriculture and Fisheries, 2015). It was first reported in Florida in late 1957 (native area being the Caribbean and Central America) and since then it has expanded its range to most regions around the world including North America, South America, Asia, Africa, Australia and several Pacific Islands (Russell, 1965; Waterhouse and Norris, 1987; Reddy, 2015). In the South Pacific, the pest has been reported in Majuro (1986) (Marshall Is), Cook Islands (1984), Fiji (1985), Nauru (1987), Papua New Guinea (1987), Kiribati (June 1988), Tokelau (late 1988) and Tonga (November 1988) (Waterhouse and Norris, 1987).

The lifecycle stages of *A. dispersus* are eggs, four larval instars and adults. The eggs (0.3 mm long) are usually smooth surfaced, yellow and tan elliptical in shape (Reddy, 2015). These are laid at an angle of 90° with Spiralling deposits of white flocculence on underside of the leaves. The first instars are mobile and called crawlers. They can travel to a short distance to select their feeding sites (Martin, 1987). They are usually 0.32 mm long and settle near the spiral pattern of the eggs from which they hatched. As the crawler grows, they develop mid-dorsal waxy tufts and the secretion of wax is usually from the narrow band of sub-margin. The second and third instars are 0.5-0.65 mm long and remains feeding at same place. The distinguishing feature about the third instar larvae is the presence of glass-like rods of wax (usually short and evenly-spaced) lined along the body. These cottony secretion is much less abundant than on the fourth instar [Russell (1965) cited in The Centre for Agriculture and Bioscience International (CABI, 2015)].

The fourth instar or puparium is 1.06 mm long and covered with numerous amounts of white materials and long glass-like rods (~8 mm in length); due to fragmentation some are shorter (Fig. 1). The second to fourth instars are protected by waxy secretions making them sessile and scale-like (Martin, 1987; Banjo, 2010). The adults are mobile and most active during the morning. The bodies of males are



Glassy wax rods emanating from each compound spores. These glassy rods are whitish in colour, translucent and longer (3-4 times) than the width of the body

From the dorusm (extending upwards and outwards) of mature puparium, a copious amount of white cottony substance is secreted

Fig. 1 - Mature puparium of Spiralling whitefly

usually 2.28 mm and females are usually 1.74 mm (3-4 times longer than the body width). The adults develop white translucent powder covering on their bodies. These whiteflies also have a pair of antenna. The males have several pores on the abdomen scattered dorsally, laterally and ventrally on the first 2 segments posterior to wax plates while the females are without pores [Russell (1965) cited in The Centre for Agriculture and Bioscience International (2015)]. The eye is reddish-brown in colour. The Spiralling whiteflies also have two characteristic dark spots on their forewings (Fig. 2). After mating adult females lay eggs in irregularly spiralling patterns and it is where whiteflies derived their common name, Spiralling whitefly (Reddy, 2015).

Heavy occasional rains and cool temperatures result in a temporary reduction in *A. dispersus* population (Mani, 2010). The mortality rate of immature stages increase between 40-45°C, and for adults the mortality rate increases at 35-40°C. Temperature below 10°C also lead to increased mortality (Cherry, 1979; Waterhouse and Norris, 1987). However, population of Spiralling whiteflies will increase during droughts and presumably when the natural enemies decline. The spread of crawler and winged adult whiteflies usually occurs with short-distance movements whereby the crawlers are walking on the plant on which it hatched and the plants that touch it and adults fly to other parts of the same plant or to other nearby host plants. Instead, the movement to long distance involves the international horticultural trade (Pacific Pests and Pathogens, 2016).

Whitefly-plant interactions - stomatal conductance

Whiteflies cause damage to plant productivity in terms of photosynthesis, respiration and transpiration performance (Shannag and Freihat, 2009). The whitefly *A. dispersus* is considered to be a major pest causing damages to crops (Boopathi *et al.*, 2015). These insect pests are reported to alter the chemical processes, growth and photosynthesis (Schröder *et al.*, 2005; Fatouros *et al.*, 2012). Moreover the plants primary metabolism is also altered by this particular infestation. For example, egg deposition by a particular species of moth (*Podoptera frugiperda)* demonstrated inhibitory effect towards the production of herbivore induced plant volatiles in maize plants (Fatouros *et al.*, 2012). The egg deposition has also shown to influence the rate of photosynthesis by reducing the amount of carbon dioxide diffusion in the mesophyll cells (Fig. 3) (Schröder *et al.*, 2005; Fatouros *et al.*, 2012). For instance, the study by Shannag and Freihat (2009) concluded that gas exchange in cucumber plants infested by whiteflies (*Bemisia tabaci*) caused photosynthesis to decline by up to 30%. The feeding behaviour of whiteflies also

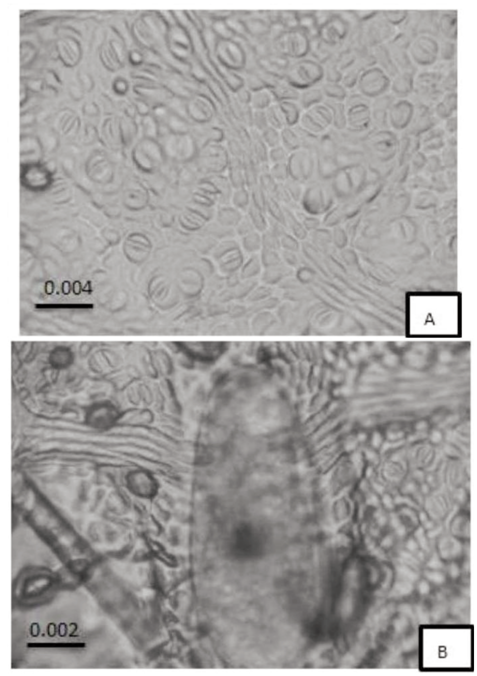


Fig. 3 - (A) the stomatal underneath the normal leaf surface and (B) the egg attached on the stomata of infected leaf.

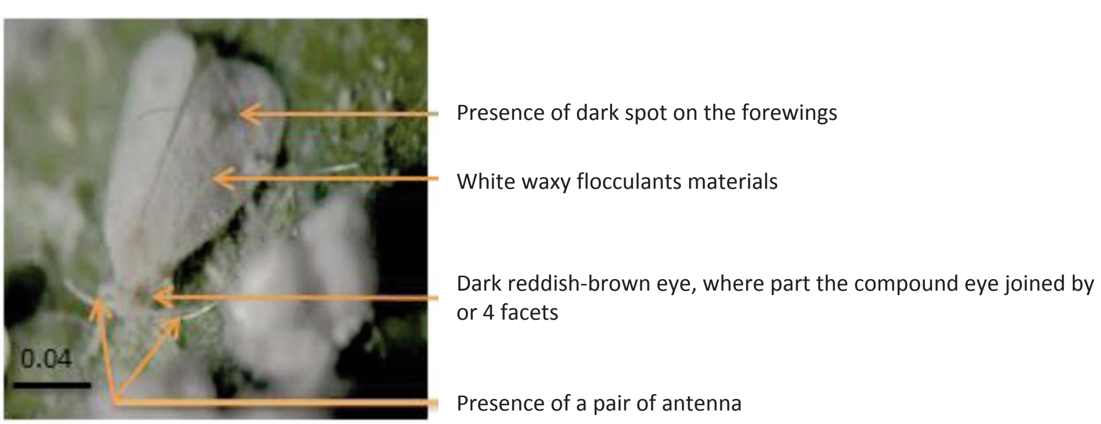


Fig. 2 - Adult of Spiralling whitefly.

increased the respiration rate by 24-78% and the rate of water loss from infested leaves was 3-32% greater than that of control leaves. Likewise, the increasing number of whiteflies (Tobacco whitefly) led to increase in transpiration rate (Shannag and Freihat, 2009). The artificial infestation of Spiralling whiteflies described by Pitan (2003) on pepper led to increase in the damage of leaves. This simply means that the chlorophyll, sugar, protein and crude fibre contents of the leaves decreased as with the level of infestation.

3. Status and damage in the South Pacific

The reproduction and dispersal rate of Spiralling whiteflies are relatively high, posing great threat to vegetable, tropical fruit tree and ornamental industries around the globe (Pacific Pests and Pathogens, 2016). The host range of these Spiralling whiteflies covers at least a range of 27 plant families, 38 genera and more than 100 species (Waterhouse and Norris, 1989). Despite there is no evidence in the measurement of economic loss by these Spiralling whiteflies in the South Pacific. Heavy infestations on plants is more likely to result in economic loss (Pacific Pests and Pathogens, 2016). Being a polyphagous, Spiralling whitefly has been recorded on many plant species in different countries. According to surveys conducted from 1996 to 1997 (Waterhouse and Norris, 1989; Barro *et al.*, 1997), Spiralling whitefly is an exotic pest in American Samoa, Cook Islands, Fiji, Kosrae (FSM), Pohnpei (FSM), Truk (FSM), Yap (FSM), Guam, Kiribati, Marshall Islands, Nauru, New Caledonia, Northern Mariana Islands, Palau, Papua New Guinea, Solomon Islands, French Polynesia, Tonga and Western Samoa (Table 1). The survey also revealed the absence of *A. dispersus* from Niue, Tuvalu and Vanuatu.

4. Management of *Aleurodicus dispersus* Russell

The management of whitefly has been difficult as a result of its many host plants. Perennial plants such as ornamentals, fruit trees and shade trees were probably used successfully throughout the year by this coloniser (whiteflies) which sucks the sap of leaves (Kajita *et al.*, 1991). According to Chandel *et al.* (2010), whiteflies must be dealt with a combination of environmental manipulations, natural enemy enhancement and area-wide control programme. This technique is known as Integrated Pest Management (IPM) which uses a combination of different strategies to control pests. The IPM program uses current, comprehensive information on the life cycles of pests and their interaction, combination with pest control methods to manage pest by the most economical means with least hazardous to the environment and the people (Boopathi, 2013; EPA, 2017). However, based on the literature available in the South Pacific, there are no reported studies on chemical and physical or combined (IPM) control practices on *A. dispersus*. The only data available is on biological control (Waterhouse and Norris, 1987). There are various methods of biological control of whitefly techniques utilised in the South Pacific, such as the use of parasitic wasps, predators (lacewings, big-eyed bugs and minute pirate bugs) and the use of entomopathogenic fungi of the genus *Aschersonia.*

Biological control

Natural predators and parasitoid. Spiralling whitefly is not regarded as a pest in its native area of Caribbean and Central America where it is assumed that populations are kept low by natural enemies (Prathapan, 1996). Biological control is perhaps the safest and most sound approach to pest control. It is an effective tool in programmes of Integrated Pest

Table 1 - Distribution of *Aleurodicus dispersus* whiteflies in the Pacific found during the whitefly survey (1996-1997)

Aleurodicus dispersus	AmS	CoI	Fij	Kos	Poh	Tru	Yap	Gua	Kir	MaI	Nau	Niu	NMI	Pal	PNG	SI	FrP	Ton	Tuv	Van	WSa
Distribution in 1996/7	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x			x
Exotic	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x			x
Serious Pest potential	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x			x

AmS= American Samoa; CoI= Cook Islands; Fij= Fiji; Kos= Kosrae (FSM); Poh= Pohnpei (FSM); Tru= Truk (FSM); Yap= Yap (FSM); Gua= Guam; Kir= Kiribati; MaI= Marshall Islands; Nau= Nauru; NCa= New Caledonia; Niu= Niue; NMI= Northern Mariana Islands; Pal= Palau; PNG= Papua New Guinea; SI= Solomon Islands; FrP= French Polynesia; Ton= Tonga; Tuv= Tuvalu, Van= Vanuatu and WSa= Western Samoa.
The letter 'x' indicates *A.dispersus* indication for presence and concern.
Information retrieved from Barro *et al*. (1997).

Management. Utilising natural enemies reduces the risk of pesticide usage that results into environmental pollution increase. The importation of parasitoids of genera *Encarsia* or *Eretmocerus* and of various predators have been successfully used in greenhouses for whitefly control (Gerling *et al.*, 2001). The three parasitic species, *Eretmocerus mundus* (Mercet), *Eretmocerus eremicus* Rose and Zolnerowich and *Encarsia formosa* Gahan (*Hymenoptera: Aphelinidae*) have been used against whiteflies in Japan (Sugiyama *et al.*, 2011).

In Fiji, the common predators of *A. dispersus* are *Coccinellids Megalocarla* (= *Archaioneda tricolor*) *fijiensis*, *Serangiella* and the *Neuropteran cbrysop* [Kumar *et al.* (1987) cited in Waterhouse and Norris (1989)]. Some common predators of whiteflies are lacewings, big-eyed bugs, minute pirate bugs and several lady beetles (For example; *Scymnus* or *Chilocorus* species). A major outbreak of Spiralling whiteflies on Papaya was reported in Samoa (Pestnet, 2005). The outbreak of whiteflies usually happens when their natural enemies are disturbed or destroyed by pesticides, dust build-up and other factors. These outbreaks commonly affected guavas, palms, ground orchids, and poinsettias (ornamental). A recent study showed that *A. swirskii* (mite) is increasingly used for the biological control of thrips and whiteflies in many crops (Messelink *et al.*, 2008). Three major predators that have been found to be most effective in attacking Spiralling whiteflies are *Megalocaria fijiensis*, *Serangiella* and the neuropteran *chrysopa* species (Waterhouse and Norris, 1989).

According to Waterhouse and Norris (1989), the establishment of Pacific bridgehead in Hawaii (1978) led to spread of Spiralling whiteflies to many of the Pacific nations. Parasitic wasps *Encarsia* (?) *haitiensis*, *Encarsia* species and three coccinellid predators from Trinidad were used as biological agents to reduce the damaging populations of whitefly in Guam. It was noted in Lanai Island (Hawaii) that *E.* (*?*) *haitiensis* and *Encarsia* species could effectively control the Spiralling whitefly. Adult whiteflies were observed on buses, cars and near parking areas. By late 1979, *A. dispersus* was considered to be an economic pest and initiated a search for natural enemies in the Caribbean. Different species of coccinellid predators and aphelinid parasite were introduced in 1979 and 1980 to reduce the population of *A. dispersus*. In Fiji, *A. dispersus* spread rapidly and became a serious pest since 1986. The introduction of *Encarsia* (?) *haitiensis, N. oculatus* and *N. bieolor* from Guam and Hawaii became well established up to 2 km from their release sites. The three predators found to attack *A.dispersus* prior to 1986 were the coccinellids *Megalocarla fijiensis, Serangiella* sp. and the neuropteran *Chrysopa* sp. Similarly, in American Samoa, *A. dispersus* were found in 1981 on a wide range of plants including ornamentals, citrus and other fruit trees. The introduction of coccinellid predators and the parasite *Encarsia* (?) *haitiensis* in 1984 from Hawaii rapidly reduced the *A. dispersus* population. Table 2 provides detail as where the Spiralling whiteflies were discovered, their host range and biological control in Hawaii, American Samoa, Cook Islands, Fiji, Pohnpei, Guam, Kiribati, Palau, Papua New Guinea, Tonga and Western Samoa.

Physical control of A. dispersus - removal and traps. Removal of leaves may be an environmental friendly approach, but it does not completely remove the pest, it rather lessens the level of whitefly population from the plant. A slight infestation can quickly spread to other plants. The removal of leaves is a good approach to get rid of non-mobile nymphal and pupal stages of whiteflies from highly dense leaves.

In addition, yellow sticky traps are used to trap adults since whiteflies are attracted to yellow (Barbedo, 2014). It is where a trap consisting strips of paper and sticky substances such as petroleum are placed in the greenhouse. The insects are caught as they fly. The drawback of this type of approach is that it only captures specimens that can fly. However it is generally ineffective for the insects that are in their early stages since they are not able to fly (Barbedo, 2014). This method is not a full-proof control method for farmers since it does not eliminate damaging population, but aims to reduce the whitefly population (Nakamura *et al.*, 2007).

Botanicals. Natural pesticides such as plant essential oils would represent an alternative in crop protection (Coats, 1994; Isman, 2000; Koul *et al.*, 2008). Different plants have been used for the control of pests and the research has worked out well (Gonzalez-Coloma *et al.*, 2010). Medicinal plants can be an alternative to a lot of synthetic chemicals for human health and agriculture. However, people in Fiji and the rest of the South Pacific countries are not very cognizant of the presence of the great plant diversity surrounding them. The only study published in relation to botanical effects against *A. dispersus* were carried by Chand *et al.* (2016). Plant extracts such as essential oils from these medicinal plants could possibly be used in agriculture in the form of pest controls. The study focused

Table 2 - Introductions for the biological control of *A.dispersus*

Country	*Aleurodicus dispersus*		
	Discovered place and Year	Plants on which they were found first	Introduction of biological Control
Hawaii	Honululu in September 1978	Tropical almond	Coleoptera. *Delphastus pusillus* (1980) from Trinidad. *Nephaspis oculatus* (1979) from Trinidad. *Nephaspis oculatus* (1979) from Honduras. *N. bicolor*(1979) from Trinidad. Hymenoptera. E. ? haitiensis (1979) from Trinidad. *Encarsia sp.* (1980) from Trinidad.
American Samoa	Tutuila in 1981	Guava, ornamentals, citrus and other fruit trees. Infestations were noted on banana leaves and later in vegetable gardens	Coleoptera. *Delphastus pusillus* (1984) from Hawaii. *Nephaspis oculatus* (1984) from Hawaii. *Nephaspis bicolor* (1984) from Hawaii. Hymenoptera. *E.? haitiensis* (1984) from Hawaii.
Cook Islands	Rarotonga in 1984	Frangipani, guava, hibiscus and mango	Coleoptera. *? Nephaspis bicolor* (1985) from Hawaii. Hymenoptera. *?Encarsia ? haitiensis* (1985) from Hawaii. *Encarsia ? haitiensis* (1988) from Fiji.
Fiji	Suva in April 1986	--	Coleoptera. *Nephaspis oculatus* (1987) from Guam. *Nephaspis oculatus* (1987) from Hawaii. *N. bicolor* (1987) from Hawaii. Hymenoptera. *E. ? haitiensis* (1987) from Guam. *E. ? haitiensis* (1987) from Hawaii.
Pohnpei	--	--	Hymenoptera. *E.? haitiensis* (1986) from Guam.
Guam	Guam in 1981	Coconut, frangipani, guava and mango	Coleoptera. *Nephaspis oculatus* (1981) from Hawaii. Hymenoptera. Encarsia ? haitiensis (1981) from Hawaii
Kiribati	Bikenibeu, Tarawa, in June 1988	Chillies, bell peppers, tomatoes, pawpaw, guava, breadfruit, banana, ornarnentals -including, frangipani and Coleus	A biological control project is to be commenced in the near future
Palau	--	--	Hymenoptera. *E.? haitiensis* (1986) from Guam.
Papua New Guinea	October 1987 in the Port Moresby	Guava, mango leaves and coconut palms	*Coccinellids and spiders were seen preying on them
Tonga	In November, 1988	--	*attacked by Unidentified wasps.
Western Samoa	First recognised in 1985	--	--

on five common medicinal plants randomly selected and screened for the insecticidal properties (fumigant and repellent test). These medicinal plants were Makosoi (*Cananga odorata*), Lemon grass (*Cymbopogon. citratus*), Curry Leaves [*Murraya. koenigii* (L.) Spreng] Tulsi [*Ocimum tenuiflorum* (L.)] and Uci (*Euodia. hortensis forma hortensis*). The results revealed that Tulsi essential oils showed strong fumigant toxicity (100% mortality in 3 hours) while Lemon grass and Curry leaves showed the best repellent effect with LC_{50} value of 0.004 and 0.113, respectively (Chand *et al.*, 2016).

Chemical control. The use insecticides are widespread among farmers. For instance the paper by Kajita *et al.* (1991) provides a description of several insecticides against *A. dispersus* on soya beans in Indonesia. However, because the whitefly has wide host-plant ranges in addition to the fact that insecticides also have impact on natural enemies, chemical control is usually considered impractical and uneconomic in the long-term (Kajita *et al.*, 1991; Lambkin, 1998). Carmichael *et al.* (2008) also discourage the use of chemical control for managing Spiralling whiteflies, suggesting that soaps and detergents can provide effective control in small scale planting. In the South Pacific, there are no recorded publications dealing with chemical control measures of *A. dispersus* yet (Waterhouse and Norris, 1987).

According to Reddy (2015), the common chemicals used for controlling whiteflies are *dimethoate 30 EC* at 0.05% and insecticidal soap at 2.5%, which deterred the adults. Likewise, the following chemicals imidacloprid, buprofezin and pyridaben are also used to manage whiteflies (Bi *et al.*, 2002). Spiromesifen, a novel insecticide inhibited egg hatching in green house by 80% to 100% at the concentrations of 3.1, 3.0, and 10.0 µg mL^{-1}. The insecticide also showed mortality of 100% for the first, second, and third instar nymphs of whiteflies (Toscano and Bi, 2007).

Chemical approach mostly kills those that come in contact with the insecticides (chemicals). The use of the chemical approach showed efficiency towards controlling pests in small and in large scale farms. For instance, farmers in Colombia intensify the use of insecticides, as the whiteflies reduced the crop yield by 79% (Carabalí *et al.*, 2010). Although, plant productions may have increased due to pesticidal applications at the same time these chemicals may have raised detrimental concerns for so many (Aktar *et al.*, 2009). Chemical pollution is a major concern to the environment and to human health due to the bioaccumulation of chemicals through food chains, resulting in severe physiological disorders and diseases (Oliva *et al.*, 2001; Baldi *et al.*, 2003; Briggs, 2003; Saiyed *et al.*, 2003; Lemaire *et al.*, 2004). The extensive use of synthetic chemicals has led to pests in developing resistance to chemicals and at the same time resulting into the accumulation of harmful chemical pollutants in the environment. These pollutants gradually affect the quality of air and water, on which many organisms rely on.

5. Conclusions and future perspective

Whiteflies are considered serious pests to vegetation and ornamental plants in many countries and as such, Spiralling whiteflies are fast becoming a concern for many farmers in the Pacific and other parts of the world. In the South Pacific the pest has been reported in Majuro (1986) (Marshall Is), Cook Islands (1984), Fiji (1985), Nauru (1987), Papua New Guinea (1987), Kiribati (June 1988), Tokelau (late 1988) and Tonga (November 1988) (Waterhouse and Norris, 1987). These whiteflies feed exclusively on leaves, which eventually damage the plant leading to diseases or plant death. Whiteflies are very difficult to manage as a result of multi host plants that support their lifespan. The most common management practices used for the control of whiteflies are biological control using the parasitic wasps, predators and entomopathogenic fungi, physical method using removal and traps, botanicals such as plant extracts and essential oils, and chemical control. At present there is no available literature in the South Pacific that provides data on chemical and physical practices. The only data available were on biological control methods and the use of botanicals (essential oils) for the management of *A. dispersus* (Waterhouse and Norris, 1987; Chand *et al.*, 2016). The salient findings gathered from this review indicate a general lack of information and research on the current status and the degree of damages that the Spiralling whitefly has inflicted on agricultural and ornamental crops in the South Pacific.

Acknowledgements

The authors are grateful to Mrs Reema Prakash for the support provided throughout the research journey especially in collecting data on whiteflies. The authors are also thankful to the University of the South Pacific for providing research funding in form of Graduate Assistantship to the corresponding author.

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Adv. Hort. Sci., 2019 33(1): 133-138 DOI: 10.13128/ahs-22530

Short note

AHS
Advances in Horticultural Science

Impact of partial root-zone drying on growth, yield and quality of tomatoes produced in green house condition

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Key words: conventional irrigation, fruit size, growth, tomato, yield.

OPEN ACCESS

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Citation:
HAKIM A., GULLO S., KHATOON M., QINYAN Z., 2019 - *Impact of partial root-zone drying on growth, yield and quality of tomatoes produced in greenhouse condition*. - Adv. Hort. Sci., 33(1): 133-138

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 16 January 2018
Accepted for publication 5 October 2018

Abstract: Water resources are limited for irrigation worldwide especially for the arid and semi-arid regions; therefore, there is an urgent need to reassess an alternative technique for conventional irrigation. Partial root-zone drying (PRD) is considered a new water-saving irrigation technique which has been tested for some crop species. The PRD technique simply requires wetting half of the rooting zone and leaving the other half dry, thereby utilizing reduced amount of irrigation water. The wetted and dry sides are interchanged in the subsequent irrigations. The focus of this article is to evaluate the effect of PRD on growth, yield and quality of tomatoes as compared to conventional irrigation. To evaluate the effect of PRD, a greenhouse experiment was conducted where two irrigation treatments were tested during a 160-day growing period: (1) control treatment where drip irrigation was applied to both sides of the plants; (2) PRD treatment in which half of the irrigation water in drip irrigation was given alternately only to one side of the root system with each irrigation. PRD treatment had 15% and 7% decreases in shoot fresh weight and leaf area of plant, respectively; however, PRD had 20% higher fruit per cluster and 18% increase in fruit production in comparison to the control treatment. No significant difference was detected on fruit size between PRD treated plants and control plants. But, fruits from PRD treated plant exhibited better appearance, higher lycopene content, firmness, total soluble solid (TSS), and TSS/titratable acidity (TA) ratio than control ones. Fruit from control treatment contained higher chlorophyll content than fruit from the PRD treatment. Postharvest storage results indicated that higher percentage of rot and chilling injury were observed in control fruits than PRD treated fruits. The results of this study indicated that PRD is a promising water saving irrigation technique which is able to produce higher yield and better quality tomatoes than conventional drip irrigation.

1. Introduction

Worldwide, agriculture accounts for 70% of all water consumption, as compared to 20% for industry and 10% for domestic use. The world's population is growing by roughly 80 million people each year. More than

99% of the world's food supply comes from the land. As the world population continues to grow geometrically, great pressure is being placed on irrigation water to provide an adequate food supply. Therefore, there is a big challenge on how to increase food production with limited water resources. This is especially true for tomato (*Lycopersicon esculentum* Mill), which is the second most important vegetable crop with a total world production of 130 million tons in 2016 (Euro-fresh, 2016).

The challenge becomes even more severe in arid and semi-arid regions where water availability is decreasing and competition for water is increasing between agriculture and industry. Therefore, water resources should be used with a higher efficiency as well as a higher productivity.

Partial root-zone drying irrigation (PRD) is one of the new efficient and productive water-saving irrigation methods that can save irrigation water up to 50% in processing tomatoes (Casa and Rouphael, 2015). This technique has the potential to significantly reduce crop water use (El-Sadek, 2014), reduce canopy vigor, but able to maintain crop yields and quality of crops (Sun *et al.*, 2014) as compared to conventional irrigation methods. Although processing tomatoes accounts for the majority of tomato tonnage, while the comparatively higher prices of fresh market tomatoes make them higher ranked in terms of value. To evaluate the effect of PRD on fresh market tomatoes, an experiment was conducted in greenhouse with the objective to compare the effect of conventional drip irrigation (CDI) to PRD drip irrigation on the growth, yield and quality of the fresh market tomatoes.

2. Materials and Methods

Experimental conditions

The experiment was conducted in a greenhouse (approximately 24°C in day time and 15°C at night time, Relative Humidity (RH) 65%, 15 hour's photoperiod and ambient light condition) at the Chateau Fresno Nursery, 13505 South Fresno, California 93609, USA from April to September 2015. Seeds of the fresh market tomato (*Lycopersicon esculentum* Mill cv. Vibelco) were sown on March 1st, 2015. Thirty days after seeding, uniform plants were transplanted into 12 wooden boxes (2.53 m length × 0.65 m width × 0.20 m height each). Each box had 4 compartments (0.50 m length × 0.50 m width × 0.20 m height) with one experimental plant per compartment. To avoid lateral water movement, a small piece of plastic (0.50 m length × 0.025 m width × 0.04 m height) was placed centrally on the base of each compartment. Plants were grown in a vermicast and coconut fiber mixture (70:30 v/v). Plants were fertilized with 15-15-15 (NPK) at 120 kg/ha, purchased from Lowes, Elk Grove, California, USA. Bees were used for pollination.

Irrigation treatments

Two weeks after transplanting two irrigation treatments were tested. Treatments were: control (conventional drip irrigation, CDI) to both sides of the root system, and half of irrigation water in drip irrigation given alternately only to one side of the root system with each irrigation (PRD) (Fig. 1). Irrigation treatment was given 0.10 m away from the main stem and on both sides of the row. Irrigation covered a total area and soil volume of 0.24 m^2 and 0.048 m^3, respectively. But half of it at 10:00 h and the other half at 16:00 h by manual drip irrigation system. Two irrigation lines were set up and operated separately for the PRD treatment. Two emitters per plant (one on each line) each emitting 4 L/h were placed 0.15 m away from the main stem of each plant. Irrigation in CDI treatment covered a total area and soil volume of 0.018 m^2 and 0.004 m^3, but half of irrigated area and soil volume was wetted in PRD treatment at each irrigation. There was some drainage in all treatments, but this was not measured. However, water losses by drainage were minimized by adjusting the amount of water as the crop developed. So, values of the irrigation use efficiency

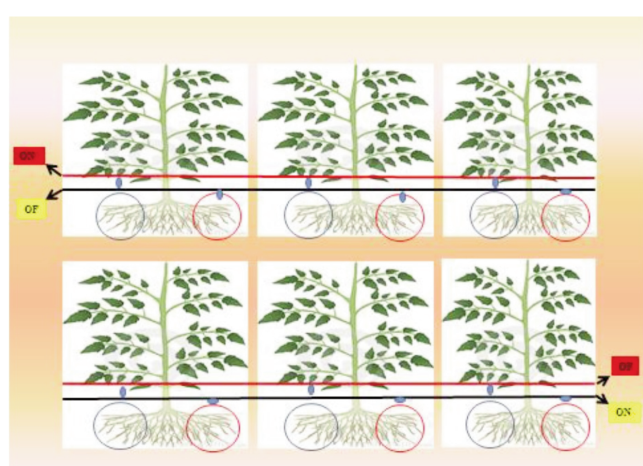

Fig. 1 - Schematic diagram of partial root-zone drying (PRD) irrigation in tomato plants.

presented here might have been under-estimated considering the water losses by drainage.

Growth, yield, yield component, fruit firmness, fruit water content and blossom-end rot measurements

Growth, yield, yield components and blossom-end rot were measured from twelve (12) randomly selected plants/fruits from each treatment. The plant heights were measured with a tape from the base of the plant to the tip of the plant. Plant growth and development data were taken on the sampled tagged plants monthly for three months. Leaf area was measured using a destructive method. Total leaf area (cm^2) was measured by leaf area meter (Model, Delta-T, Cambridge, UK). The numbers of clusters were counted per plant from the first to the last cluster during the growing period. The numbers of fruits were counted when the plants started fruiting. The fruit weights were determined after harvesting the tomato using a weighing balance. After 150 days, one plant per treatment per replication was destroyed and the total vegetative fresh weight was assessed and expressed as kg/plant. Mature green tomato fruit firmness was measured using an Instron Universal Testing Machine with a 0.5 cm^2 plunger, measurement was taken at the mid-section of the fruit. Water content of fruit was expressed on a dry weight basis. Fruit blossom-end rot incidence was recorded and calculated in percentage of fruit affected per plant.

Fruit quality at harvest or postharvest

For postharvest quality evaluation, six (6) replicates of five (5) mature green (Cascio, 2017) fruits from each treatment were randomly chosen approximately 130 days after transplanting and were stored in a dark refrigerated room at 3°C with 95% RH. After a storage period of 2 weeks, all fruits were moved to a ventilated room without supplemental light at 24°C with 65% RH and held for 7 days. The following attributes were checked for quality measurements: weight loss, chilling injury, decay, appearance/color change, total soluble solids (TSS), pH, titratable acidity (TA), chlorophyll and lycopene content.

Fruit weight loss was determined prior to and after storage. It was calculated as the percentage of initial fresh weight. Color development was observed visually using a subjective scale with mature green (MG) = 1, breaker (B) = 2, pink (P) = 3, light red (LR) = 5 and Red (R) = 6 (USDA, 2005). Chilling injury (surface pitting) was rated visually by estimating the percentage of the injured fruits. Decay (unidentified) was rated visually and calculated as a percentage of fruit affected.

Chlorophyll and lycopene content were determined from three randomly selected fruits from each treatment by grinding pericarp tissue (about 5 g) in 15 ml acetone. The extract was taken for centrifugation at 35,000 rpm for 10 minutes. Before centrifugation, the tubes were covered with aluminum foil to prevent light-induced lycopene oxidation. After centrifugation, the supernatant was decanted and adjusted to 20 ml with acetone. Absorbance of the extracts at 664 nm for chlorophyll and 503 nm for lycopene was measured with a spectrometer (Model 160 A). Total chlorophyll content in milligram per 100 gram of tissue was calculated according to the formula developed by Holden (1976). Lycopene content was calculated using the molecular extension coefficient of 3240 (Davis, 1976) and expressed as micrograms per gram of fresh weight.

Total soluble solid (TSS), pH and titratable acidity (TA) were measured on juice extracted from fruit. TSS content was determined with a digital refractometer (Atago, Model 1, Tokyo, Japan). TA was determined by a Metler Auto titrator (Model V 20) and pH was measured with an autocal pH meter (Model PHM 83).

Experimental design and data analysis

A completely randomized design was used with the two treatments replicated six times with four plants per replication for each treatment. Data were analyzed by a complete randomized model using the GLM procedure of SAS software version 8.2 (SAS Institute, Cary, NC, USA). Student's t-tests were used to determine significant effects between two treatment means.

3. Results and Discussions

Mean plant height over the 3 months are illustrated in figure 2. The maximum height was obtained from tomato plant irrigated with CDI/full water regime. PRD treated plants resulted in the lower height. Similar results were reported by Pal *et al*. (2016) who grown tomato plant under deficit irrigated conditions plus paclobutrazol application.

PRD treatment had 15% and 7% decreases in shoot fresh weight and leaf area of plant, respectively. However, PRD had 20% higher fruit per cluster and 18% increase in fruit production in comparison to the CDI treatment (Table 1). The difference between CDI and PRD treated plant's shoot fresh weight, leaf area, and fruit production were significantly different at

0.001, 0.01 and 0.05% level respectively. The CDI treated plant appeared to have excess moisture in root zone, which causes root inactivity contributing to lower yield and delayed maturity of the crop as compared with the PRD treated plant.

Mean cluster per plant, fruit per cluster, total fresh weight of fruit per plant and fruit weight (individual) were lower in CDI treated plants as compared to PRD treated plants. The difference was statistically significant only in case of cluster per plant and total fresh weight per plant (Table 1). The lower cluster number, less fruit per cluster and less weight of fruit per plant in CDI treated plant might be due to the excessive vegetative growth as a result of luxurious amount of water application.

PRD treated plant's fruit exhibited significantly higher percentage of blossom-end rot as compared to the CDI treated ones. The blossom-end rot is a physiological disorder of tomato fruit caused by calcium deficiency or excessive soil moisture fluctuation which reduce uptake and movement of calcium into

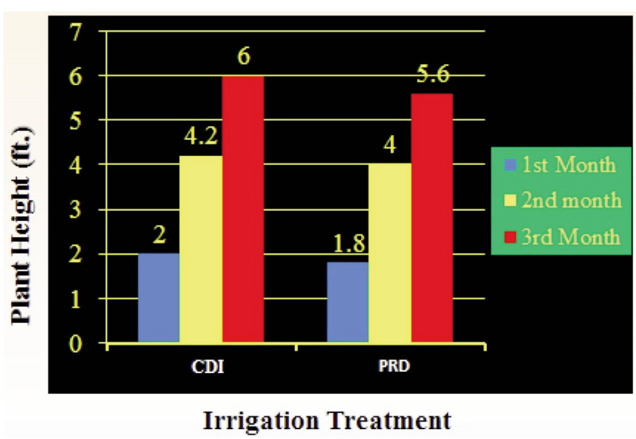

Fig. 2 - Effect of CDI (conventional dripping irrigation) and PRD (partial root-zone irrigation) on plant height over 3 months.

the plant (Mathew and Salvadore, 2007). The higher percentage of blossom-end rot in PRD treated fruit might be due to the reduced movement of calcium to the PRD treated plants. However, no calcium was analyzed either from leaf or from fruits in this study.

Fruit size, water content and fruit weight were influenced by PRD treatment. There were some differences in fruit size, weight and water content in PRD and CDI treated plant's fruit; however, the difference were not statistically significant (Table 1).

Reduced weight loss in the PRD treated tomatoes during the storage is a positive quality attribute in tomato fruit especially for distant market (Table 2). Yadav and Singh (2014) indicated that the weight loss of fruits in storage condition is mainly from water loss and from solid constituents. The lower water loss in PRD treated fruit might be due to lesser incident of micro-cracks in the skin. However, no skin micro-cracks were examined in this study.

In this study, when compared to fruits from CDI treated plants, fruits from PRD treated plants exhibited significantly lower chlorophyll and higher lycopene content on the 7th day at 24°C followed by a 2-week storage period at 3°C (Table 2). Klunklin and Savage (2017) also detected significantly higher lycopene content in PRD treated tomato fruit.

According to Gindi *et al*. (2016), 63 percent of consumers' purchase interest depends on color of fruits. Table 2 demonstrated that fruits from PRD treated plants exhibited less visible chilling injury (fruit surface pitting) and decay, but similar color change as fruits from CDI treated ones. In the regions of the world where irrigation water is expensive, such as arid and same-arid regions, PRD treatment would be beneficial in terms of economic return, where water supply is crucial.

Soluble solids are an important tomato quality

Table 1 - Effect of PRD on tomato fruit size, total fresh weight, water content, fruit firmness, cluster/plant, fruit/cluster, shoot fresh weight, leaf area, and blossom-end rot

Physiological parameters	Treatment		Difference (PRD-CDI)
	CDI Group	PRD Group	
Fruit size, diameter (mm)	68.4	67.8	0.6 NS
Total fresh weight of fruit (kg/plant)	5.1	7.03	1.93 ***
Fruit water content (%)	95.2	94.8	0.4 NS
Fruit weight (g)	95.2	95.0	0.2 NS
Fruit firmness after harvest (kg/cm^2)	9.1	9.0	0.1 NS
Cluster/plant	8.1	10.2	2.1 ***
Fruit/cluster	6.1	6.3	0.2 ns
Shoot fresh weight (kg)	10.6	9.1	1.5 ***
Leaf Area (cm^2)	451.54	418.26	33.28 *
Blossom end rot (%)	5.5	6.0	0.5 ***

NS, *, **, *** Non-significant or significant at t≤0.05, 0.01 or 0.001 respectively.

parameter. Tomato flavor is generally determined by the content of soluble solids and acid (titratable acid). According to Aoun *et al*. (2013), tomato flavor impact is co-related to total sugar and acid. In this study, total soluble solid (TSS), TSS/TA and pH increased, while TA decreased in PRD treated fruits (Table 2). In an earlier study, Sun *et al*. (2014) also detected higher TSS in tomato fruit produced under PRD condition. The higher TSS and lower TA in fruit from PRD treated plants were probably due to the less retained water in fruit from PRD treated plant than fruit from CDI treated plant. The higher pH in fruit from PRD treated plant was compatible with the lower TA in fruits from PRD treated plant than in fruits from CDI treated plant.

4. Conclusion

The result of this study demonstrated that "Vibelco" tomato plant treated with PRD slowed its vegetative growth, but produced higher yield and better quality fruit at the same time saved water by 50%. This study proved that PRD is a noble water saving method which can prevent excess water use and increases economic returns due to the reduction of water use.

Acknowledgements

The author wishes to thank Dr. Abu Helal Ibrahim for his experimental assistance.

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Table 2 - Effect of PRD on weight loss, chilling injury, decay, chlorophyll content, lycopene content, color change, pH, titratable acidity (TA), total soluble solid (TSS), and TSS/TA in mature green tomatoes on the 7th day at 24°C following a 2-week storage period at 3°C

Physiological parameters	Treatment		Difference (PRD-CDI)
	CDI Group	PRD Group	
Weight loss (%)	2.15	1.96	0.19 ***
Chilling injury (%)	15.00	10.00	5.0 NS
Decay (%)	13.00	8.00	5.0 ***
Chlorophyll (mg/100 g fresh weight)	3.90	3.20	0.7 **
Lycopene (µg/g fresh weight)	7.10	7.70	0.6 *
Color Change	4.50	4.50	0.0 NS
pH	4.40	4.55	0.15 NS
TA (% Citric Acid)	0.49	0.46	0.03 *
TSS	4.30	4.50	0.2 NS
TSS/TA	8.77	9.80	1.01 ***

NS, *, **, *** Non-significant or significant at t≤0.05, 0.01 or 0.001 respectively.

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Adv. Hort. Sci., 2019 33(1): 139-143 DOI: 10.13128/ahs-22813

Short note

AHS
Advances in Horticultural Science

Effects of packing materials and ethanol concentrations on removal of astringency of 'Rojo Brillante' at room temperature

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Key words: calcium carbide, de-astringency, *Diospyros kaki*, ethanol dipping.

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Citation:
HEJAZI Z., SADAT M.I., KARIMI B.K., FAWAD M., MUNEER A., 2019 - *Effects of packing materials and ethanol concentrations on removal of astringency of 'Rojo Brillante' at room temperature*. - Adv. Hort. Sci., 33(1): 139-143

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 17 April 2018
Accepted for publication 5 October 2018

Abstract: Most of the persimmons grown in Afghanistan are astringent, hence their fruits require de-astringency treatments to become edible at harvest time. The CO_2 with or without ethanol treatment provides an optimal method for the rapid removal of the persimmon astringency. However, this method is sophisticated and currently not feasible for most of the farmers. The present study is therefore carried out to assess a simple and suitable technique for de-astringency of the persimmons at farmer level. The fruits of 'Rojo Brillante', a Pollination Variant Astringent (PVA) Spanish cultivar, were quickly dipped in 0, 10, 20, and 40% ethanol. The treated fruits were packed and sealed in the paper cartons, polyethylene bags or left open at the room temperature. The firmness, total soluble solids and astringency level of fruits were measured after every three days for nine days. The astringency was removed when fruits treated with 20% or 40% ethanol and packed in the polyethylene bags for nine days. The total soluble solids content of the fruits treated with ethanol was lower in any of the used concentrations than the untreated fruits. The commercial firmness of the fruits (1.5-2.5 kg) obtained by packing the fruits in the polyethylene bags or treated with 20% ethanol concentration.

1. Introduction

Persimmon (*Diospyros kaki* Thunb.) is one of the fruits cultivated in the East of Afghanistan. The subtropical climate of this region provides a favorable environment for the persimmon production. On the other hand, the naturally grown wild population of *Diospyros lotus* can be seen in the mountainous area, lies at an altitude between 1500 and 1900 m asl (Samadi *et al.*, 2009).

In the frame of Perennial Horticulture Development Project (PHDP), an *ex situ* germplasm of 24 persimmon accessions was established in Farme-Jaded, Jalalabad (Masini and Giordani, 2016). Currently, this center preserved 22 different varieties, of which ten were donated by University of Florence, Italy (afghanhorticulture.org; Samadi *et al.*, 2009). These cultivars contained both astringent and non-astringent types of the persimmons. Because of the good adaptation with the climate and affinity with a more common rootstock of *Diospyros lotus*, the astringent type is widely spread in the country. Non-astringent persimmons are normally edible at harvest, whereas fruit of the astringent cultivars as 'Rojo Brillante' (Pollination Variant Astringent - PVA) can be consumed after astringency is removed. Natural loss of the astringency of the latter type is achieved by leaving the fruits on the tree until they are overripe, with soft flesh (Taira, 1995). However, it is not desirable for the fruit growers, because it takes a long time, attracts birds eating the fruits and increases protection expenses; furthermore, the handling and marketing of soft fruits is very time consuming and expensive. Due to these limitations, the ripening of the astringent persimmons by the artificial method is required. In Afghanistan, the persimmon producers used Calcium Carbide (CaC_2) to hasten ripening process of the fruits (Samadi *et al.*, 2009). This agent is also commonly used in ripening of mangoes and bananas in several other developing countries (Asif, 2012). Although CaC_2 is widely available, easy to use and cheap, it is associated with health hazards. Recent findings have proved that calcium carbide is carcinogenic (Per *et al.*, 2007; Asif, 2012). Moreover, fruits ripened with CaC_2 have negative quality attributes. They are often soft and less tasty, and have a shorter storage life. Therefore, secure and simple methods that can be used by farmers to remove astringency of persimmons are needed.

Today, the world top persimmon producers make use of either CO_2 or ethanol (EtOH) for the removal of the persimmon astringency (Kato, 1984, 1990; Eaks, 1967). The use of CO_2 is safe and induces rapid ripening, but maintaining a strict control condition of its application wouldn't be easy for the persimmon growers, particularly in remote countryside orchards. De-astringency of the 'Rojo Brillante' persimmons by CO_2 with or without ethanol is already established (Salvador *et al.*, 2007; Novillo *et al.*, 2015), but the removal of astringency using alcohol without CO_2 has not been explored. The aim of the present study was to assess the use of EtOH without CO_2 in different packaging material.

2. Materials and Methods

The fruits of 'Rojo Brillante' from two clones of 5-year-old trees grown in Jalalabad Perennial Horticulture Development and Research Center, formerly named PHDP, were harvested at the yellow-orange color on October 2017. After sorting, fruits (n=180) were mixed for uniformity of size and used for the experiments. The fruits were quickly immersed in 0 (distilled water), 10, 20, and 40% aqueous ethanol solutions and then air dried. Sixty fruits were packed and sealed in the paper catons (2 kg net weight), another 60 fruits were sealed in transparent polyethylene bags (0.018 mm thickness), and the remaining fruits were left open at the ambient temperature. The fruits within each group were further sub-divided into five per pack. Subsequently, all the packaged fruits left at the room temperature. Room temperature measured at 15 min interval using a data logger with thermo-hygro sensor (RC-4HC, Elitech, Jiangsu Jingchuang Electronics Co., Ltd, China). The data were recorded after every three days for the fruit firmness, total soluble solids (TSS) and astringency levels. Fruit firmness was determined for each fruit at two pared equatorial positions after peeled, using a hand-held penetrometer equipped with an 8 mm tip. To measure the astringency and TSS, fruits were cut into two halves. One half was used to estimate astringency and the remaining halve was used to express the juice for the brix with a digital Atago refractometer. The astringency was estimated by tannin print method (Eaks, 1967), where the freshly cut surface of the fruit soaked in 5% ferric chloride and then the black color scored from 0 (non-astringent) to 5 (very astringent).

The data were subjected to repeated measures ANOVA of General Linear Model of SPSS (16.0) statistical software and mean separation was performed using Tukey's (HSD) method at $P \leq 0.05$.

3. Results and Discussion

The room naturally kept almost a consistent temperature that averaged 20.9°C during the experiments. This degree is recommended if faster de-astringency of the persimmons is aimed with CO_2 or CO_2-ethanol treatment (Besada *et al.*, 2010; Novillo *et al.*, 2015).

The results of the ANOVA showed that firmness of the 'Rojo Brillante' fruits were significantly affected by packing and ethanol concentrations; however, the interaction between packing and ethanol was found insignificant (Fig. 1). The fruits packed and sealed nine days in the polyethylene bags were softer (2.5 kg) than of paper cartons (3.0 kg) and unpacked persimmons (2.9 kg). Likewise, fruits treated with 20% ethanol was significantly softer (2.5 kg) than the fruits treated with distilled water (3.1 kg). Kato (1990) stated that high-quality persimmon fruit has a firmness of 1.5 to 2.5 kg; fruit firmer than 2.5 kg is too hard and fruit with a firmness <1.5 kg are too soft to tolerate the physical handling. Hardness of the persimmon fruits is a concern both for the consumers and fruit shelf life, but people in our region are not yet familiar with eating of crispy persimmons.

The content of soluble solids was significantly affected by the ethanol concentrations, while the effects of packing and the interaction were not considerable (Fig. 2). The TSS of the fruits immersed in 20% ethanol solution were significantly lower (17.9) than of distilled water (19.5). More in a similar pattern, ethylene treatment decreased total soluble solids of the astringent type of 'Hiratanenashi,' but increased in fruits of 'Fuyu,' which is a non-astringent persimmon (Takata, 1975).

Except for the ethanol concentrations, the astringency of the 'Rojo Brillante' fruits was significantly influenced by packing and the interaction between packing and ethanol concentration at $P \leq 0.002$. Despite that after six days of the treatment, recurrence of the astringency (Edagi *et al.*, 2009; Sestari *et al.*, 2009) was evident in some treatments, fruits treated with 20% or 40% ethanol concentrations and placed nine days in the polyethylene bags remained slightly to non-astringent (Fig. 3 and 4). These findings are in accordance with Kato (1987) who attained de-astringency of the persimmons by ethanol vapor method. In our study, packing alone was not effective to remove the astringency, because the fruits treated with distilled water and placed nine days in the paper cartons or polyethylene bags were similar as those exposed to the room ambient. Although the anaerobic condition of the package or chamber promoted de-astringency of the persimmons (Eaks, 1967; Novillo *et al.*, 2015; Monteiro *et al.*, 2017), no effect of the polyethylene bags or paper cartons

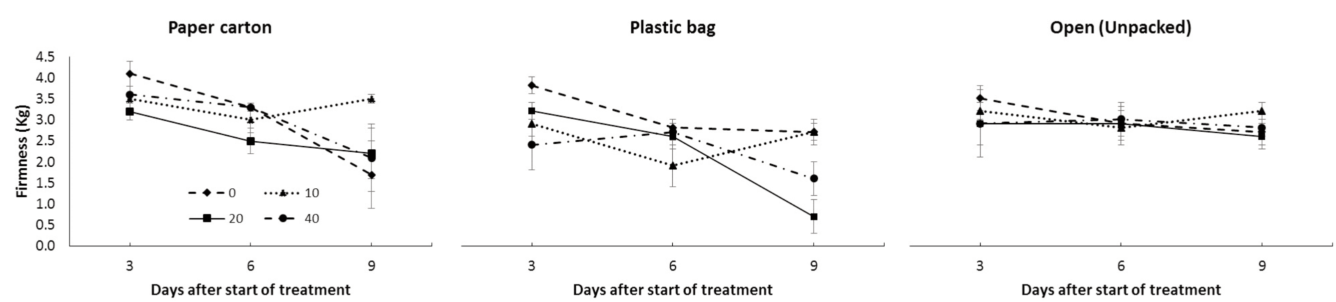

Fig. 1 - Changes in the firmness of 'Rojo Brillante' fruit treated with 0, 10, 20, and 40% ethanol and then packed and sealed in paper cartons, polyethylene bags or left open during three days at the room ambient. Bars indicate SE (n= 5).

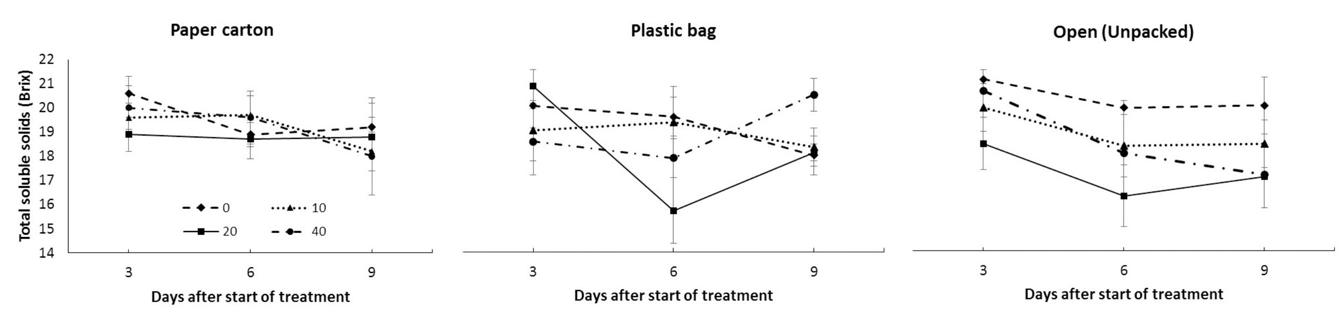

Fig. 2 - Changes in total soluble solids of 'Rojo Brillante' fruit treated with 0, 10, 20, and 40% ethanol and then packed and sealed in paper cartons, polyethylene bags or left open during three days at the room ambient. Bars indicate SE (n= 5).

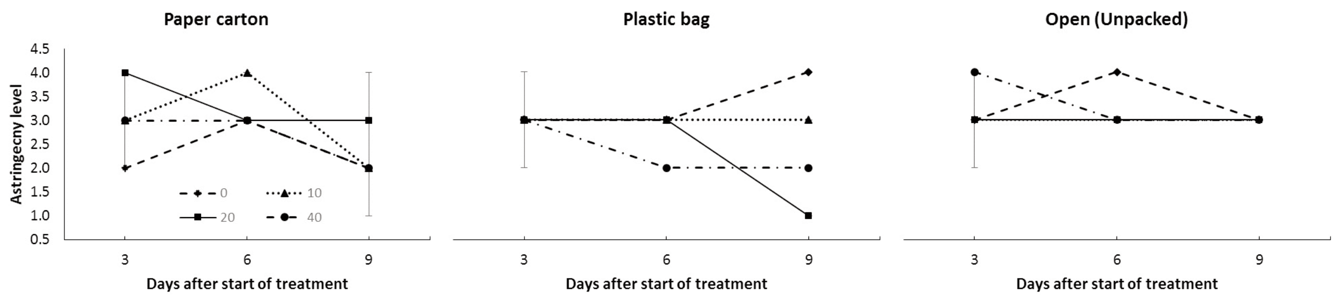

Fig. 3 - Changes in astringency level of 'Rojo Brillante' fruit treated with 0, 10, 20, and 40% ethanol and then packed and sealed in paper cartons, polyethylene bags or left open during three days at the room ambient. Bars indicate SE (n= 5).

would be associated with materials permeability. Monteiro *et al*. (2017) reported that among three different polyethylene packages, polyethylene-polyamide succeeded to remove 'Giombo' astringency faster, whereas low-density polyethylene and polypropylene didn't work because of their greater permeability to gases. Moreover, the result of de-astringency of the closed chamber with no-addition was alike with the chamber added ethyl alcohol (Eaks, 1967).

Astringency will be removed when soluble tannins are becoming insoluble because of the polymerization with acetaldehyde in the fruit flesh during the treatments (Taira, 1995). Since acetaldehyde is known to be generated *in situ*, it can be triggered by exogenous ethanol application (Novillo *et al.*, 2015). Kato (1984) revealed that during de-astringency of the persimmons, acetaldehyde increased with the increase of ethanol concentration. He further explained that alcohol treatment caused a rise in ethylene evolution and induced carbon dioxide. Thus, the effectiveness of the polyethylene bags to astringency removal in higher doses (20%, 40%) of the ethanol solutions of this study might be referred to ethylene and through CO_2 production, which probably preserved better by the polyethylene bags than of paper cartons.

Fig. 4 - Tannin prints of the freshly cut surface of 'Rojo Brillante' fruits treated with 20% ethanol solution, then left open at the room ambient (A), or packed and sealed in paper cartons (B), or polyethylene bags for nine days (C).

4. Conclusions

This study suggests that it is feasible to remove astringency of the 'Rojo Brillante' persimmons by packing them nine days in the polyethylene bags after immersed in 20% ethanol solution. However, to assure complete sweetness, time might be extended for two or three more days, depending on fruit maturity, temperature, and package permeability. Future researchers may test polyethylene bags in different thickness along with some modifications to improve this protocol for a more rapid loss of the persimmon astringency.

Acknowledgements

We would like to appreciate the effort of the European Commission (EC), in the particular University of Florence for granting the elite cultivars of persimmons to the national fruit collection of Jalalabad, Afghanistan.

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Adv. Hort. Sci., 2019 33(1): 145-150

DOI: 10.13128/ahs-24881

Short note

AHS
Advances in Horticultural Science

Kinetics of volatile organic compounds (VOCs) released by roasted Coffee during the first ten days after processing

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Citation:
COLZI I., MARONE E., MAGNELLI S., MANCUSO S., AZZARELLO E., TAITI C., 2019 - *Kinetics of volatile organic compounds (VOCs) released by roasted Coffee during the first ten days after processing.* - Adv. Hort. Sci., 33(1): 145-150

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 30 November 2018
Accepted for publication 15 March 2019

Key words: *Coffea arabica*, *Coffea canephora*, PTR-ToF-MS, volatile organic compounds.

Abstract: The quality of coffee is linked to the aroma created by the chemical reactions that occur during the roasting process. While it is generally thought that roasted coffee is a stable product with a relatively long shelf-life, little information is available on the evolution (kinetic) of the volatile organic compounds (VOCs) in the days immediately following the process. The aim of this study is to determine the evolution of VOCs released by coffee beans, on samples of *Coffea arabica* (three different origins) and *Coffea canephora* (1 single origin), by using a Proton Transfer Reaction Time-of-Flight Mass Spectrometer (PTR-ToF-MS) 24 hours after roasting, and for the next 9 days. Results confirmed the differences already highlighted in previous studies between the VOCs *spectra* of the two species. There were also significant differences in the intensity of emissions for the different origins of *Coffea arabica*, with the highest VOCs amount over time always detected in the Honduras Arabica samples. The involved detected protonated ions were grouped into three classes: compounds (ppbv) present with decreasing quantity; weakly increasing; almost constant trend; or always increasing. A complex dynamic emerged for the different protonated ions over time, which not only affects the mass *spectra* of the different species but also influences the configuration of the mass *spectra* of the different geographical zones of production.

1. Introduction

Two species contribute to the production of roasted beans, *Coffea arabica* (commercially known as Arabica) and *Coffea canephora* var.

Robusta (commercially known as Robusta). These two species are characterized by different economic value, with Arabica having the higher costs, due to both the different aromatic properties and the higher production costs. The two species are distinguishable by aromatic profiles (Gloess *et al.*, 2014; Yener *et al.*, 2014; Colzi *et al.*, 2017), and our previous work (Colzi *et al.*, 2017) demonstrated that the Proton Transfer Reaction - Time of Flight - Mass Spectrometry (PTR-ToF-MS) can be successfully used to distinguish between the aromatic profiles of these two species in each step of the processing chain (green beans, roasted beans, ground coffee, brews).

Little known are the changes in volatile organic compounds (VOCs) detected through headspace analyses, that could be used to recognize the genetic origin of the coffee stock and, within the same species, the geographical provenience as macro zone of production.

The quality of coffee is linked to the particular aroma created by the chemical reactions that occur during the roasting process (Maillard reaction). In addition to the roasting conditions, the quality of the product also depends on the genetic origin of the raw material. Roasted coffee is generally considered a stable product with a relatively long shelf-life. Nevertheless, little is known about the possible changes in the days immediately following the roasting process, which could alter the complex kinetics of VOCs determining the coffee aroma and therefore its value. Indeed, evidence indicates that chemical changes can still occur following the roasting process (Nicoli *et al.*, 2009), affecting the aroma of the brews. Confirming this, coffee made from freshly ground beans was found to have a stronger aroma, less bitterness and was preferred by expert panelist when compared with beans stored for 1 or 2 weeks (Ross *et al.*, 2006).

The present study has two goals: 1) to define the VOCs emission in coffee beans of different species and origins in the first days after roasting; 2) to ascertain if differences in the VOCs kinetics depend on the physical parameters of the roasted bean, such as porosity and the surface; 3) to explore the variation of the VOCs *spectra* emitted over time as a way to improve the possibility of distinguishing the different species and, within them, the origins of the different stocks.

2. Materials and Methods

Coffee samples

Coffee was sampled from medium-dark roasted beans of 3 certified commercial stocks of *C. arabica* having different origin (Honduras, India, and Nicaragua) and from one of *C. canephora* var. Robusta (Congo). All just roasted coffee samples were provided by Caffè Magnelli S.r.l. (Florence, Italy) and stored in laminate packaging in the dark at room temperature (21-23°C) before the analysis. Mass spectrometry analyses were thus performed on 120 samples, subdivided in 90 *C. arabica* and 30 *C. canephora*.

VOCs analysis

VOC measurements on roasted beans were performed by using a commercial PTR-ToF 8000 model, from Ionicon Analytik GmbH (Innsbruck, Austria). The analyses were carried out starting approximately 25 hours after roasting, and proceeded for the next 9 days, with 24-hour intervals. Three 5 g samples were taken each day from the different coffee stocks for the analyses. The analyses were performed following the methodology reported by Colzi *et al.* (2017). Each sample was put into a 0.75 l glass container plugged with a cover in which two Teflon tubes are inserted, respectively connected to a zero-air generator (Peak Scientific, Glasgow, Scotland) and to the PTR-ToF-MS. Before each analysis, the jar was cleaned for 60 seconds with free VOCs air and after was incubated for 60 s. The measurement was carried out for 60 seconds using a dynamic headspace flushing flow rate of 0.75 l per minute (lpm). Since, the "temperature" is critical for the release of VOCs, the analisys was performed in a climatized room (22°C, 90% RH). The raw data were acquired by the TOFDAQ Viewer® software (Tofwerk AG, Thun, Switzerland) and subsequently VOCs were converted in ppbv using the formula described by Lindinger *et al.* (1998) and constant values for the reaction rate coefficient (kR=2.109 cm^3 s^{-1}) to calculate concentration for each VOC (Cappellin *et al.*, 2011).

All detected compounds were tentatively identified by comparing the measured protonated ions with those reported in the extensive literature regarding the composition of VOCs released from coffee and in other previous PTR-MS studies (Colzi *et al.*, 2017; Flament and Bessière-Thomas, 2002; Gloess *et al.*, 2014; Lindinger *et al.*, 2008; López *et al.*, 2016; Maeztu *et al.*, 2001; Mondello *et al.*, 2005; Romano *et al.*, 2014; Yener *et al.*, 2014; Yeretzian *et al.*, 2002).

Statistical data analysis

One-way analyses of variance (ANOVA) was performed to compare the total VOCs content in 4 coffee stocks over 10 days; the separation of means was calculated by the Fisher's least significant difference (LSD) test. Computations were performed by Statgraphics Centurion XV v. 15.0.04.

On 90 Arabica and 30 Robusta samples, a multivariate partial least square-discriminant analysis (PLS-DA) was applied, as supervised classification method, on the spectral data (n = 129) obtained from a total of 120 coffee samples taken from 4 different stocks (Arabica Honduras, Arabica India, Arabica Nicaragua, and Robusta Congo), to determine whether it is possible to distinguish the genetic and geographical origin of the samples based on the VOC profiles. For a more detailed description of the method, see (Colzi *et al.*, 2017; Taiti *et al.*, 2017). Data were pre-processed by a logarithmic transformation (log+1). The statistical analysis was performed by PLS-Toolbox v. 8.0.2 (Eigenvector Research Inc., West Eaglerock Drive, Wenatchee, WA) for MATLAB® R2015b (Mathworks Inc., Natick, MA, USA).

To monitor VOC trend in real-time in each coffee batch over the 10 days after roasting, a Batch Statistical Process Control (BSPC) was performed after logarithmic transformation (log+1) on spectral (PTR-ToF-MS) data of the 120 samples. BSPC is the analysis of process data as a function of both correlation among the measured variables and correlation in time (also known as the "batch trajectory"). In this analysis the "measured variables" were 54 protonated ion signals selected on a total of 129 as tentatively identified in previous studies (Maeztu *et al.*, 2001; Flament and Bessière-Thomas, 2002; Yeretzian *et al.*, 2002; Mondello *et al.*, 2005; Lindinger *et al.*, 2008; Gloess *et al.*, 2014; Romano *et al.*, 2014; Yener *et al.*, 2014; López *et al.*, 2016; Colzi *et al.*, 2017), while the "time" was represented by the 10 days of surveys. The VOCs data were subdivided into 4 "batches" (experiments) (Arabica Honduras, Arabica India, Arabica Nicaragua, and Robusta Congo) that were subsequently subdivided into "Steps" (10 steps = 10 days). A summary Principal Component analysis (PCA) was then applied, after mean centering, summarizing the change in measured variables over the batch progress based on the "mean" as a reference statistic, sufficiently sensitive to the trajectory profile, to capture the trends in the trajectories of the variables.

As unsupervised classification method a hierarchical clustering (Ward method, Euclidean distance) was performed on the data expressed as percentage of the highest value of each protonated ions, related to the 90 Arabica samples, which represent all the samples unequivocally related to the same species. Computations were performed by XLSTAT 2014.5.03 software.

3. Results and Discussion

Figure 1 shows the amount of the VOCs emitted from the 4 stocks during the 10 days of testing, and the related trend curves (2nd grade polynomials: for Arabica Honduras $y = -61.937x^2 + 656.19x + 4604$; for Arabica India $y = -95.22x^2 + 1126x + 455.4$; for Arabica Nicaragua $y = -108.42x^2 + 1250.8x + 1382.7$; for Robusta Congo $y = -7.1514x^2 + 111.47x + 1705.4$). The compared samples of the 4 batches were strongly differentiated both for amounts of emitted VOCs and for trends (Table 1). The Robusta (Congo) recorded the lowest emissions, that did not statistically change during the considered time period. By contrast, the comparison between the Arabica samples highlighted key differences between the 3 batches. Coffee samples from Honduras were very rich in VOCs. The Nicaraguan samples had a less intense emission, and quantitatively increased only until the 5th day. Although the amount of VOC emitted resulted lower compared with the Nicaraguan samples, the Arabica samples of Indian origin had a similar VOC emission trend with a peak on the 5th day after roasting (Fig. 1) (Table 1).

The data represented in figure 1 were explored through two different methods of multivariate analysis: a PLS-DA (Fig. 2), and a PCA applied to the results of a batch process (Fig. 3). These approaches allow to highlight the two different aspects related to the fac-

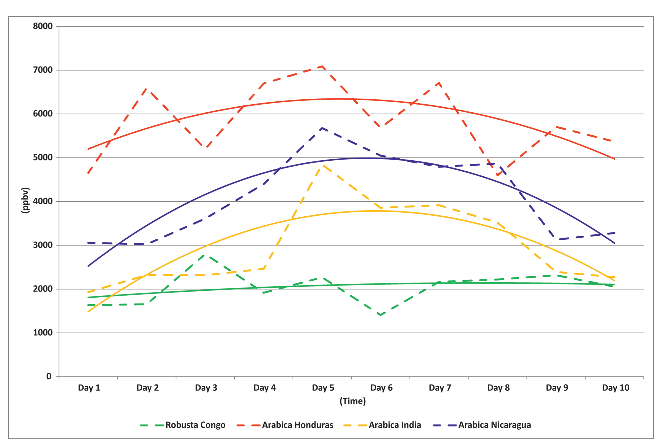


Fig. 1 - Total VOCs emission (ppbv) in the different Coffee stocks over the time (dotted line) and related trend curves (whole line).

Table 1 - Trend of total VOCs emission (ppbv) in 4 coffee batches over 10 days

Time	Total VOCs (ppbv)				Average *
	Arabica Honduras	Arabica India	Arabica Nicaragua	Robusta Congo	
Day 1	4654.6 ± 340.3	1928.8 ± 353.9	3055.9 ± 125.0	1634.7 ± 249.7	2818.5 ± 1261.3 a
Day 2	6588.9 ± 516.63	2321.3 ± 392.1	3023.0 ± 465.0	1653.1 ± 267.0	3396.5 ± 2022.5 ab
Day 3	5200.3 ± 92.8	2315.7 ± 5.19	3609.9 ± 250.3	2797.1 ± 123.0	3480.8 ± 1150.8 ab
Day 4	6694.6 ± 2105.3	2461.9 ± 756.2	4398.1 ± 931.3	1916.7 ± 491.7	3867.8 ± 2223.7 abc
Day 5	7087.2 ± 2509.9	4844.2 ± 314.3	5677.0 ± 912.4	2267.03 ±197.3	4968.8 ± 2162.8 c
Day 6	5680.3 ± 1146.0	3856.5 ± 449.9	5048.2 ± 906.3	1408.3 ± 148.6	3998.4 ± 1826.5 abc
Day 7	6706.1 ± 576.0	3915.7 ± 1614.9	4792.3 ± 747.9	2166.0 ± 684.7	4395.0 ± 1907.6 bc
Day 8	4600.6 ± 522.9	3517.5 ± 481.2	4867.8 ± 632.7	2218.1 ± 355.4	3801.0 ± 1173.7 abc
Day 9	5704.1 ± 1286.2	2393.8 ± 681.6	3127.7 ± 890.6	2315.0 ± 586.2	3385.2 ± 1630.0 ab
Day 10	5368.0 ± 862.5	2271.3 ± 646.7	3281.8 ± 934.5	2055.4 ± 459.3	3244.1 ± 1510.8 ab
Average *	5828.5 ± 1345.7 d	2982.7 ± 1102.6 b	4088.2 ± 1126.8 c	2043.1 ± 513.0 a	3735.6 ± 1764.0

Average ± S.D.

*Different lower case letters within a row or a column indicate difference by the LSD test at the 95.0% confidence level (p=0.05).

tors influencing the process: 1) species and origins (PLS-DA applied to 120 coffee samples based on 129 detected signals), and 2) the processes that develop over time expressed as coefficients of 54 selected variables with PC1 during the 10 days of surveys after roasting (PCA applied to a BSPC).

The score plot from PLS-DA shown in figure 2 confirmed that VOC analysis using the PTR-ToF-MS technique can indeed be used to distinguish Robusta from Arabica samples. Furthermore we found that the evaluation of the whole spectrum of VOC emission during 10 days of surveys can also be used to determine within the same species, albeit with some overlapping, the different origins (Fig. 2).

In the figure 3, where the dynamics of the single 54 tentatively identified protonated ions related to VOCs found in the Arabica samples of the three different origins are reported, a downward shift of the VOCs pool on the 4th day is observed (Fig. 3, red circle) while, in general, a progressive slight decrease and compression of the VOCs complex can be seen in the time, up to the 10th day.

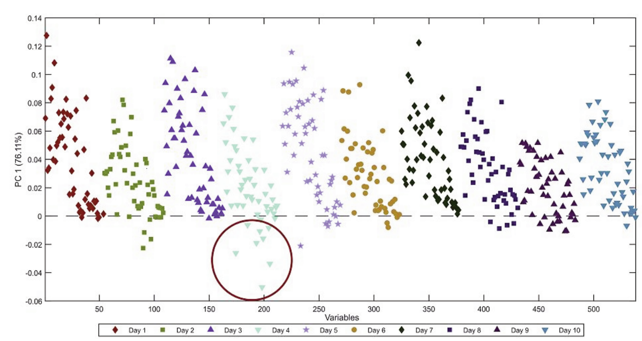
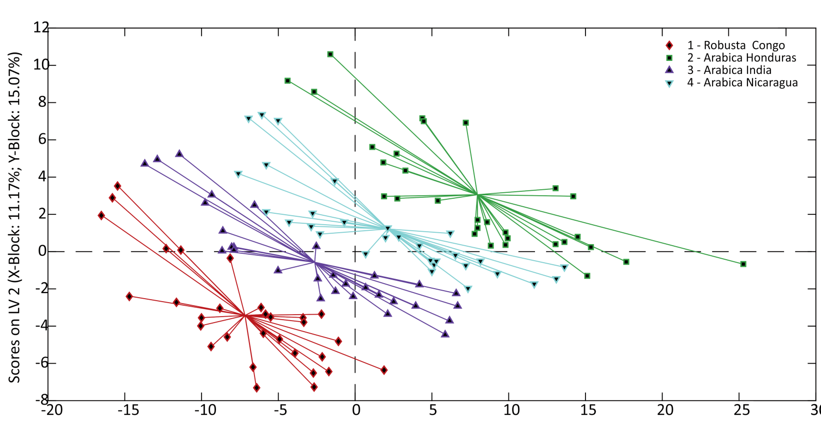


Fig. 3 - Score plot from a PCA applied to a BSPC, showing the dynamic over the 10 days of surveys evidenced by the VOCs complex (54 protonated ion signals tentatively identified) related to 120 Coffee samples. Outlier samples were highlighted by a red circle.



Statistics	Robusta Congo	Arabica Honduras	Arabica India	Arabica Nicaragua
LVs	5	5	5	5
SE (Cal)	1.000	1.000	0.846	1.000
SP (Cal)	0.987	1.000	0.908	0.961
SE (CV)	0.962	0.917	0.654	0.923
SP (CV)	0.961	0.974	0.803	0.895
SE (P)	1.000	1.000	0.750	0.750
SP (P)	1.000	1.000	0.786	1.000
Class. error (Cal)	0.006	0.000	0.122	0.019
Class. error (CV)	0.038	0.054	0.271	0.091
Class. error (P)	0.000	0.000	0.232	0.125
RMSEC	0.186	0.159	0.300	0.254
RMSECV	0.246	0.246	0.402	0.343
RMSEP	0.251	0.154	0.365	0.263

Fig. 2 - Score plot of PLS-DA model and summary statistics for the 4 Coffee stocks samples based on 129 protonated m/z.

In the dendrogram obtained by the hierarchical classification of the three Arabica stocks (90 samples) based on 54 protonated ions, two main clusters (A and B) were identified and, within the cluster B, a further division into two subgroups of compounds (B1 and B2) emerged (Fig. 4). Each group was characterized by a particular trend in the 10 days of the test. Cluster A combined compounds characterized by a decreasing trend over time. On the other hand, cluster B grouped compounds with increasing trend (B1), or compounds at first increasing and then decreasing their amount over the time, in slightly different way (B2).

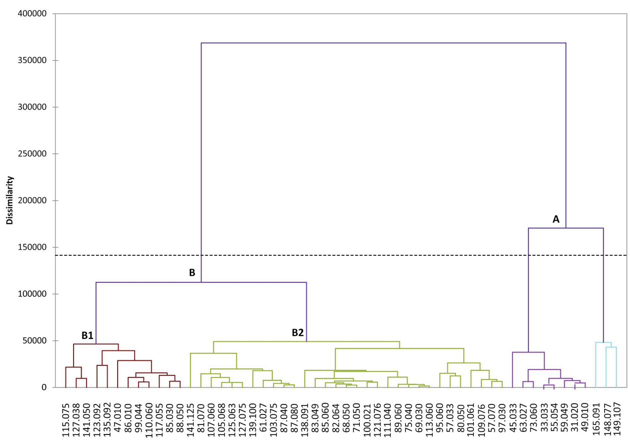


Fig. 4 - Dendrogram from cluster analysis of 90 Arabica sample based on 54 selected protonated m/z.

Figure 5 shows the trends, for the three Arabica stocks, of three protonated ions chosen as representative of the groups emerged by the dendrogram reported in figure 4. In particular, mass m/z = 45.030, representative of the group A, was always decreasing, while mass m/z = 99.040 (B1) showed an upward trend and mass m/z = 82.060 (B2) initially increased and and then decreased to finally stabilize on intermediate values.

4. Conclusions

Roasted coffee beans are considered resistant to spoiling phenomena, and as such are marketed, or considered for subsequent treatments. Nevertheless, little is known about the possible changes in the days immediately following the roasting process, which can determine complex variations in the kinetics of VOC emission within the different species and of different origins. The data set collected in this work highlights that samples of Arabica and those coming from Honduras production area have the highest level of VOC emissions and the greater dynamism in the kinetics of change. These changes in the VOC kinetics in the 10 days following the roasting process found within the Arabica samples also improved the separation of the samples based on their geographical origin.

The Arabica species samples hierarchical classification applied to the 54 selected protonated ions related to VOCs and/or fragment of VOCs allowed to pool them together, independently of the chemical family they belong to, in relation to their kinetics over time.

Three groups of VOCs were highlighted from the dendrogram (Fig. 4). The first, which clustered together those that decrease over time. On the other hand, the second and third group respectively combined VOCs that either had an upward trend but then stabilized over time, or those that constantly increased.

The analysis of the VOCs highlighted dynamics that changed over time. This was associated with the simultaneous presence of VOC whose amount

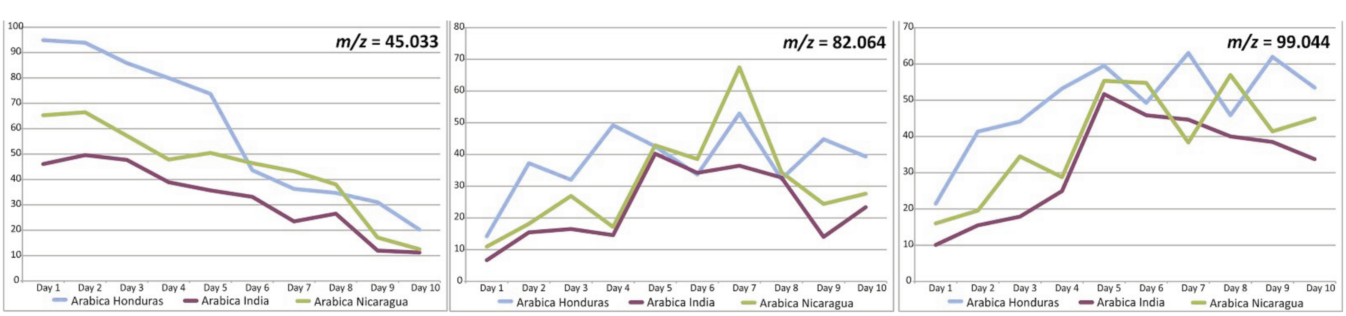


Fig. 5 - Trend of m/z = 45.030, m/z = 99.040, and m/z = 82.060, representative of group A, subgroup B1, and subgroup B2, respectively. Average values for each of the 4 stocks from normalized data.

in the *spectrum* decreased while others tended to increase. The combined resulting variability determined changes of coffee aromas in the 10 days immediately following the roasting.

Acknowledgements

The Authors would like to thank Caffè Magnelli S.r.l. for providing coffee materials and relative information.

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