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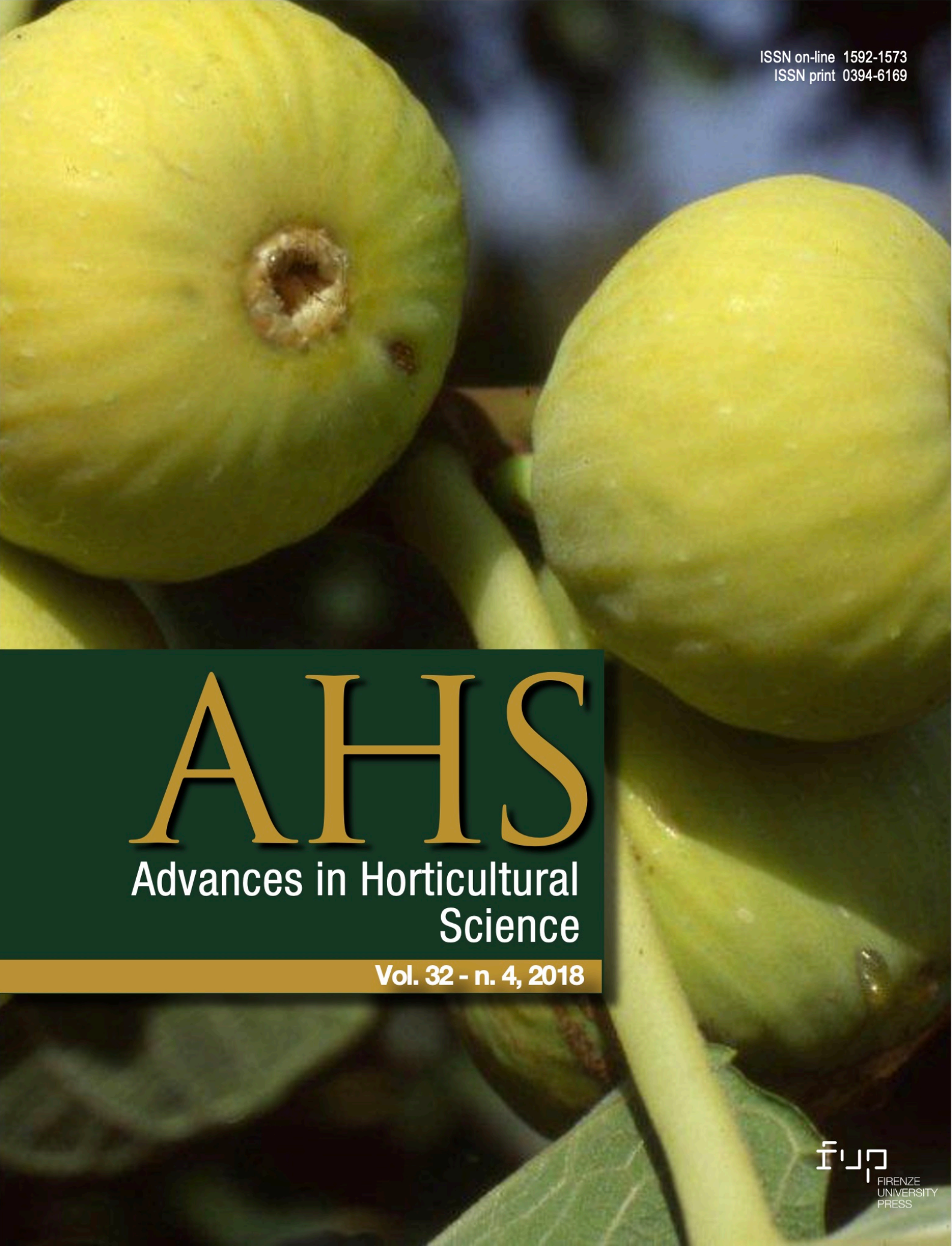

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SHORT NOTES

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Advances in Horticultural Science

Yield, fruit quality and physiological responses of melon cv. Khatooni under deficit irrigation

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Key words: antioxidant enzyme, irrigation, melon, proline, water use efficiency.

Abstract: To evaluate the effect of water deficit stress on growth, yield, fruit quality and physiological traits of melon cv. Khatooni, field experiments were conducted in split plot randomized complete block design with three replications. In 2014, irrigation treatments consisted of two deficit irrigation regimes, 33% and 66% of ETc (crop evapotranspiration), and 100% ETc as the control (DI_{33}, DI_{66} and I_{100}). In 2015, irrigation treatments applied were: 40, 70 and 100% ETc (DI_{40}, DI_{70} and I_{100}). The results showed that plant height and leaf area decreased from treatment I_{100} to DI_{40} and DI_{33}. The highest average fruit weigh and yield were obtained from irrigation 100% ETc for both years. The water use efficiency (WUE) significantly increased in response to increase water deficit stress. Deficit irrigation treatments significantly decreased leaf relative water content, vitamin C and fruit firmness, whereas antioxidant enzymes activity, proline and total soluble solid contents increased. These results suggest that the crop is sensitive to water deficits, that moderate water stress (DI_{70} and DI_{66}) reduced yield by about 28.5-38.2% and severe water stress (DI_{40} and DI_{33}) had a much more marked effect, reducing yield by 48.1-61.4%.

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The authors declare no competing interests.

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1. Introduction

Melon (*Cucumis melo* L.) is an important horticultural crop in Iran, generally cultivated in arid and semi-arid regions. Iran is the third largest melon-producing country in the world with more than 1476801 tonnes (FAO, 2014) of production. Melon plants are highly productive under adequate irrigation conditions; however water for irrigation is not always available at the time and amount needed by the crop, so water scarcity is a major constraint to horticultural production in arid and semiarid regions (Sharma *et al.,* 2014). Deficit irrigation regime, a practice that supplies water below evapotranspiration (ET) demands, can optimize water productivity when full irrigation is not possible (Fereres and Soriano, 2007). When water supply is limited, plant growth and yield is reduced and plant structure is modified by decreasing in leaf size (Kirnak *et al.,* 2002; Chaves *et al.,* 2003).

The effect of deficit irrigation on fruit yield and quality has been reported by numerous researchers with different results. In melon, deficit

irrigation reduced marketable fruit number and yield, average fruit weight, fruit diameter and did not affect rind thickness and seed cavity, but increased total soluble solids content (Sharma *et al.,* 2014). Although deficit irrigation reduce crop yield, may be able to save a significant amount of irrigation water (Sharma *et al.,* 2014). Fabeiro *et al.* (2002) stated that deficit irrigation during blooming stage affected mainly fruit yield, at setting stage both quantity and quality, and the deficit imposed at ripening stage affected sugar content.

Rouphael *et al.* (2008) indicated that water deficit significantly reduced yield, biomass production and leaf water status of mini-watermelon, but increase the water use efficiency.

The soluble solids concentration (SSC) is probably the most important quality parameter that is commonly evaluated by consumers (Cabello *et al.,* 2009). Water deficit studies in melon have been reported to increase (Sharma *et al.,* 2014), decrease (Long *et al.,* 2006), or had no effect (Hartz, 1997) on soluble solid content. Vitamin C content, as a secondary metabolite of plants, did not change with deficit irrigation in watermelons (75% ETc) (Leskovar *et al.,* 2004) and melons (50% ETc) (Sharma *et al.,* 2014).

Oxidative stress is one of the major causes of cellular damage in plants during stress (Miller *et al.,* 2010). However, plants can avoid the drought damage by promoting antioxidant enzymes activity, such as superoxide dismutase (SOD), peroxidases (POD), and catalase (CAT), to scavenge for free radicals and, or accumulate osmotic regulators such as soluble sugar, and proline may play a role in protection of cellular machinery against photo-oxidation by reactive oxygen species (ROS) that increase the drought resistance of plants under water stress (Foyer and Noctor, 2005; Veljovic-Jovanovic *et al.,* 2006).

Although the effects of water stress have been studied on growth and yield of different crops during the last years, recent information on the response of Iranian melon yield and quality to deficit irrigation remains limited, particularly about the results of restricted water distributions in arid and sub-arid environments. The main goal of this study was to evaluate the effect of controlled deficit irrigation on the physiological parameters and yield of the Khatooni melon cultivar.

2. Materials and Methods

Experimental site

Two field experiments were conducted during the growing season of 2014 and 2015 from June to September at Research farm of Agriculture faculty, University of Zanjan (Iran), to study the effect of water deficit on fruit yield and quality, antioxidant enzymes activities, water use efficiency (WUE), proline and vitamin C content. The soil texture was silty loam with 7.8 pH. Some soil characteristics and irrigation water chemical properties were showed in Table 1 and 2. The daily climate data during the growing seasons (2014 and 2015) was shown in Table 3.

Table 1 - Soil physical and chemical properties at the experiment site

pH	EC (dS m^{-1})	N (%)	Ca (g kg^{-1})	Na (g kg^{-1})	K (g kg^{-1})	OM (%)	Soil texture	Sand (%)	Silt (%)	Clay (%)
7.40	1.49	0.07	0.12	0.13	0.2	0.94	Silt loam	25	38	37

OM= Organic matter.

Table 2 - Irrigation water chemical properties at the experiment site

Bicarbonate (mg L^{-1})	Carbonate (mg L^{-1})	Cl (mg L^{-1})	Mg (mg L^{-1})	Ca (mg L^{-1})	K (mg L^{-1})	Na (mg L^{-1})	EC (dS m^{-1})	pH
195.2	0.0	582.2	103.7	258.45	0.0	50	2.35	6.5

Table 3 - Climatic parameters during the growing seasons

Climatic parameters	June		July		August		Sept	
	2014	2015	2014	2015	2014	2015	2014	2015
Minimum air temperature (°C)	7.60	12.90	10.70	18.53	13.10	16.14	6.80	12.58
Maximum air temperature (°C)	35.80	31.90	39.50	34.46	39.10	35.50	35.40	30.28
Rainfall (mm)	7.30	0.33	17.30	1.13	0.10	0.00	4.00	2.93
Relative humidity (%)	41.50	44.00	43.40	42.00	37.00	39.00	41.40	52.00

Plant materials and irrigation treatments

The experiment was done on a completely randomized block design whit three irrigation levels and three replications. 'Khatooni', yellow-green netted skin color and chimeric stripes, an Iranian melon from the Inodorous group widely cultivated in Iran, was selected for study. The seeds were sown on 1th July 2014 and 23th May 2015 at recommended spacing of 50 cm in row with 200 cm between rows. The irrigation system consisted of one drip line every crop row. Fertilizers was delivered as a pre-plant base comprising 80 kg N/ha, 50 kg p/ha and 80 kg K/ha. At a very early stage, plants were pruned (removing the apex of the main stem), and trained to have two lateral branches.

Three irrigation levels were calculated, based on actual evapotranspiration (ETc). In 2014, irrigation treatments were control or irrigation at 100% ETc (I_{100}), deficit irrigation at 66% ETc (DI_{66}) and at 33% ETc (DI_{33}) of control. According to 2014 results, when water deficit stress treatments strongly reduced fruit yield, in 2015 deficit irrigation treatments were changed, and the irrigation treatments were: 100% ETc (I_{100}), 70% ETc (DI_{70}) and 40% ETc (DI_{40}). Before starting the differential irrigation at five-leaf stage, all treatments were supplied with similar amount of water to maximize stands and uniform crop establishment. All other necessary operations such as pests and weeds control were performed according to recommended package of practices during the crop growth.

Measurements. Plant growth and leaf area

After 30 days of irrigation treatments, the average of leaf area was recorded whit leaf area measurement (DELTA-T Device Ltd, England). After fruit harvest, vine length of each plant was measured. For estimate leaf dry weight, at first fresh weight of leaf was measured; then they were dried in a hot-air oven for 2 days at 72°C, after which the dry weights (%) of leaf was recorded.

Yield and productivity components

The fruits were harvested when color changed from green to yellow and after the appearance of the netted pattern. Each melon fruit was weighed to determine mean fruit weight (FW). The fruit number per plant and fruit yield per plant was measured to determine of total yield, expressed in t ha^{-1}. Fruit yield was calculated by the mean fruit weight (kg), fruit number per plant and the density (20,000 plants/ha).

Fruit quality

Immediately after harvest, flesh ratio (FR), fruit firmness (ff), total soluble solid (TSS) and vitamin C (VC) were determined. The flesh ratios were calculated using the formulae:

$$FR\ (\%)=[(a+b)^2-(a'+b')^2/(a+b)^2]\times 100$$

where a is the fruit length, a' is the seed cavity length, b is the fruit diameter and b' is the seed cavity diameter.

From the liquid extract obtained by liquefying the mesocarp of each fruit, TSS content was determined by a handheld refractometer and expressed as °Brix. Fruit firmness was measured on the mesocarp tissue at three random locations per fruit using a digital penetrometer (Mc Cormic-FT 327) and recorded as kg cm^{-1}.

Proline content

Proline content in leaf tissue was determined according to the method of Bates *et al*. (1973). Mature leaves of plant were sampled 30 days after the onset of the deficit irrigation treatments. Proline was extracted from a sample of 0.5 g fresh leaves material samples in 3% (w/v) solution sulphosalycylic acid and estimated using the ninhydrin reagent. After reading the absorbance of fraction at a wave length of 520 nm, proline concentration was determined using a calibration curve and expressed as mg g^{-1} FW.

Catalase and peroxidase enzymes activity

Samples were taken from the fully expanded leaf and transferred to the laboratory in the ice. Leaf sample (0.5 g) was frozen in liquid nitrogen and ground using a porcelain mortar and pestle.

Catalase (CAT) activity was measured by following the decomposition of H_2O_2 at 240 nm with a UV spectrophotometer (Havir and McHale, 1987). Samples without H_2O_2 were used as blank. The activity of CAT was calculated by the differences obtained at OD_{240} values at 30 second interval for 2 min after the initial biochemical reaction. Peroxidase (POD) activity was measured using modified method of the Tuna *et al.* (2008) with guaiacol at 470 nm. A change of 0.01 units per minute in absorbance was considered to be equal to one unit POD activity, which was expressed as unit g^{-1} FW min^{-1}.

Leaf relative water content

The relative water content (RWC) in leaves was determined whit sampling fully expanded young leaves at noon according to Yamasaki and Dillenburg, (1999). Leaf relative water content was calculated

using the following formula:

$$RWC\ -(\%) = [(FW-DW)/(SW-DW)] \times 100$$

where FW stands for fresh weight, DW for dry weight, and SW for saturated weight.

Water use efficiency

Water use efficiency (WUE) was calculated for all treatments based on total crop yield and amount of water applied during growth period. WUE was estimated as the ratio of fruit yield (Y, kg ha^{-1}) and irrigation water applied (W, m^{-3}) (Stanhill, 1986). WUE=Y/W.

Statistical analysis

All data were analyzed statistically using a one-way ANOVA. Because of differences in the treatments, the data for each year were submitted to ANOVA separately. For data analysis, a completely randomized block design was used (3 Irrigation levels × 3 replications × 10 observations per experimental unit). Data were analyzed using the SAS statistical program (SAS Institute Inc., Cary, NC, USA), and means were compared by Duncan's multiple range tests at the 5% and 1% probability levels.

3. Results and Discussion

Plant growth

Leaf area, vine length and leaf dry weight (LDW) data of the treatments were presented in Table 4. Leaf area significantly decreased in the water deficit stress treatments in both years, reduction 20.38% (DI_{33}) and 30.4% (DI_{40}) in 2014 and 2015, respectively. In 2014, deficit irrigation stress had no effect on LDW. On the contrary, in 2015, LDW was affected significantly by the irrigation treatments, decreasing 22.05% in I_{100} treatment. Also, water deficit stress significantly reduced vine length in 2014, but no significant effect was observed by water deficit stress in 2015. These findings are similar the results obtained by Pew and Gardner (1983) and Ribas *et al.* (2001) who found that vegetative growth was higher under full irrigation instead of limited irrigation. Growth is an irreversible increase in volume, size, or weight, which includes the phases of cell division, cell elongation, and differentiation. A decrease in plant growth may be due to the limitation of cell division, cell enlargement caused by loss of turgor and inhibition of various growth metabolisms (Farooq *et al.,* 2012), and also decrease in photosynthesis (Huang *et al.,* 2011).

Yield, productivity components and water use efficiency

Fruit yield was affected significantly by the irrigation treatments in both years (Table 4). The highest value of fruit yield (40.37 and 43.43 t h^{-1}) was obtained in the irrigation 100% ETc in 2014 and 2015, respectively. Fruit number and fruit weight significantly reduced under deficit irrigation (Table 4).

The mean fruit number per plant was lower in 2014 (1.8, I_{100}) compared to 2015 (2.7, I_{100}). In contrast, fruit mean weight was higher in 2014 (2.18 kg) against 2015 (1.60 kg) that was obtained under irrigation 100% ETc. The lowest fruit number and fruit weight (1.25 kg) was observed respectively, with irrigation 33% ETc in 2014 and irrigation 40% ETc in 2015. This result agrees with the findings of Ribas *et al.* (2001), Cabello *et al.* (2009) and Sharma *et al.* (2014), who reported that limited irrigation reduced fruit yield of melon.

Table 4 - Effect of deficit irrigation on average leaf area (LA), vine length (VL), fruit weight (FW), number of fruits per vine (FN), yield (Y), and water use efficiency (WUE) in 2014 and 2015 seasons

Year	Irrigation (% ETc)	LA (cm^2)	LDW (%)	VL (cm)	FN	FW (kg)	Y (t ha^{-1})	WUE (kg m^{-3})
2014	100	151.53 a	16.24 a	185.6 a	1.8 a	2.18 a	40.37 a	14.14 ab
	66	130.11 b	16.45 a	133.3 ab	1.3 ab	1.91 ab	24.94 b	15.11 ab
	33	120.64 b	17.23 a	116.33 b	1.1 b	1.36 b	15.55 c	17.65 a
2015	100	183.74 a	16.4 b	148.33 a	2.7 a	1.60 a	43.43 a	14.24 b
	70	151.72 b	19.59 a	138.33 a	2.2 ab	1.42 ab	31.03 b	14.53 b
	40	127.88 b	21.04 a	115.5 a	1.8 b	1.25 b	22.50 c	18.45 a

I_{33}, I_{40}, I_{66}, I_{70} and I_{100} represent the irrigation treatments that received 33, 40, 66, 70 and 100% of ETc, respectively. Values are the average of 10 observation of each replication per irrigation level. Within each column, values followed by the same letters are not significantly different at $p<0.05$.

The reduction in fruit yield under deficit irrigation treatments compare to I_{100} treatment can be explained by the decrease in both mean fruit weight and numbers of fruits per vine (Table 4). Cabello *et al.* (2009) and Sharma *et al.* (2014) also reported the reduction in fruit number and fruit weight under deficit irrigation. Previous studies indicated that fruit weight in melon is more sensitive to water stress than fruit number (Long *et al.,* 2006; Dogan *et al.,* 2008).

Figure 1 presents the correlation between irrigation and fruit yield, fruit weight and fruit number per vine. Correlation between irrigation and fruit yield (R^2= 0.93) was stronger than the correlation with fruit weight (R^2= 0.58) and fruit number per vine (R^2= 0.51) which indicates that the reduction in fruit yield with deficit irrigation was attributed to the significant decrease in average fruit weight and fruit number per vine (Fig. 1 b and c).

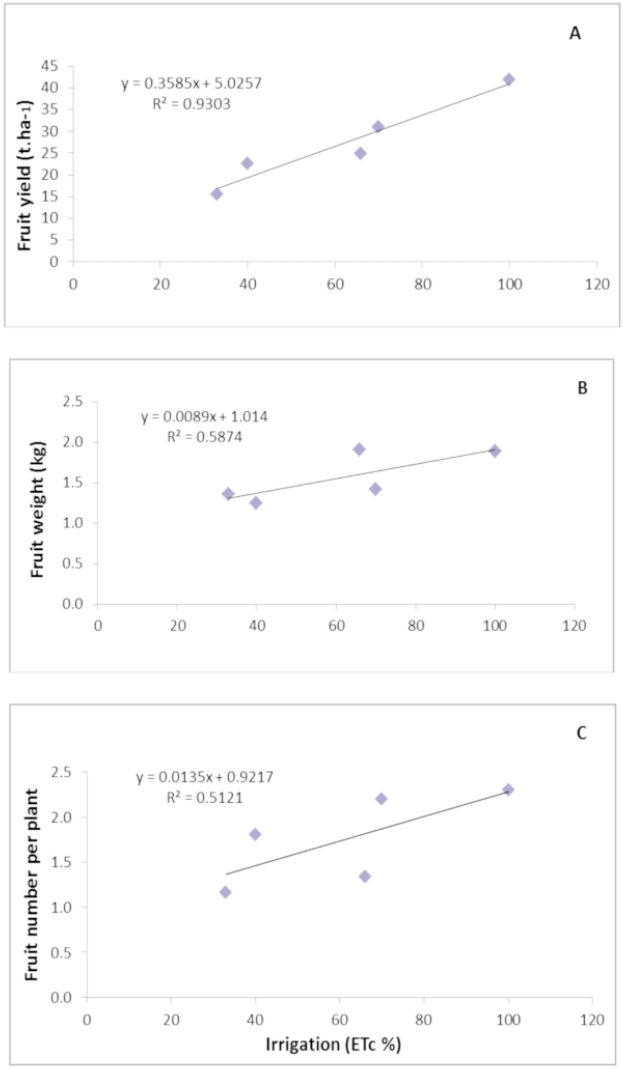

Fig. 1 - Relationship between irrigation by fruit yield (a), fruit weight (b) and fruit number per plant (c) in 2014 and 2015. Values are the mean of 3 replications/10 observations each irrigation level, in two years.

WUE is the relation between yield and the quantity of irrigation water (Zeng *et al.,* 2009). In both years, WUE was lowest for irrigation 100% ETc. Overall; deficit irrigation resulted in 19.88% and 22.81% WUE increased in DI_{33} and DI_{40}, respectively (Table 4). WUE had negative correlation (R^2 = 0.64) with irrigation water amount (Fig. 2). Higher WUE has also been achieved in watermelon (Leskovar *et al.,* 2004), muskmelon (Kirnak *et al.,* 2005; Zeng *et al.,* 2009), Mission and Da Vinci melon cultivars (Sharma *et al.,* 2014) in response to deficit irrigation.

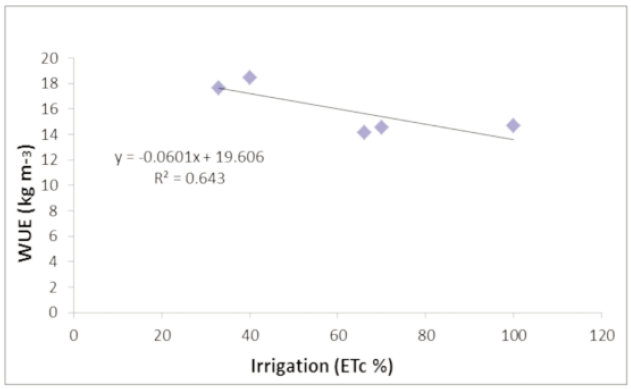

Fig. 2 - Relationship between irrigation by water use efficiency (WUE) in 2014 and 2015. Values are the mean of 3 replications/10 observations each irrigation level, in two years.

Fruit quality

Fruit quality as indicated with fruit firmness, flesh ratio, total soluble solid (TSS) and vitamin C was presented in Table 5. In both years, fruit firmness decreased as the irrigation was restricted. The lowest fruit firmness was 1.49 kg cm^{-1} under irrigation 33% ETc, although there was no significant difference

Table 5 - Effect of deficit irrigation on fruit firmness (FF), flesh ratio (FR), total soluble solid (TSS) and vitamin C (VC) in 2014 and 2015 seasons

Year	Irrigation (% ETc)	FF (kg cm^{-1})	FR (%)	TSS (°Brix)	VC (mg 100 ml^{-1})
2014	100	2.38 a	49.55 a	10.06 b	10.002 a
	66	1.79 ab	49.53 a	11 ab	8.082 b
	33	1.49 b	48.29 a	12.06 a	6.98 c
2015	100	3.15 a	54.07 a	9.03 b	10.68 a
	70	3.00 a	49.04 ab	10.7 ab	9.21 b
	40	2.1 b	45.77 b	11.76 a	7.88 c

I_{33}, $I4_{40}$, I_{66}, I_{70} and I_{100} represent the irrigation treatments that received 33, 40, 66, 70 and 100% of ETc, respectively. Values are the average of 10 observation of each replication per irrigation level. Within each column, values followed by the same letters are not significantly different at p<0.05.

between I_{100} whit DI_{66} and DI_{70} in 2014 and 2015, respectively. These results was agreement with Cabello *et al.* (2009) in melon, who reported that increasing irrigation water improved flesh firmness, but obtained a reduction in flesh firmness when irrigation water increased in following year. Also, Sharma *et al.* (2014) did not obtain a significant difference of irrigation treatments on fruit firmness with approximately positive effect of optimal irrigation.

The flesh ratio was unaffected by the irrigation rates in 2014 season. However, flesh ratio varied significantly in 2015. The largest flesh ratio (54.07%) was obtained under irrigation 100% ETc in 2015. These results are in agreement with the results of Dogan *et al.* (2008) in melon. The results indicated that optimal irrigation water could increase flesh thickness while water stress has a negative effect on it. It is not in accordance with Ribas *et al.* (2003) who reported that the flesh and skin ratios are not usually affected by the irrigation levels.

TSS is a very important index of quality in melon fruits (Zeng *et al.,* 2009). In both years, larger amounts of irrigation water resulted in lower TSS. The highest TSS was recorded with 12.06 and 11.76 °Brix in irrigation 33 and 40% ETc, respectively. The similar results were also observed by some other researchers (Lester *et al.,* 1994; Fabeiro *et al.,* 2002). Dogan *et al.* (2008) showed that fruit sugar content affected positively by water stress. Furthermore, other studies have shown that in muskmelon, TSS decreased with the decrease in irrigation water levels (Long *et al.,* 2006; Zeng *et al.,* 2009; Li *et al.,* 2012). Gonzalez *et al.* (2009) found no significant differences for watermelon fruit soluble solids between well-watered and regulated deficit irrigation treatments, although it was 9.5% higher, for the regulated deficit irrigation treatment.

Deficit irrigation markedly (P<0.05) reduced vitamin C content. The highest value of vitamin C was found in treatments I_{100} in both years (Table 5), which high decrease value (30.21%) was recorded in irrigation 33% ETc. The results indicated that vitamin C content was highly sensitive to deficit irrigation. Our results are agreement with Li *et al.* (2012) and Wang *et al.* (2017) who showed that severe water deficit stress reduced significantly the fruit vitamin C content, but these results differ from the findings of Cui *et al.* (2008) who stated that water deficit during the fruit growth and maturation stages increased significantly vitamin C content.

Proline accumulation

The exposure to water deficit stress significantly (*P*<0.05) increased proline content (Table 6). The maximum value of proline content was 1.97 and 1.8 mg g^{-1} FW under irrigation 33 and 40% ETc, respectively. Accumulation of proline plays an important role in plants to adaptive on environmental stresses, particularly low water stress (Kavas *et al.*, 2013). The proline that accumulated in the leaves under water-limited environment is a cellular regulator that helping to sustain the activity of the cell and tissue in water deficit condition by preventing injuries in the internal apparatus of cell (Ahmed *et al.,* 2009).

Catalase and peroxidase enzymes activity

Significant differences among treatments were observed for CAT enzyme activity (Table 6). CAT activity was the highest (7.47 and 6.97 µmol H_2O_2 g^{-1} FW min^{-1}) with DI_{33} and DI_{40} treatments. Similar to CAT, the POD activity in both seasons increased in response to an increase in water deficit stress (Table 6), which high POD activity was found by irrigation 33% ETc in 2014.

In present study, the antioxidant enzyme activates increased with the decrease of irrigation water

Table 6 - Effect of deficit irrigation on proline, catalase enzyme activity (CAT), peroxidase enzyme activity (POD) and relative water content (RWC)

Year	Irrigation (% ETc)	Proline (mg g^{-1}FW)	CAT (µmol H_2O_2 g^{-1} FW min^{-1})	POD (unit g^{-1} FW min^{-1})	RWC (%)
2014	100	0.77 b	4.52 b	0.422 b	78.63 a
	66	1.5 a	5.62 b	0.5 b	67.45 ab
	33	1.97 a	7.47 a	0.789 a	55.19 b
2015	100	0.97 c	4.4 b	0.356 b	73.13 a
	70	1.302 b	5.19 b	0.486 a	64.26 ab
	40	1.808 a	6.97 a	0.511 a	58.74 b

I_{33}, $I4_{40}$, I_{66}, I_{70} and I_{100} represent the irrigation treatments that received 33, 40, 66, 70 and 100% of ETc, respectively. Values are the average of 10 observation of each replication per irrigation level. Within each column, values followed by the same letters are not significantly different at p<0.05.

applied. As found by Kavas *et al.* (2013) in melon and Huseynova (2012) in wheat, the antioxidant activity of CAT significantly increased by drought stress. Antioxidative enzymes like POD and CAT play a major role in conferring drought tolerance and CTA and POD activity of drought tolerance genotypes were higher than sensitive genotypes under drought stress (Hameed *et al.,* 2013).

Relative water content

As applied irrigation water decreased, the relative water content of leaf decreased (Table 4). The results showed that different irrigation treatments had similar effects on RWC in both seasons. The highest value of RWC was recorded in irrigation 100% ETc. The decrease in RWC being respectively, 29.8 and 19.67% for DI_{33} and DI_{40} compared to I_{100}. RWC decreased linearly in response to an increase in water deficit stress in melon (Kavas *et al.,* 2013), watermelon (Kirnak and Dogan, 2009) and mini-watermelon (Rouphael *et al.,* 2008). The results indicated that the RWC was improved by the increasing irrigation water. Kirnak and Dogan (2009) stated the higher leaf relative water content values are generally indication of enough soil water in root zone.

4. Conclusions

Water deficit has been shown to adversely affect leaf area, yield, and leaf water status of melon, but led to increase the WUE and TSS. Since the water scarcity is a key factor for plant production under arid and semi-arid regions, thus achieving great values of WUE is more reasonable than maximum yield. WUE in DI40 and DI33 was greater than full irrigation treatment. Irrigation water increased yields not only by increasing the mean weight of the fruits, but also by increasing fruit number per vine. In both years, the physiological parameters showed significant differences. Results indicated that the change of CAT and POD activity and proline accumulation cooperated with water deficit; indeed CAT and POD activities and proline content (60.1% and 46% in DI33 and DI40, respectively) increased with enhancement of drought intensity, and in stressed plants were significantly higher than full irrigated plants. The results suggested that antioxidant enzyme activities (CAT and POD) as well as proline accumulation may play an important role in protecting 'Khatooni' melon plants against drought stress.

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Combining ability and gene action of some tomato genotypes under low light condition

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Abstract: Limitations in access to electricity in rural areas and substantial cost of supplemental lightning necessitate breeding as response to low light conditions. Seven inbred lines of tomato (*Solanum lycopersicum* L.) and their F1 hybrids, including reciprocals, developed through a 7×7 full diallel cross were evaluated under two different levels of light. Mean square for light (L) effect was significant for total yield, average fruit weight and days to first flower. Variation attributable to Genotypes and genotype × light (G×L) interaction had significant effect on all studied traits except days to ripening for which G×L interaction was not significant. Diallel analysis across two environments indicated that general (GCA), specific (SCA) and reciprocal combining ability (REC) were significant for all characters implying importance of additive and non-additive gene action along with cytoplasmic effects on genetic expression of yield, yield components and earliness. Ratio of SCA variance to SCA variance and estimates of narrow sense heritability ($h^2_{n.s}$) demonstrated higher weight of additive effects in inheritance of yield, fruit number and days to ripening, while indicating predominance of non-additive effects for fruit weight and early flowering. Interactions GCA×L and SCA×L were significant for almost all studied features. A particular genotype could not be recommended for all traits, but variation among genotypes in response to ambient light was promising for feasibility of plant breeding for non-optimal light intensity and duration.

1. Introduction

Tomato (*Solanum lycopersicum* L.), a member of solanaceae family, is a wide cultivated vegetable used for fresh and processing market. This day neutral plant is grown in various regions of the world with different climates (Mizoguchi *et al*., 2007; Gerszberg *et al.*, 2015). Iran is among the top 10 countries producing tomato (FAOSTAT, 2014), but it performs very poorly in terms of tomato seed production and a large portion of the seeds, particularly hybrid cultivars, is imported to the country. The major obstacle to seed production in Iran is the absence of breeding programs

for most of the vegetables including tomato; therefore, more attention to vegetables breeding in order to produce high quality seeds is required.

Hybrid breeding technique is one of the most important methods used for crop improvement. The information needed to develop proper F_1 hybrid cultivars via hybrid breeding could be achieved through different methods including diallel analysis, a method to analyze crosses made among (n) lines in all possible combinations (Griffing, 1956 a, b). The analysis is mainly adopted when dealing with limited number of parental lines and determines genetic parameters such as heterosis, general combining ability (GCA), specific combining ability (SCA), heritability, and nature of gene action. Heterosis demonstrates the superiority of F1 progenies compared to the average of their parents. General combining ability (GCA) shows the average performance of a parental line while specific combining ability (SCA) refers to the best combination of crosses. General combining ability (GCA) and specific combining ability (SCA) are the indicators of additive and non-additive gene effects, respectively. These parameters help plant breeders in the selection of suitable parental lines and appropriate breeding method (Sprague and Tatum, 1942).

It should be noted that despite of the advantages of hybrid cultivars over open pollinated ones, the high price of hybrid seeds developed via hybridization programs makes the use of them more economical for intensive and indoor cultivation (Zengin *et al.*, 2015). One of the major problems affecting plants growth in greenhouses is the decreased level of light received by plants due to significant loss of solar radiation caused by reflection and absorption by greenhouse covering material (Baeza and López, 2012) and high plant density, typically executed in greenhouse cultivations (Laurent *et al.*, 2017). Since light is one of the most crucial factors influencing growth rate, production quality and quantity of plant, supplying plants with adequate light intensity with suitable quality is of importance in greenhouses particularly during autumn and winter seasons (Hangarter, 1997).

In developing countries such as Iran, most of the greenhouses do not benefit from high technology and suffer from lack of accessibility to electricity, hence; supplemental lightning is not applied. Moreover, substantial price of energy resources and tendency toward lower energy consumption (Oz and Atilgan, 2015) necessitate hybrid breeding for low light conditions. To our knowledge breeding for low light condition has not been conducted in tomato, therefore, this study aimed to investigate if there existed any differences among various tomato genotypes in response to two different light conditions while considering the introduction of suitable parental lines and hybrids for each condition as well as determination of stable genotypes over two environments. Genetic parameters including combining ability, gene action and heritability were estimated to help breeders in choosing the best approach for the improvement of tomatoes regarding increased yield and earliness.

2. Materials and Methods

Plant material

Seven inbred lines of tomato consisted of 'Perimoga', 'La1793', 'AC06', 'CT6', 'MC3', 'C20' and 'Kingstone' (Table 1) were cultivated in a research greenhouse of Ferdowsi University of Mashhad, Mashhad, Iran under optimum conditions. The lines were crossed in all possible two-way combinations (full diallel cross system) to develop F1 hybrids with their reciprocals.

Experimental design

The experiment was performed in research greenhouse of Ferdowsi University of Mashhad with computerized temperature control system. A year round was divided into two growing seasons: the first grow-

Table 1 - Description of parental inbred lines crossed in 7×7 full diallel cross system

Plant material	Abbreviation	Origin	Growth habit	Leaf type	Fruti shape
Perimoga	P1	Russia	determinate	vulgare	oval
La1793	P2	USA	indeterminate	vulgare	round
AC06	P3	Iran	indeterminate	vulgare	round
CT6	P4	Russia	semi-indeterminate	grandifolium	oval
MC3	P5	Russia	indeterminate	vulgare	round
C20	P6	Russia	indeterminate	vulgare	oval
Kingstone	P7	Italy	semi-indeterminate	vulgare	oval

ing season was from March to August including warm seasons with high light intensity and long photoperiod (more sunny hours a day) and the second season covering cold seasons with low light intensity and short photoperiod (less sunny hours per day) started in September and ended up in January. To investigate the performance of tomato plants under two different light conditions, 21 F1 progenies together with reciprocals (42 F1 hybrid progenies) developed via a 7×7 full diallel cross system were cultivated during each of the two aforementioned growing seasons. Daily average of light intensity of experimental greenhouse during each month is represented in Table 2. The experiment was conducted in a completely randomized design with three replications per genotype in two different time span mentioned above (split plot).

Measurement of characters

The observations concerning the following characteristics were recorded as described below.

Total yield per plant (Kg)

It was calculated by summing up the weight of fruits obtained from all pickings during 8 weeks from each plant.

Average fruit weight (g)

The average fruit weight was an index of fruit size. All fruits collected from each harvest were weighted and the total weight of the fruits was divided into the number of the weighted fruits.

Number of fruits per plant

Harvested fruits of each plant in a period of 8 weeks were counted.

Days to first flower

The number of days from seeding until the formation of first flower on the plants was recorded.

Days to ripening

The number of days from flower anthesis to fruit ripening stage was determined through the date tagging of five flowers per plant at the time of anthesis. Fruit ripening was considered the time when genetically red fruits turned pink and yellow ones turned yellow (Garg *et al.*, 2008).

Statistical analysis

Obtained data in each environment were analyzed using Excel software (Microsoft office 2010) using Griffing's (1956, *a*, *b*) Method 3, Model 1 (fixed effects) formula. Combined analysis of data over two seasons was performed based on modified Method 3, Model 1 for several environments proposed by Singh (1973) as described below:

Where Y_{ijk} = observation of trait value of parents *i*

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + r_{ij} + l_k + (gl)_{ik} + (gl)_{jk} + (sl)_{ijk} + (rl)_{ijk} + e_{ijk}$$

and *j* in year *k*; μ = population mean; g_i / g_j = GCA effect of parent *i* / *j*; s_{ji} / s_{ji} = SCA effect of the hybrid developed from parent *i* × parent *j* / parent *i* × parent *j*; r_{ij} = REC effect of the hybrid produced by parent *i* × parent *j*; l_k=effect of environment *k*; $(gl)_{ik}$ / $(gl)_{jk}$ = interaction between GCA effect of parent *i* / *j* with environment *k*; $(sl)_{ijk}$ = interaction between SCA effect of cross *ij* with environment *k*; and e_{ijk} = error of observation *ijk*.

Broad-sense heritability ($H^2_{b.s}$) and narrow-sense heritability ($h^2_{b.s}$) over environments were estimated using following formula (Sharifi *et al.*, 2010):

$$H^2_{b.s}\% = \frac{2\sigma_g^2 + \sigma_s^2}{2\sigma_g^2 + \sigma_s^2 + (2\sigma_{gl}^2 / L) + (\sigma_{sl}^2 / L) + (\sigma_e^2 / RL)} \times 100$$

$$h^2_{b.s}\% = \frac{2\sigma_g^2}{2\sigma_g^2 + \sigma_s^2 + (2\sigma_{gl}^2 / L) + (\sigma_{sl}^2 / L) + (\sigma_e^2 / RL)} \times 100$$

Where L indicated light condition; σ^2g, σ^2s and σ^2r stand for variance components of GCA, SCA and REC, respectively; σ^2gl, σ^2sl and σ^2rl represent variance components of GCA×L, SCA×L and REC×L, respectively.

$$\text{Standard heterosis \%} = \frac{F_1 - \bar{Y}_{ij}}{\bar{Y}_{ij}} \times 100$$

Where F_1 and Y_{ij} are the mean performances of hybrids and parents, respectively.

Table 2 - Average of daily light intensity for each months (foot candle intensity/24h)

Average light intensity											
First growing season with high light intensity and duration						Second growing season with low light intensity and duration					
Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
680.7	694	750.5	863.3	893	760	650.7	547.3	550	527	535.5	655.7

The average of light intensity per day was calculated based on 24 hours a day including night hours (n= 24, one measurements every hour).

3. Results

ANOVA analysis

The variance analysis of genotypes for each season separately showed that genotypes were highly significant (Table 3); therefore, the compound analysis of variance over two environments was conducted (Table 4). Light condition was considered as main plot; therefore, error A refers to whole plot error. Some genotypes, namely 42 F1 hybrids grown under each light treatment along with the interaction between genotypes and environments, were the sub plot of the experiment and error B represent whole plot error. Compound ANOVA analysis for two seasons represented in Table 4 showed that light (L) effect was highly significant for total yield per plant, average fruit weight and days number to first flower but not significant for fruit number per plant and days number for fruit ripening. Genotypes and the interaction of genotypes with light (G×L) were highly significant for all studied traits except for days to ripening in which genotypes did not show any interaction with environment.

Estimation of genetic parameters

Compound analysis of variance for general combining ability (GCA), specific combining ability (SCA) and reciprocal combining ability (REC) over two seasons for yield, yield components and earliness varied (Table 4). The results indicated that variances due to

Table 3 - Mean squares of ANOVA analysis over two light conditions for yield, yield components and earliness of F1 hybrids with their reciprocals developed via a 7×7 full diallel cross

Sources of variation	Degree of Freedom	Total yield per plant (Kg)	Average of fruit weight (g)	Fruit number per plant	Days to first flower	Days to ripening
Light (L)	1	16.69 **	14654.49 **	913.52	359.53 **	5.43
error A	4	0.74	261.6	140.91	0.61	8.8
Genotypes (G)	41	2.05 **	1944.73 **	1593.50 **	34.74 **	23.38 **
G×L	41	0.17 **	146.54 **	96.24 **	2.50 **	3.99
error B	164	0.07	34.01	18.33	0.97	2.81

** Significant at P<0.01 level.

Table 4 - Compound analysis of variance for combining ability over two environments for yield, yield components and earliness of F1 hybrids with their reciprocals developed via a 7×7 full diallel cross and estimates of heritability in broad and narrow senses as well as heterosis

Sources of variation	Degree of Freedom	Total yield per plant (Kg)	Average of fruit weight (g)	Fruits number per plant	Days to first flower	Days to ripening
GCA	6	1.28 **	3343.91 **	2063.32 **	54.66 **	23.20 **
SCA	14	1.35 **	430.82 **	644.60 **	9.55 **	8.68 **
REC	21	0.07 **	23.00 **	17.79 **	0.62 *	2.80 **
GCA×L	6	0.14 **	120.47 **	70.33 **	2.86 **	1.95
SCA×L	14	0.06 **	53.49 **	42.07 **	0.94 **	1.68 *
REC×L	21	0.03	25.29 *	14.49 **	0.19	0.92
error	164	0.02	11.34	6.11	0.32	0.94
Variance components						
$\sigma^2 g$	-	0.06	166.63	102.86	2.72	1.11
$\sigma^2 s$	-	0.33	104.87	159.62	2.31	1.94
$\sigma^2 r$	-	0.01	2.92	2.92	0.07	0.47
$\sigma^2 g/ \sigma^2 s$	-	0.19	1.59	0.64	1.18	0.57
$\sigma^2 gl$	-	0.01	10.91	6.42	0.25	0.1
$\sigma^2 sl$	-	0.02	21.08	17.98	0.31	0.37
$\sigma^2 rl$	-	0	6.98	4.19	-0.07	-0.01
H^2 broad-sense (%)	-	94.77	94.94	95.7	94.38	90.38
h^2 narrow-sense (%)	-	26.02	72.22	53.89	66.25	48.34
Heterosis (%)		37.75	-22.95	55.04	3.05	-6.38

* Significant at P<0.05 level, ** Significant at P<0.01 level.

GCA, SCA and REC were highly significant for all studied characters. The significance of both GCA and SCA variances implies that all studied characters are controlled by either of additive or non-additive gene action. The effect of interaction GCA×L and SCA×L on all evaluated features were significant but fruit ripening period, which showed non-significant variance of GCA×L. The interaction of REC×L showed remarkable effect only on average fruit weight and fruits number per plant. For total yield, fruits number and days to ripening, the GCA (σ^2g) was less than variance component of SCA (σ^2s) in a way that σ^2g/σ^2s was less than unity, demonstrating the predominance of non-additive gene action in controlling mentioned traits (Baker, 1978). The ratio of σ^2g/σ^2s for fruit weight and days to first flower was higher than unity, illustrating the more important role of additive gene action for inheritance of these attributes.

Estimates of broad-sense heritability percentage (H^2 %) over environments was high for all studied traits ranged from 90 to 95% (Table 4). Narrow-sense heritability percentage (h^2 %) was low for total yield, fruits number and days to ripening while relatively high for fruit weight and days to flowering. Total heterosis percentage was high for yield and fruit number while negative for fruit weight. Unlike yield and yield components, in earliness characters negative values show superiority but heterosis for early flowering was positive. Negative but low heterosis was recorded for early maturity.

Estimation of mean values

Mean values of F1 hybrids for plant yield showed that hybrid 'CT6×MC3' (P4×P5) had the highest rate under both light conditions, while lowest amount was for hybrid 'C20×MC3' (P6×P5) (Table 5). 'Perimoga×Kingstone' (P1×P7) cross and its reciprocal produced heaviest fruits over two environments. Lightest fruits produced under normal light were for 'MC3×La1793' (P5×P2), but under low light, the fruits of hybrid 'MC3×AC06' (P5×P3) had the least weight. The Highest number of fruits per plant was produced by hybrid 'La1793×C20' (P2×P6) and the lowest was for 'Kingstone×Perimoga' (P7×P1). Hybrid 'Perimoga×CT6' (P1×P4) commenced flowering earlier than other progenies. Latest flowering under adequate light intensity was for 'MC3×C20' (P5×P6), while under low light, it was for hybrid 'La1793×AC06' (P2×P3). Overall, according to pooled data over two seasons, hybrid 'C20×MC3' (P6×P5) took more days to flower compared with other progenies. Longest fruit ripening duration was for hybrid 'MC3×LA1793' (P5×P2) and shortest period for ripening was observed in the cross of 'ACO6×Kingstone' (P3×P7).

Estimation of GCA, SCA and REC effect

For yield and yield components, positive values of GCA, SCA and REC indicate the superiority of genotypes while for earliness characters negative values are desired (Table 6). Most of the parental lines were not stable during two growing seasons and superior parents for each character differed with environmental changes. Parental line of 'CT6' (P4) was the best combiner for achieving higher yield in both seasons, and the lowest GCA in low light and high light condition was for 'Kingstone' (P7) and 'La1793' (P2), respectively. In total 'Kingstone' (P7) was the weakest genitor for yield across two environments. For fruit weight, although 'Perimoga' (P1) had the highest GCA in warm season, parental line 'Kingstone' (P7) acted as the best combiner in both seasons. The best donors for increased fruits number during sunny and cloudy seasons were 'La1793' (P2) and 'MC3' (P5), respectively and 'La1793' (P2) had the best GCA over both seasons. For days to first flower, parental genotype 'Perimoga' (P1) possessed the highest negative GCA and 'MC3' (P5) had the highest positive and significant GCA under each ambient light and over both of them. The best combiner concerning days to ripening in both tested environments was 'La1793' (P2) and the weakest performance was for 'Kingstone' (P7).

In yield and related trait, a positive value of SCA is repetitive of a successful cross between parental lines of that hybrid, while a negative value demonstrates that parental lines did not make up a good couple. For earliness, negative values of SCA are indicative of prosperity .Estimation of SCA for each evironment and over two environments indicated that the best combination for total yield was 'La1793×C20' (P2×P6) and the worst was for 'MC3×C20' (P5×P6) (Table 7). For fruit weight, Hybrid 'AC06×Kingstone' (P3×P7) had the highest SCA during sunny seasons, and the hybrid developed from 'Perimoga×Kingstone' (P1×P7) had the highest magnitude during cloudy seasons and over two seasons. The highest and lowest SCA estimations for fruits number were similar to total yield. The cross between 'MC3×C20' (P4×P6) was the most successful cross for decreased days to flowering during sunny months. 'La1793×MC3' (P2×P5) not only had the highest negative SCA during cold months, but also possessed the best SCA in total. For this trait,

Table 5 - Means values and standard deviation for yield components and earliness of F1 hybrids developed via 7×7 diallel cross over two light conditions (part A)

Genotypes ♀ × ♂	Total yield per plant (Kg)			Average of fruit weight (g)			Fruits number per plant		
	L1	L2	Pooled	L1	L2	Pooled	L1	L2	Pooled
P1×P2	2.72(±0.28)	1.48(±0.44)	2.10(±0.36)	70.13(±8.70)	45.90(±9.34)	58.02(±8.83)	38.93(±3.02)	31.90(±3.92)	35.42(±3.07)
P1×P3	3.54(±0.36)	2.87(±0.48)	3.20(±0.42)	85.63(±10.48)	55.17(±8.89)	70.40(±8.53)	41.33(±1.64)	52.27(±7.01)	46.80(±2.69)
P1×P4	3.81(±0.14)	3.50(±0.06)	3.65(±0.10)	88.53(±2.05)	56.47(±4.97)	72.50(±1.63)	43.00(±2.55)	62.20(±5.20)	52.60(±1.96)
P1×P5	3.61(±1.02)	3.18(±0.20)	3.40(±0.60)	84.73(±7.22)	57.33(±3.14)	71.03(±2.79)	42.10(±8.98)	55.70(±6.58)	48.90(±7.65)
P1×P6	2.24(±0.19)	2.23(±0.36)	2.24(±0.26)	40.60(±4.06)	43.80(±4.88)	42.20(±2.08)	55.27(±2.15)	51.87(±13.90)	53.57(±5.97)
P1×P7	2.47(±0.36)	2.13(±0.33)	2.30(±0.34)	114.20(±14.97)	96.80(±10.83)	105.50(±8.28)	21.80(±3.97)	21.87(±2.20)	21.83(±2.05)
P2×P1	2.29(±0.18)	1.58(±0.22)	1.94(±0.20)	64.33(±16.06)	42.17(±2.35)	53.25(±7.55)	36.60(±5.97)	37.50(±6.41)	37.05(±0.52)
P2×P3	2.69(±0.17)	1.60(±0.04)	2.14(±0.08)	58.53(±7.25)	33.77(±2.03)	46.15(±2.64)	46.20(±3.10)	47.27(±2.75)	46.73(±1.05)
P2×P4	3.27(±0.31)	2.77(±0.23)	3.02(±0.27)	46.13(±2.06)	39.83(±4.11)	42.98(±2.72)	70.70(±5.05)	70.07(±9.36)	70.38(±7.17)
P2×P5	2.81(±0.25)	2.55(±0.20)	2.68(±0.19)	36.90(±3.33)	35.03(±2.84)	35.97(±1.21)	76.10(±4.58)	72.67(±3.25)	74.38(±3.01)
P2×P6	4.20(±0.33)	2.77(±0.31)	3.49(±0.32)	44.63(±1.65)	32.63(±3.39)	38.63(±2.32)	94.03(±5.54)	84.80(±3.99)	89.42(±4.13)
P2×P7	3.24(±0.33)	2.74(±0.33)	2.99(±0.33)	51.80(±4.54)	56.80(±4.54)	54.30(±4.54)	62.57(±1.72)	48.17(±2.29)	55.37(±1.94)
P3×P1	3.33(±0.31)	2.42(±0.31)	2.87(±0.31)	76.60(±8.52)	54.03(±11.95)	65.32(±10.03)	43.50(±3.21)	45.27(±4.35)	44.38(±2.98)
P3×P2	3.14(±0.30)	1.98(±0.25)	2.56(±0.19)	61.00(±2.11)	37.07(±3.62)	49.03(±1.37)	51.33(±3.18)	53.17(±2.90)	52.25(±2.83)
P3×P4	3.34(±0.24)	2.62(±0.19)	2.98(±0.21)	68.20(±8.89)	54.23(±7.73)	61.22(±8.29)	49.17(±3.73)	48.57(±3.27)	48.87(±3.48)
P3×P5	2.97(±0.25)	2.44(±0.30)	2.70(±0.27)	40.07(±1.75)	33.47(±4.46)	36.77(±3.07)	73.97(±3.10)	72.83(±1.05)	73.40(±1.15)
P3×P6	2.81(±0.24)	2.48(±0.12)	2.65(±0.18)	61.50(±1.71)	37.90(±3.73)	49.70(±2.66)	45.63(±3.85)	65.67(±6.37)	55.65(±4.15)
P3×P7	3.03(±0.18)	2.50(±0.20)	2.77(±0.19)	99.03(±5.56)	78.47(±2.90)	88.75(±1.66)	30.63(±0.59)	31.93(±3.61)	31.28(±1.89)
P4×P1	4.27(±0.34)	3.69(±0.15)	3.98(±0.16)	83.03(±12.08)	64.67(±4.61)	73.85(±3.83)	51.77(±3.88)	57.20(±3.72)	54.48(±0.33)
P4×P2	2.86(±0.2)	2.66(±0.20)	2.76(±0.09)	47.97(±4.71)	38.27(±3.84)	43.12(±1.66)	59.60(±1.73)	69.50(±1.85)	64.55(±0.15)
P4×P3	3.18(±0.36)	3.01(±0.22)	3.09(±0.29)	66.53(±1.46)	54.90(±6.05)	60.72(±3.02)	47.80(±6.17)	54.83(±1.93)	51.32(±2.63)
P4×P5	4.57(±0.26)	3.80(±0.34)	4.19(±0.27)	63.20(±4.30)	47.50(±3.76)	55.35(±1.30)	72.40(±3.41)	80.00(±5.73)	76.20(±3.21)
P4×P6	3.32(±0.32)	3.14(±0.25)	3.23(±0.24)	63.83(±3.40)	50.20(±7.27)	57.02(±3.95)	52.27(±7.31)	62.77(±4.40)	57.52(±2.45)
P4×P7	3.03(±0.31)	2.75(±0.17)	2.89(±0.21)	87.20(±11.27)	62.80(±3.24)	75.00(±5.56)	34.90(±2.80)	43.77(±0.51)	39.33(±1.57)
P5×P1	3.37(±0.31)	2.97(±0.35)	3.17(±0.58)	76.83(±8.81)	47.27(±5.53)	62.05(±1.91)	44.03(±3.81)	62.77(±2.74)	53.40(±3.14)
P5×P2	2.52(±0.40)	2.35(±0.27)	2.44(±0.58)	30.27(±4.99)	29.53(±2.17)	29.90(±1.41)	83.33(±3.32)	79.47(±7.33)	81.40(±5.24)
P5×P3	3.29(±0.21)	2.10(±0.22)	2.69(±1.53)	44.13(±4.23)	26.87(±2.87)	35.50(±0.79)	75.00(±9.37)	77.90(±3.30)	76.45(±3.36)
P5×P4	4.00(±0.20)	3.60(±0.15)	3.80(±2.08)	56.17(±4.99)	45.23(±1.63)	50.70(±2.92)	71.53(±8.22)	79.47(±0.49)	75.50(±4.29)
P5×P6	1.87(±0.23)	1.51(±0.18)	1.69(±0.58)	42.17(±4.32)	37.67(±2.97)	39.92(±1.66)	44.33(±1.12)	40.10(±6.67)	42.22(±3.82)
P5×P7	2.34(±0.25)	1.97(±0.35)	2.16(±0.58)	54.40(±3.10)	32.77(±3.08)	43.58(±3.09)	42.87(±2.34)	59.70(±5.28)	51.28(±3.73)
P6×P1	2.57(±0.29)	1.97(±0.34)	2.27(±0.58)	55.63(±3.27)	36.90(±3.24)	46.27(±3.25)	46.10(±3.57)	53.10(±5.48)	49.60(±4.45)
P6×P2	3.79(±0.39)	3.06(±0.25)	3.43(±1.53)	44.53(±2.34)	37.33(±4.52)	40.93(±3.41)	84.90(±4.27)	82.13(±4.31)	83.52(±1.90)
P6×P3	2.99(±0.15)	2.59(±0.18)	2.79(±1.53)	55.77(±2.89)	43.10(±4.45)	49.43(±0.78)	53.70(±5.11)	60.27(±2.29)	56.98(±1.75)
P6×P4	2.96(±0.18)	2.69(±0.24)	2.82(±1.53)	51.57(±0.46)	40.37(±6.80)	45.97(±3.35)	57.23(±3.45)	67.10(±5.23)	62.17(±0.94)
P6×P5	1.77(±0.16)	1.24(±0.22)	1.51(±0.58)	50.93(±2.78)	40.23(±9.32)	45.58(±5.95)	34.67(±1.35)	31.03(±1.76)	32.85(±0.88)
P6×P7	3.27(±0.35)	3.17(±0.25)	3.22(±0.58)	69.43(±3.52)	54.47(±3.54)	61.95(±3.53)	46.97(±2.77)	58.43(±8.39)	52.70(±3.27)
P7×P1	2.18(±0.16)	1.83(±0.20)	2.00(±0.58)	116.40(±13.25)	99.53(±8.03)	107.90(±9.74)	18.97(±3.54)	18.30(±1.01)	18.63(±2.14)
P7×P2	2.94(±0.12)	2.47(±0.17)	2.70(±1.00)	48.90(±0.35)	45.97(±3.68)	47.43(±1.95)	59.97(±2.03)	53.73(±2.06)	56.85(±1.55)
P7×P3	3.25(±0.33)	2.62(±0.18)	2.93(±0.58)	108.60(±11.61)	72.67(±1.29)	90.62(±6.45)	29.93(±1.27)	35.90(±2.20)	32.92(±1.70)
P7×P4	2.86(±0.09)	2.60(±0.16)	2.73(±0.58)	70.83(±2.00)	73.50(±2.56)	72.17(±1.24)	40.40(±2.40)	35.27(±0.99)	37.83(±1.63)
P7×P5	2.67(±0.45)	2.18(±0.21)	2.42(±0.58)	60.07(±12.53)	39.57(±1.89)	49.82(±7.03)	44.73(±3.43)	54.97(±3.85)	49.85(±0.39)
P7×P6	2.82(±0.27)	2.77(±0.27)	2.80(±1.15)	65.97(±8.25)	54.10(±5.91)	60.03(±1.24)	42.77(±1.37)	51.57(±7.46)	47.17(±3.05)
F	11.42 **	16.70 **	16.47 **	25.61 **	28.12 **	45.39 **	49.16 **	33.15 **	76.95 **
LSD 0.05	0.50	0.41	0.40	11.51	8.69	7.52	6.80	8.11	5.23

P1= Perimoga, P2= La1793, P3= AC06, P4= CT6, P5= MC3, P6= C20, P7= Kingstone. Values in parenthesis represent standard errors.
** Significant at P<0.01.

To be continued

Continued

Table 5 - Means values and standard deviation for yield components and earliness of F1 hybrids developed via 7×7 diallel cross over two light conditions

Genotypes ♀ × ♂	Days to first flower			Days to ripening		
	L1	L2	Pooled	L1	L2	Pooled
P1×P2	56.00(±1.00)	54.33(±0.58)	55.17(±0.76)	14.00(±1.00)	15.67(±0.58)	14.83(±0.29)
P1×P3	56.67(±0.58)	54.00(±1.00)	55.33(±0.58)	16.67(±2.08)	18.67(±1.53)	17.67(±1.61)
P1×P4	54.00(±1.00)	50.67(±0.58)	52.33(±0.58)	18.33(±1.15)	18.33(±1.15)	18.33(±1.15)
P1×P5	62.67(±0.58)	60.33(±0.58)	61.50(±0.50)	19.00(±2.65)	20.67(±1.15)	19.83(±1.76)
P1×P6	58.00(±1.00)	54.67(±0.58)	56.33(±0.58)	18.00(±3.00)	18.00(±3.00)	18.00(±3.00)
P1×P7	57.33(±0.58)	53.33(±1.53)	55.33(±0.76)	19.33(±2.08)	18.67(±1.53)	19.00(±1.00)
P2×P1	56.67(±0.58)	54.67(±0.58)	55.67(±0.58)	19.00(±1.00)	16.67(±1.53)	17.83(±1.15)
P2×P3	60.33(±1.15)	60.67(±1.53)	60.50(±0.87)	18.33(±1.53)	17.33(±1.53)	17.83(±1.44)
P2×P4	60.67(±0.58)	58.67(±0.58)	59.67(±0.58)	16.33(±1.15)	15.67(±1.15)	16.00(±1.00)
P2×P5	60.67(±0.58)	58.67(±0.58)	59.67(±0.58)	15.67(±2.08)	15.67(±2.08)	15.67(±2.08)
P2×P6	61.67(±1.53)	60.33(±0.58)	61.00(±1.00)	17.00(±2.00)	17.00(±1.00)	17.00(±0.50)
P2×P7	58.33(±0.58)	58.00(±1.00)	58.17(±0.29)	16.33(±1.15)	17.00(±2.00)	16.67(±1.53)
P3×P1	57.33(±0.58)	54.67(±0.58)	56.00(±0.00)	18.33(±2.08)	18.33(±2.08)	18.33(±2.08)
P3×P2	60.00(±0.00)	59.67(±0.58)	59.83(±0.29)	20.33(±2.52)	19.33(±2.31)	19.83(±2.36)
P3×P4	56.00(±1.00)	55.67(±2.31)	55.83(±1.61)	16.67(±2.08)	16.67(±2.08)	16.67(±2.08)
P3×P5	60.00(±1.00)	56.33(±1.53)	58.17(±1.04)	20.00(±1.73)	17.33(±2.08)	18.67(±1.53)
P3×P6	60.67(±0.58)	59.00(±1.00)	59.83(±0.76)	17.00(±1.73)	17.00(±1.73)	17.00(±1.73)
P3×P7	58.33(±0.58)	56.33(±0.58)	57.33(±0.58)	22.67(±2.08)	22.67(±2.08)	22.67(±2.08)
P4×P1	54.67(±1.15)	51.33(±1.15)	53.00(±0.00)	17.00(±2.00)	17.00(±1.15)	17.00(±2.00)
P4×P2	60.00(±1.00)	58.00(±1.00)	59.00(±1.00)	15.00(±1.00)	14.00(±1.00)	14.50(±0.87)
P4×P3	58.00(±1.73)	57.00(±1.00)	57.50(±0.50)	14.67(±0.58)	15.33(±1.00)	15.00(±0.00)
P4×P5	61.67(±1.15)	59.67(±1.15)	60.67(±1.15)	18.00(±2.00)	18.00(±1.15)	18.00(±2.00)
P4×P6	56.33(±0.58)	54.33(±0.58)	55.33(±0.58)	20.00(±2.65)	21.00(±0.58)	20.50(±1.80)
P4×P7	57.67(±0.58)	55.67(±0.58)	56.67(±0.58)	18.67(±1.53)	18.67(±0.58)	18.67(±1.53)
P5×P1	62.00(±1.00)	59.33(±0.58)	60.67(±0.76)	20.33(±1.53)	19.33(±0.58)	19.83(±1.04)
P5×P2	61.67(±0.58)	58.33(±0.58)	60.00(±0.00)	13.00(±1.00)	12.67(±0.58)	12.83(±1.04)
P5×P3	61.00(±0.00)	57.33(±1.53)	59.17(±0.76)	19.67(±2.52)	16.00(±1.53)	17.83(±1.04)
P5×P4	60.67(±2.08)	58.67(±2.08)	59.67(±2.08)	19.00(±3.00)	20.33(±2.08)	19.67(±2.08)
P5×P6	63.67(±1.53)	58.67(±0.58)	61.17(±0.58)	21.00(±1.00)	20.33(±0.58)	20.67(±1.15)
P5×P7	61.00(±0.00)	58.33(±0.58)	59.67(±0.29)	21.33(±1.53)	15.00(±0.58)	18.17(±0.29)
P6×P1	58.33(±1.53)	55.33(±0.58)	56.83(±0.76)	17.67(±2.52)	17.67(±0.58)	17.67(±2.52)
P6×P2	62.33(±1.53)	59.67(±1.53)	61.00(±1.32)	15.00(±2.00)	15.33(±1.53)	15.17(±1.76)
P6×P3	59.67(±0.58)	59.33(±1.53)	59.50(±0.87)	16.00(±1.73)	19.67(±1.53)	17.83(±1.04)
P6×P4	56.67(±0.58)	54.33(±1.53)	55.50(±0.87)	20.00(±2.65)	18.33(±1.53)	19.17(±2.02)
P6×P5	63.33(±0.58)	60.33(±0.58)	61.83(±0.58)	18.67(±1.53)	18.67(±0.58)	18.67(±1.53)
P6×P7	59.00(±1.00)	55.33(±0.58)	57.17(±0.29)	20.67(±1.53)	21.33(±0.58)	21.00(±1.50)
P7×P1	58.00(±1.00)	52.67(±0.58)	55.33(±0.76)	20.67(±2.08)	20.67(±0.58)	20.67(±1.76)
P7×P2	60.00(±0.00)	59.00(±1.00)	59.50(±0.50)	20.00(±1.00)	18.00(±1.00)	19.00(±0.00)
P7×P3	58.33(±0.58)	56.33(±0.58)	57.33(±0.58)	20.67(±0.58)	20.67(±0.58)	20.67(±0.58)
P7×P4	58.33(±0.58)	56.33(±0.58)	57.33(±0.58)	17.33(±1.15)	18.67(±0.58)	18.00(±0.00)
P7×P5	62.00(±0.00)	59.33(±0.58)	60.67(±0.29)	20.00(±1.00)	17.67(±0.58)	18.83(±1.61)
P7×P6	58.67(±1.15)	53.33(±1.15)	56.00(±1.00)	19.00(±1.00)	19.00(±1.15)	19.00(±1.00)
F	18.69 **	19.82 **	28.14 **	4.20 **	19.82 **	4.82 **
LSD 0.05	1.52	1.66	1.28	2.98	2.59	2.53

P1= Perimoga, P2= La1793, P3= AC06, P4= CT6, P5= MC3, P6= C20, P7= Kingstone. Values in parenthesis represent standard errors.
** Significant at P<0.01.

Table 6 - General combining ability (GCA) effects of parental lines for yield, yield components and earliness in a 7×7 diallel cross over two light conditions

Parental lines	Total yield per plant (Kg)			Average of fruit weight (g)			Fruits number per plant			Days to first flower			Days to ripening		
	L1	L2	Pooled	L1	L2	Pooled	L1	L2	Pooled	L1	L2	Pooled	L1	L2	Pooled
P1	-0.02	-0.06	-0.04	18.32 **	10.97 **	14.64 **	-13.46 **	-11.38 **	-12.42 **	-1.93 **	-2.70 **	-2.31 **	-0.06	0.42	0.18
P2	-0.02	-0.24 **	-0.13 **	-16.82 **	-11.61 **	-14.22 **	14.63 **	6.67 **	10.65 **	0.74 **	1.77 **	1.25 **	-1.90 **	-2.11 **	-2.00 **
P3	0.09	-0.12 **	-0.02	5.22 **	-0.87	2.17 **	-2.98 **	-1.78 *	-2.38 **	-0.46 **	0.40 *	-0.03	0.2	0.36	0.28
P4	0.48 **	0.64 **	0.56 **	1.98	3.76 **	2.87 **	3.28 **	6.70 **	4.99 **	-1.63 **	-1.20 **	-1.41 **	-0.80 *	-0.34	-0.57 **
P5	-0.08	-0.06	-0.07 **	-13.35 **	-11.79 **	-12.57 **	8.71 **	10.29 **	9.50 **	2.94 **	2.30 **	2.62 **	0.67 *	-0.38	0.15
P6	-0.20 **	-0.08	-0.14 **	-12.68 **	-8.17 **	-10.42 **	3.99 **	4.51 **	4.25 **	0.74 **	0.24	0.49 **	0.1	0.79 **	0.45 *
P7	-0.25 **	-0.07	-0.16 **	17.34 **	17.71 **	17.52 **	-14.15 **	-15.01 **	-14.58 **	-0.40 *	-0.83 **	-0.61 **	1.77 **	1.26 **	1.51 **
LSD 0.05															
$g_i - g_j$[a]	0.16	0.13	0.09	3.56	2.68	2.07	2.1	2.51	1.52	0.47	0.51	0.35	0.92	0.8	0.6

P1= Perimoga, P2= La1793, P3= AC06, P4= CT6, P5= MC3, P6= C20, P7= Kingstone.
* Significant at P<0.05 level, ** Significant at P<0.01 level.
[a] difference between GCA of two parental lines at P<0.05 level.

Table 7 - Specific combining ability (SCA) effects of F1 hybrids for yield, yield components and earliness in a 7×7 diallel cross over two light conditions

Genotypes ♀ × ♂	Total yield per plant (Kg)			Average o fruit weight (g)			Fruits number per plant			Days to first flower			Days to ripening		
	L1	L2	Pooled	L1	L2	Pooled	L1	L2	Pooled	L1	L2	Pooled	L1	L2	Pooled
P1×P2	-0.51 **	-0.70 **	-0.60 **	1.29	-4.52 *	-1.62	-14.90 **	-15.90 **	-15.40**	-1.72 **	-1.43 **	-1.58 **	0.21	-0.1	0.06
P1×P3	0.31 **	0.29 **	0.30 **	-6.87 **	-4.69 **	-5.78 **	7.36 **	6.62 **	6.99**	0.14	-0.23	-0.04	-0.89	-0.23	-0.56
P1×P4	0.53 **	0.48 **	0.50 **	1.03	-3.36	-1.16	6.07 **	9.07 **	7.57**	-1.36 **	-1.97 **	-1.66 **	0.28	-0.37	-0.04
P1×P5	0.55 **	0.66 **	0.60 **	11.36 **	3.93 *	7.64 **	-3.68 **	5.01 **	0.67	2.08 **	3.37 **	2.72 **	0.81	2.00 **	1.41 **
P1×P6	-0.42 **	-0.29 **	-0.36 **	-21.97 **	-11.65 **	-16.81 **	8.66 **	4.04 *	6.35**	0.11	0.6	0.36	-0.46	-1.33 *	-0.89 *
P1×P7	-0.45 **	-0.43 **	-0.44 **	15.16 **	20.30**	17.73 **	-3.51 *	-8.84 **	-6.17**	0.74 *	-0.33	0.21	0.04	0.03	0.04
P2×P3	-0.21 *	-0.38 **	-0.30 **	6.92 **	-1.3	2.81 *	-14.38 **	-9.97 **	-12.18**	0.64 *	1.13 **	0.89 **	2.78 **	2.13 **	2.46 **
P2×P4	-0.46 **	-0.22 *	-0.34 **	-2.56	-2.3	-2.43	-4.25 **	1.11	-1.57	1.98 **	0.90 **	1.44 **	0.11	-0.67	-0.28
P2×P5	-0.29 **	0.21 *	-0.04	-0.69	6.48 **	2.90 *	4.88 **	3.80 *	4.34**	-1.76 **	-2.43 **	-2.09 **	-2.69 **	-1.30 *	-1.99 **
P2×P6	1.16 **	0.70 **	0.93 **	9.64 **	5.56 **	7.60 **	19.35 **	16.98 **	18.17**	1.28 **	1.13 **	1.21 **	-0.46	-0.47	-0.46
P2×P7	0.31 **	0.38 **	0.35 **	-14.61 **	-3.91 *	-9.26 **	9.29 **	3.99 *	6.64**	-0.42	0.70 *	0.14	0.04	0.4	0.22
P3×P4	-0.37 **	-0.24 **	-0.30 **	-4.28	2.48	-0.9	-3.31 *	-8.53 **	-5.92**	-0.16	0.27	0.06	-1.99 **	-1.97 **	-1.98 **
P3×P5	0.07	-0.09	-0.01	-14.22 **	-6.37 **	-10.29 **	17.26 **	11.55 **	14.41**	-1.22 **	-2.73 **	-1.98 **	0.71	-1.27 *	-0.28
P3×P6	-0.04	0.20 *	0.08	1.65	0.34	1	-2.84 *	4.93 **	1.05	0.64 *	1.67 **	1.16 **	-2.06 **	-0.77	-1.41 **
P3×P7	0.25 *	0.22 *	0.23 **	16.80 **	9.54 **	13.17 **	-4.09 **	-4.60 **	-4.34**	-0.06	-0.1	-0.08	1.44 *	2.10 **	1.77 **
P4×P5	0.83 **	0.58 **	0.71 **	6.60 **	5.20 **	5.90 **	8.48 **	7.43 **	7.96**	0.61	1.20 **	0.91 **	0.38	1.93 **	1.16 **
P4×P6	-0.2	-0.18 *	-0.19 **	3.95	0.49	2.22	-4.01 **	-1.59	-2.80**	-1.86 **	-1.57 **	-1.71 **	2.44 **	1.27 *	1.86 **
P4×P7	-0.34 **	-0.43 **	-0.38 **	-4.75 *	-2.51	-3.63 **	-2.98 *	-7.48 **	-5.23**	0.78 *	1.17 **	0.97 **	-1.22 *	-0.2	-0.71
P5×P6	-0.95 **	-1.02 **	-0.98 **	8.13 **	9.71 **	8.92 **	-24.69 **	-34.54 **	-29.62 **	0.58	0.1	0.34	0.81	1.13	0.97*
P5×P7	-0.21	-0.33 **	-0.27 **	-11.20 **	-18.95 **	-15.07 **	-2.26	6.75 **	2.24 *	-0.29	0.5	0.11	-0.02	-2.50 **	-1.26 **
P6×P7	0.45 **	0.59 **	0.52 **	-1.4	-4.45 *	-2.93 *	3.53 *	10.19**	6.86 **	-0.76	-1.93 **	-1.34 **	-0.29	0.17	-0.06
LSD 0.05															
$S_{ij} - S_{ik}$[a]	0.29	0.23	0.17	6.44	4.87	3.75	3.81	4.54	2.75	0.85	0.93	0.63	1.67	1.45	1.08
$S_{ij} - S_{kl}$[b]	0.25	0.2	0.14	5.58	4.21	3.25	3.3	3.93	2.38	0.74	0.81	0.55	1.44	1.26	0.93

P1= Perimoga, P2= La1793, P3= AC06, P4= CT6, P5= MC3, P6= C20, P7= Kingstone. *Significant at P < 0.05 level.
** Significant at P<0.01 level.
[a] Difference between two SCA of two hybrids, with a common parent.
[b] Difference between two SCA of two hybrids, with non-common parent.

'Perimoga×MC3' (P1×P5) was the weakest combination across both environments. The most negative value of SCA for ripening period under low light belonged to 'La1793×MC3' (P2×P5) and under high light was for 'Kingstone×MC3' (P7×P5). Pooled value of SCA in this character showed that generally 'La1793×MC3' (P2×P5) and 'AC06×La1793' (P3×P2) had the highest and the lowest negative values, respectively.

The results of REC indicated that the best reciprocal combinations over two environments for total yield, average of fruit weight, fruits number, days to flowering and days to ripening was for 'AC06×La1793' (P3×P2), 'Kingstone×MC3' (P7×P5), 'MC3×La1793' (P5×P2), 'Kingstone×C20' (P7×P6) and 'MC3×La1793' (P5×P2), respectively (Table 8). The Lowest pooled REC in foregoing characters was for 'Kingstone×C20' (P7×P6), 'C20×CT6' (P6×P4), 'C20×MC3' (P6×P5), 'MC3×AC06' (P4×P3) and 'La1793×Perimoga' (P2×P1), respectively

4. Discussion and Conclusions

The results indicated that total yield, average fruit weight and flowering time were influenced by the amount of received light, while fruit number and fruit ripening period were not affected. Genotype effect was highly significant for all studied traits implying the feasibility of breeding. Despite of simultaneous influence of light and genotype on yield, fruit weight and days to flower, a comparison between magnitude of environment and genotype effects revealed that the genotype variation played more important in the expression of studied traits.

The significance of interaction genotype × light condition (G×L) for almost all characters except days to ripening revealed that there is a genotype variation in response to light intensity as regards yield, yield components and early flowering. Previous studies reported genotype variation regarding reaction to environmental light in different species (Stratton,

Table 8 - Reciprocal effect (REC) for yield, yield components and earliness in a 7×7 diallel cross over two light conditions

Genotypes ♀×♂	Total yield per plant (Kg)			Average of fruit weight (g)			Fruits number per plant			Days to first flower			Days to ripening		
	L1	L2	Pooled	L1	L2	Pooled	L1	L2	Pooled	L1	L2	Pooled	L1	L2	Pooled
P2×P1	-0.22	0.05	-0.08	-2.9	-1.87	-2.38	-1.17	2.8	0.82	0.33	0.17	0.25	2.50 **	0.5	1.50 **
P3×P1	-0.11	-0.23 *	-0.17 *	-4.52	-0.57	-2.54	1.08	-3.5	-1.21	0.33	0.33	0.33	0.83	-0.17	0.33
P4×P1	0.23	0.1	0.17 *	-2.75	4.1	0.67	4.38 *	-2.5	0.94	0.33	0.33	0.33	-0.67	-0.67	-0.67
P5×P1	-0.12	-0.11	-0.11	-3.95	-5.03 *	-4.49 **	0.97	3.53	2.25	-0.33	-0.5	-0.42	0.67	-0.67	0
P6×P1	0.16	-0.13	0.02	7.52 *	-3.45	2.03	-4.58 **	0.62	-1.98	0.17	0.33	0.25	-0.17	-0.17	-0.17
P7×P1	-0.15	-0.15	-0.15 *	1.1	1.37	1.23	-1.42	-1.78	-1.6	0.33	-0.33	0	0.67	1	0.83
P3×P2	0.22	0.19	0.21 **	1.23	1.65	1.44	2.57	2.95	2.76 *	-0.17	-0.5	-0.33	1	1	1.00 *
P4×P2	-0.21	-0.06	-0.13	0.92	-0.78	0.07	-5.55 **	-0.28	-2.92 *	-0.33	-0.33	-0.33	-0.67	-0.83	-0.75
P5×P2	-0.14	-0.1	-0.12	-3.32	-2.75	-3.03	3.62 *	3.4	3.51 **	0.5	-0.17	0.17	-1.33	-1.50 *	-1.42 **
P6×P2	-0.21	0.14	-0.03	-0.05	2.35	1.15	-4.57 **	-1.33	-2.95 *	0.33	-0.33	0	-1	-0.83	-0.92
P7×P2	-0.15	-0.14	-0.15 *	-1.45	-5.42 *	-3.43 *	-1.3	2.78	0.74	0.83 *	0.5	0.67 *	1.83 *	0.5	1.17 *
P4×P3	-0.08	-0.19	0.06	-0.83	0.33	-0.25	-0.68	3.13	1.23	1.00 *	0.67	0.83 **	-1	-0.67	-0.83
P5×P3	0.16	-0.17	-0.01	2.03	-3.3	-0.63	0.52	2.53	1.52	0.5	0.5	0.5	-0.17	-0.67	-0.42
P6×P3	0.09	0.06	0.07	-2.87	2.6	-0.13	4.03 *	-2.7	0.67	-0.5	0.17	-0.17	-0.5	1.33 *	0.42
P7×P3	0.11	0.06	0.08	4.77	-2.9	0.93	-0.35	1.98	0.82	0	0	0	-1	-1	-1.00 *
P5×P4	-0.29 *	-0.1	-0.19 **	-3.52	-1.13	-2.33	-0.43	-0.27	-0.35	-0.5	-0.5	-0.5	0.5	1.17	0.83
P6×P4	-0.18	-0.22 *	-0.20 **	-6.13 *	-4.92 *	-5.53 **	2.48	2.17	2.32	0.17	0	0.08	0	-1.33 *	-0.67
P7×P4	-0.09	-0.08	-0.08	-8.18 **	5.35 *	-1.42	2.75	-4.25 *	-0.75	0.33	0.33	0.33	-0.67	0	-0.33
P6×P5	-0.05	-0.13	-0.09	4.38	1.28	2.83	-4.83 **	-4.53 *	-4.68 **	-0.17	0.83 *	0.33	-1.17	-0.83	-1.00 *
P7×P5	0.17	0.1	0.13	2.83	3.4	3.12	0.93	-2.37	-0.72	0.5	0.5	0.5	-0.67	1.33 *	0.33
P7×P6	-0.23	-0.2	-0.21 **	-1.73	-0.18	-0.96	-2.1	-3.43	-2.77*	-0.17	-1.00 *	-0.58 *	-0.83	-1.17	-1.00 *
LSD 0.05 $R_{ij} - R_{ik}$ [a]	0.31	0.25	0.18	7.04	5.32	4.1	4.16	4.96	3.01	0.93	1.02	0.69	1.82	1.59	1.18

P1= Perimoga, P2= La1793, P3= AC06, P4= CT6, P5= MC3, P6= C20, P7= Kingstone.

* Significant at P<0.05 level, ** Significant at P<0.01 level.

[a] Difference between two RCA of two hybrids.

D.A., 1998; Martínez-Ferri *et al.*, 2001) promising for plant improvement regarding maintenance of high yield and earliness under lower level of light intensity. The remarkable effect of interaction G×L on studied traits except ripening period demonstrated that genotypes were not stable across two environments and should be evaluated in a range of environments. Importance of genotype selection across different environments for tomato improvement concerning yield and earliness attributing traits was reported by Chadha and Kumar (2001), and Biswas *et al.* (2011).

According to mean performances of hybrids, superior genotypes for various characteristics differed and none of them could be considered as the best for all of the attributes. In this regard, in order to commercialize F1 hybrids, breeding programs should be conducted to collect suitable features in one plant (Breseghello and Coelho, 2013).

Either of general combing ability (GCA) or specific combining ability (SCA) was highly significant for all of evaluated features illustrating both additive and non-additive gene action were involved in controlling yield, yield components and earliness. Our findings supports additive-dominance model reported by Chishti *et al.* (2008) and Biswas *et al.* (2011) for production and earliness traits. Significant contribution of REC to total sum squire is indicative of inter-allelic interactions in the expression of studied traits. Similarly, REC effect on fruit weight and number was reported by Hannan *et al.*, (2007 b).

Higher magnitude of SCA variance (σ^2s) in comparison with GCA variance (σ^2g) for total yield, fruit number and days to ripening indicated that these traits are mainly under the control of dominant effects. Similar findings were reported by Solieman (2009) and El-Gabry *et al.* (2014) for fruit yield and number. The predominance of non-additive gene action over additive effects for days to ripening was in agreement with Hannan *et al.* (2007a) but inconsistent with Garg *et al.* (2008) who found additive gene action to be more effective on days to ripening over two environments.

The ratio of σ^2g/σ^2s over two light conditions was greater than unity for fruit weight and days to flowering indicative of more weight of additive effects in inheritance of these features. Garg *et al.* (2008), Rai and Asati (2011) and Nadeem *et al.* (2013) also contributed the expression of fruit weight and early flowering to both additive and dominance gene actions with preponderance of additive effects. Biswas *et al.* (2011) who examined tomato genotypes across two environments, different in terms of temperature and light intensity, reported more important role of additive gene action in control of fruit weight.

The interaction GCA×L was significant for all characters except for days to ripening indicating the sensibility of additive effects to light condition. The significant interaction SCA×L was indicative of instability of dominance effects under different environmental light. REC×L varied for fruit weight and number demonstrating the susceptibility of cytoplasmic effects to environment in some traits and necessity of reciprocal crosses for choosing the most suitable genotypes for target environment.

Estimates of broad-sense heritability percentage ($H^2_{b.s}$%) across two different light conditions was high, demonstrating the low effect of environment and high response of studied traits to breeding programs. Relatively high narrow sense heritability percentage ($h^2_{n.s}$%) for fruit weight and early flowering indicated that these traits are largely controlled by additive effects; while, low $h^2_{n.s}$% of yield, fruit number and early maturity demonstrated higher weight of non-additive effects in inheritance of these traits. These findings agree with earlier work of Biswas *et al.* (2011) and Dutta *et al.* (2013) who reported low narrow sense heritability for yield and fruit number over two environments.

Importance of both additive and non-additive gene action with predominance of additive effects in expression of fruit weight and days to flowering revealed that selection breeding programs could be an effective strategy for genetic improvement of tomato for these characters, while exploitation of hybrid vigor should not be neglected (Grilli *et al.*, 2003). Yield, fruit number and early ripening were controlled by additive-dominance effects with higher weight of dominance effects implying hybrid breeding could be adopted for improvement of these characters (Gul *et al.*, 2010). Abd El-Maksoud *et al.* (2013) proposed recurrent selection program for improvement of traits controlled by both additive and non-additive effects. For such traits, hybridization in segregating generations followed by selection for outperforming genotypes has been recommended (Dutta *et al.*, 2013; Bhattarai *et al.*, 2016).

Limitation in access to clean energy resources in rural areas and global interest toward lower energy consumption necessitate breeding for low energy input. In the current study, genetic variation among tomato genotypes under different light conditions was observed and some genotypes showed more stability than others. None of parental lines or F1

hybrids exhibited high performance for all studied features, therefore, a particular genotype cannot be recommended. However, genetic knowledge obtained from this research could be used in planning tomato breeding programs. More important role of additive gene action in inheritance of fruit weight and early flowering indicate the effectiveness of selection breeding, while predominance of non-additive effects in genetic expression of plant yield, fruit number and early maturity suggest adoption of bi-parental mating for improvement of mentioned traits.

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Essential oil production of *Murraya paniculata* (L.) Jack at different harvest times

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Key words: β-methylesculetin, caryophyllene, murralongin, solvent extraction.

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Abstract: *Murraya paniculata* (L.) Jack has a fragrant flower, from which the fragrance is due to the essential oil. The study aimed to investigate the production of essential oil and its chemical compounds at different harvest times. The research was conducted at an organic experimental farm, Bogor Agricultural University, Bogor, Indonesia (6°30'-6°45' S, 106°30'-106°45' E) from October 2016 to February 2017 using randomized complete block design. The experiment consisted of one factor, namely the harvest times, comprised of harvest at 05.00-07.00 and 07.00-09.00 a.m. *M. paniculata* flowers were collected at three different flower ages, comprised of two days before anthesis, one day before anthesis and the day of anthesis (blooming). The different flower ages indicated by the flower size. Ethanol extraction method was used to extract the essential oil of the flowers from different harvesting times and then chemical compounds were analyzed by Gas Chromatography-Mass Spectrometry. The result showed that flower number and weight were not affected by harvesting times. The flower collected on the day of anthesis had the highest flower number and weight. Harvesting flowers at anthesis can be done at 05.00-09.00 a.m. The highest quantity and quality of essential oils were obtained by harvesting the flowers at anthesis. β-methylesculetin and murralongin were the primary compounds in *M. paniculata* flowers that harvested at 05.00-09.00 a.m.

1. Introduction

Murraya paniculata (L.) Jack well known as orange jessamine is an ornamental plant and belongs to family Rutaceae (Shah *et al*., 2014), it has white flowers with sweet fragrance (Gilman, 1999). The plants are native to Southeastern Asia, i.e. Cambodia, Laos, Myanmar, Thailand, Vietnam, Indonesia, Malaysia, and Philippine (Dosoky *et al*., 2016). *M. paniculata* has been used in traditional medicine because the plant has anti-amnesic, anti-inflammatory, anti-diabetic, anti-fungal, anti-bacterial, anti-helminthic, anti-cancer, and anti-oxidative properties (Sharma and Arora, 2015). Beside as a source for perfumery, *M. paniculata* is also used as a source of flavors (El-Sakhawy *et al*., 1998) because the flowers are highly aromatic and contain sufficient amount of essential oil (Naseem *et al*., 2015). Plants essential oils are aromatic components that composed

of different chemical compounds (Younis *et al*., 2011).

Different compositions of chemical compound of *M. paniculata* essential oils have been studied. Rout *et al*. (2007) found that pentane extraction was the best method to obtain the highest amount of essential oil of *M. paniculata* flowers in India. This study also found that manool and (*E*)-nerolidol were the major component of essential oil (Rout *et al*., 2007). The chemical compounds of *M. paniculata* flowers can also be extracted with liquid CO_2 (Rout *et al*., 2010). Different compounds from *M. exotica* flowers were found, namely (*E,E,E*)-α-springene, (*E*)-nerolidol, (*E,E*)-α-farnesene, methyl palmitate and germacrene B (Raina *et al*., 2006).

The variation of chemical composition in the essential oil from *Murraya* flowers is affected by the place where the plants are planted (El-Sakhawy *et al*., 1998) and harvesting time. Concerning the latter, harvesting time may influence the quantity and quality of essential oils of *M. paniculata* flowers; this has been reported in four *Rosa* cultivars (Younis *et al*., 2009) and *Jasminum sambac* flowers (Younis *et al*., 2011). Younis *et al*. (2011) reported that the best time to collect *J. sambac* flowers was in the morning before sunrise because highly volatile of jasmine oil. Therefore, the purpose of this research was to investigate the effect of harvest time on the production and chemical compounds of *M. paniculata* essential oil.

2. Materials and Methods

Plant material

The field experiment was conducted from October 2016 to February 2017. The experiment used 30 (62 month-old) plants that were planted in 1 m x 1 m on latosol soil, at the organic experimental farm, Bogor Agricultural University, Bogor, Indonesia (6°30'-6°45' S, 106°30'-106°45' E) at 250 m above sea level. A voucher specimen was deposit at The Herbarium Bogoriense, Bogor, Indonesia. Type-A climate based on Schmidt-Ferguson with the average monthly rainfall, temperature and humidity of 305 mm, 26°C and 85%, respectively (MCGA, 2017). Before treatment, each plant was fertilized with 3.0 kg rice-hull ash, followed Eliazar and Aziz (2015).

Experimental design

The experiment was arranged in randomized complete block design, with single factor (Petersen, 1994) with harvest times as treatments, comprised of harvest at 05.00-07.00 and at 07.00-09.00 a.m. The flowers were collected at three different ages, comprised of two days before anthesis, one day before anthesis, and at the day of anthesis. The two ages of flower before anthesis were indicated by the size of flower buds which have been observed in the preliminary study. The anthesis of the flower bud with 1.00±0.06 cm length and 0.48±0.06 cm width will occur two days later; while flower bud with 1.16±0.06 cm length and 0.56±0.04 cm width will occur in the next day. The observations include developmental stages, number, fresh weight, the content of essential oils and chemical compounds of *M. paniculata* flowers. Data were analyzed using t-student with α= 5% (Petersen, 1994).

Essential oil extraction

The analysis of essential oils was conducted at Tropical Biopharmaca Research Center, Bogor Agricultural University, Bogor, Indonesia. All harvested flowers (from different harvesting times) were extracted with solvent extraction method of ethanol. The flowers were immersed in ethanol until all samples were submerged, for two days. This mixture was filtered, then, the extract was separated from the solvent by rotary evaporator with a temperature of 40°C. The oil yield percentage was calculated from the weight of extract (g) divided by weight of fresh flower (g).

Gas chromatography-mass spectrometry analysis

Chemical compounds of essential oils were analyzed at Health Laboratory of DKI Jakarta Province. The essential oils were injected into Gas Chromatography-Mass Spectrometry (GCMS). An Agilent Technologies 7890 Gas Chromatography instrument with an Auto Sampler and 5975 Mass Selective Detector and Chemstation Data System, equipped with a 30 m x 0.25 mm HP Ultra 2 capillary column with 0.25 µm film thickness. Helium was used as the carrier gas. The initial temperature was programmed at 80°C and then increased at the rate of 3°Cmin^{-1} to 150°C held for 1 min and finally raised to 280°C at the rate of 20°C min^{-1} held for 26 min. The injector and interface temperatures were 250°C and 280°C, respectively. The ionization voltage was 70eV and a sample injection volume 5 µL. The individual peaks were identified by retention times, compared with those of compounds in Health Laboratory of DKI Jakarta Province database. The percentage of each compound detected from samples was calculated according to the area of the chromatographic peaks.

3. Results

The results showed that the average of flower number (regardless the flower ages) harvested at 05.00-07.00 AM was not significantly different to those harvesting at 07.00-09.00 a.m. (Table 1). At both harvest times, it was found the following order of flower number based on flower stages: flowers at anthesis, flowers that would bloom two days later, and the least was flowers that would bloom in the next day. The differences in flower number between flower stages were not always significant. The difference of flower number between harvesting time was 8.96%.

Table 1 - Number of orange jessamine flowers at different harvesting times

Harvesting time	Average of flower number/plant	P-value	Percentage of flower number (%)
At 05.00-07.00 AM, flower ages	29.40 (k)		
2 days before anthesis	24.67	x: 0.3349 NS	27.97
1 day before anthesis	14.07	y: 0.1742 NS	15.95
At anthesis	49.47	z: 0.0397 *	56.08
At 07.00-09.00 AM, flower ages	31.11 (k)		
2 days before anthesis	35.53	x: 0.0150 *	38.82
1 day before anthesis	12.87	y: 0.5056 NS	14.06
At anthesis	44.93	z: 0.0282 *	47.12

If P-value>α; α= 0.05, then means between the treatments were statistically equal; (k) = average of flower number at three flower age criteria; x= comparison between the flower 2 days before anthesis with 1 day before anthesis; y= comparison between the flower 2 days before anthesis with anthesis; z= comparison between the flower 1 day before anthesis with anthesis.

The average flower weight (regardless the flower stages) of *M. paniculata* was not significantly different between the times of harvesting (Table 2). A similar trend as of flower number was also found in flower weight based on flower stages. The highest flower weight was blooming flowers (flowers at anthesis), and the lowest was flowers at the stage of one day before anthesis. The difference in flower weight between harvesting time was 16.59%.

Table 2 - Weight of orange jessamine flowers at different harvesting times

Harvesting time	Average of flower weight (g/plant)	P-Value	Percentage of flower weight (%)
At 05.00-07.00 AM, flower ages	3.43 (k)		
2 days before anthesis	2.05	x: 0.4572 NS	19.92
1 day before anthesis	1.34	y: 0.0884 NS	13.21
At anthesis	6.88	z: 0.0355 *	66.86
At 07.00-09.00 AM, flower ages	4.72 (k)		
2 days before anthesis	5.70	x: 0.0819 NS	40.35
1 day before anthesis	1.33	y: 0.4398 NS	9.38
At anthesis	7.13	z: 0.0029 **	50.27

If P-value>α; α= 0.05, then means between the treatments were statistically equal; k: average of flower number at three flower age criteria; x: comparison between the flower 2 days before anthesis with 1 day before anthesis; y: comparison between the flower 2 days before anthesis with anthesis; z: comparison between the flower 1 day before anthesis with anthesis.

The extraction of *M. paniculata* flowers at different harvesting times with ethanol resulted in a yellowish-brown solution called concrete. These results were in line with that of Paibon *et al*. (2011), that reported how the extraction of *J. Sambac* flowers with ethanol produced a solution of yellowish brown to reddish. On the other hand, *M. paniculata* flowers that extracted with pentane produced a deep yellow waxy residue (Rout *et al*., 2007).

There was an indication that the essential oil percentage between harvest times was different. The percentage of essential oils from flowers harvested at 07.00-09.00 was higher than that at 05.00-07.00 a.m., regardless the flower stages, the difference was 0.38% (Table 3). Comparing among flower stages, it was found that anthesis flowers harvested at 05.00-07.00 AM had the highest percentage of essential oils. On the other hand, the highest percentage of essential oils at 07.00-09.00 AM was obtained from flowers at the stage of one day before anthesis. From this calculation, the production of essential oils from flowers harvested at 07.00-09.00 was higher than that from 05.00-07.00 AM. Based on flower stages, blooming flowers (at anthesis stage) produced the highest amount of essential oils at both harvesting times, this related to the highest fresh flower weight.

Table 3 - The percentage and production of essential oil of orange jessamine flowers at different harvesting times

Treatment	Essential oil (%)	Production of essential oil (g/g fresh flower) *
At 05.00-07.00 AM, flower ages	3.13 (k)	4.83
2 days before anthesis	2.06	0.63
1 day before anthesis	3.40	0.69
At anthesis	3.94	4.07
At 07.00-09.00 AM, flower ages	3.51 (k)	7.47
2 days before anthesis	3.49	3.00
1 day before anthesis	3.80	0.76
At anthesis	3.25	3.47

Data were not analyzed statistically;
(k) = average of essential oils at three flower age criteria;
* = Production of essential oils based on the weight of the harvested flowers.

The analysis of chemical compounds showed 41 types that were contained in *M. paniculata* flowers at different harvesting times. The highest number of chemical compound types was found in flowers at anthesis when they were harvested at 07.00-09.00 AM. This result was in line with Younis *et al*. (2011), where *J. Sambac* flowers harvested at anthesis had

more chemical compounds than those in flower bud. The analysis on *M. paniculata* flowers revealed the presence of coumarins, esters, fatty acids, phenolics, triterpenes, sesquiterpenes and other compounds (Table 4). Coumarin was the most common compound found in all treatments. Harvesting time at 05.00-07.00 a.m. gave the highest number of coumarins, esters, fatty acids, and sesquiterpenes.

(Table 4). The percentage of triterpenes was higher than sesquiterpenes, this finding was different from the previous study which showed that sesquiterpenes were the main compound in essential oils. The current study showed that sesquiterpenes derivatives found in the essential oils of *M. paniculata* flowers and found at both harvesting times were α-zingiberene, α-bergamotene, and caryophyllene

Table 4 - Chemical compounds of orange jessamine essential oils at different harvesting times

Treatment	% Peak area						
	Coumarin	Ester	Fatty acid	Phenolic	Triterpene	Sesquiterpene	Other compound
At 05.00-07.00 am, flower ages	71.29 k	1.80 k	9.12 k	4.34 k	3.27 k	0.77 k	9.42 k
2 days before anthesis	69.68	3.16	12.39	5.52	1.55	0.47	7.24
1 day before anthesis	74.33	1.41	10.09	4.43	1.33	0.55	7.87
At anthesis	69.85	0.83	4.89	3.06	6.93	1.30	13.14
At 07.00-09.00 am, flower ages	68.07 k	1.60 k	10.40 k	5.09 k	1.93 k	0.72 k	12.20 k
2 days before anthesis	66.98	2.15	11.91	6.20	1.03	0.41	11.33
1 day before anthesis	70.52	1.21	5.87	5.13	1.21	0.71	15.34
At anthesis	66.72	1.44	13.41	3.94	3.55	1.04	9.92

Data were not analyzed statistically; k= average of chemical compounds at three flower age criteria.

Coumarin was the highest amount of bioactive compound found in *M. paniculata* essential oils, (Table 4) and the dominant compounds in the coumarin group were β-methylesculetin and murralongin (Table 5). The amount of β-methylesculetin from flowers harvested at 07.00-09.00 AM was higher than those from 05.00-07.00 a.m. Different flower stages had a different dominant compound. Harvesting flowers at the stage of one day before anthesis gave the highest percentage of β-methylesculetin at both harvesting times, but harvesting at anthesis delivered the highest percentage of murralongin.

The analysis of chemical compounds showed that terpenoid groups found in the essential oil of *M. paniculata* flowers were triterpenes and sesquiterpenes

Table 5 - Coumarins compounds of orange jessamine essential oils at different harvesting times

Treatment	% Peak area	
	β-methylesculetin	Murralongin
At 05.00-07.00 am, flower ages	60.65 (k)	7.36 (k)
2 days before anthesis	59.46	5.36
1 day before anthesis	61.59	7.76
At anthesis	60.89	8.96
At 07.00-09.00 am, flower ages	62.07 (k)	5.71 (k)
2 days before anthesis	61.00	5.12
1 day before anthesis	65.51	5.01
At anthesis	59.71	7.01

Data were not analiyzed statistically; k: average of chemical compounds at three flower age criteria.

(Table 6). Flowers harvested at 05.00-07.00 AM had a higher percentage of α-zingiberene, α-bergamotene, and caryophyllene compare to those harvested at 07.00-09.00 a.m. There was an indication that different flower ages have different compositions of chemical compounds, except caryophyllene that was found at the same flower age at both harvesting times.

Table 6 - Sesquiterpenes compounds of orange jessamine essential oils at different harvesting times

Treatment	% Peak area		
	α-ZBN	α-BGN	CP
At 05.00-07.00 AM, flower ages	0.75 (k)	0.55 (k)	0.27 (k)
2 days before anthesis	0.47	0.00	0.00
1 day before anthesis	0.00	0.55	0.00
At anthesis	1.03	0.00	0.27
At 07.00-09.00 AM, flower ages	0.71 (k)	0.41 (k)	0.17 (k)
2 days before anthesis	0.00	0.41	0.00
1 day before anthesis	0.57	0.00	0.15
At anthesis	0.85	0.00	0.19

Data were not analyzed statistically; k: average of chemical compounds at three flower age criteria. ZBN, zingiberene; BGN, bergamotene; CP, caryophyllene.

4. Discussion and Conclusions

The above results showed that there were no significant differences between the number and weight of flowers harvested at 05.00-07.00 and 07.00-09.00 AM. The different harvesting times reflected the posi-

tion of sunrise where higher light intensity was found at 07.00-09.00 AM. The current study showed that the anthesis of *M. paniculata* flowers occurred before 05.00 AM, therefore there was no increase in the number and weight of flower after that time. De Souza *et al*. (2004) reported that the anthesis of *Metrodorea nigra* St. Hill. flowers, belonging to Rutaceae family, occurs in the morning. Time of harvesting is important because it is related to the amounts of essential oils produced. Filho *et al*. (2006) reported that harvest at 08.00 AM resulted in the highest yield of the essential oil from fresh leaves of basil (*Ocimum basilicum* L.). The importance of harvesting time is also shown by Dobreva and Kovacheva (2010) where the essential oils content of *Rosa damascena* Mill. and *R. alba* L. drops dramatically when the flowers collected after noon.

Besides investigating the effect of harvesting time, this study also observed the essential oil production at different flower developmental stages of *M*. *paniculata*. The results showed that harvesting flowers at anthesis stage (blooming flower) yielded the highest percentage of essential oils. Flowering plants released diverse blends of volatile to attract pollinator and seed disseminators. The floral scent is a signal, which pollinators can use to discriminate a particular flower. It may contain from one to 100 volatile substances, but most species emit between 20 and 60 different compounds, so there won't be any identical floral scents (Dudareva *et al*., 2006). Therefore, the presence of essential oil at anthesis will ensure the reproductive success. Azam *et al*. (2013) also reported that the highest amounts of volatile compounds were present in fully opened flowers of *Citrus reticulata* Blanco, *C. unshiu* Marc., *C. sinensis* (L.) Osbeck, *C. limon* (L.) Burm., *C. medica* (L.), and *C. changshanensis* Chen et. Fu.

In general, essential oils are a mixture of compounds belonging to different chemical entities such as terpenes, phenols, aliphatic compounds, benzenoid, and heterocyclic compounds (Shakeel-u-Rehman *et al*., 2018). Chemical compounds found in *M. paniculata* flowers in India were monoterpenes, sesquiterpenes, benzenoids, diterpenes, and fatty acids (Rout *et al*., 2007). Different from those study, the current experiment showed that coumarins were the dominant compounds in *M. paniculata* flowers. Coumarins are also present in leaves of *M. paniculata* in Indonesia (Kinoshita and Firman, 1996) and Taiwan (Kinoshita *et al*., 1996). Furthermore, from the current study, it was found the presence of two coumarins derivatives in essential oils of *M. paniculata*, β-methylesculetin and murralongin. β-methylesculetin compounds can function as antioxidants (Kontogiorgis and Hadjipavlou-Litina, 2005) and anti-inflammatory (Kontogiorgis and Hadjipavlou-Litina, 2005; Zuoqi *et al*., 2008). Murralongin is thought to be a chemical compound identifier of *M. paniculata* essential oils. Harvesting at anthesis produced the highest percentage of murralongin. The previous study reported that murralongin was found in essential oils from leaves and flowers of *M. paniculata* (Gill *et al*., 2014), and leaves of *M. omphalocarpa* in Taiwan (Chen *et al*., 2003).

Terpenoids compounds that were identified in this study were triterpenes and sesquiterpenes. In general, terpenoids are the dominant compounds in essential oils (Sangwan *et al*., 2001). The current study showed that the percentage of triterpenes compounds is higher than sesquiterpenes (Table 4). This was not in line with Butu *et al*. (2014) who reported that the basic compound in the essential oil was sesquiterpenes. Terpenoids have many volatile compounds that have high enough vapor pressures at normal atmospheric conditions to allow significant release into the air (Dudareva *et al*., 2004). Therefore, despite the same plant, may have different types of compounds. Sesquiterpenes compounds that could be identified in this current study were α-zingiberene, α-bergamotene, and caryophyllene. There were similarity and difference between this finding and the previous study. The similarity was reported by Raina *et al*. (2006) where those three compounds were also found in *M. exotica* essential oils from flowers. Raina *et al*. (2006) found that caryophyllene had the highest percentage, on the contrary, the current study showed that caryophyllene had the lowest percentage. The different finding indicates that the chemical composition and yield of essential oils are affected by many factors, such as provenance, weather, soil conditions, time of harvest, and the extraction method (Boira and Blanquer, 1998). Caryophyllene is one of the compounds in perfume ingredient (Salvador-Carreno and Chisvert, 2005), but it also used as a mixture of spices, citrus scents, soaps, detergents, lotions as well as in various food products (Sabulal *et al*., 2006). Flamini *et al*. (2007) and Darjazi (2012) reported that caryophyllene is also present in flower of *C. limon* and *C. nobilis* Lour var. deliciosa swingle.

It can be concluded that the harvest of *M. paniculata* flowers can be done at 05.00-09.00 AM to obtain the highest quantity and the best quality of essential oil. Flowers must be harvested at anthesis stage to reach the highest production of essential oil with β-

methylesculetin and murralongin, as the main compounds of *M. paniculata* flowers.

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Factors affecting *in vitro* propagation of some genotypes of Himalayan cedar [*Cedrus deodara* (Roxb. ex Lamb) G. Don.]

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Key words: auxin, conifers, cytokinins, micropropagation, pinaceae.

Abstract: Four genotypes of Himalayan cedar were grown *in vitro* for assessing shoot proliferation. This experiment consist three parts. Initially, the explants (leafy and defoliated shoot-tips) of mature plants were disinfected and cultured on different basal media (LP, MS and WPM) that supplemented with benzyladenine (BA) at different concentrations for 6 weeks. Leafless explants produced the highest number of shoots and the longest shoots for four genotypes. There was no significant difference between the culture mediums and benzyladenine concentrations. In second phase, the influence of benzyladenine (2.5, 5, 10, 20 µM) and thidiazuron (TDZ) (0.4, 0.8, 1.6 µM) with combination of different auxin (NAA) concentrations (0, 1, 2, 3 µM) was determined on axillary shoot proliferation of the leafless explants of four genotypes grown on WPM. For all thidiazuron concentrations, significant differences between genotypes were detected. In general, with all genotypes, the use of 0.8 µM thidiazuron in the absence or presence of auxin (2 µM) led to the highest length and number of axillary shoots per explant, respectively. Finally, in another experiment, the following cytokinin treatments were investigated for axillary shoot multiplication of the CD1 genotype: thidiazuron (0, 0.1, 0.2, 0.4, 0.8 µM) and N6-[2-Isopentenyl] adenine (2iP) (0, 0.1, 0.2, 0.3, 0.4, 0.5 µM) in combination with benzyladenine (2.5 µM). The best results were obtained in thidiazuron (0.8 µM) with combination of benzyladenine (2.5 µM). This protocol is considered as the first successful report on culture establishment of some genotypes of mature *C. deodara* trees.

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The authors declare no competing interests.

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1. Introduction

The Himalayan cedar [*Cedrus deodara* (Roxb. ex Lamb) G. Don.], which belongs to the Pinaceae family, is a beautiful, evergreen, and ornamental tree growing widely on the gradient of the Western Himalayas (Champion *et al.*, 1965). Commercial seed bearing of *C. deodara* begins about 30 to 45 years of age, and good seed crops are borne every 3 years (Tewari, 1994). Regeneration through seeds in *Cedrus deodara* is quite slow and undependable. Generally, there is a preference for propagation of mature trees, which helps improve afforestation management, breeding projects and production of elite tree genotypes. However, maturation of most tree species is a major limiting factor for the use of micropropagation in

afforestation projects (Lin *et al.*, 1991). There are a few reports on *in vitro* propagation of mature conifers in the last 20 years (Gupta and Durzan, 1985; Dumas and Monteuuis, 1995; Parasharami *et al.*, 2003; Andersone and Ievinsh, 2005; Malabadi and van Staden, 2005; Cortizo *et al.*, 2009; De Diego *et al.*, 2010). Few reports on *in vitro* propagation of cedars (*Cedrus libani* A. Rich. and *C. atlantica* Manetti) are available (Piola and Rohr, 1996; Piola *et al.*, 1998, 1999; Renau-Morata *et al.*, 2005). There are only two reports on the *in vitro* culture of deodar cedar with the sole use of the seeds (Bhatnagar *et al.*, 1983; Tamta and Palni, 2004).

Among regeneration methods, axillary bud induction is preferable for most maintenance of genetic stability and less mutation risk (Vasil and Vasil, 1980; Pierik, 1987). The enhancement of reliability of tissue culture system is being achieved through improving of medium component or correction of the conditions of environment, or both. However, the genotype has a significant impact on the accuracy and repeatability of tissue culture and its effect must be evaluated (Sul and Korban, 1994). In this domain, cytokinins and auxins play important roles for maintenance and acceptable growth of cultures. During the recent decades, several synthetic compounds have been introduced for induction of regeneration potential of plants (Guo *et al.*, 2011). Among the compounds, TDZ (Tang and Newton, 2005) and BA (Datta *et al*., 2006) are highly effective on Pinaceae family such as Eastern white pine and lodgepole pine, Virginia pine, red spruce, and *Taxus wallichiana* Zucc., respectively.

To our best knowledge, no report document the *in vitro* vegetative regeneration of this species from mature tissues. Our goal was to build methods for the *in vitro* propagation of *C. deodara* from adult trees, and to show the effect of genotype on its micropropagation.

2. Materials and Methods

Experiment 1. Plant materials

Actively growing shoots (4-6 cm long) were collected from mature (20-25 years old) *C. deodara* trees (genotype not specified) in a seed orchard near the School of Agriculture, Shiraz University, Iran. This was done from September 2015 to November 2016. The shoots were wrapped with wet paper toweling, enclosed in plastic bags and then kept at 4°C until 1 day before use.

Surface sterilization of explants

In this experiment, two kinds of explants with the similar length were used. The first type of explants retained their needles (leaves) to full length (Fig. 1 a). In the second type of explants, the leaves were trimmed to a quarter of their initial length (Fig. 1 a). Both types of explants were soaked in tap-water for at least 2 h. Then, they were submerged for 30 minutes in an aqueous solution of 2% benomyl to reduce fungal contamination. Afterwards, they were treated with 70% ethanol for 2 minutes followed by 15% Clorox (containing 5.25% sodium hypochlorite) with 0.2% 'Tween-20' for 15 minutes for surface sterilization. Finally, they were rinsed three times with sterile distilled water. Both kinds of explants were cut into 1-2 cm pieces under sterile conditions and subsequently cultured on different nutrient media.

Culture establishment

The explants were cultured on three different culture media: LP (Quoirin and Lepoivre, 1977), woody plant medium [WPM] (Lloyd and McCown, 1980) and MS (Murashige and Skoog, 1962), that were supplemented with benzyladenine (BA) at different concentrations (0, 0.63, 1.25, 2.5, 5 µM). Then, the cultures were placed in a growth chamber at 25±2°C under a 16 h photoperiod provided by cool white fluorescent lamps (30 µm m^{-2} s^{-1}) for 6 weeks. At the end, the number and length of proliferated shoots were measured.

Experiment 2. Plant materials and aseptic culture

This experiment consisted of two phases. In the first phase, the effect of different growth regulators on axillary shoot proliferation of four genotypes was

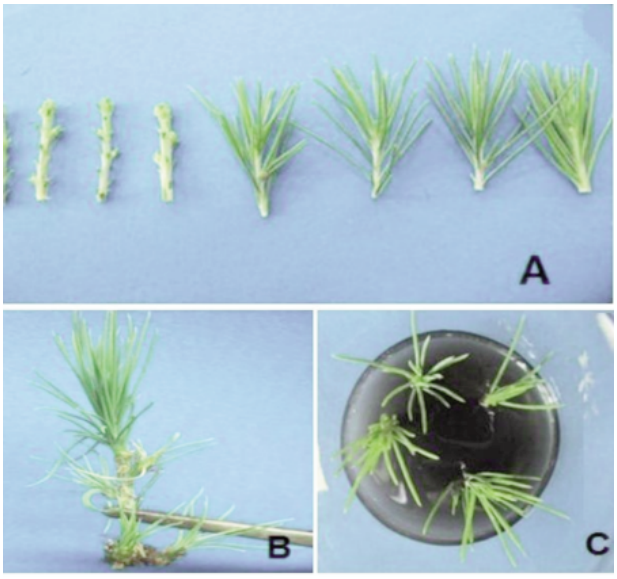

Fig. 1 - *In vitro* proliferation of *Cedrus deodara* (Roxb. ex Lamb) G. Don. (A) Different types of explants: explants retained their needles (leaves) and, defoliated explants; (B) Axillary shoot proliferation on WPM medium containing 0.8 µM TDZ and 2 µM NAA (after 4 weeks); (C) Elongation of axillary bud on EM medium after 90 days.

studied. In the second phase, axillary shoot multiplication of genotype CD1 was studied. To examine the effect of genotype on proliferation rate, 110 to 130 actively growing shoots with 4-6 cm length were collected from four mature 20-25 year-old *C. deodara* trees (genotypes CD1 to CD4) in a seed orchard near the School of Agriculture, Shiraz University, Iran. The shoots were defoliated, cut into 1-2 cm in length pieces and used as initial explants (n=114 per genotype). Surface sterilization was carried out in the same manner as in experiment 1. For culture establishment, the explants were cultured on WPM medium supplemented with growth regulators [BA, NAA and thidiazuron (TDZ)] for shoot induction and proliferation. The medium was supplemented with BA (0, 2.5, 5, 10, 20 µM) and TDZ (0, 0.4, 0.8, 1.6 µM) alone or in combination with NAA (1, 2, 3 µM). The effect of TDZ and BA on shoot proliferation of four genotypes (CD1 to CD4) was investigated. The conditions, under which the cultures were incubated, were same to those of experiment 1.

Elongation of induced shoots

Axillary shoots formed on explants (from genotype CD1) were isolated and transferred to elongation media (EM). The EM was growth regulator-free half strength WPM, and it was supplemented with 3 g L^{-1} activated charcoal (AC), 15 g L^{-1} sucrose and 8 g L^{-1} agar. The explants were maintained in the culture medium, and then subcultured into glass jars (150 ml) containing 40 ml of the fresh EM. The environmental conditions were same as that in culture establishment. The subculturing procedure was repeated every 4 weeks for 3 months. The culture conditions (temperature and light) were same as those used for culture establishment experiment.

Shoot multiplication

After 3 month, the shoots grown on EM were cut to the same length and then transferred to shoot multiplication medium containing TDZ (0, 0.1, 0.2, 0.4, 0.8 µM) and 2iP (0, 0.1, 0.2, 0.3, 0.4, 0.5 µM) in combination with 2.5 µM BA for 6 weeks.

Statistical analysis

All experiments were conducted as factorial based on a completely randomized design with four replications, each replicate comprised of four jam glasses, four explants per glass. All experiments were repeated twice. Shoot proliferation rate and shoot length were recorded at the end of the fourth week. SPSS statistical software was used for analyzing data, and the one-way ANOVA with Tukey's test (*P<0.05*) was used for comparing means. Three-way ANOVA was applied to examine the interactions of TDZ, BA and genotype.

3. Results

Experiment 1

The effects of different culture media (LP, MS and WPM) on two types of explants were investigated. The defoliated explants showed the best results, and there was a significant difference for shoot proliferation between defoliated and leafy explants, in all three culture media. The lowest frequency in shoot proliferation belonged to the leafy explants in MS medium (0.06). According to the results, WPM medium showed the highest number and length of proliferated shoots, but without significant difference as compared with LP. Moreover, leafless explants produced higher and longer proliferated shoots than leafy explants. There was no significant difference between different amounts of BA on length and number of proliferated shoots (data not shown).

Experiment 2

When all four genotypes (CD1, CD2, CD3, CD4) were grown at different levels of cytokinin treatments, shoot proliferation from leafless explants was observed. The highest mean number of shoots per explant was observed at 0.8 µM thidiazuron for genotypes CD1 (4.6), CD2 (4.4), CD3 (2.4), and at 0.4 µM thidiazuron for CD4 (2.4) (Fig. 2). Genotypes CD1 and CD2 were the most responsive genotypes over all cytokinin treatments. Furthermore, the highest

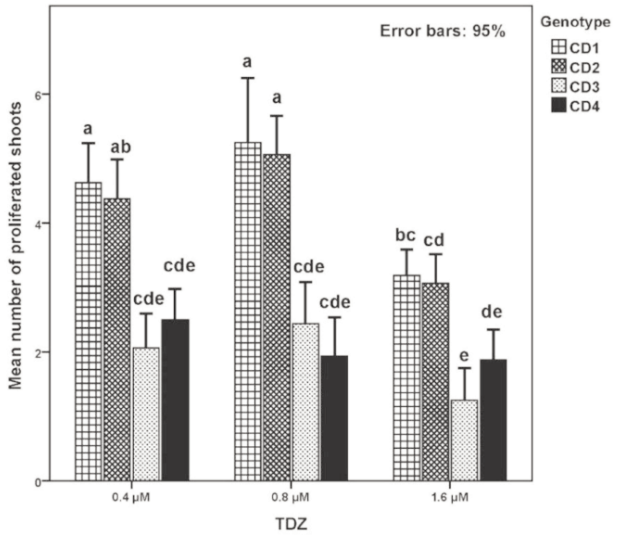

Fig. 2 - Effect of TDZ concentrations on shoot proliferation of four genotypes of *Cedrus deodara*. Bars with the same letters indicate no significant difference at Tukey test (P=0.05).

mean number of axillary shoots per explant of all genotypes (4.31) was obtained when 0.8 µM TDZ was used in combination with 2 µM NAA and the highest mean length of axillary shoots of all genotypes (11.2 mm) was obtained when 0.8 µM TDZ was applied alone (Tables 1, 2, Fig. 1 b). NAA had a positive effect on quality attributes (like color, vitality, and size). However, increasing the concentration of NAA to 3 µM reduced the number and length of proliferated shoots (Tables 1, 2). Then, axillary shoot multiplication of genotype CD1 was studied (Fig. 3). The highest mean number of axillary shoots per explant (4.13) and mean length of axillary shoots (10.38 mm) were obtained when 0.8 µM TDZ was applied (Table 3).

The best result of shoot proliferation obtained for CD1 genotype on WPM medium supplemented with 0.8 µM TDZ (Fig. 2). The same result was obtained for CD1 genotype on WPM medium supplemented with 2.5 µM BA (Fig. 4).

4. Discussion and Conclusions

Propagation of conifers from mature explants has always been difficult. In general, most studies on the induction of embryogenesis and/or organogenesis in conifers includes the culture of seed or zygotic tis-

Table 1 - Effects of different concentrations of TDZ, BA and NAA on the number of proliferated shoots in *Cedrus deodara*

Treatments	NAA				Mean values
	0	1	2	3	
Control	2.41± 0.02 lm†	2.16±0.02 pq	2.31±0.05 mno	1.97±0.01 r	2.21 F
TDZ (µM)					
0.4	3.75±0.00 b	3.31±0.03 e	3.44±0.03 de	3.06±0.03 f	3.39 B
0.8	3.38±0.03 de	3.50±0.00 cd	4.31±0.03 a	3.63±0.03 bc	3.70 A
1.6	2.25±0.00 nop	2.50±0.00 jkl	2.63±0.03 ij	2.00±0.00 r	2.34 EF
BA (µM)					
2.5	2.69±0.03 hi	3.06±0.03 f	3.40±0.03 de	2.50±0.00 jkl	2.91 C
5	2.38±0.03 lmn	2.44±0.03 klm	2.44±0.03 klm	2.56±0.03 ijk	2.45 E
10	2.44±0.03 klm	2.88±0.03 g	2.81±0.03 gh	2.31±0.03 mno	2.61 D
20	2.25±0.00 nop	2.38±0.03 lmn	2.19±0.03 opq	2.06±0.03 qr	2.22 F
Mean values	2.66 B	2.71 AB	2.87 A	2.45 C	2.67

Means with the same letters (small letters for interactions and capital letters for main effects) indicate no significant difference at Tukey's test (P=0.05).

Table 2 - Effects of different concentrations of TDZ, BA and NAA on the length of proliferated shoots in *Cedrus deodara*

Treatments	NAA				Mean values
	0	1	2	3	
Control					
0.0	5.80±0.06 h	5.55±0.06 hij	4.88±0.04 klm	4.50±0.09 mn	5.18 D
TDZ (µM)					
0.4	8.75±0.05 c	6.92±0.01 fg	8.12±0.09 d	7.12±0.12 fg	7.73 B
0.8	11.20±0.14 a	7.75±0.07 de	9.56±0.05 b	6.81±0.22 g	8.83 A
1.6	4.96±0.07 klm	5.24±0.08 ijk	5.20±0.08 i-l	4.67±0.02 lm	5.02 D
BA (µM)					
2.5	6.82±0.16 g	7.32±0.10 efg	5.57±0.10 hij	5.08±0.08 jkl	6.20 C
5	7.15±0.19 fg	7.43±0.10 ef	5.93±0.11 h	5.18±0.15 i-l	6.42 C
10	5.65±0.16 hi	5.93±0.08 h	4.07±0.09 no	3.12±0.08 q	4.69 D
20	3.44±0.12 pq	3.84±0.17 op	3.90±0.13 op	2.16±0.07 r	3.34 E
Mean values	6.62 A	6.17 AB	5.79 B	4.79 C	5.84

Means with the same letters (small letters for interactions and capital letters for main effects) indicate no significant difference at Tukey's test (P=0.05).

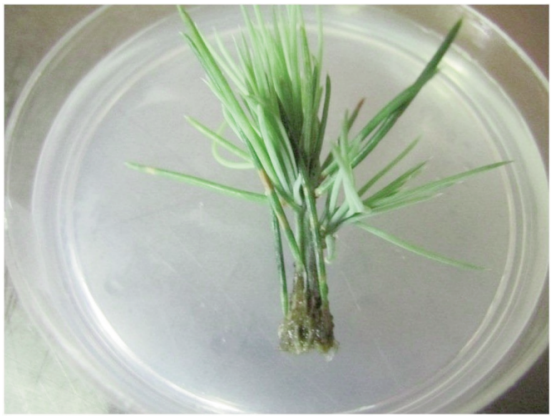

Fig. 3 - Multiple shoot formation on subculture of *Cedrus deodara* on WPM with 0.8 µM TDZ in combination with 2.5 µM BA.

Table 3 - Effects of different concentrations of TDZ and 2iP in combination with 2.5 µM BA on shoot multiplication of *Cedrus deodara*

Treatments	Number of proliferated shoots	Length
Control		
0.0	2.44± 0.07 e	6.69± 0.12 e
TDZ (µM)		
0.1	3.38± 0.16 bc	7.26± 0.16 de
0.2	3.44± 0.21 bc	7.80± 0.27 cd
0.4	3.88± 0.16 ab	9.93± 0.24 a
0.8	4.13± 0.13 a	10.38± 0.11 a
2iP (µM)		
0.1	2.50± 0.20 e	6.58± 0.11 e
0.2	2.69± 0.12 de	7.38± 0.18 de
0.3	3.00± 0.10 cde	7.93± 0.21 cd
0.4	3.25± 0.18 bcd	8.38± 0.15 bc
0.5	3.56± 0.12 abc	8.88± 0.14 b

In each column, means with the same letters indicate no significant difference at Tukey's test (P=0.05).

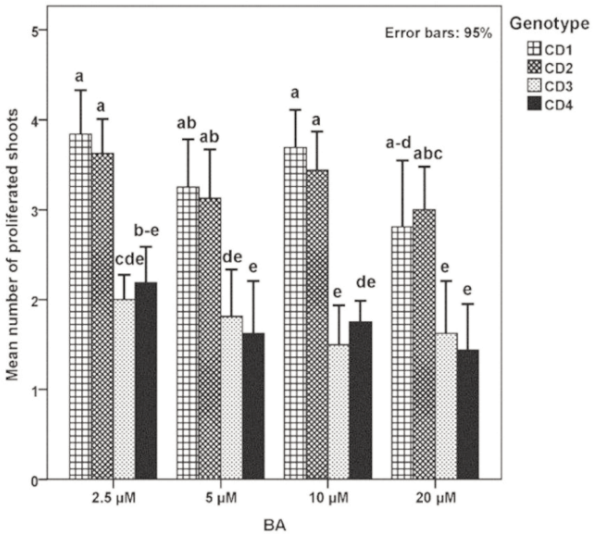

Fig. 4 - Effect of BA concentrations on shoot proliferation of four genotypes of *Cedrus deodara*. Bars with the same letters indicate no significant difference at Tukey test (P=0.05).

sues (Tang *et al.*, 2006; Humánez *et al.*, 2011). To our best knowledge, this is the first report of successful induction of axillary shoots on explants taken from adult *C. deodara.* There have been few reports on the effect of different kinds of basal media on the axillary shoot proliferation in cultures of explants from mature conifer trees (Andersone and Ievinsh, 2002; De Diego *et al.*, 2010; Renau-Morata *et al.*, 2005). In the present investigation, shoot proliferation rates were generally greater on basal WPM than on basal LP and MS media. Shoot buds cultured on MS medium presented the lowest organogenic response, and this was perhaps as a result of the comparatively high nitrate concentration as compared to the LP medium or WPM. Tuskan *et al.* (1990) showed that the extra nitrate had a negative effect on the organogenic response during micropropagation. Therefore, low nitrogen content of the medium is a major factor for promoting organogenesis in conifer species (Tang *et al.*, 2001; Schestibratov *et al.*, 2003). Explant type was an important factor affecting axillary bud proliferation in *in vitro* culture. Piola *et al.* (1998) demonstrated that the accumulation of ABA in needles seems to be the major cause of bud dormancy in micropropagation of *C. libani*. In this experiment, defoliated explants of *C. deodara* also showed better response on proliferation medium.

In *in vitro* bicentennial cedar micropropagation, it was found that the accomplishment of the protocol depends on genotype (Renau-Morata *et al.*, 2005). Among two cytokinin treatments examined, TDZ induced the highest number of proliferated shoots for all genotypes. After investigating the effect of three TDZ concentrations on shoot proliferation of four genotypes, the significant difference for their interaction was found. However, in our investigation, BA showed no significant genotypic differences for shoot proliferation. TDZ has gotten more attention in recent years due to its ability to assist *in vitro* regeneration of conifers (Mathur and Nadgauda, 1999; Sul and Korban, 2004; Renau-Morata *et al.*, 2005; Tang and Newton, 2005; Cortizo *et al.*, 2009; De Diego *et al.*, 2010; Humánez *et al.*, 2011). TDZ can decrease the enzyme activity related to oxidative stress during formation of adventitious shoots (Tang and Newton 2005). Recent reports on mature stone pine displayed the superiority of TDZ over other cytokinins in advancing axillary shoot proliferation (Cortizo *et al.*, 2009). It was shown that high concentrations of cytokinin in the medium, particularly BA led to the low regeneration response, which may be attributed to the toxic effects of high concentrations of cytokinins (Sarmast *et al.*, 2012). In this report, the presence of NAA with either TDZ or BA improved the incidence of shoot organogenesis in adult tissues of *C. deodara*. This has been also observed in other conifer species (Sul and Korban, 2004; Zhu *et al.*, 2010).

As this study shows, the type of explants and culture media has a very important role in success of culture establishment of *Cedrus deodara*. It was proven that defoliation of the explants has positive effect on shoot proliferation. The effects of genotype and growth regulators on proliferation of axillary

shoots have been studied and low concentration of TDZ combined with NAA has positive effects on the number of proliferated axillary shoots. Furthermore, two of the four genotypes showed better response to cytokinins.

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In vitro activity of some essential oils against *Penicillium digitatum*

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Key words: cinnamon, citrus, decay, postharvest, savory, summer.

Abstract: Natural plant essential oils (EOs) can be used instead of synthetic fungicides because of human health concerns and environmental protection. In this study, the *in vitro* activity of some plants EOs against *Penicillium digitatum*, the cause of citrus green mold was evaluated during 8 days of incubation at 25°C. The EOs extracted from sweet orange (*Citrus sinensis*), lemon (*Citrus limon)*, lime (*Citrus aurantifolia*), and sour orange (*Citrus aurantium*) fruit peel (500, 1000 and 2000 µl l^{-1} concentrations), cinnamon (*Cinnamomum cassia*) bark and summer savory (*Satureja hortensis*) aerial parts (400, 500 and 600 µl l^{-1} concentrations) were used on *Penicillium digitatum* mycelium. None of the EOs extracted from tested citrus in this study could inhibit mycelial growth completely even at concentration of 2000 µl l^{-1}. The best results were obtained with cinnamon and summer savory EOs at concentration of 500 and 600 µl l^{-1}. So, based on the results, cinnamon and summer savory EOs can be ideal candidates to replace the synthetic fungicides to control postharvest green mold of citrus fruit. GC-MS analysis showed that the most abundant of all constituents in EO extracts were carvacrol and γ-terpinene in summer savory and (E)-cinnamaldehyde in cinnamon.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

Citrus spp. are the most important produced fruits in the world (Sharma and Saxena, 2004), due to their good taste, useful nutrients, and widespread availability (Liu *et al*., 2012). Nevertheless, the high water content and nutrient composition make them also very susceptible to decay by pathogens after harvest (Tripathi and Dubey, 2004). One of the most common diseases that infects citrus fruit is green mold caused by *Penicillium digitatum* (Zheng *et al*., 2005). The yield losses and the worsening of the quality caused by the fungus are economically important. This pathogen infects the fruit through wounds on the peel inflicted during harvest, transportation, handling or commercialization. *Penicillium digitatum* is one of the most important pathogen in citrus industry, because one generation of green mold complete during 7-10 days in rotten fruit at 20-25°C, and the large amounts of spores are disseminated easily by air currents (Palou, 2014).

Currently, the use of synthetic fungicides is the primary and most sim-

ple method for the control of postharvest diseases of citrus fruit (Palou *et al*., 2008). However, fungicides consumption is strongly becoming restricted because of residual toxicity, carcinogenicity, long degradation period and increasing human health concerns (Tripathi and Dubey, 2004; Palou *et al*., 2008).

Recently, researchers have been interested in development of alternative methods to manage postharvest decay. The essential oils (EOs) are one of non-chemical and useful control options for the management of fungal postharvest diseases (Sassi *et al*., 2008). Essential oils are complex compounds that are natural and environmentally friendly, having antioxidant, antimicrobial and medicinal properties (Bakkali *et al*., 2008). So, they can be ideal candidates to replace synthetic antimicrobials for maintenance of harvested horticultural crops (Tripathi and Dubey, 2004).

Many studies reported the beneficial effects of EO treatments for the control of postharvest decay caused by *P. digitatum*, such as *Thymus vulgaris* at concentration of 1000 ppm (Fatemi *et al.*, 2012), *Mentha spicata* and *Lippia scaberrima* at concentrations of 1000 and 3000 µl l^{-1}, respectively (Du Plooy *et al*., 2009), *Bubonium imbricatum* at concentration of 1000 ppm (Alilou *et al*., 2008), *Citrus* spp. at concentration of 10% (Badawy *et al*., 2011), and *Cinnamomum zeylanicum* at concentration of 0.5% (Kouassi *et al*., 2012), thereby enhancing shelf life of fruits and vegetables.

The purpose of this study was to investigate the *in vitro* activity of EOs obtained from sweet orange (*Citrus sinensis*), lemon (*Citrus limon)*, lime (*Citrus aurantifolia*), and sour orange (*Citrus aurantium*) fruit peel, cinnamon (*Cinnamomum cassia*) bark and summer savory (*Satureja hortensis*) aerial parts for the control of green mold caused by *P. digitatum* as a preliminary study to find a suitable and effective EO as alternative to synthetic fungicides to control green mold in citrus postharvest management.

2. Materials and Methods

Extraction of essential oils

Plant materials used in this study are shown in Table 1. The air-dried plants material (300 gr) were cut into pieces, grounded into powder by blender, then the EOs extracted through hydro-distillation for 3-4 hours using a clevenger apparatus (Miquel *et al*., 1976). Then the EOs were dehydrated with anhydrous sodium sulfate and stored in dark bottles at -20°C before using for antifungal study.

Isolation of fungus

The fungus used throughout this study was *P. digitatum*, the cause of citrus green mold. For isolation of fungus colony, *P. digitatum* spores were isolated from a decayed orange and cultured on potato dextrose agar (PDA) by the single spore procedure at 25°C. The isolates were maintained on PDA until needed.

In vitro antifungal assay

The antifungal assay was performed on PDA plates amended with three concentrations (500, 1000 and 2000 µl l^{-1}) of sweet orange, lemon, lime and sour orange EOs and three concentrations (400, 500 and 600 µl l^{-1}) of cinnamon and summer savory EOs. Tween 80 (Merck-KGaA, Germany) as an emulsifier was mixed with 80 ml of sterilized and molten PDA media, cooled to about 45°C, and then enriched with EOs. There were four 80 mm plates/replicates per treatment. After one day, the mycelia of *P. digitatum* from 4-days-old cultures were put in the center of amended PDA petri plates with a cork borer. All of the plates were sealed with parafilm. Inoculated plates were kept at 25°C for 8 days. Colony diameter was determined daily by measuring the average radial growth (Obagwu and Korsten, 2003). In order to evaluate its effect on fungal growth, tween 80 (emulsifier) was also considered as a treatment in the experiment.

Table 1 - Plant materials used for EOs extraction

Name	Family	Used part	Origin
Sweet orange (*Citrus sinensis* cv. Thomson navel)	Rutaceae	Fruit rind tissue (flavedo and albedo)	Fars-Iran
Lemon (*Citrus limon* cv. Lisbon)	Rutaceae	Fruit rind tissue (flavedo and albedo)	Fars-Iran
Lime (*Citrus aurantifolia* cv. Mexican lime)	Rutaceae	Fruit rind tissue (flavedo and albedo)	Fars-Iran
Sour orange (*Citrus aurantium* cv. amara)	Rutaceae	Fruit rind tissue (flavedo and albedo)	Fars-Iran
Cinnamon (*Cinnamomum cassia*)	Lauraceae	Tree bark	China
Summer savory (*Satureja hortensis*)	Lamiaceae	Aerial parts	Fars-Iran

Inhibition percentage (IP) of fungal growth was calculated as the radial growth of treated fungus (T) relative to the growth in control (C) treatment (plates without EO and Tween 80) according to the following formula:

$$IP\ (\%) = (\frac{C-T}{C}) \times 100$$

Essential oils analysis

At the end of the study, the main components of the most effective EOs on *P. digitatum* were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The GC analysis was carried out by the use of Agilent GC (7890-A, PerkinElmer, USA) and a flame ionization detector. It was done on fused silica capillary HP-5 column. The temperatures of injector and detector were held at 250°C and 280°C, respectively. Nitrogen was selected as carrier gas; oven temperature was 60-210°C at a rate of 4°C/min, which was then increased to 240°C at a rate of 20°C/min, and finally, kept for 8.5 min.

The GC-MS analysis was performed using an Agilent GC series 7890-A (PerkinElmer, USA) with a fused silica capillary HP-5MS column and 5975-C mass spectrometer (UNICO, USA). Carrier gas was helium. Ion source and interface temperatures were set at 230°C and 280°C, respectively. Mass range was programmed from 45 to 550 amu. Oven temperature was 60-210°C at a rate of 4°C/min. N-alkanes was used as a standard to determine the retention indices for all constituents. The constituents were recognized by comparing their retention indices with literature reports, and their mass spectra comparison with the Wiley, Adams and Mass Finder 2.1 Library data (Adams, 1997).

Statistical analysis

The experiment was distributed according to a split plot in time design. The analysis of variance (ANOVA) was performed. Mean comparisons were conducted by LSD (least significant difference) at P≤ 0.01. Data were analyzed by SAS software (v. 9.1).

3. Results and Discussion

Inhibitory effects of different treatments on Penicillium digitatum growth

The *in vitro* activity of the tested EOs on colony diameter of *P. digitatum* during 8 days of incubation is summarized in Table 2.

Our results indicated that colony radial growth of *P. digitatum* was inhibited completely (100%) under *in vitro* condition by both cinnamon and summer savory EOs at 500 and 600 µl l^{-1} concentrations during 8 days of incubation. Also, the mycelial growth

Table 2 - Inhibition percentage (%) of plant essential oils on *in vitro* radial growth of *Penicillium digitatum*

Treatment	EO Concentration (µl l^{-1})	Time (day)							
		1	2	3	4	5	6	7	8
Control	-	0.00 ^{g} * $_{S}$ ^	0.00 ^{g} $_{S}$	0.00 ^{h} $_{S}$	0.00 ^{g} $_{S}$	0.00 ^{e} $_{S}$	0.00 ^{g} $_{S}$	0.00 ^{e} $_{S}$	0.00 ^{e} $_{S}$
Tween 80	-	0.80 ^{g} $_{Q\text{-}S}$	0.43 ^{g} $_{RS}$	0.38 ^{h} $_{RS}$	1.29 ^{g} $_{P\text{-}S}$	1.24 ^{e} $_{P\text{-}S}$	5.09 ^{fg} $_{N\text{-}S}$	7.08 ^{e} $_{L\text{-}S}$	8.13 d ^{e} $_{K\text{-}S}$
Sweet orange	500	22.42 ^{f} $_{W\text{-}i}$	5.81 ^{fg} $_{L\text{-}S}$	10.34 ^{gh} $_{I\text{-}S}$	12.73 ^{fg} $_{D\text{-}Q}$	6.59 ^{e} $_{L\text{-}S}$	9.37 ^{fg} $_{J\text{-}S}$	6.55 ^{e} $_{L\text{-}S}$	0.00 ^{e} $_{S}$
Sweet orange	1000	23.81 ^{f} $_{W\text{-}g}$	5.75 ^{fg} $_{M\text{-}S}$	18.02 $^{e\text{-}g}$ $_{B\text{-}K}$	23.07 ^{ef} $_{W\text{-}h}$	12.56 ^{e} $_{D\text{-}R}$	16.26 $^{e\text{-}g}$ $_{C\text{-}N}$	11.55 ^{e} $_{G\text{-}S}$	0.00 ^{e} $_{S}$
Sweet orange	2000	75.80 ^{b} $_{B}$	54.74 ^{b} $_{E\text{-}I}$	48.24 ^{c} $_{I\text{-}O}$	50.72 ^{c} $_{G\text{-}K}$	32.09 ^{cd} $_{R\text{-}b}$	38.42 ^{cd} $_{L\text{-}S}$	41.76 ^{c} $_{J\text{-}S}$	36.56 ^{bc} $_{O\text{-}V}$
Lemon	500	32.81 ^{ef} $_{R\text{-}a}$	17.41 $^{d\text{-}g}$ $_{C\text{-}M}$	20.79 $^{e\text{-}g}$ $_{Z\text{-}j}$	23.18 ^{ef} $_{W\text{-}h}$	13.03 ^{e} $_{D\text{-}Q}$	12.79 ^{fg} $_{D\text{-}Q}$	12.19 ^{e} $_{F\text{-}S}$	0.00 ^{e} S
Lemon	1000	46.91 $^{c\text{-}e}$ $_{L\text{-}P}$	24.46 $^{c\text{-}f}$ $_{V\text{-}E}$	38.18 ^{cd} $_{M\text{-}S}$	41.15 ^{cd} $_{J\text{-}S}$	17.42 $^{c\text{-}e}$ $_{C\text{-}M}$	22.27 $^{d\text{-}f}$ $_{X\text{-}i}$	20.14 ^{de} $_{B\text{-}K}$	12.50 $^{c\text{-}e}$ $_{E\text{-}R}$
Lemon	2000	61.38 $^{b\text{-}d}$ $_{D\text{-}H}$	23.54 $^{c\text{-}f}$ $_{W\text{-}H}$	45.84 ^{c} $_{I\text{-}Q}$	50.87 ^{c} $_{G\text{-}K}$	34.39 ^{c} $_{Q\text{-}X}$	44.26 ^{c} $_{I\text{-}R}$	34.66 ^{cd} $_{P\text{-}W}$	25.31 $^{b\text{-}e}$ $_{T\text{-}c}$
Lime	500	36.98 ^{ef} $_{M\text{-}U}$	20.71 $^{d\text{-}f}$ $_{A\text{-}I}$	21.95 $^{e\text{-}g}$ $_{Y\text{-}i}$	17.98 ^{f} $_{C\text{-}M}$	15.74 ^{de} $_{C\text{-}N}$	16.98 $^{e\text{-}g}$ $_{C\text{-}N}$	17.25 ^{de} $_{C\text{-}N}$	0.00 ^{e} $_{S}$
Lime	1000	54.54 $^{b\text{-}e}$ $_{E\text{-}I}$	30.49 $^{c\text{-}e}$ $_{S\text{-}b}$	44.03 ^{c} $_{I\text{-}R}$	48.19 ^{c} $_{I\text{-}O}$	30.72 ^{cd} $_{S\text{-}B}$	33.27 $^{c\text{-}e}$ $_{R\text{-}Y}$	33.22 ^{cd} $_{R\text{-}Y}$	30.85 $^{b\text{-}d}$ $_{S\text{-}b}$
Lime	2000	70.43 ^{bc} $_{B\text{-}D}$	42.48 ^{bc} $_{I\text{-}S}$	49.22 ^{c} $_{H\text{-}M}$	50.62 ^{c} $_{G\text{-}L}$	34.11 ^{c} $_{Q\text{-}Y}$	41.72 ^{c} $_{J\text{-}S}$	40.39 ^{c} $_{K\text{-}S}$	32.50 $^{b\text{-}d}$ $_{R\text{-}a}$
Sour orange	500	48.92 $^{c\text{-}e}$ $_{I\text{-}N}$	36.82 $^{b\text{-}d}$ $_{N\text{-}U}$	14.64 ^{fg} $_{C\text{-}O}$	12.61 ^{fg} $_{D\text{-}R}$	11.36 ^{e} $_{H\text{-}S}$	16.16 $^{e\text{-}g}$ $_{c\text{-}n}$	17.89 ^{de} $_{C\text{-}M}$	0.00 ^{e} $_{S}$
Sour orange	1000	50.94 $^{c\text{-}e}$ $_{G\text{-}K}$	24.00 $^{c\text{-}f}$ $_{W\text{-}f}$	30.77 ^{de} $_{S\text{-}b}$	33.67 ^{de} $_{Q\text{-}Y}$	13.36 ^{e} $_{C\text{-}P}$	15.97 $^{e\text{-}g}$ $_{C\text{-}N}$	14.85 ^{de} $_{C\text{-}O}$	9.37 ^{de} $_{J\text{-}S}$
Sour orange	2000	61.44 $^{b\text{-}d}$ $_{D\text{-}H}$	33.01 $^{c\text{-}e}$ $_{R\text{-}Z}$	44.12 ^{c} $_{I\text{-}R}$	53.39 ^{c} $_{F\text{-}J}$	33.12 ^{c} $_{R\text{-}Y}$	37.28 ^{cd} $_{M\text{-}T}$	41.08 ^{c} $_{J\text{-}S}$	40.94 ^{b} $_{K\text{-}S}$
Cinnamon	400	40.52 $^{d\text{-}f}$ $_{K\text{-}S}$	12.63 $^{e\text{-}g}$ $_{D\text{-}R}$	24.77 ^{ef} $_{U\text{-}d}$	22.22 ^{ef} $_{X\text{-}I}$	3.34 ^{e} $_{O\text{-}S}$	6.48 ^{fg} $_{L\text{-}S}$	9.40 ^{e} $_{J\text{-}S}$	3.44 ^{e} $_{O\text{-}S}$
Cinnamon	500	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$
Cinnamon	600	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$
Savory	400	100.00 ^{a} $_{A}$	89.61 ^{a} $_{A}$	74.01 ^{b} $_{BC}$	70.91 ^{b} $_{B\text{-}D}$	66.01 ^{b} $_{B\text{-}E}$	65.25 ^{b} $_{B\text{-}F}$	61.83 ^{b} $_{C\text{-}G}$	51.25 ^{b} $_{G\text{-}K}$
Savory	500	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.0 0 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$
Savory	600	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$	100.00 ^{a} $_{A}$

* For each column, similar letters (lower case, superscript) are not significantly different according to LSD (P≤0.01) test.

^ Means followed by similar letters (subscript), are not significantly different according to LSD (P≤0.01) test.

was decreased by cinnamon and summer savory EOs at concentrations lower than 500 µl l^{-1}, but it was not suppressed completely.

The main activity of the EOs in the postharvest fruit are derived from their ability to inhibit pathogen growth (Periago *et al*., 2004). Cinnamon EO has the potential to be employed as a natural antifungal agent for fruit disinfectation, as cinnamaldehyde is its main constituent (Xing *et al*., 2010). Furthermore, it has been reported that summer savory contains some substances with antibacterial properties (Deans and Svoboda, 1989). In this research, cinnamon and summer savory EOs have the strongest effect on *P. digitatum* growth (Table 2). It has been reported that eucalyptus and cinnamon (*Cinnamomum zeylanicum*, Blume) oil vapour (500 ppm) reduced decay almost by 50% in tomatoes after 10 days of storage (Tzortzakis, 2007). Moreover, Win *et al*. (2007) presented that EOs from cinnamon at the concentration of 5.0 g l^{-1} completely inhibited conidial germination and mycelial growth of all fungi on banana (*Colletotrichum musae*, *Fusarium* spp. and *Lasiodiplodia theobromae*). In addition, Lopez-Reyes *et al*. (2010) showed that summer savory, oregano and thyme EOs at 10% showed significant inhibitory effect (similar to chemical control) against *P. expansum* and *Botrytis cinerea* on four cultivars of apples.

The mechanism by which EOs suppress the microbial growth is not fully understood, but a number of possible explanations have been postulated. Essential oils are lipophilic and this property enables them to preferentially move from an aqueous phase into fungi membrane. This action leads to membrane expansion, increasing in membrane fluidity and permeability, membrane proteins disorder, respiration rate control, change of ion transportation in fungi and induced cellular contents leakage (Burt, 2004; Oonmetta-Aree *et al*., 2006; Khan *et al*., 2010; Fadli *et al*., 2012).

In the present study, the lowest inhibition was observed in control plates that contained only PDA (0%); however, this was not significantly different from plates containing PDA and the tween 80 without the EOs during 8 days. So, results indicated that the tween 80 used as an emulsifier had no effect on the mycelial growth (Table 2).

We observed an increase of antifungal effects of the tested citrus fruits peel EOs such as sweet orange, lemon, lime and sour orange as the EOs concentration increased, but the fungi growth was not inhibited completely even at concentration of 2000 µl l^{-1}. So, as the results showed, none of the tested concentrations of citrus fruits EOs in this study could inhibit radial growth completely (Table 2).

Essential oils are present in great quantities in the flavedo of citrus fruit (Caccioni *et al*., 1998). The citrus fruits EO consists a mixture of components such as terpenes, hydrocarbons, ketones, aldehydes, alcohols, acids, and esters. The amount of them depends on the citrus cultivar, the extraction and separation techniques (Fisher and Phillips, 2008).

The positive effect of the volatile components of citrus fruit essential oils on *P. digitatum* and *italicum* growth has been reported (Caccioni *et al*., 1998). The spore germination and mycelium growth of *P. italicum* and *digitatum* were stimulated by the essential oil of *Citrus reticulata* Blanco at concentration of more than 2.5 µl ml^{-1} (Wang *et al*., 2012). Moreover, Badawy *et al*. (2011) reported that *Citrus aurantifolia* EOs had the antifungal effects against *P. digitatum* pathogens at concentration of 10% (v/v). However, in our study the application of *Citrus* spp. could not provide acceptable control of green mold disease.

Analysis of the summer savory and cinnamon EOs

The analysis of the volatile profiles in summer savory and cinnamon EOs are listed in Table 3 and 4,

Table 3 - Chemical composition of the summer savory essential oil

Number	Component	RI*	(%)
1	α- Thujene	924	1.15
2	α-Pinene	932	0.64
3	Camphene	946	0.06
4	Hepten-1-ol	958	0.05
5	Sabinene	969	0.01
6	β-Pinene	974	0.21
7	3- Myrcene	988	1.15
8	Phellandrene	1002	0.23
9	α-Terpinene	1014	3.75
10	p-Cymene	1020	2.19
11	Sylvestrene	1025	0.37
12	E-β- Ocimene	1044	0.07
13	γ-Terpinene	1054	31.98
14	Terpinolene	1086	0.05
15	trans-α Sabinene hydrate	1098	0.07
16	Isoborneol	1155	0.06
17	Terpinene-4-ol	1174	0.2
18	α-Terpineol	1186	0.1
19	carvacrol methyl ether	1241	0.09
20	Thymol	1289	0.8
21	Carvacrol	1298	55.66
22	Thymol acetate	1349	0.03
23	Carvacrol acetate	1370	0.07
24	Caryophyllene	1417	0.36
25	Aromadendrene	1439	0.08
26	α–Humulene	1454	0.01
27	Bicyclogermacrene	1500	0.18
28	Bisabolene	1505	0.21
29	Unknown	-	0.01
30	Spathulenol	1577	0.04

* Retention index

Table 4 - Chemical composition of the cinnamon essential oil

Number	Component	RI*	(%)
1	α-Pinene	932	0.57
2	Camphene	946	0.34
3	Benzaldehyde	958	0.49
4	β -Pinene	975	0.16
5	α-Phellandrene	1004	0.02
6	p-Cymene	1023	0.07
7	Limonene	1026	0.13
8	1,8-Cineole	1029	0.07
9	γ-Terpinene	1056	0.04
10	Benzenepropanal	1160	0.26
11	Borneol	1163	0.19
12	α-Terpineol	1189	0.03
13	(Z)-Cinnamaldehyde	1217	0.73
14	(E)-Cinnamaldehyde	1271	70.04
15	Unknown	1333	0.06
16	Cyclosativene	1367	0.59
17	α-Copaene	1373	10.82
18	Unknown	1387	0.18
19	β-Elemene	1389	0.14
20	Sativene	1393	0.44
21	(E)-Caryophyllene	1416	0.20
22	β-Gurjunene	1426	0.06
23	α -Humulene	1450	0.20
24	γ-Muurolene	1474	1.25
25	ar-Curcumene	1480	0.15
26	Viridiflorene	1492	0.28
27	α -Muurolene	1497	4.23
28	β-Bisabolene	1506	0.15
29	γ-Cadinene	1511	0.26
30	δ-Cadinene	1521	5.35
31	(E)-ortho-Methoxy cinnamaldehyde	1528	0.27
32	trans-Cadina-1(2),4-diene	1529	0.96
33	α -Calacorene	1540	0.50
34	epi-a-Muurolol	1639	0.40
35	α -Muurolol	1643	0.20
36	α -Cadinol	1651	0.03
37	Cadalene	1671	0.10

* Retention index

respectively. A total of 30 different components of summer savory, and 37 components of cinnamon were identified and isolated by GC and GC-MS from the EOs. The principal components of the summer savory EO were carvacrol (55.66%), γ-terpinene (31.98%), α-terpinene (3.75%), p-cymene (2.19%), 3-myrcene (1.15%), and α-thujene (1.15%). The major components of the cinnamon EO were (E)-cinnamaldehyde (70.04%), α-copaene (10.82%), δ-cadinene (5.35%), α-muurolene (4.23%), and γ-muurolene (1.25%). Other constituents which were less than 1% have been shown in Table 3 and 4.

As shown in Table 2, both summer savory and cinnamon EOs were equally effective in inhibiting the growth of *P. digitatum*. This is in accord with the reported in vitro inhibitory effect of carvacrol against pathogens (Periago *et al*., 2004). In fact, the main component of summer savory EO is a phenol (Sacchetti *et al*., 2005), and its most important mechanism of antimicrobial activity is connected with the phenolic ring in its chemical structure (Ultee *et al*., 2002). Furthermore, it has been reported that the toxicity rate of the phenol ring is due to the site (s) and number of hydroxyl groups (Cowan, 1999). Concerning cinnamon EOs, its major volatile compound is cinnamaldehyde. Moreover, it has been reported that cinnamon EO had potent anti-bacterial and anti-fungal activities due to cinnamaldehyde (Ooi *et al*., 2006), because it acts as membrane irritants (Nabavi *et al*., 2015).

4. Conclusions

In this study, the *in vitro* activity of plants EOs against *P. digitatum* were tested at different concentrations during 8 days of incubation at 25°C. As showed by the results, the stronger inhibitions were obtained by cinnamon and summer savory EOs at concentration of 500 and 600 µl l^{-1}. None of the citrus EOs could inhibit fungus radial growth completely compared with cinnamon and savory EOs. GC-MS analysis showed that the most abundant of all constituents in EO extracts were carvacrol and γ-terpinene in summer savory and (E)-cinnamaldehyde in cinnamon.

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Onion crop response to different irrigation and N-fertilizer levels in dry Mediterranean region

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Abstract: Due to the water scarcity in dry Mediterranean condition, determination of water and nitrogen (N) fertilizer needs is a major challenge for crop production and environment protection. Pot experiments under open field conditions were conducted for two consecutive years (2016 and 2017) to assess the effects of various levels of N-fertilizer and irrigation on onion crop, following a 4×3 factorial experiment arranged in a randomized block design with four N-fertilizer rates (0, 40, 80, and 120 kg N ha^{-1}), and three irrigation levels (100, 80, and 60% of the seasonal water use), with three replications. Results indicated that the initial soil N-content (about 60 kg N ha^{-1}) was sufficient to meet crop nitrogen requirements. However, results indicated that onion crop was sensitive to water stress, so that the highest total bulb yield (BY, 19.1 t ha^{-1}), dry matter in bulbs (DM, 2.97 t ha^{-1}), and water productivity (WP, 1.9 kg m^{-3}) were found under full irrigation compared to the deficit conditions. BY, DM, and WP were predicted to be increased linearly with increasing levels of irrigation. The developed equations could be used for predicting onion crop yields under similar agro-pedo-climatic context, and as a tool for rational management of limited irrigation water.

1. Introduction

Onion (*Allium cepa* L.) is one of the most important horticultural crops worldwide. Many studies have been carried out regarding the water and nitrogen fertilizer requirements of onion crop and the effects of deficit irrigation on yield and yield components (Abdissa *et al.*, 2011; Igbadun *et al.*, 2012; Patel and Rajput, 2013; Tsegaye *et al.*, 2016). A nitrogen fertilizer level of less than 100 kg ha^{-1} was found to be sufficient for onion crop production as in Abdissa *et al.* (2011) and Tsegaye *et al.* (2016). Russo (2008) reported that nitrogen fertilizer had no significant effect on onion yield. Moreover, the onion crop was found to be more moderately responsive to water deficit during the total growing season; and it is better to partition the water stress throughout the growing season (Regulated deficit irrigation, RDI) rather than creating a stress during the

critical stages of crop growth (Kirda, 2000; Kadayifci *et al.*, 2005; Patel and Rajput, 2013). The deficit irrigation given at different levels (up to 40%) was found to be economically recommended. This wide range in allowable deficit levels could be due to the various agro-pedo-climatic context of their studied regions. In other word, onion grown in different soil and crop management factors responded differently to the application of both deficit irrigation and N-fertilizer. Therefore, there is a continuous need to select both optimum N-fertilizer rate and irrigation level for onion crop in ever changing agro-pedo-climatic conditions.

In dry areas of the Mediterranean region, scarcity of water is the most limiting factor for onion crop production, due to the lack of rainfall over the production period between April and August (Ragab and Prudhomme, 2002; Turner, 2004). The onion crop is grown in arid and semi-arid area in that region, with many cultivars. In the eastern Mediterranean, as in Syria, the oval- to elongated-shape onion, referred to as 'Selmouni red', is the famous variety used for its long-day storage capacity. Growers have targeted the larger bulb size and the higher marketable yield. They imagined the greater yields would require increased N fertilizer and water amount. High nitrogen fertilizer and irrigation water amounts may allow nitrate and other components to be most likely deeply percolated. So, research findings are an urgent need to determine water and N-fertilizer requirements for the onion crop in the production areas. In this context, the study reported herein has been designed to predict onion crop response to various levels of regulated deficit irrigation and N-fertilizer rates in dry Mediterranean areas. Results may contribute to introduce practical alternatives that would sustain onion productivity while using less water and fertilizer in the context of water scarcity and environmental protection.

2. Materials and Methods

Pot experiments under open field conditions were carried out at the Deir Al-Hajar Agricultural Experiment Station, Damascus, Syria (33°20′ N, 36°26′ E, altitude 600 m), for two consecutive growing seasons 2016 and 2017. Table 1 shows some climatic data for the studied site during the course of these experiments. No rainfall was recorded during both growing seasons. The chemical and physical soil properties are: pH 8.0; ECe 0.58 ds m^{-1}; organic matter 1.19%; available P 6 ppm; total N 0.06%; NO_3^- 51.8 ppm; NH_4^+ 39.2 ppm. Particle-size distribution analysis showed that the soil contains on average 27.8% sand, 42.7% silt and 29.5% clay, and therefore classified as a clay loam. Average volumetric soil water contents at field capacity (*FC*) and permanent wilting point (*PWP*) are 0.36 and 0.18 m^3 m^{-3}, respectively.

Table 1 - Some climatic data of the experimental site during both growing seasons

Season	Variable	Apr.	May	Jun.	Jul.	Aug.
2016	T_{min} (°C)	11.6	14.9	18.7	19.9	21.0
	T_{max} (°C)	29.2	30.5	36.6	38.1	37.8
	$T_{average}$ (°C)	21.2	23.6	30.6	28.9	29.5
	RH (%)	67.0	58.0	69.0	64.0	65.0
	Precipitation (mm)	0.0	0.0	0.0	0.0	0.0
2017	T_{min} (°C)	9.7	14.4	17.3	20.6	20.0
	T_{max} (°C)	26.2	31.6	35.8	40.6	38.5
	$T_{average}$ (°C)	19.2	24.9	28.4	31.1	28.9
	RH (%)	63.1	57.9	56.3	55.6	59.3
	Precipitation (mm)	0.0	0.0	0.0	0.0	0.0

T_{min}= minimum temperature, T_{max}= maximum temperature, $T_{average}$= average temperature, RH= relative air humidity.

Each pot was of diameter and depth of 25×30 cm, and contained 8 kg of natural soil from the field. Three small bulb sets (also called as bulbils or bulblets) of onion (*Allium cepa* L. 'Selmouni red') were grown in each pot. After germination, plants were thinned to two bulb sets per pot, making a plant density of about 400000 plants ha^{-1}. The pots were set outdoors under natural climatic conditions. The experiment was started on the planting day (March 31st and April 2nd for the 2016 and 2017 seasons, respectively) with the soil water content (SWC) of all pots at field capacity (measured by pot's weight).

The experiment was laid out following a 4×3 factorial experiment arranged in a randomized complete block design (RCB design) with four N-fertilizer rates (N0, N40, N80, and N120), and three irrigation levels (FI, DI80, and DI60), with three replications, making a total of 36 pots. The N-fertilizer rates composed of N0, N40, N80, and N120 with 0, 40, 80, and 120 kg N ha^{-1} added to the soil, respectively. The three distinct irrigation treatments were: FI treatment (full irrigation) in which plants received 100% of accumulated crop evapotranspiration (100% of ETc) and the root zone was replenished to field capacity; DI80 and DI60 treatments (regulated deficit irrigation) were irrigated at the same frequency as FI treatment but with

water amounts equal to 80 and 60% of the accumulated ETc, respectively. In other words, the three watering treatments received at each irrigation event 1.0, 0.8 and 0.6 times the amount of soil water depleted under FI conditions, respectively. Irrigation was applied 3 times per week. The pots were weighed before and after each irrigation event. The water amounts were regulated by weight (Eq. 1). The depleted water amount (crop evapotranspiration, ETc) (mm) between two successive irrigations was calculated as:

$$ETc = \frac{W_1 - W_2}{p_w \, x \, S} \qquad [1]$$

where W_1 was the weight of the pot (kg) after irrigation; W_2 was the weight of the pot (kg) just before the next irrigation; ρ_w is the water density (g cm^{-3}); and S is the soil surface area in the pot (m^2). The daily crop evapotranspiration (mm day^{-1}) was estimated by dividing the crop evapotranspiration estimated from Eq. (1) by the number of days between two successive irrigation events. The seasonal crop evapotranspiration, i.e., the total crop water use during the growing season, was the summation of the daily ETc.

Full doses of phosphorous and potassium were applied as basal dose at the time of planting. Nitrogen was divided into two equal doses (according to the studied N-fertilizer rate) and applied with the irrigation water during early vegetative growth stage. Irrigation was stopped at the end of July when over 50% dropping of leaf-tops was observed as signs of maturity. The onions were lifted to field cure about two weeks after (up to mid-August). After the leaves were completely dried, they were cut leaving about 2.0 cm above the bulb. The length, diameter, and weight of both matured onion bulbs from each pot were measured. The total bulb yield (BY, t ha^{-1}) was estimated. The dry matter in bulbs (DM, t ha^{-1}) was also estimated by drying bulbs at 50°C to a constant weight. Water productivity (WP, kg m^{-3}) was computed by dividing the total bulb yield by the seasonal evapotranspiration. Bulb shape index (Sh I) was also calculated as the relationship between bulb length and diameter. No Multi-centred bulbs were observed at harvest even under deficit irrigation.

With two factors (N-fertilizer rate and irrigation level), the two-way analysis of variance (ANOVA) was conducted using the DSAASTAT add-in version 2011 (Onofri, 2007). A combined analysis of data over both years was performed to verify if N-fertilizer rate and irrigation level may have a significant and stable effect over year. Mean comparison was made only for data after combined analysis using Duncan's Multiple Range test (DMRT) at the 1% level of significance. Trend analysis (regression analysis) was also used to examine the relationship between measured variables and the quantitative factors showing a significant effect. The trend analysis was done based on the method of orthogonal polynominals as described by Gomez and Gomez (1984).

3. Results and Discussion

The effects of years, N-fertilizer rates, and irrigation levels on the measured variables of onion crop (BY, DM, WP, and Sh I) were summarized in Table 2. Since no significant interaction year × treatments was observed, data are shown as the averages of the two years (Table 3 and Figs. 1-3).

Table 2 - Analysis of variance of the combined data of measured variables as affected by years, N-fertilizer rates, and irrigation levels (F-test values)

Source of variation	*df*	BY	DM	WP	Sh I
Year (Y)	1	*b*	*b*	*b*	*b*
Reps. within year	4				
Irrigation level (I)	2	213.4 **	75.44 **	41.35 *	12.30 NS
N-fertilizer rate (N)	3	<1	<1	<1	1.90 NS
Y x N	3	2.20 NS	1.24 NS	1.65 NS	1.30 NS
Y x I	2	<1	<1	1.03 NS	<1
N x I	6	<1	1.82 NS	<1	<1
Y x N x I	6	2.89 NS	1.47 NS	2.58 NS	2.61 NS
Residual (Pooled error)	44				
Total	71				
CV (%)		18.6	22.8	18.9	14.9

* = significant at 5% level, ** = significant at 1% level, NS = non-significant at 5% level.
b = the degree of freedom of Reps. within year is not adequate for valid test of significance (Gomez and Gomez, 1984).
df = degree of freedom, BY = total bulb yield, DM = dry matter in bulbs, WP = crop water productivity, and Sh I= shape index.

Total bulb yield

The combined analysis of data over years indicated that only the main effect of irrigation was significant, and highly influenced BY (p<0.01) (Table 2 and 3).

No significant change in BY was recorded with the addition of N fertilizer (Table 3), indicating that the initial soil nitrogen content (about 60 kg N ha^{-1}) was sufficient to meet the crop nitrogen requirements, and no extra N-application was needed. This is in agreement with the results of Abdissa *et al.* (2011) who found that a rate of 69 kg N ha^{-1} was sufficient

for onion crop production. Tsegaye *et al.* (2016) reported that a N level of 100 kg ha^{-1} was economically recommended for onion in southern Ethiopia. According to Russo (2008), nitrogen fertilizer did not affect onion yield.

The effect of irrigation level resulted in the highest BY (19.1 t ha^{-1}) in FI treatment, while under DI80 conditions, total bulb yield decreased by 36.4%, reaching 63.0% of decrease (7.1 t ha^{-1}) under DI60 conditions (Table 3). Similar results were also obtained by Kumar *et al.* (2007) and Bekele and Tilahun (2007). Trend analysis indicated that the relationship between total bulb yield and irrigation level

Table 3 - Effect of N-fertilization rate and irrigation level on total bulb yield (BY), dry matter in bulbs (DM), crop water productivity (WP), and shape index (Sh I) in onion 'Selmouni red' (data are the averages of the two years of experimentation)

Studied factor	BY (t ha^{-1})	DM (t ha^{-1})	WP (kg m^{-3})	SH I (cm cm^{-1})
N-fertilization rate				
N0 (0 kg N ha^{-1})	13.47 a	2.26 a	1.59 a	1.78 a
N40 (40 kg N ha^{-1})	12.26 a	2.14 a	1.47 a	1.86 a
N80 (80 kg N ha^{-1})	12.73 a	2.06 a	1.52 a	1.86 a
N120 (120 kg N ha^{-1})	12.61 a	2.08 a	1.47 a	1.65 a
Irrigation level				
Full irrigation (100% ETc)	19.08 a	2.97 a	1.90 a	1.72 a
Deficit irrigation at 80%of ETc	12.14 b	2.16 b	1.50 b	1.67 a
Deficit irrigation at 60%of ETc	7.08 c	1.27 c	1.14 c	1.98 a

In each column and for each studied factor, means followed by different letters are significantly different according to the DMR test.

was linear within the range of irrigation levels tested (R^2=0.991 with p<0.01) (Fig. 1). A significant linear decrease in the total bulb yield was predicted with increasing water deficit. In other word, BY increased with increasing irrigation level (Fig. 1). This is in agreement with other published findings (Kadayifci *et al.*, 2005; Kumar *et al.*, 2007; Bekele and Tilahun, 2007; Nagaz *et al.*, 2012). For instance, Nagaz *et al.* (2012) observed that applying 60% of crop evapotranspiration (ETc) caused significant decreases in fresh yield, dry matter, bulbs per hectare and bulb weight, compared to full irrigation (100% ETc). This result confirmed the sensitivity of the onion crop to water deficit during the total growing season. On the contrary, other studies showed that onion crop is responsive to deficit conditions as compared with full irrigation. For example, Tsegaye *et al.* (2016) and Igbadun *et al.* (2012) found that deficit irrigation given at 75% of ETc was economically recommended. Also, Nagaz *et al.* (2012) found no significant differences between regulated deficit irrigation at 80% ETc and full irrigation (100% ETc). These differences in onion crop response to deficit irrigation levels could be due to the various agro-pedo-climatic conditions.

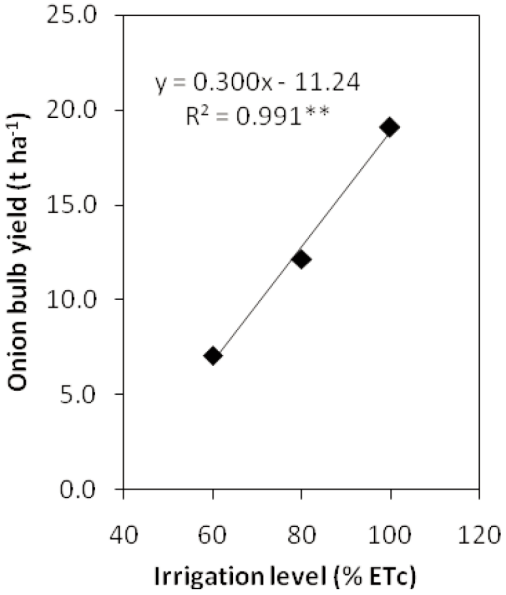

Fig. 1 - Response of onion total bulb yield (BY) to irrigation levels. A regression equation is fitted and coefficient of determination (R^2) is given. ** = significant at 1% level. Each experimental point represents the data of BY averaged over both years and all levels of N-fertilizer, at the specific irrigation level.

Dry matter yield in onion bulbs (DM)

Also DM was found to be highly affected only by the main effects of irrigation levels (Table 2 and 3). The mean values of DM were 2.97, 2.16, and 1.27 t ha^{-1} under FI, DI80 and DI60, respectively. That is to say, significant decreases of 27.2 and 57.1% in DM could be attained when onion crop was under DI80, and DI60, respectively, compared with FI (p<0.01) (Table 3). Trend analysis designated that the response of DM to various irrigation levels followed a linear relationship (R^2=0.999 with p<0.01). Dry matter yield increased with increasing levels of irrigation as can be seen in figure 2. Thus, dry matter yield could be maximized whatever the N-fertilizer rate used in this study, when full irrigation was applied. Several studied reported similar results, showing that dry matter yield was maximized under full irrigation rather than under deficit conditions (e.g., Nagaz *et al.*, 2012).

Concerning fertilizer rates, as mentioned above, ANOVA did not detecte any significant change in DM yield with the addition of nitrogen fertilizer (Table 3). This is in agreement with the findings of Russo

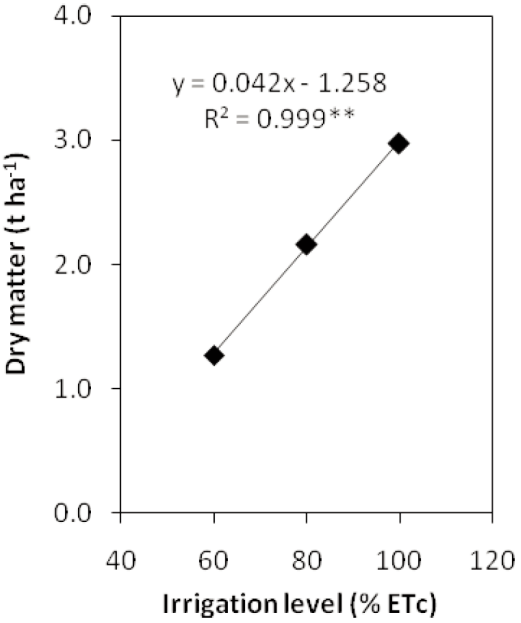

Fig. 2 - Response of onion dry matter yield in bulbs (DM) to irrigation levels. A regression equation is fitted and coefficient of determination (R^2) is given. ** = significant at 1% level. Each experimental point represents the data of DM averaged over both years and all levels of N-fertilizer, at the specific irrigation level.

(2008). This result newly indicated that the initial soil nitrogen content was sufficient to meet the N-fertilizer needs of onion crop.

Irrigation water applied and water productivity

With no rainfall received during both growing seasons (Table 1), large water amounts were applied to meet the high crop water demand. The irrigation water applied to FI, DI80, and DI60 were, 1014, 822, and 634 in the 1st season, and 1039, 849, and 566 mm in the 2nd one, respectively. Seasonal crop evapotranspiration (ETc), as calculated using Eq. (1), during the 2016 season was 993, 803, and 615 mm, and during the 2017 season was 1013, 823, and 630 mm, for FI, DI80, and DI60, respectively. As it can be seen, the seasonal ETc values were very close to the irrigation water amounts even in DI80 and DI60 treatments. Therefore, WP can be considered also a good estimate of irrigation water use efficiency (IWUE).

The combined analysis over years detected that WP was highly significantly influenced by the irrigation levels. It was unchanged with the addition of N fertilizer (Table 2 and 3). The mean value of WP under FI (1.90 kg m^{-3}) was significantly higher than both deficit irrigation levels, i.e., DI80 (1.50 kg m^{-3}) and DI60 (1.14 kg m^{-3}) (Table 3). Trend analysis indicated that the relationship between WP and irrigation level was linear with values of R^2 of 0.998 at the 1% level (Fig. 3). Results indicated that WP was not ameliorated under deficit irrigation. This could be due to high onion crop sensitiveness to the water deficit during the total growing season. Thus, the water savings under deficit irrigation could not balance the huge decrease in the total bulb yield under the dry conditions. This result is in agreement with similar results obtained by Kadayifci *et al.* (2005), who found that high water use efficiencies were observed with increasing levels of irrigation. Contrariwise, this result was in disagreement with the findings of other researchers (Fereres and Soriano, 2007; Kumar *et al.*, 2007; Bekele and Tilahun, 2007; Patel and Rajput, 2013; Tsegaye *et al.*, 2016). For example, Patel and Rajput (2013) reported that with 40% deficit irrigation throughout the growing season, water productivity can be significantly improved with 272-mm water saving which may be used to irrigate additional cropped area (half a hectare). Kumar *et al.* (2007) found that irrigation water use efficiency and water productivity both were highest under 80% of ETc and then declined with the increase in irrigation with microsprinkler irrigation system. Igbadun *et al.* (2012) showed that higher water productivity in terms of water supplied could be obtained by irrigating onion crop at 50 and 75% of ETc. These different results could be due to

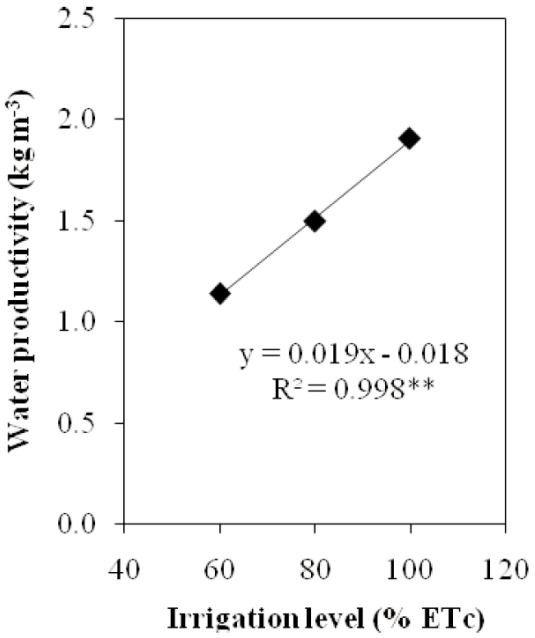

Fig. 3 - Response of onion crop water productivity (WP) to irrigation levels. A regression equation is fitted and coefficient of determination (R^2) is given. ** = significant at 1% level. Each experimental point represents the data of WP averaged over both years and all levels of N-fertilizer, at the specific irrigation level.

the differences in tested onion cultivars, soil type, and climatic conditions. They indicated that onion crop produced in different agricultural managements responded differently to water stress.

Bulb shape index

No significant effects of both tested factors were observed on this variable (Table 2 and 3). As above mentioned, the oval- to elongated-shape onion, referred to as 'Selmouni red', was tested. Its shape index generally varies from 1.5 to 3.0, according to several environmental conditions including planting dates, plant density, planting depth, and soil water availability. The lower the shape index, the better the bulb shape for marketing purposes (appearance and ease of packaging). The mean values of Sh I, found in this study, were 1.72, 1.67, and 1.98 under FI, DI80, and DI60, respectively (Table 3). The average Sh I under severe water stress (DI60) was 14.7% larger than that under non-water stress conditions (FI). In other word, sharp deficit irrigation tended to increase the bulb length, although this increase not significant, compared to its diameter. Thus, the recommended agricultural management to produce better shape index of onion bulbs, is to irrigate onion plants using irrigation level of 100 or 80% of ETc.

4. Conclusions

The following conclusions can be drawn from the results obtained in onion subjected to different N-fertlizer rates and irrigation levels in the studied agro-pedo-climatic context:

No additional N application over the initial soil N content (about 60 kg N ha^{-1}) was found to be required for the tested onion cultivar.

The highest total bulb yield, dry matter, and water productivity were recorded under full irrigation compared to the deficit conditions, indicating that onion crop was sensitive to water stress.

Total bulb yield, dry matter, and water productivity were predicted to be increased linearly with the increment in irrigation water amount. The developed equations could be useful for predicting onion crop yields under similar agro-pedo-climatic context and for rational management of limited irrigation water.

With huge crop water requirements, further studies should focus on how to improve the regulated deficit irrigation practices in order to address water shortage and sustainable crop production in dry areas of the Mediterranean region.

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Allelopathic activities of celery extract and its fractions against *Corchorus olitorius*, *Echinochloa crusgalli* and *Portulaca oleracea* weeds

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Abstract: Aqueous extract of celery (2.5-20 g l^{-1}, w/v) was evaluated for its phytotoxicity against three weed species, under laboratory and greenhouse conditions. Celery extract had a strong inhibitory effect on germination and seedling growth of *Corchorus olitorius, Echinochloa crusgalli* and *Portulaca oleracea* seeds. From dose response curves of tested seeds, LC_{50} were calculated to be in the range from 6.3 to 8.3 g l^{-1} for germination percent, from 7.2 to 8.0 g l^{-1} for shoot length and from 1.7 to 3.6 g l^{-1} for root length. Completely inhibition of root growth was exhibited *C. olitorius* and *P. oleracea* at 7.5 g l^{-1} corresponded with 15 g l^{-1} for *E. crusgalli* seed. Total phenolics in celery extract at 20 g l^{-1} constituted 201 mg l^{-1}. Ten phenolic acids were identified in extract by HPLC, among of them *p*-coumaric acid and *p*-hydroxybenzoic acid were presented in high amounts. Aqueous extract was partitioning between three solvents, hexane, methylene chloride, ethyl acetate. Generally, water residue after partitioning aqueous extract with the three solvents had the most phytotoxic effect on seedling growth of target seeds. In greenhouse trial, foliar spray of aqueous extract of celery (30, 60 and 90 g l^{-1}) and its fractions did not produce any significant effect on growth of two-weeks-old *C. olitorius,* or *E. crusgalli* or *P. oleracea* weeds.

1. Introduction

Worldwide, weeds caused about 34% yield losses among the major crops. Herbicide application is the most reliable weed control methods. Negative impacts of herbicides on environmental human health and herbicide resistant weeds were considered the two problems faced in weed management with herbicides (Jabran *et al*., 2015). Thus, manipulating the allelopathy can help to improve weed control in agriculture and increase the acceptance of agricultural products in today´s demanding consumer markets (Trezzi *et al*., 2016).

Allelopathy has been used as the basis for identifying plant species which may contain phytotoxic chemicals. These natural compounds can

offer excellent potential for new herbicidal solutions, or lead compounds for new natural herbicides (Duke *et al*., 2000; Vyvyan, 2002). The main purposes of research on allelopathy include the application of the allelopathic effects to agricultural production, reduction of the input of chemical pesticides and consequent environmental pollution, and provision of effective methods for the sustainable development of agricultural production and ecological systems (Han *et al*., 2013; Jabran *et al*., 2015). Knowledge concerning allelopathy can also be a key component in supporting organic farming, for which weed control is a major problem (Trezzi *et al*., 2016) Organic farming can involve reduced weed infestation by using plant extracts or intercropping plant species with an allelopathic potential (Bajwa *et al*., 2015). Plant extracts are the way of using allelochemicals for weed control in agroecosystems, as they have been already used as post-emergence natural herbicides in some countries. In Pakistan, for example, an aqueous extract deriving from sorghum shoots with a 10% concentration was left to ferment for several weeks and was subsequently sprayed post emergence for weed control. This fermented water extract, known as "Sorgaab", reduced weed density and weed dry weight up to 50% in field trials (Cheema and Khaliq, 2000; Cheema *et al*., 2002).

Celery (*Apium graveolens* L.; Apiaceae) has been cultivated for the last 3000 years, notably in Egypt, and was known in China in the fifth century BC. It has been used as a popular aromatic herb and spice (Chevallier, 1998). Earlier studies of *A*. *graveolens* led to isolation of some phenolic compounds as phthalides (Tang *et al*., 1990; Momin and Nair, 2001) and furocoumarins (Garg *et al*., 1979). These compounds are reported for their insecticidal, nematicidal, antifungal and phytotoxic activities (Kato *et al*., 1977; Momin and Nair, 2001; Pavela and Vrchotová, 2013). Recently, Sbai *et al*. (2017) reported that the aqueous extract (10-50 g/L) of *A*. *graveolens* had great inhibitory effect on root growth of germinated seeds of lettuce (in the range between 80% and 90%). They isolated six compounds which included three phthalides [senkyunolide A, (3S)-butylphthalide and sedanolide], two furanocoumarins (bergapten and scopoletin) and one phenyl propanoid (p-hydroxyphenethyltrans ferulate). Senkyunolide A compound was the most toxic on lettuce germination and shoot growth, however, p-hydroxyphenethyl trans-ferulate was the most toxic on root growth.

However, in spite of the wide range of biological activity of celery extract, but information concerning the herbicidal activity of this extract is rarely available. Therefore, the main objective of this study was to evaluate the herbicidal activity of the aqueous celery extract and its fractions against *Corchorus olitorius*, *Echinochloa crusgalli* and *Portulaca oleracea* weeds, with the goal of developing an effective plant derived herbicide. Moreover, the phenolic acids that considered the main source of all bioactive phenolic substances were identified in celery extract via HPLC.

2. Materials and Methods

Plant material

Plants of celery (*A*. *graveolens* L. var. dulce) were purchased from a local market in Cairo, Egypt. Identification of celery based on morphological traits that extensive observation of mature plants. The leaves located at the top of the leaf stalks were collected and dried in hot-air oven at 50°C for 72 h, powdered and used for extraction.

Preparation of aqueous extract

Different concentrations (w/v) of extracts were prepared by soaking known weight of dried leaves in known volume from distilled water at room temperature, and shaken for 24 h. The extracts were filtered through a Whatman No. 1 filter paper and kept at 4°C in the dark until use.

Germination bioassay

Mature seeds of *Corchorus olitorius*, *Echinochloa crusgalli* and *Portulaca oleracea* were collected from plants growing in fields of the experimental station of the National Research Centre (Research and Production Station, Nubaria region, Behaira Governorate, Egypt. Uniform healthy seeds were selected. Seeds were surface-sterilized with sodium hypochlorite (0.1%, w/v) for 2 min, washed under running tap water for 5 min followed by distilled water for 2 min, and stored for further use. Twenty seeds were placed in a 9-cm plastic Petri dish lined with a single Whatman No. 1 filter paper, and then 4 mL of each extract concentration (2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 g l^{-1}) was added onto filter paper. Distilled water (4 mL) was applied to a Petri dish to serve as a control. Four replicates of Petri dishes of each treatment were placed, in a completely randomized manner, in a growth chamber at 25°C. After 5 days, germination percent, shoot length and root length of germinated seeds were determined.

Extract concentrations required to cause 50% inhibition of different germination criteria (germination percentage, root length and shoot length) were calculated by Probit analysis.

Determination of total phenolics in aqueous extract

Extract of celery at 20 g l^{-1} was subjected to determine total contents of phenolics using Folin-Ciocalteu reagent (Singleton and Rossi, 1965).

Analysis of phenolic acids in aqueous extract by High-Performance Liquid Chromatography (HPLC)

Phenolic acids in aqueous celery extract at a concentration 20 g l^{-1} were hydrolyzed with sodium hydroxide (McKeehen *et al*., 1999) and subjected to HPLC analysis. Approximately 15 ml of aqueous extract was added to 15 ml of 8 N NaOH in a 50 ml Pyrex centrifuge tube, purged with nitrogen, shaken for 2 h in the dark with a shaker and acidified with ice-cold 6 N HCl to reduce pH to 2. Sample was centrifuged at 3000g, and the supernatant was decanted into separatory funnel. The supernatant was extracted with ethyl acetate (3 x 50 ml) with shaking for 10 s, and the mixture was allowed to settle for 5 min between extractions. The phenolic acids rich ethyl acetate fraction was dried by addition of anhydrous sodium sulfate and concentrated using rotary evaporator at 40°C to dryness. The residue was re-solubilized in 2.5 ml of methanol and filtered through a 0.2 µm PTFE filter prior to analysis. HPLC analysis was performed using equipment from Shimadzu (Japan): a Shimadazu LC-2010A liquid chromatograph, a Shimadazu SPDM10A Diode Array Detector and a Shimadzu Class-vp V6.12 SP4 offline processing system. Phenolics were analyzed using a Luna RP-C18 (2) column (250×4.6 mm i.d, 5 µm, Phenomenx). The mobile phase consisted of a mixture of acetate buffer: acetonitrile (9:1, v/v). Acetate buffer was prepared by dissolving 6.35 g sodium acetate in one-liter H_2O and 20 ml acetic acid. The detecting wavelength was 260 nm. Standard phenolic acids (gallic, protocatechuic, *p*-hydroxybenzoic, syringic, ferulic and *p*-coumaric) were purchased from Sigma Aldrich and vanillic, caffeic, salicylic and cinnamic were purchased from Fluka.

Greenhouse bioassay

Another experiment was performed to study the effect of the aqueous. celery extract on the growth of two-weeks-old *C*. *olitorius*, *E*. *crusgalli* and *P*. *oleracea* weeds grown under controlled conditions in an experimental greenhouse (25±3°C, 12 h photoperiod). Aqueous extracts at four concentrations (0, 30, 60 and 90 g l^{-1}) were prepared as previously mentioned and used in a greenhouse bioassay. Plants were raised from the collected seeds in 12-cm diameter earthenware pots. The pots were filled with 750 g soil (sand:peat moss = 3:1, w/w) and 20 seeds of each plant species were sown per pot. Two weeks after emergence, plants were sprayed with plant extracts at 0, 30, 60 and 90 g l^{-1} concentrations. Extract solutions were applied to shoots of tested weeds using a Epoca sprayer (Italy). The solution was sprayed evenly over the entire surface of the plant, including the adaxial and abaxial surface of leaves. A total of 48 pots were maintained (i.e. 4 extract concentrations × 3 weeds × 4 replicates) in a completely randomized design. Seven days after extract spraying, the plants were examined for visible injury levels and the percent of chlorotic and necrotic areas were recorded.

Fractionation of extract

One hundred milliliter of high extract concentration that used either in germination trial (20 g l^{-1}, w/v) or in greenhouse trial (90 g l^{-1}, w/v) was subsequently partitioned with organic solvents with increasing polarity. The extract was partitioned three times with 200 mL aliquots of each solvent: hexane, methylene chloride ($MeCl_2$) and ethyl acetate (EtOAc) using separatory funnel. Solvents were dried by addition of anhydrous, sodium sulfate, filtered and evaporated using Buchi Rotary Evaporator at 40°C to dryness. The residues of hexane, $MeCl_2$ and EtOAc fractions were dissolved in 100 mL of DMSO-water solution (0.1%, v/v). These three fractions and water residue (H_2O fraction) were subjected to bioassay as previously mentioned.

Statistical analysis

All data was subjected to ANOVA analysis using costat software to evaluate the effect of extract rates upon germination and growth parameters of tested weeds. After ANOVA, the parameters that were statistically significant (*A*. *graveolens* extracts with $P \leq 0.05$), were subjected to Pro-bit analysis using LdP line. Pro-bit curves were derived by plotting the extract concentration (on the x-axis) and inhibition % (on the y-axis). With the curves obtained, LC_{50} value was calculated for each parameter. This value represents the extract concentration at which 50% inhibi-

tion in different germination parameter occurs.

3. Results

Effect of celery extract on germination percentage of seeds

As shown in Table 1, aqueous extract of celery had a strong inhibitory effect on germination percentage of the three tested seeds and the inhibition increased with concentration. Increasing extract concentration above 5 g l^{-1} produced gradual decrease in germination percentage of *C. olitorius*. The highest reduction effect was produced by extract at highest concentrations, 17.5 and 20 g l^{-1} (89% and 93%, respectively). Also, germination percentage of *E. crusgalli* and *P. oleracea* was reduced by the extract at all tested concentrations. The reduction percentage varied from 37% and 9%, respectively at the lowest concentration to 96% and 73%, respectively at the highest ones.

Table 1 - Effect of aqueous celery extract on germination percentage of three weed species

Concentration (g l^{-1})	Germination %		
	C. olitorius	*E. crusgalli*	*P. oleracea*
Control	100±0 a	100±0 a	100±0 a
2.5	95±5 a	63±7 b	91±9 b
5	93±2 a	67±4 b	84±4 b
7.5	49±5 b	52±2 c	73±3 c
10	41±4 c	43±5 c	74±7 c
12.5	23±3 d	39±7 c	60±2 d
15	11±5 e	25±4 d	40±2 e
17.5	11±5 e	14±5 d	37±2 e
20	7±2 e	4±1 e	27±3 f

Values are given as means of three replicates ± standard error. Means with the same letters in a column are not significantly different at P<0.05.

Effect of celery extract on Seedling growth of seeds

Aqueous extract of celery exhibited a great inhibitory effect on shoot length of the three germinated seeds (Table 2). The extract improved the shoot growth of *E. crusgalli* and *P. oleracea* at the lowest concentrations. The progressive increase in extract concentration followed by progressive reduction in shoot length of all tested seeds. More than 90% reduction in shoot length of three germinated seeds was obtained by soaking seeds in celery extract at 17.5 and 20 g l^{-1}.

An inhibition of root growth was observed in presence of all extract concentrations and roots more sensitive than shoots (Table 2). Root length of three target seeds varied in their response to different extract concentrations and *E. crusgalli* was less effective one. Soaking seeds in celery extract at the lowest concentration produced great reduction in root elongation of all treated seeds ranged between 36% and 71%, relative to control. Using extract at 7.5 g l^{-1} and above completely inhibited root growth of both *P. oleracea* and *C. olitorius*, whereas complete inhibition of *E. crusgalli* roots was obtained at 15 g l^{-1}.

Extract concentrations required to cause 50% inhibition (LC50)

Inhibition percent and levels of LC_{50} of all germination parameters of tested weeds were calculated and dose-response curves were illustrated in figure 1. The three tested weeds varied in their inhibition percentages as affected with celery extract. Among tested seeds, germination percent of *P. oleracea* was con-

Table 2 - Effect of aqueous celery extract on shoot and root lengths of three weed species

Rate (g l^{-1})	Shoot length (cm)			Root length (cm)		
	C. olitorius	*E. crusgalli*	*P. oleracea*	*C. olitorius*	*E. crusgalli*	*P. oleracea*
Control	2.56±0.14 a	3.35±0.35 a	2.17±0.13 b	2.41±0.08 a	1.83 ±0.26 a	1.95±0.23 a
2.5	2.74±0.25 a	2.91±0.34 b	2.50±0.23 a	0.81±0.17 b	1.17 ±0.12 b	0.57±0.07 b
5	2.21±0.19 b	2.43±0.19 c	2.19±0.60 b	0.51±0.11 c	0.65 ±0.20 c	0.29±0.06 c
7.5	1.38±0.40 c	1.73±0.33 d	0.58±0.40 c	0.00 d	0.51 ±0.17 c	0.00 d
10	0.76±0.28 d	0.79±0.20 e	0.60±0.06 c	0.00 d	0.25 ±0.06 d	0.00 d
12.5	0.36±0.06 e	0.77±0.20 e	0.56±0.31 c	0.00 d	0.22 ±0.01 d	0.00 d
15	0.10±0.02 e	0.53±0.31 ef	0.15±0.08 d	0.00 d	0.00 e	0.00 d
17.5	0.05±0.03 e	0.27±0.14 ef	0.14±0.14 d	0.00 d	0.00 e	0.00 d
20	0.00 f	0.06±0.03 f	0.11±0.06 d	0.00 d	0.00 e	0.00 d

Values are given as means of three replicates ± standard error. Means with the same letters in a column are not significantly different at P<0.05.

sidered a less sensitive to celery extract. Since, value of LC_{50} of *P. oleracea* constituted 13.4 g l^{-1} corresponded with 8.3 and 6.3 g l^{-1} for *C. olitorius* and *E. crusgalli*, respectively. Whereas, inhibition percent of shoot length did not produce a great variation among tested seeds and LC_{50} calculated to be 7.8, 7.2 and 8 g l^{-1} for *C. olitorius*, *E. crusgalli* and *P. oleracea* seeds, respectively. As shown in figure 1, root length exhibited the maximum inhibition effect as affected by celery extract when compared with germination percent and shoot length. Moreover, root length of tested seeds varied in their inhibition percent as affected with celery extract. Depending on LC_{50} levels, root length of *P. oleracea* (1.65 g l^{-1}) was considered the most sensitive, followed by *C. olitorius* (1.8 g l^{-1}) and *E. crusgalli* (3.6 g l^{-1}).

Total phenolics and phenolic acids content in aqueous extract

In this study, we determine total phenolics for celery extract at a concentration 20 g l^{-1} and found that the extract contained high amounts of phenolics con-

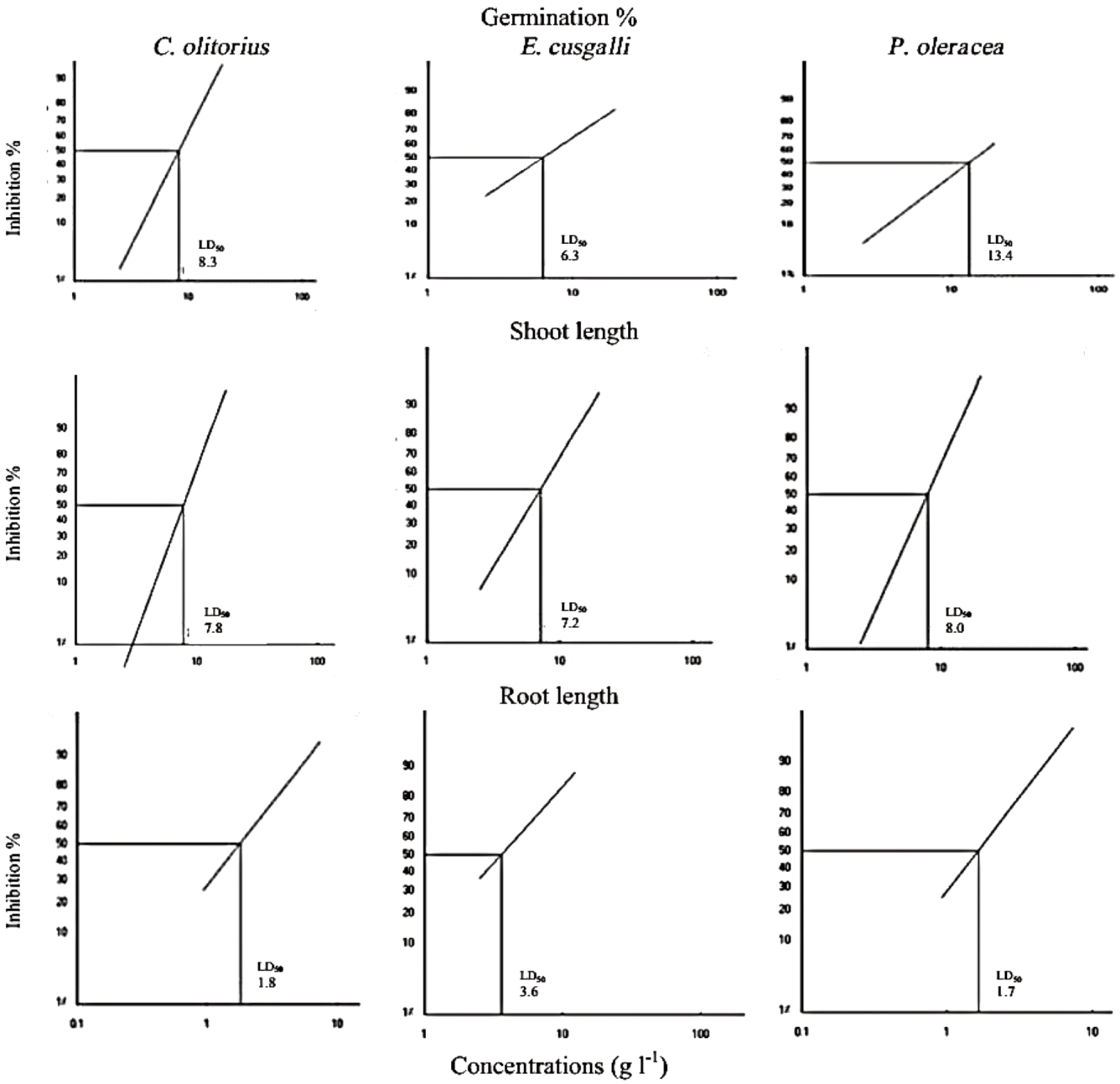

Fig. 1 - Dose-response curves showing the effect of celery extract (inhibition % and LC50) on percent germination and seedling growth of three tested seeds.

stituted 201 mg l^{-1}, as gallic acid. Using HPLC, ten phenolic acids were detected in this aqueous. extract, of which six compounds consisted of benzoic acid derivatives and four of cinnamic acid derivatives (Table 3). Among the identified phenolic acids, *p*-hydroxybenzoic acid, *p*-coumaric acid and ferulic acid were presented in high amounts (18.72, 30.32 and 11.12 mg l^{-1}, respectively). Except the minor quantity of cinnamic acid, moderate concentrations from other phenolic acids were determined in celery sample.

Table 3 - Phenolic acids in aqueous extract of celery at concentration 20 g l^{-1}

Acid derivatives	Concentration (g l^{-1})
Benzoic acid derivatives	
p-Hydroxybenzoic acid	18.72
Gallic acid	8.56
Vanillic acid	8.8
Syringic acid	6.4
Protocatechuic acid	9.28
Salicylic acid	7.84
Cinnamic acid derivatives	
Cinnamic acid	0.64
Caffeic acid	9.52
p-Coumaric acid	3.032
Ferulic acid	11.12

Effect of fractions on germination of weeds

As shown in Table 4, fractions obtained from the aqueous extract of celery varied in their effects on germination criteria of target seeds. As for germination percentage, the significant reduction effect was recorded only for $MeCl_2$ fraction on *P. oleracea* (15%) as well as for H_2O residue on both *C. olitorius* (30%) and *P. oleracea* (36%). But, all examined fractions did not produce any significant effect on germination percentage of *E. crusgalli* seeds. Whereas, H_2O fraction induced a great reduction on shoot elongation of *C. olitorius*, *E. crusgalli* and *P. oleracea* seeds, reached 64%, 47% and 74%, respectively relative to control. With less extent, hexane and $MeCl_2$ fractions reduced shoot length of *E. crusgalli*. With few exceptions, all fractions reduced root elongation of target seeds. Water fraction was the most toxic, inducing an inhibition of 99%, 94% and 50% in root length of *P. oleracea*, *C. olitorius* and *E. crusgalli*, respectively. Whereas, the other fractions, hexane, $MeCl_2$ and EtOAc fractions reduced root length of *E. crusgalli* and *P. oleracea* between 17% and 43%, relative to control.

Effect of celery extract and its fraction on growth of weeds

Data presented Table 5 revealed that spraying aqueous celery extract and its fractions did not produce any significant effect on growth of two-weeks old of either *C. olitorius,* or *E. crusgalli* or *P. oleracea* weeds.

Table 5 - Effect of aqueous celery extract and its fractions on the growth of two-weeks-old of three weeds

Treatments	Injuries (% of control)		
	C. olitorius	*E. crusgalli*	*P. oleracea*
30 g l^{-1}	2±0 a	0±0 a	1±0 a
60 g l^{-1}	3±1 a	2±5 a	3±2 a
90 g l^{-1}	3±2 a	3±1 a	3±1 a
Hexane fraction	2±1 a	3±2 a	4±2 a
$MeCl_2$ fraction	4±2 a	1±0 a	1±0 a
EtOAc fraction	3±1 a	2±1 a	3±1 a
H_2O residue	4±1 a	1±0 a	1±0 a

Values are given as means of three replicates± standard error. Means with the same letters in a column are not significantly different at P<0.05.

Table 4 - Effect of fractions obtained from aqueous extract of celery on germination of three weeds (% of control)

Criteria	Weed species	Fractions				
		Control	Hexane	$MeCl_2$	EtOAc	H_2O
Germination (%)	*C. olitorius*	100 a	104 a	96 a	102 a	70 b
	E. crusgalli	100 a	98 a	104 a	102 a	95 a
	P. oleracea	100 a	107 a	85 b	109 a	64 c
Shoot length	*C. olitorius*	100 a	97 a	102 a	102 a	36 b
	E. crusgalli	100 a	86 b	83 b	95 a	53 c
	P. oleracea	100 a	95 a	100 a	87 a	26 b
Root length	*C. olitorius*	100 a	101 a	74 b	103 a	6 c
	E. crusgalli	100 a	57 c	83 b	79 b	50 c
	P. oleracea	100 a	61 b	76 b	63 b	1 c

Means with the same letters in a raw are not significantly different at P<0.05.

4. Discussions and Conclusions

Aqueous extract of celery was evaluated for its phytotoxicity against three plant species typically present as weeds in summer crops, under laboratory and greenhouse conditions. The extract displayed a great inhibition on germination percentage and seedling growth of target seeds. In line of these results, Sbai *et al*. (2017) reported that lettuce germination was completely inhibited by celery extract at concentration above 20 g l^{-1}. They extracted and identified the allelochemicals compounds that responsible of toxicity namely phthalides, among of them senkyunolide A was the most toxic in lettuce germination. Current study revealed that the inhibitory effect of celery extract on germination percent varied between tested seeds, and *P*. *oleracea* possessed the least sensitivity. Variation between different plant species in their sensitivity to plant extracts was previously observed by many investigators (Al-Humid and El-Mergawi, 2014; Han *et al*., 2008). This study showed that root growth was the most sensitive to extract than shoot growth. Since, LC_{50} of shoot length of three target seeds ranged between 7.2 g l^{-1} and 8 g l^{-1} whereas, values of LC_{50} for root growth are calculated to be in the range of 1.7 g l^{-1} and 3.6 g l^{-1}. These results are in agreement with Sbai *et al*. (2017), who reported that celery extract had more pronounced effects on roots of lettuce, rather than shoots. Generally, in germination bioassay, water extract of allelopathic plants have more pronounced effect on root rather than shoot growth (Inderjit and Dakshini, 1995; Muhammad *et al*., 2011). This may be attributed to the fact that roots are the first to absorb the phytotoxic compounds (Turk and Tawaha, 2002).

In order to identify and distribute the chemical groups of toxic allelochemical constituents, celery aqueous. extract was partitioning between three organic solvents varied in their polarities. Generally, the most phytotoxic compounds were represented water residue (H_2O fraction). It can be suggested that the high toxicity compounds in celery extract may be related to presence of more polar compounds. These results are in general agreement with the results obtained by Sbai *et al*. (2017). Who observed that extraction of celery with non-polar solvents, petroleum ether or chloroform had no significant effect on lettuce germination, contrarily to the high toxicity effect of methanol extract (polar solvent). The high toxicity of celery extracts may be attributed to the present water soluble compounds as saponins, glycosides, hormones or enzyme which could affect growth directly or by altering the mobilization of storage compounds during germination (Chaves and Escudero, 1997; El-Khatib, 1997).

For the greatest inhibition effect of aqueous celery extract, we analyzed phenolics and phenolic acids in the extract at a concentration 20 g l^{-1}. The results indicated the presence of high amount of phenolic compounds (201 mg l^{-1} as gallic acid) may explain the greatest effect of celery extract. In line of these results, Jung *et al*. (2011) found high levels of phenolic in celery (51.09 mg g^{-1} dw, as gallic acid). Phenolic acids are the precursor of all phenolic constituents in plants as well as the bioactive constituents in celery extract. Identification and determination the concentration of the phenolic acids in the aqueous extract was conducted by using HPLC. Ten phenolic acids were identified in celery extract, among of them *p*-hydroxybenzoic acid, *p*-coumaric acid and ferulic acid were presented in relatively high amounts. In line of these results, Yang *et al*. (2010) identified caffeic acid, *p*-coumaric acid, and ferulic acid in celery extract. Presence of high concentration of total phenolic and phenolic acids in areal parts of celery was previously reported by many investigators (Yang *et al*., 2010; Sbai *et al*., 2017).

In greenhouse trial, we evaluated the effects of foliar s pray of aqueous extract of celery and its fractions, hexane, $MeCl_2$, EtOAc and H_2O-residue on growth of two-weeks-old *C. olitorius,* or *E*. *crusgalli* or *P*. *oleracea* weeds. Celery extract at 30, 60 and 90 g l^{-1} as well as the obtained fractions did not produce any significant effect on growth of three examined weeds. In general, the growth of two-weeks-old weeds tended to be less sensitive to the test fractions than the weed germination process. These results are in agreement of those obtained by Inderjit and Weston (2000), they found that greenhouse bioassays do not adequately predict the responses observed in laboratory bioassay. Hence, weed germination might be the most sensitive index with which to judge allelopathy of plant extracts and its fractions under natural conditions (Corrêa *et al*., 2008).

In conclusion, in this study aqueous extract of celery was evaluated for its phytotoxicity against three weed species under laboratory and greenhouse conditions. The extract displayed a great inhibition on germination percentage and seedling growth of target seeds. Root growth of *C. olitorius* and *P. oleracea* was completely inhibited by extract at 7.5 g l^{-1}, corresponded with 15 g l^{-1} for *E*. *crusgalli*. Celery aqueous extract was fractionated by using three less polar sol-

vents; however, water extract displayed the strongest inhibition effect on germination of three target seeds. Celery extract had a relatively high amount of phenolics and phenolic acids. High concentrations of water celery extract 30, 60, 90 g l^{-1}) and the obtained less polar fractions did not produce any significant effect on growth of two weeks-old of tested weeds. Water extract of celery may be a useful source for the future development of pre-emergence bio- herbicide.

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Effects of cold stratification and chemical treatments on seed germination in four hazelnut cultivars

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Key words: chemical treatment, cold stratification, *Corylus avellana* L., filbert, germination.

Abstract: Propagation of European hazelnut by seed is influenced by some seed treatments. In this investigation, effect of stratification period and some chemicals on seeds of four hazelnut cultivars were studied. GA_3 and four months of stratification, each individually resulted in the highest germination percentage at 82.73% and 83.75%, respectively. There were significant differences between cultivars and treatments in terms of germination percentage and rate. The highest germination percentage and rate were observed in the local cultivar Gerd under GA_3 treatment at 100 mg/L and also after four months of stratification.

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1. Introduction

Propagation by seeds is a conventional method to produce new plants. This is one of the most recognized efficient methods which is widely applied for different plant species. Although sexual reproduction do not result in true to type plants, in breeding programs it is inevitable to apply it to grow hybrid seedlings. For example, in hazelnut, interspecific hybridization is necessary to transfer superior characters from wild species to the commercial European hazelnut (*Corylus avellana* L.) (Erdogan and Mehlenbacher, 2000).

Seed germination is a main step in plant life cycle, and is influenced by various biotic and abiotic factors (Yuan and Wysocka-Diller, 2006). It is essential to investigate different aspects in sexual propagation for all plant species. However, there are some common difficulties in using such approach to propagate many plants such as hazelnut, including seed dormancy and inconsistent seed germination which make some problems and retard improvement programs and sometimes end in hybrids loss. Therefore, studying beneficial treatments to remove dormancy and subsequent uniform seed germination is considered of great importance

(Wang and Berjak, 2000; Copeland and McDonald, 2001). Generally, germination process is controlled through a balance between inducing and inhibiting factors. Provided that concentration of inducers is higher than inhibitors, seed dormancy will predominate. Some stimulants such as temperature and light are necessary to lower the effect of inhibitors in seed. In such case, an inducing factor such as gibberellic acid (GA_3) could have cumulative influence so that germination process will commence (Bradbeer, 1988).

In many temperate zone species, dormancy prevents the seeds from germinating (Derkx, 2000). A dormant seed would not germinate even in favorable environmental conditions. Several approaches have been suggested in literature to overcome this phenomenon, including cold stratification (Bewley and Black, 1994), and seed treatment by some chemicals such as gibberellic acid (GA_3), polyamines and thiourea (Frankland, 1961; Çetinbaş and Koyuncu, 2006; Mello *et al.*, 2009). Cold stratification plays a major role as a stimulant to break seed dormancy. Also its effect is accelerated in combination with chemicals or physical removing of seed coat (Bewley and Black, 1994). This technique is usually performed at temperatures between 0 and 10°C; which vary depending on different species. However, the best reported temperature for this kind of seed treatment is 5°C (Bewley and Black, 1994). Aygun *et al.* (2009) suggested that hazelnut seeds need two to six months of pre-germination cold stratification. Dormancy in hazelnut seeds is diminished through cytological, hormonal and biochemical changes during cold stratification period. For example, mobilization of phytic acid and phosphate was observed during this treatment (Vasilios *et al*., 2005).

GA_3 treatments could remove various seed physiological dormancies and induce germination of dormant seeds (Frankland, 1961). Aygun *et al.* (2009) showed that in hazelnut seeds treated by GA_3 at concentrations from 0 to 200 mg/L, the highest seed germination percentage was obtained by 100 mg/L GA_3. The main polyamines existing in plant cells are putrescine, spermine and spermidine (Davies, 2004). Based on some evidences, polyamines have a role in seed dormancy process. Sinska and Lewandowska (1991) found that putrescine, spermine and spermidine decreased in apple seeds during cold stratification. In fact, putrescine and spermidine had inducing effect and spermine had inhibiting effect on apple seed germination. Although thiourea is not applied commonly in seed germination experiments, it is able to enhance germination of some kinds of seeds (Gul and Weber, 1998; Çetinbaş and Koyuncu, 2006). Stidham *et al.* (1980) showed that thiourea had an inducing effect on germination of 18 shrub species. According to Cetinbaş and Koyuncu (2006), this property of thiourea is attributed to its cytokinin-related effect in removing inhibitors.

This investigation aimed to study the effects of some treatments including some chemicals and cold stratification on percentage and rate of seed germination in four hazelnut cultivars grown in Iran.

2. Materials and Methods

Seeds of four hazelnut cultivars including a local cultivar Gerd and three introduced cultivars Barcelona, Ronde (= Ronde du Piemont) and Segorbe were collected from Astara Hazelnut Research Station, in Astara, Guilan province, Iran. Defected seeds were discarded and proper seeds were separated to study.

Treatments

Treatments applied in this study included GA_3 (100 and 200 mg/L), putrescine (0.01 and 0.1 mM), thiourea (1000 and 2000 mg/L) and stratification for two and four months. The control treatment was distilled water.

Germination test

The seeds were soaked for 24 hours in GA_3 (100 and 200 mg/L), putrescine (0.01 and 0.1 mM) and thiourea (1000 and 2000 mg/L), and also some seeds were soaked in distilled water. In order to prevent seeds from rotting, the treated seeds were surface sterilized with sodium hypochlorite (10% v/v for 5 min.) and then rinsed by sterilized water. Afterwards, the seeds were cultured in plastic pots containing pre-autoclaved sand as the medium (diameter of 2 mm). Germination test was conducted in a factorial completely randomized design with three treatments including “no stratification”, “two months of stratification” and “four months of stratification”. Emerging radical was considered as the index of germination. In chemical treatments, the number of germinated seeds up to 40 days from the culture date, and in stratification treatments, the number of germinated seeds up to 40 days from outing from refrigerator were recorded. Stratification treatments were just performed by keeping them in refrigerator (5°C) for two to four months. Cultured seeds were inspected regularly and in case of moisture decrease of media,

autoclaved water was sprayed on them. The measured characteristics were percentage and rate of seed germination. The following formula was used to calculate germination percentage.

Germination percentage = (number of germinated seeds / total number of seeds) × 100

Germination rate is defined as the time to reach 50 percent of germination, which was calculated by the formula below:

Germination rate = (1/time of reaching 50 percent of germination)

For each cultivar, 210 seeds were used. The data were analyzed by analysis of variance (ANOVA) and the software Germin-g was applied to measure the target parameters (Soltani *et al*., 2004).

3. Results and Discussion

According to the analysis of variance, there was a significant difference among cultivars and chemical treatments with regard to germination percentage ($P < 0.01$) (Fig. 1). Even so, no significant difference was observed among cultivars and chemical treatments regarding germination rate (Fig. 2). In addition, effect of stratification on germination rate and percentage was significantly different between the cultivars ($P<0.01$) (Fig. 3 and 4).

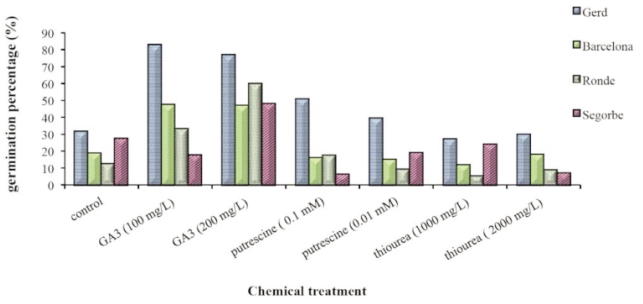

Fig. 1 - Effect of chemical treatments and cultivars on seed germination percentage of hazelnut cultivars.

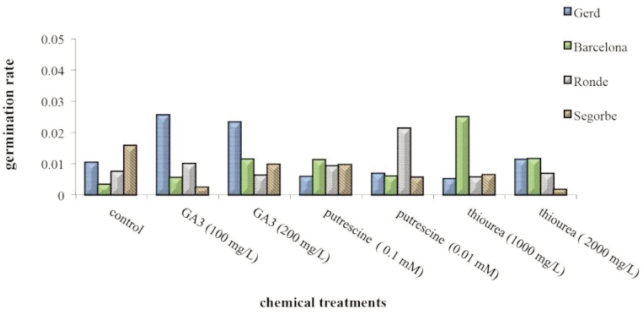

Fig. 2 - Effect of chemical treatments and cultivars on seed germination rate of hazelnut cultivars.

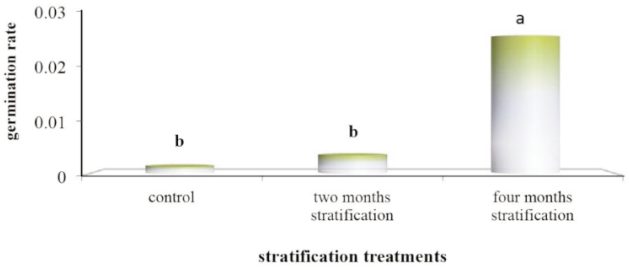

Fig. 3 - Effect of stratification treatments on seed germination rate of hazelnut cultivars.

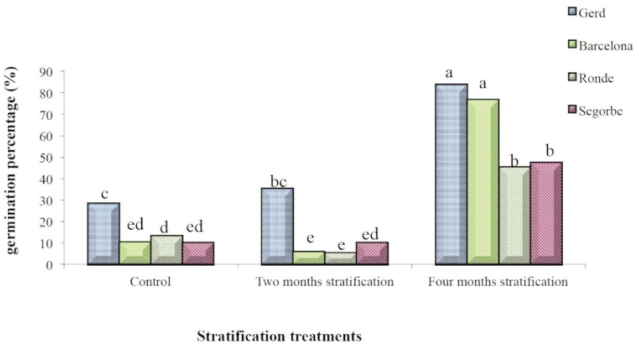

Fig. 4 - Effect of stratification treatments and cultivar on seed germination percentage of hazelnut cultivars.

For germination percentage, the difference between mutual effects of "cultivar × chemical treatment" ($P<0.05$) (Fig. 1), "cultivar × stratification" ($P<0.05$) (Fig. 4) and "chemical treatment × stratification" ($P<0.01$) (Fig. 5) was significant.

For germination rate, the mutual effect of "cultivar × chemical treatment" showed a significant difference ($P<0.05$) (Fig. 2).

Of the four hazelnut cultivars, 'Gerd' had the highest germination percentage (82.73%) at 100 mg/L GA_3, which showed 50.76% increase compared to the control (31.97%). Although applying both GA_3 concentrations was resulted in higher germination percentages, but no difference was observed between these two levels (Fig. 1).

Between stratification and cultivar, the highest germination percentage was exhibited after four

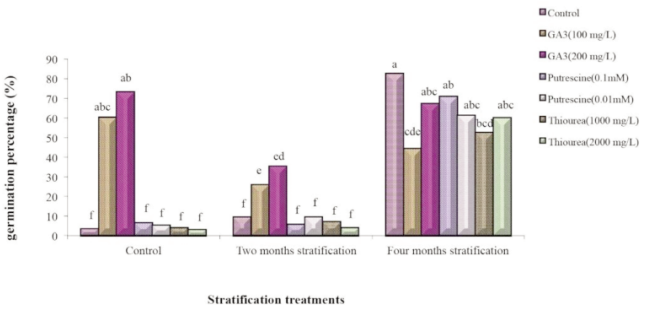

Fig. 5 - Effect of stratification and chemical treatments on seed germination percentage of hazelnut cultivars.

months of stratification in the local cultivar Gerd. No difference existed in four months of stratification between two local cultivars and Barcelona (Fig. 6).

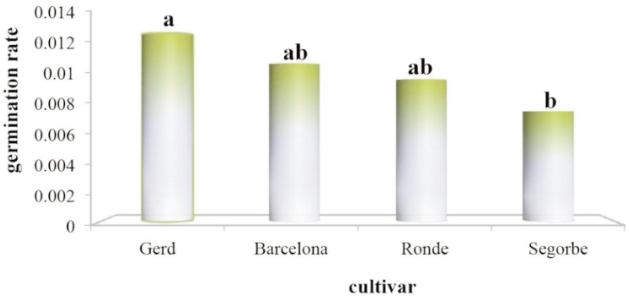

Fig. 6 - Seed germination rates in four hazelnut cultivars.

Figure 5 reveals that between mutual effect of chemicals and stratification treatments, the highest germination percentage occurred through four months of stratification and in the control treatment. Nevertheless, in treatments of two months of stratification and no stratification, both levels of GA_3 resulted in the highest germination percentage.

In all cultivars, the highest germination rate was recorded in 'Gerd' (Fig. 6). Four months of stratification had the greatest influence on germination rate (Fig. 3). Of chemical treatments, the fastest germination was obtained by using the first level of GA_3 (100 mg/L) in the cultivar Gerd (Fig. 2).

Various studies have demonstrated a direct relationship between cold stratification length and increasing in germination percentage. As can be clearly seen in figures 4 and 5, when stratification period increased, as a result, germination percentage of hazelnut seeds increased in all treatments and all cultivars. Similar results were observed by Aygun *et al*. (2009) which suggested that 120 days of cold stratification ended in higher germination percentage in hazelnut seeds in comparison with control. Furthermore, Bradbeer (1988) reported that three months of cold stratification led to a rise in hazelnut seed germination percentage. In other studies, three months of stratification without any warming improved germination percentage in seeds of *Jasminum fruticans* (Pipinis *et al.*, 2009). Another example for such impact has been reported by Chin *et al*. (1992) in kiwifruit.

In addition, there are many other studies that support the influence of applying some chemicals and plant growth regulators on rising germination percentage of seeds, which could be used singly or in combination with chilling. As a matter of fact, these chemicals are considered as substitutes for chilling requirement of seeds, and also can decrease length of chilling period.

Overall, gibberellins and cytokinins are able to promote seed germination in plants (Davies, 2004; Miransari and Smith, 2014). In contrast, abscisic acid (ABA) plays an inhibiting role in seed germination (Miransari and Smith, 2014). Among types of gibberellins, GA_3, GA_4 and GA_7 have the most impact on germination enhancement. However, cytokinins and auxins have so lower effect compared to gibberellins and cytokinins in terms of stimulating germination. In fact, the effect of all these growth regulators depends upon other factors such as light, temperature and oxygen; and also there are some mutual effects between them. Gibberellins and cytokinins are capable of neutralizing the inhibiting effect of abscisic acid. Since all these growth regulators naturally exist in seed in different ratios, so their observed effects on seed germination could be interpreted by the state of hormone balance (Kucera *et al.*, 2005).

Gibberellins are able to enhance seeds of different species to germinate through different ways. That is, external use of gibberellins could induce germination in seeds in which lack of germination is due to seed coat (e.g. legumes), or seed dormancy is because of seed embryo (e.g. apple, birch, hazelnut) (Davies, 2004; Miransari and Smith, 2014). Besides, in seeds that their germination depends on exposure to light (e.g. Arabidopsis, lettuce), GA_3 could promote seed germination even in the dark (Cao *et al.*, 2005). Based on the results obtained in this study, the highest germination percentage and rate were gained through GA_3 treatment at 100 mg/L (Fig. 1, 2), which corresponded with results reported by Aygun *et al.* (2009) that showed higher germination percentage after treatment by 100 mg/L gibberellin. In addition, there are some reports on gibberellin application on hazelnut seeds in order to enhance germination results (Bradbeer and Pinfield, 1967; Jarvis and Wilson, 1977; Pinfield and Stobart, 2006).

In addition to gibberellins, other substances such as thiourea can also overcome seed dormancy. Several investigations have demonstrated the effect of thiourea on dormancy breaking and increasing germination percentage of seeds in different plant species (Gul and Weber, 1998; Çetinbaş and Koyuncu, 2006). Also Ojha *et al.* (2010) showed that after using four treatments including gibberellin, potassium nitrate, ascorbic acid and thiourea on seeds of *Abrus precatorius*, despite increasing germination percentage in all treatments, the highest and

lowest germination percentage were obtained by gibberellin and thiourea treatments, respectively. Polyamines are also a group of growth regulators and could improve seed germination in some species (Szczotka and Lewandowska, 1989; Sinska and Lewandowska, 1991). Furthermore, in this research, putrescine had a significant difference compared to the control, in terms of germination percentage and germination rate of hazelnut seeds (Fig. 2).

4. Conclusions

The results of this study revealed that the hormone GA_3 and four months of cold stratification resulted in the highest germination percentage in hazelnut seeds at 82.73% and 83.75%, respectively. The cultivars and chemical treatments had significant effect on seed germination which were the highest in the local cultivar Gerd under GA_3 treatment at 100 mg/L and also after four months of cold stratification.

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Stability analysis of fruit yield of some olive cultivars in semi-arid environmental condition

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Key words: adaptability, AMMI, *Olea europaea* L., olive, stability parameters, yield.

Abstract: This study was conducted to evaluate yield stability of 12 Iranian and foreign olive cultivars in Dalaho Olive Research Station during 2006-2008. According to the variance analysis, significant variation ($p<0.01$) was observed between cultivars and years. Classification based on Duncan ($p<0.05$) showed that Konservolia was superior variety and Sevillano, Koroneiki and Zard were placed in the second group. Cultivars were divided into 3 groups based on cluster analysis using Ward method. The first principal component of the interaction between olive cultivars and the year's show 69.25% of the variance and was statistically significant at 1% level based on AMMI analysis. According to regression coefficient (*bi*) deviation from regression (S^2_{di}), Wricke's ecovalence (W_i), coefficient of determination (Ri^2) and Shukla's stability variance (δ_i^2) methods 'Mission' and 'Zard' had the higher stability. According to the AMMI stability (ASV) ranking, the following cultivars were the most stable, Mission, Amigdalolia and Koroneiki, while the most unstable were 'Konservolia', 'Sevillano', 'Roghani', 'Arbequina' and 'Abou-Satal'. 'Konservolia' even showed the lowest stability but its stability in all parameters was significant different in terms of performance. Generally 'Konservolia', 'Sevillano', 'Koroneiki' and 'Zard' were appropriate for fruit yield and will be introduced for breeding programs in semi-warm climate.

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The authors declare no competing interests.

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1. Introduction

Olive (*Olea europaea* L.) tree is an evergreen native to the Mediterranean region. Some olive wild genotypes are present in different region of Iran like Kermanshah province in the west of Iran. There are more than 40 native olive genotypes in subtropical regions of Kermanshah province like Sarpool-e-Zahab, Gilan-e-Gharb and Paveh. Marone and Fiorino (2012) reported that olive (*Olea europaea* L.) distributed across three continents from South Africa to the central part of the Africa and Horn Africa, from Egypt and Red Sea to the Mediterranean areas and Asia from Palestine, Syria, Mesopotamia and western and eastern areas of Himalaya Chain to the Southwestern of China. This report

revealed that there are some olive genotypes in three continents. In recent years, due to higher olive oil demand, the cultivation of olive has been expanded in various regions of Iran. However, the cultivation of olive tree is limited because of harsh environmental conditions and water scarcity in most of the new olive plantation areas (Arji and Arzani, 2008). The limitation of water as well as long hot summers in the regions lead to poor fruit and oil quality (Saadati *et al.,* 2013; Khaleghi *et al.,* 2015). Cheng *et al.* (2017) stated that low temperatures would be improved olive oil quality by increasing unsaturated fatty acid amounts in the fruit. Temime *et al.* (2006) reported that more unsaturated fatty acid of Chetoui olive variety was recorded in cooler regions than dry and warm regions. Despite of good vegetative growth, some of the olive varieties do not show good performance as production in warm regions. This is due to lack of adapted and stable cultivars in such environmental conditions. Check-adapted varieties and optimal stability are essential for the fruit yield. It is assumed that the stability of a genotype is very important over time in each region (Finlay and Wilkinson, 1963).

Homeostatic and agronomic are two genotypic stabilities. In homeostatic stability a certain genotype shows constant response under different conditions. But in agronomic stability, genotype yield is linked to productivity potential (Hayward *et al.,* 1993). Generally, the stability is defined as the actual performance of a genotype under changing environmental conditions. Reliable stability of production efficiency under environment changing is very important (Kan *et al.,* 2010). Stability analysis methods are categorized in two parametric and non-parametric groups (Sabaghnia *et al.,* 2006). Several methods such as regression coefficient (Finlay and Wilkinson, 1963), sum of squared deviations from regression (Eberhart and Russel, 1966), stability variance (Shukla, 1972) and additive main effects and multiplicative interaction (AMMI) (Gauch and Zobel, 1988) have been commonly used to parametric stability analysis.

Environmental sustainability of individual genotypes can also be estimated by regression analysis and cultivar will be stable when the deviation of regression was zero or at least (Hayward *et al.,* 1993). It is mentioned that regression analysis in bilinear models and analysis of variance in biadditive models have limitations in genotype and environment interaction. This restriction reduced by multiplicative components for interactions in generalized linear models (GLM) such as additive effects and multiplicative interaction (AMMI) (Gauch, 1992). In this model the main additive effects was calculated by variance analysis and then genotypes and environment interaction, which is known as multiplicative interaction, are analyzed by principal components analysis (Romagosa and Fox, 1993).

Olive is one of the fruit trees with alternate bearing tendency in which it not bear regularly (Lavee, 2007). This phenomenon is affected by different factors like genetic and physiological traits (Goldschmidt, 2005). The degree of alternate bearing in olive is highly dependent on environmental conditions (Lavee, 2007). Fruit production in olive is more irregular by climate change where adverse environmental conditions are frequent (Lodolini and Neri, 2012). For this purposes stability of olive production is very important in new olive growing region like Sarpool-e-Zehab environmental conditions. AMMI analysis was used to evaluate the stability of different crops (Esmaeilzadeh-Moghaddam *et al*., 2011), but there is lack of research in horticultural crops. Weather conditions are variable during different years in new olive cultivation regions so that we need to find out more stable olive cultivars. In the present work, the year was considered as environmental variable. Generally, the main goal of this study was the evaluation of yield stability of different olive cultivars in warm condition of Kermanshah province.

2. Materials and Methods

Material, site characterization and experimental design

This experiment was conducted in Dalahv Olive Research Station of Sarpool-e-Zahab (longitude: 45° 51´ E, latitude: 34° 30´ N, altitude: 570 m asl) to verify the yield stability of 12 Iranian and foreign olive cultivars (Table 1). Two years old self-rooting plantlets were planted in the year 2000, with 6x6 m spacing

Table 1 - Name and codes of genotypes

Genotype	Name
1	Amphisis
2	Konservolia
3	Zard
4	Amigdalolia
5	Koroneiki
6	Roghani
7	Manzanillo
8	Abou-Satal
9	Mission
10	Arbequina
11	Sevillano
12	Shenge

distance in a randomized complete block design with three replications. Each experimental unit consisted of 5 trees so that 15 trees of each cultivar were evaluated. Trees were pruned as vase shape and irrigated each three days with drip irrigation system. Climate of Sarpool-e-Zahab is warm with relatively low humidity during summer as shown in figure 1. Also soil and water analysis were reported (Tables 2 and 3).

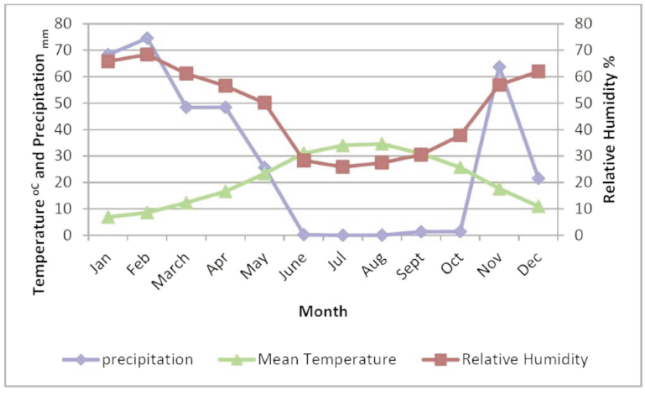

Fig. 1 - Precipitation, mean temperature and relative humidity during five years of experiment.

Table 2 - Physical and chemical soil characteristics

Soil depth (cm)	Particle-size distribution (%)			OC (%)	pH	TNV (%)	Ava. K (mg/kg)	Ava. P (mg/kg)	Total N (%)
	Clay	Silt	Sand						
0-30	34	52	14	2.25	7.7	41	520	6.2	0.18
31-60	40	37	23	0.78	7.7	45	275	2.6	0.06

Table 3 - Irrigation water chemical characteristics

EC (dS/m)	TDS (mg/l)	pH	Meq/L								S.S.P (%)	S.A.R
			CO_3^{-2}	CO_3H	Cl^-	SO_4^{-2}	Sum Anions	Ca^{2+}+ Mg^{2+}	Na^+	Sum Cations		
550	352	7.28	0	4.6	0.3	1.9	6.8	6.6	0.2	6.8	2.94	0.11

Data analysis methods

Fruit yield was measured during 5 years from 2004 to 2008. As fruit yield was low in the years 2004 and 2005, therefore 3 years (2006, 2007 and 2008) were analyzed to determine yield stability. SPSS, IRRISTAT and Excel were used for statistical analysis and the mean comparison was done by Duncan's multiple range test at p<0.05. The model of AMMI analysis is presented in equation (1).

$$Y_{ger} = \mu + \alpha_n + \beta_e + \Sigma_n \lambda_n \alpha_{gn} \gamma_{en} + \rho_{ge} + \varepsilon_{ger} \qquad (1)$$

Where α_n is the main effect of genotype; βe is the main effect of environment; n is the number of main components in AMMI model; λ_n is a single value related to the n remained main components in the model; α_{gn} is thespecific vector for the g genotype from n main component; γ_{en} is the specific vector for the e environment from n main components; ρ_{ge} is the noise and g_{er} is the error (Clay *et al*., 1995).

The following parameters were calculated to analyze yield stability, coefficient of variability (CV_i) (Francis and Kannenberg, 1978), Wricke's (1962) ecovalance (W_i2), Shukla's (1972) stability variance (σ_i^2), Pinthus's (1973) coefficients of determination (R^2), and Finlay and Wilkinson (1963) regression coefficient (*bi*).

Alternate bearing index (ABI) was calculated during three successive years from 2006 till 2008, using the following equation (2) (Monselise and Goldschmidt, 1982):

$$ABI = \frac{1}{n-1} \times \left\{ \frac{a2-a1}{a2+a1} + \frac{a3-a2}{a3+a2} + \ldots . \frac{an-an-1}{an+an-1} \right\} \qquad (2)$$

Where n = number of years, and a1, a2... an = yields in the corresponding years.

3. Results and Discussion

Fruit yield analysis of variance

The results of variance analysis for yield of olive (kg/tree) show that the genotype, environment (year) and interaction effects were significant (p<0.01) (Table 4). Specific response of the cultivars to ecological factors over a 3-year period were confirmed by the results of Duncan Multiple Range-Test,

Table 4 - Analysis of variance for olive fruit yield

S.O.S	df	SS	MS
Replication	2	2083	0.115 NS
Cultivar	11	3026.53	275.14 **
Error	22	199.59	9072
Year	4	1023.13	511.567 **
Cultivar x year	44	1423.3	64.696 **
Error	96	285115	5.94
CV%			19.18%

which proved that cultivar and year interaction effect was also significant (Table 5). It is evident from data in Table 5, for 3 study years, 'Konservolia' had the-highest mean yield, 24.69 kg/tree, while 'Roghani' had the lowest mean yield, 4.87 kg/tree. Fruit yield variability was depending on the year but olive varieties show different responses (Table 5). So, this indicates that the genotypes present different behavior in that environment. This may be due to differences in genetic basis of cultivars (Rakonjac and Živanovic,

Table 5 - Fruit yield (Kg tree^{-1}), mean yield (Kg tree^{-1}) and Alternate Bearing Index of olive cultivars during 2006-2008

Cultivar	2006	2007	2008	Mean	Alternate bearing index
Amphissis	7.03 hij	3.5 j	16.37 cdef	8.97 efg	0.16
Konservolia	26.03 b	14.57 c-g	33.47 a	24.69 a	0.06
Zard	17.37 cd	8.23 hij	16.67 cde	14.09 cd	0.01
Amigdalolia	15.39 cdef	6.533 ij	10.43 fghi	10.78 def	0.1
Koroneiki	23.8 b	13.33 d-h	16.7 cde	17.94 bc	0.08
Roghani	7.1 hij	4.5 ij	3 j	4.87 g	0.21
Manzanillo	24.53 b	7.86 hij	6.27 ij	12.89 de	0.31
Abou-Satl	7.78 hij	7.17 hij	9.1 ghij	8.02 fg	0.04
Mission	14.53 c-g	8.07 hij	14.33 c-g	12.31 def	0.003
Arbequina	7.36 hij	10.18 fghi	16.08 cdef	11.21 def	0.19
Sevillano	25.13 b	10.8 e-i	20.48 bc	18.81 b	0.04
Shenge	10.34 fghi	6.4 ij	7.13 hij	7.96 fg	0.09
Mean	15.53	8.43	14.17	12.71	

2008). Olive varieties with yield stability are important for sustainable production. Stable cultivars have high yield with lower variation during the years. Based on the results, 'Konservolia', 'Zard', 'Koroneiki', 'Amigdalolia', 'Arbequina' and 'Sevillano' have higher fruit yield with moderate yield fluctuation during the years.

Analysis of variance is only able to express the presence or absence of interaction and is not possible to interpret yield stability. For this reason, using univariate and multivariate nonparametric interpret better interaction of cultivars and years in the sustainability debate (Gauch, 1992; Falconer and McKay, 1996; Arciniegas-Alarcon *et al.*, 2011; Gauch, 2013).

AMMI analysis

The ANOVA for fruit yield using the AMMI method is presented in Table 6. There were significant differences among the genotypes, environments (Years) and G × E interaction. In this experiment environments were the years based on Citadin *et al.* (2013) method. Combined analysis of variance (ANOVA) for fruit yield of olive cultivars indicated that genotypes, year and genotype-by-year interactions (GEI) were the most important source of fruit yield variation (Table 6). The contribution of variation caused by the cultivar, year and GEI were 52.56%, 17.77%, and 24.72%, respectively. This result showed that olive cultivars had different yield performance across years. The high share of interaction in the total sum of squares is very important to use stability analysis for fruit yield of olive varieties. Similar results were reported in yellow passion fruit by Oliveira *et al.* (2014) and peanuts (Oliveira and Godoy, 2006). Maulión *et al.* (2014) stated that the significance of

Table 6 - Analysis of variance for fruit yield of 12 olive cultivars by AMMI during 2006 -2008

S. O. V	df	SS	SS%	MS
Genotypes	11	3026.52	52.56	275.14 **
Year	2	1023135	17.77	511.57 **
Cultivar x year	22	1423305	24.72	64.7 **
IPC1	12	985614	69.25	82.13 **
Noise	10	437688	30.75	43.77 NS
Error	96	285115	4.95	2.97
Total	35	5758075		

the environmental effect and GEI were used as a starting point to study yield stability among peach accessions.

AMMI analysis indicated that two first IPCA were significant (P<0.01). The IPCA1 accounted for 69.25% of the GE interaction (Table 6). However, based on these results most information can be graphically displayed using IPCA1. Biplot graph of the model (IPCA1 *vs.* yield) is presented in figure 2. According to figure 2, 'Zard' and 'Mission' showed greater yield stability by values near the origin of the IPCA1 axis. However, mean yield of 'Mission' was lower than total mean yield. 'Konservolia' with highest fruit yield and 'Roghani' with the lowest fruit yield were unstable cultivars and the others were in the intermediate stability.

One of the most important parameter in olive stability is alternate bearing. This index seems to be useful in determining the sustainability of production in fruit trees. Based on biplot AMMI1 analysis, 'Konservolia' was more productive (Fig. 2) in all years than the others and its alternate bearing index was low (Table 5). So it is recommended to use this parameter in stability evaluation. In this experiment, variability due to the year was greater than variability

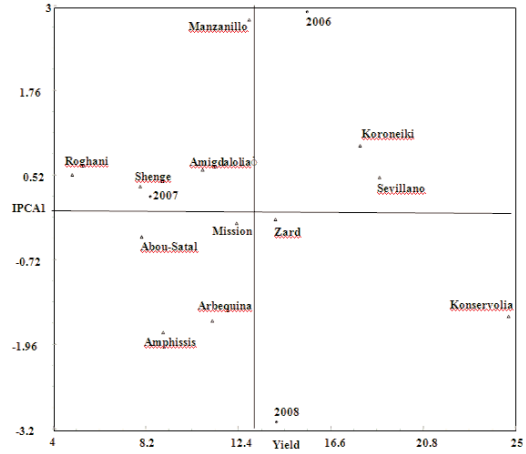

Fig. 2 - Biplot AMMI1 (means *vs* PC1) for the data on the yield of olive (Ton ha^{-1}) with 12 cultivars (•) and five years (Δ).

caused by varietal effects based on scattered effect (Fig. 2). AMMI analysis method is highlighted to study G x E interaction which combines a univariate method for the additive effects of genotypes and years with a method for the multiplicative effects of the G x E interaction (Zobel *et al.*, 1988; Citadin *et al.*, 2013). Gauch and Zobel (1996) stated that this method can contribute to the identification of widely adapted genotypes with high yields, as to the agronomic zoning for regional cultivar recommendation. A genotype will be ideal with high yields and IPCA1 values near zero. In general, according to the results of AMMI analysis Zard was the most stable cultivar with high yield and IPCA1 values near zero. 'Konservolia' and 'Sevillano' had high yield but higher IPC1 values than zero, therefore we recommend them as superior cultivars for pickling purpose. Ferreira *et al*. (2006) reported that an undesirable genotype has low stability as well as low yields.

Cluster analysis

According to the obtained dendrogram from cluster analysis using Ward method, genotypes were divided in three groups (Fig. 3). This result is confirmed by Biplot AMMI1 (Fig. 2).

Stability analysis results

Eberhart and Russell's (1966) stated that a stable cultivar is considered to be the one that has regression coefficient approximating 1.0 and standard error of regression as low as possible. According to this model a genotype with the higher mean fruit yield has general adaptability. In the present research, regression coefficients ranged from 0.02 to 2.11 for fruit yield (Table 7). This variation in regression coefficients indicates that cultivars had different responses to year's fluctuations. A genotype would be adapted to favorable conditions when regression coefficient is higher than one and other would be adapted to unfavorable conditions when regression coefficient is less than one. A genotype with regression coefficient equal to one would have an average adaptation to all environments.

According to Table 7, 'Amphissis', 'Mission' and 'Amigdalolia' with regression coefficients near to one are most stable all the years. 'Koroneiki', 'Zard', 'Manzanillo', 'Sevillano' and 'Konservolia' with regression coefficients higher than one were stable (Table 7, Fig. 2), while other cultivars like Abequina,

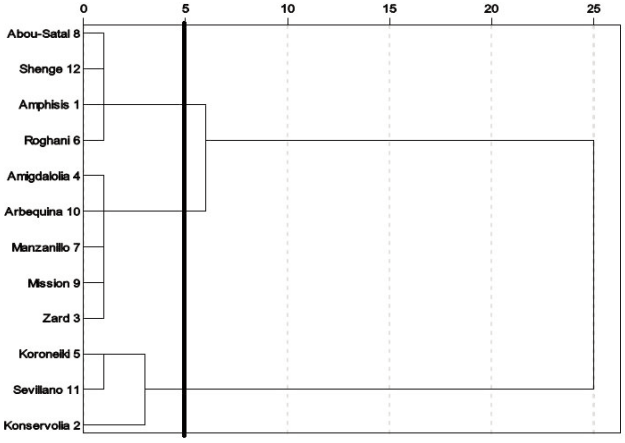

Fig. 3 - Dendrogram from cluster analysis based on Ward method.

Table 7 - Mean yields (kg/tree) and various stability measurements and their ranking orders of 12 olive cultivars evaluated during five years 2006-2008

Cultivar	Fruit yield (Kg/tree)	Rank	*bi*	Rank	S^2_{di}	Rank	*Wi*	Rank	δ_i^2	Rank	CV_i	Rank	R_i^2	Rank	ASV	Rank
Amphissis	8.97	9	1.01	6	59.32	11	59.32	9	33.44	9	74.14	11	0.33	3	0.291	4
Konservolia	24.69	1	2.11	12	54.93	10	89.87	11	51.77	11	38.57	6	0.7	7	1951	12
Zard	14.09	4	1.34	9	0.66	2	3.94	2	0.21	1	36.08	5	0.99	10	0.546	6
Amigdalolia	10.78	8	1.08	7	6.27	6	6.44	3	1.71	2	41.14	9	0.84	9	0.032	2
Koroneiki	17.94	3	1.21	8	15.34	8	16.62	5	7.81	5	29.78	4	0.73	8	0.184	3
Roghani	4.87	12	0.18	3	7.67	7	26.73	8	13.88	8	42.63	10	0.11	2	1345	10
Manzanillo	12.89	5	1.57	10	134.4	12	143.68	12	84.05	12	78.49	12	0.34	4	0.5	5
Abou-Satal	8.02	10	0.16	2	1.22	3	21.27	6	10.61	6	12.33	1	0.37	5	1239	8
Mission	12.31	6	0.96	5	0.65	1	0.69	1	1.74	3	29.86	3	0.98	11	0.027	1
Arbequina	11.21	7	0.02	1	39.64	9	66.81	10	37.93	10	39.73	8	0.0004	1	1265	9
Sevillano	18.81	2	1.92	11	2.13	4	26.21	7	13.57	7	38.88	7	0.98	11	1349	11
Shenge	7.96	11	0.43	4	3.57	5	12.85	4	5.56	4	26.36	2	0.59	6	0.937	7

bi = Finlay and Wilkinson's (1963) regression coefficient; *Sdi2* = Eberhart and Russell's (1966) deviation from regression parameter; Wi = Wricke's (1962) ecovalence; δ_i^2 = Shukla's (1972) stability variance; CV% = Francis and Kannenberg's (1978) Coefficient of variability; Ri^2= Coefficient of determination; ASV = AMMI stability value

Shenge, Abou-Satal and Roghani with regression coefficients less than one were unstable (Fig. 2). 'Konservolia' (*bi*=2.11) was productive during 2006 and 2008 than the others. High yielding varieties were not found stable with regression coefficients (*bi*). Similar results were found by Maulión *et al.* (2014) in peach stability evaluation. As olives have alternate bearing, 'Konservolia' had the highest fruit yield in non-bearing year (2007) in compare to the others (Table 5).

The most stable cultivars with the lowest S^2_{di} values were Mission and Zard. The most unstable cultivarswith the highest S^2_{di} values were Manzanillo, Amphissis and Konservolia. According to the Eberhart and Russell's (1966) model, regression coefficients (*bi*) approximating 1.0 coupled with S^2_{di} of zero indicate an average stability. 'Mission' and 'Zard' with regression coefficients near to 1 and S^2_{di} near to zero were most stable than the others. Zard cultivar had higher mean yield so it has general adaptability all the years.

Concept of ecovalence was defined by Wricke (1962), where the genotypes with low eco valence have smaller fluctuations across environments and therefore are stable. The most stable cultivars according to the ecovalence method of Wricke (1962) were Mission and Zard. These cultivars were in the ranked 6 and 4 for mean yield, respectively. The most unstable cultivars according the eco valence method were Manzanillo and Konservolia with the mean yield rank of 5 and 1 respectively (Table 7). This method would not be suitable to select high-yielding cultivars but it is useful to select cultivars with the same yield of the mean yield (Table 5). For this reason, genotypes with a low *Wi* value have smaller deviations from the mean across years and are thus more stable.

Shukla's (1972) stability variance (δ_i^2) revealed that 'Zard', 'Amigdalolia' and 'Mission' had the smallest variance across the years and were stable, while Manzanillo and Konservolia cultivars had the largest δ_i^2 and were unstable. The 'Konservolia', ranked first for mean yield, showed insteadpoor stability based on Shukla's stability variance.

The mean CV analysis was proposed by Francis (1977) to study the physiological basis of yield stability. The stable cultivar is the one that provides a high yield performance and consistent low CV (Crossa *et al.,* 1990). According to this method, 'Abou-Satal', 'Shenge', 'Mission' and 'Koroneiki' were the most stable; 'Zard', 'Konservolia', 'Sevillano' and 'Arbequina' were intermediate stable, while Amigdalolia, Roghani, Amphissis and Manzanillo were the most unstable cultivars (Table 7). Moghaddam and Dehghanpour (2001) stated that the main problem with this method is that low-yielding cultivars are placed into the category of stable cultivars. In this experiment high yielding varieties were in intermediate parts of classification.

A greater coefficient of determination (Ri^2) value is desired because higher Ri^2 values indicate favorable responses to environmental changes (Sayar *et al.*, 2013). In our study, Zard, Mission and Sevillano cultivars had higher Ri^2 values for fruit yield and 'Amigdalolia', 'Koroneiki', 'Konservolia' and 'Shenge' with medium Ri^2 values have high and medium stability in yield, respectively while others with low Ri^2 values were unstable cultivars (Table 7).

According to the ASV ranking, the following cultivars were the most stable, Mission, Amigdalolia and Koroneiki, while the most unstable were 'Konservolia', 'Sevillano', 'Roghani', 'Arbequina' and 'Abou-Satal' .

Based on yield cluster analysis olive cultivars were classified into three categories. Category 1 was cultivars having high yield and medium alternate bearing ('Konservolia', 'Sevillano' and 'Koroneiki') (Fig. 3). These cultivars are widely adapted around the world (Barranco *et al.*, 2000; Therios, 2009). Barranco *et al.* (2000) reported that 'Konservolia' has a high productivity and alternate bearing but 'Sevillano' is productive with constant production in Mediterranean regions. Also, Therios (2009) stated that 'Sevillano' is cultivated in warmer regions in Spain and Italy without any problems. Our results revealed that 'Sevillano' had relatively constant production during the experiment. Koroneiki is one of the most important olive oil cultivar in the Greece with high fruit yield and good oil quality (Barranco *et al.*, 2000). Our results indicated that its productivity was relatively high and constant but oil content (data not presented) was low.

Category 2 was cultivars having medium yield and medium or high alternate bearing ('Zard', 'Manzanillo', 'Mission', 'Arbequina' and 'Amigdalolia') (Fig. 3). Results showed that 'Arbequina' had medium productivity with medium alternate bearing. Our result was not confirmed by Therios (2009) and Barranco *et al.* (2000) findings, where 'Arbequina' has a high productivity with constant yield and high oil content in the Italy. Therios (2009) stated that Manzanillo is categorized as a good performance olive cultivar in the world. In our research, 'Manzanillo' had medium productivity with high

alternate bearing. Mission is a dual-purpose commercial olive cultivars in the world (Therios, 2009). Mission's productivity was medium and alternate in our research. Amigdalolia is an olive cultivar originated from Greece with medium productivity and alternate bearing (Barranco *et al.*, 2000). Our result represent that this cultivar show medium productivity and alternate bearing.

Category 3 was cultivars having low yield and low, medium or high alternate bearing ('Abou-Satl', 'Shengeh', 'Roghani' and 'Amfissis') (Fig. 3). We do not recommend these cultivars for planting in warm environmental condition.

4. Conclusions

In conclusion, one of major purpose of yield-trial research is to select the best cultivar for a growing region. An ideal cultivar should have the highest mean performance and be highly stable. Such an ideal cultivar would have the greatest vector length of the high-yielding genotypes and zero (G × E). In this study, Zard cultivar performed as the ideal cultivar based on almost mentioned methods. Konservolia, Sevillano and Koroneiki were the highest yielding cultivars in the regional trials. Generally, 'Konservolia' and 'Sevillano' are introduced for pickling use; while 'Koroneiki' is not suitable for cultivation in hot and dry regions due to low oil content (data not presented).

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Diversity of plant growth promoting Rhizobacteria of *Rhus tripartitus* in arid soil of Algeria (Ahaggar) and their physiological properties under abiotic stresses

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Abstract: Plant Growth Promoting Rhizobacteria (PGPR) associated with Ucria *(Rhus tripartitus*) represents a good alternative for including this crop in revegetation programs in arid area. In this study, 137 bacterial strains were isolated in Tryptic Soy Agar medium (TSA) from six samples of ucria's rhizospheric soil (Ahaggar, Algeria), based on colony characteristics, Gram © reaction, oxidase and catalase tests. To evaluate their PGP activities and their physiological characteristics under stress environment, ten tests were made. Sixty strains of 16 genera were selected for their PGPR abilities, which represent 43.79% to the total of rhizobacteria isolated. The maximum bacterial population were *Bacillus* (35%). 71.66% of isolates were able to solubilize the phosphate, 31.66% were able to produce Indole Acid Acetic (IAA), 58.33% were siderophore producers, 28,33% were able to produce Cyanhydric Acid (HCN) and 70% were able to grow without any source of nitrogen. Indeed, PGPR strains have shown tolerance and/or resistance to several experimental environmental conditions. As a conclusion, the PGPR strains of Ucria's rhizosphere were shown a good potential for biofertilization and biocontrol of crops, and their tolerance to abiotic stresses is an interesting step to support their utilities.

1. Introduction

Rhus tripartitus (called Ucria, African sumac and Tahounek in tamahaq) is an important medicinal plant belonging to the Anacardiaceae fam-

ily. This species often grows in areas of marginal agricultural capacity. It encountered in Algeria, in arid areas especially in the mountains of the Ahaggar where the indigenous people (Touaregs) use it to treat gastric disorders (Chermat and Gharzouli, 2015). This plant is also found in the North-Eastern part of Saudi Arabia and in Tunisia where it spreads in the center to the far southern part of the country. It is used in the Arabian traditional medicine for centuries to treat cardiovascular and gastrointestinal disorders and inflammatory conditions (Chetoui *et al.*, 2013; Shahat *et al.*, 2016).

The study of rhizosphere bacteria from medicinal plants is very important, as they are well known to have impact on plant growth and also produce industrially important metabolites and improve quality of medicinal products (Bafana and Lohiya, 2013). Considerable numbers of studies were focused on the beneficial effects of bacterial species that colonize the rhizosphere of many plant species and proved their beneficial effects on plant growth, yield, and productivity as well as their role in the reduction of their susceptibility to diseases caused by phytopathogenic bacteria, fungi, viruses and nematodes and even against abiotic stresses. These bacteria have been called «Plant Growth Promoting Rhizobacteria» (Kloepper *et al.*, 2004; Orhan *et al.*, 2006; Miransari, 2014; Nadeem *et al.*, 2014; Gupta *et al.*, 2015). These PGPR's can enhance the plant growth by direct mechanisms such as the fixation of atmospheric nitrogen, the solubilization of minerals like phosphorus and iron, the production of siderophores and enzymes, the synthesis of phytohormones like the auxin, indole-3-acetic acid (IAA), cytokinins and gibberellins, their role in lowering of ethylene levels and the induction of systemic resistance. Indirect mechanisms are used by PGPR to benefit the plant growth by the induction of the disease resistance by producing antibiotics or hydrogen cyanide, competition for nutrients, extracellular enzymes production and others (Glick, 1995; Vessey, 2003; Adesemoye *et al.*, 2009; Saharan and Nehra, 2011; Saha *et al.*, 2016).

To the best of our knowledge, there are no studies conducted on the rhizospheric bacteria associated with *Rhus tripartitus*, a medicinal plant that grows in Ahaggar (Algeria). We hypothesize, that this plant harbors a diverse group of rhizospheric bacteria that can help Ucria to cope with harsh environmental conditions. So, the main objectives of this study were to characterize the isolated rhizobacteria associated with the rhizosphere of *Rhus tripartitus,* their propreties as plant growth promoting bacteria and their capacities to tolerate abiotics stresses.

2. Materials and Methods

Sample collection, isolation and characterization of rhizobacteria

Six soil samples are taken from the rhizosphere of the wild ucria shrubs from Ilaman region in Tamanrasset, an arid area, which is located in the National Culturel Parc of Ahaggar in the south of Algeria (22° 49' 59'' north, 5° 19' 59'' east) during March, 2017. Each soil sample was collected at a depth of 15 cm, around the root and placed in a sterile container. The samples collection was transported to the laboratory in an ice box set at 4°C.

Tenfold serial dilution of the samples was made by mixing the soil with sterile water, and plating on a Tryptic Soy Agar medium (TSA). The plates were inverted and incubated, at 30°C for five days. The maximum of bacterial colonies present on plates were purified and characterized. The Gram reaction, oxidase reaction and catalase test were performed as per standard procedure.

In vitro screening and identification of Plant Growth Promoting Rhizobacteria

The collected rhizobacterial isolates associated with *Rhus tripartitus* were tested for their capacities to produce plant growth promoting effects. The PGPR were identified by using API galeries E20, NE20, CHB, Staph and NH (API, bioMerieux sa, Lyon, France). All the strains were preserved in nutrient broth added with 20% glycerol at -80°C.

Nitrogen fixation. The fixation of molecular nitrogen is tested on a free nitrogen medium. The bacterial isolates are inoculated on the plates and incubated at 25°C for 24-48 h. The growth on this medium after being transferred ten times in the same medium reflects the ability of bacteria to fix nitrogen (Haahtela *et al.*, 1983).

Production of HCN. The strains ability to produce Hydro Cyanic Acid (HCN) is carried out according to the method of Lorck (1948) on solid bennett agar amended with 4.4 g/l glycine is inoculated with a loop of the bacterial culture. 90 mm Whatman paper are dipped in sodium picrate solution (0.5% picric acid and 2% sodium carbonate) for one minute and then placed underneath the Petri plates lids. The plated were sealed with parafilm and incubated at 30°C for four days. The appearance of an orange to

red color indicates the production of HCN.

Solubilization of phosphates. Qualitative phosphate solubilization activity was tested on NBRIP (National Botanical Research Institutes Phosphate) medium by applying a spot of 20 µl of bacterial suspension on the surface of the agar and incubated at 30°C for 15 days (Nautiyal, 1999). A clear halo zone around the colony is an indication of phosphate solubilization. The calculation of the solubilization index (S.I.) is carried out according to the formula developed by Kumar and Narula (1999):

S.I. = Diameter of the halo around the spot/Diameter of the spot

Production of siderophores. The production of siderophores on solid medium is carried out qualitatively on Chrome Azurol S (CAS) medium as described by Schwyn and Neilands (1987). The CAS plates were prepared and divided into Sectors and inoculated with bacterial culture spots (10 µl of 10^6 CFU/ml) and incubated at 25±2°C for 48-72 h. The development of an orange yellow halo around the colony was considered positive for the siderophore production. The change in color is due to the transfer of ferric ions from the CAS to the siderophores. The calculation of the ratio (halo diameter/diameter of the bacterial colony) makes it possible to compare production differences between bacterial strains.

Production of indole acetic acid (IAA). The production of IAA was determined according to the method of Holt *et al.* (1994). The principle is to inoculate the selected strains on the nutrient broth containing 0.1 g/l of L-tryptophan. The change of the solution color from yellow to pink or red when we add the reagent of Salkowski (50 ml, 35% perchloric acid; 1 ml 0.5 $FeCl_3$) is an indication of positive result.

Screening of PGPR isolates for stress tolerance

Influence of salinity. To evaluate the strains ability of these strains to grow in salinity levels, different concentrations of NaCl (1%, 5% and 8%). The salt were added to TSA medium in the liquid stage (infusion at 40°C) and deposited on the magnetic stirrer to dilute it.

Influence of pH and temperature. In order to test the ability of the bacteria to grow in alkaline and/or acidic environment, two media were prepared with the addition to 250 ml of TSA of 3 g of solid NaOH 6.4 M and 1 ml of KCl 12 N to obtain a pH of 8.8 and 6 respectively.

For studying the effect of incubation temperature on growth of the isolates, the bacterial cultures were grown on TSA medium and incubated at a variables temperatures (10, 20, 30, 40 and 50°C).

Tolerance of heavy metal. The sensitivity of the selected strains to Copper (Cu), Zinc (Zn), Bromine (Br), Cyanide (Cn), Fluorine (F) and Silicon (Si) were tested. For this test, six TSA media of 250 ml were prepared with respectively three grams of solid KBr, CaF_2, SiO_2 and one gram of K3[Fe(Cn)6], $ZnSO_4.7H_2O$ and $CuSO_4.5H_2O$. Any growth on this media reflects the ability of strains to tolerate the heavy metal toxicity.

Antibiotics resistance. The selected strains were tested for their susceptibility to antibiotics on Muller-Hinton medium. It consists of bringing the germ into contact with disks of blotting paper impregnated with a given antibiotic at concentrations determined by the standardization of the antibiogram according to the Clinical and Laboratory Standards Institute (CLSI), to determine the sensibility of this germ to antibiotics which allows classifying it in the category: R (resistant), S (sensible) or I (intermediate). Ten different antibiotics were used: Fosfomycin (FOS) 50 µg, Rifampin (RA) 5 µg, Nalidixicid (NA) 30 µg, Spiramycin (SP)100 µg, 30 µg, Novobiocin (NV) 30 µg, Teicoplycin (TEI) 30 µg, Kanamycin (K) 30 µg and Erythromycin (E) 15 µg (all from Sigma Chemical Co., St. Louis, Mo.).

3. Results

In this study, we focused on the diversity of bacterial community of ucria's rhizosphere and the evaluation of their plant growth promoting abilities, also under abiotic stresses.

Isolation and characterization of rhizobacteria

One hundred and thirty seven (137) culturable bacteria were isolated from the rhizosphere of six healthy ucria plants using TSA medium. The rhizobacteria isolates showed a diversity of phenotypic and cultural characteristics of their colonies. Infact, 54.19% of the isolated rhizobacteria were Gram negative. 100% of strains showed positive test to catalase and variability to oxidase reaction (67.88% negative).

Screening and identification of PGPR

Biochimical characterization of PGPR isolates. The isolates were grouped into 16 genera of *Bacillus*,

Pseudomonas, *Ewingella*, *Staphylococcus*, *Alcaligenes*, *Micrococcus*, *Kocuria*, *Chryseomonas, Chryseobacterium*, *Cedecea*, *Shigella*, *Yersinia*, *Providencia*, *Acinetobacter*, *Haemophilus* and *Aeromonas* based on cultural, morphological and biochemical characteristics. The maximum and minimum populations were *Bacillus* (35%), and *Haemophilus* and *Aeromonas* with a percentage of 1.66% each (Table 1).

Table 1 - Functional diversity of PGPR strains isolated from ucria's rhizosphere

Sample No	Division/strains	Functions
1	***Gamma Proteobacteria***	
	Cedecea lapagei	Solubilization of phosphate, production of HCN and siderophores
	Chryseomonas luteola	Solubilization of phosphate, production of siderophores and nitrogen fixation
	Chryseomonas luteola	Solubilization of phosphate, production siderophores and nitrogen fixation
	Firmicutes	
	Bacillus megaterium	Solubilization of phosphate, production of siderophores and nitrogen fixation
	Bacillus megaterium	Solubilization of phosphate, production of AIA and siderophores and nitrogen fixation
	Bacillus circulans	Solubilization of phosphate, production of AIA and siderophores,
2	***Gamma Proteobacteria***	
	Ewingella americana	Solubilization of phosphate, production of AIA and siderophores
	Chryseomonas luteola	Solubilization of phosphate, production of AIA and siderophores and nitrogen fixation
	Cedecea lapagei	Solubilization of phosphate, production of siderophores and nitrogen fixation
	Cedecea lapagei	Solubilization of phosphate, production of AIA and siderophores and nitrogen fixation
	Firmicutes	
	Bacillus licheniformis	Solubilization of phosphate, production of AIA and siderophores
	Bacillus subtilis	Solubilization of phosphate, production of siderophores and nitrogen fixation
	Bacillus licheniformis	Solubilization of phosphate, production of AIA and siderophores and nitrogen fixation.
	Bacillus licheniformis	Production of AIA and siderophores
	Actinobacteria	
	Kocuria varians	Production of AIA
3	***Gamma Proteobacteria***	
	Chryseomonas luteola	Solubilization of phosphate, production of AIA and siderophores and nitrogen fixation
	Providencia rattgeri	Production of AIA
	Ewingella americana	Production of siderophores
	Firmicutes	
	Bacillus subtilis	Production of HCN and fixation of azote
	Bacillus non reactiv	Production of siderophores and fixation of azote
	Bacillus licheniformis	Production of HCN and fixation of azote
4	***Gamma Proteobacteria***	
	Yersinia pestis	Solubilizationof phosphate, production of siderophores and nitrogen fixation
	Haemophilus aphrophilus	Solubilization of phosphate, production of AIA, and nitrogen fixation
	Aeromonas salmonicida	Solubilization of phosphate, production of HCN and siderophores
	Pseudomonas aeruginosa	Solubilization of phosphate, production of HCN and nitrogen fixation
	Cedecea lapagei	Solubilization of phosphate, production of siderophores and nitrogen fixation
	Acinetobacter colcoaticus	Solubilization of phosphate and production of AIA,
	Acinetobacter baumanii	Solubilization of phosphate, production of AIA and nitrogen fixation
	Chryseomonas luteola	Solubilization of phosphate, production of HCN and siderophores.
	Acinetobacter baumanii	Solubilization of phosphate and nitrogen fixation
	Firmicutes	
	Staphylococcus lentus	Solubilization of phosphate and production of AIA.
	Staphylococcus lentus	Solubilization of phosphate, production of siderophores and nitrogen fixation
	Actinobacteria	
	Micrococcus ssp	Solubilization of phosphate, production of siderophores and nitrogen fixation
	Béta Proteobacteria	
	Alcaligenes faecalis	Production of AIA and nitrogen fixation
5	***Gamma Proteobacteria***	
	Chryseomonas luteola	Production of siderophores and nitrogen fixation
	Chryseomonas luteola	Solubilization of phosphate, production of AIA and siderophores and nitrogen fixation
	Chryseomonas luteola	Solubilization of phosphate, production of HCN and AIA.

To be continued

Table 1 - Functional diversity of PGPR strains isolated from ucria's rhizosphere *continued*

Sample No	Division/strains	Functions
5	***Firmicutes***	
	Bacillus circulans	Solubilization of phosphate, production of HCN and siderophores and nitrogen fixation
	Bacillus circulans	Solubilization of phosphate, production of siderophores and nitrogen fixation
	Bacillus licheniformis	Solubilization of phosphate, production of siderophores and nitrogen fixation
	Bacillus subtilis	Solubilization of phosphate, production of siderophores and nitrogen fixation
	Bacillus licheniformis	Solubilization of phosphate, production of HCN and siderophores and nitrogen fixation
	Bacillus licheniformis	Solubilization of phosphate and nitrogen fixation
	Bacillus circulans	Production of HCN
	Bacteroidetes	
	Chryseobacterium meningosepticum	Nitrogen fixation
	Actinobacteria	
	Kocuria varians	Nitrogen fixation
	Micrococcus ssp	Solubilization of phosphate, production of HCN and siderophores and nitrogen fixation
6	***Gamma Proteobacteria***	
	Ewingella americana	Production of AIA and nitrogen fixation
	Shigella spp	Solubilization of phosphate and nitrogen fixation
	Pseudomonas aeruginosa	Solubilization of phosphate, production of siderophores and nitrogen fixation
	Ewingella americana	Solubilization of phosphate, production of siderophores and HCN and nitrogen fixation
	Ewingella americana	Solubilization of phosphate and nitrogen fixation
	Providencia rattgeri	Production of AIA
	Firmicutes	
	Bacillus subtilis	Solubilization of phosphate and nitrogen fixation
	Bacillus licheniformis	Solubilizationof phosphate, production of HCN and siderophores and nitrogen fixation
	Bacillus subtilis	Solubilization of phosphate, production of HCN and nitrogen fixation
	Bacillus non reactiv	Nitrogen fixation
	Actinobacteria	
	Micrococcus ssp	Production of HCN and siderophores and nitrogen fixation
	Béta Proteobacteria	
	Alcaligenes faecalis	Solubilization of phosphatase, production of HCN and siderophores
	Alcaligenes faecalis	Production of HCN

The Rhizobacteria were identified through six samples and five Plant Growth Promoting treatments: solubilization of phosphate, production of Cyanhydric Acid, production of Indol-Acid-Acetic, fixation of nitrogen and production of siderophores.

*Plant growth promoting traits.*The isolated rhizobacteria were screened for various PGP features responsible for plant growth promotion. The PGPR isolates represent 43.79% of the total of the rhizospheric bacteria. They belong to four divisions (Gamma-Proteobacteria, Firmicutes, Actino-bacteria and Béta-Proteobacteria). The strains mostly belonging to the Gamma-Proteobacteria, most of them affiliated to the Enterobacteriaceae. According to the figure 1, species of Gamma Proteobacteria division were predominant with 45% among the isolats of PGPR of Ucria behind firmicutes with 38.33%. Infact, the species *Cedecea lapagei, Chryseomonas luteola* and *Ewingella americana* were the most representative PGPR of Gammaproteobacteria division. The species *Chryseomonas luteola* found in five samples of six rhizospheric soils (Table 1). Different combinations of PGP effects have been found, 18.33% strains have been able to produce up to 4 PGP effects against 40% were able to produce 3 PGP effects, 25% showed 2 traits of PGP and 16.66% were able to produce only one PGP effect. In fact, the genera *Bacillus, Ewingella, Alcaligenes, Chryseomonas* and *Cedecea* have showed positive screening for all the PGP traits, according to the Table 2.

Screening and assessment of phosphate solublizers and IAA producers. Among the total isolates screened for phosphate solublization, 71.66% were able to solubilize inorganic phosphate (Fig. 2) and were identified as potential phosphate solubilizing

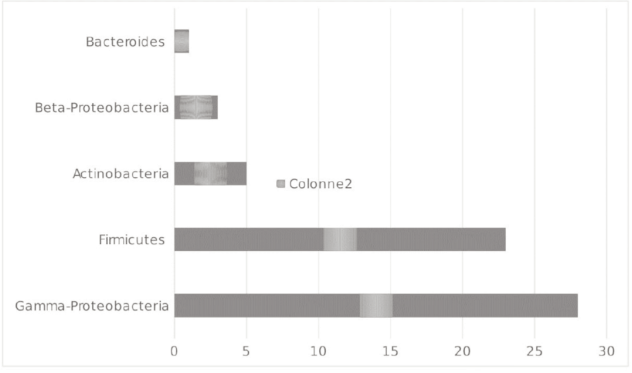

Fig. 1 - Classification of rhizospheric bacteria of Ucria on the PGP traits. The main bacteria divisions of plant growth promoting rhizobacteria from the rhizosphere of ucria.

Table 2 - Plant growth promotion activities of rhizobacteria genera isolated from ucria's rhizosphere

Genera	N_2	Fe^2	HCN	AIA	PO_2
Bacillus	+	+	+	+	+
Pseudomonas	+	+	+	-	+
Ewingella	+	+	+	+	+
Staphylococcus	+	+	-	+	+
Alcaligenes	+	+	+	+	+
Micrococcus	+	+	+	-	+
Kocuria	+	-	-	+	-
Chryseomonas	+	+	+	+	+
Chryseobacterium	+	-	-	-	-
Cedecea	+	+	+	+	+
Shigella	+	-	-	-	+
Yersinia	+	+	-	-	+
Providencia	-	-	-	+	-
Acinetobacter	+	-	-	+	+
Haemophilus	+	-	-	+	+
Aeromonas	-	+	+	-	+

N^2 = nitrogen fixation, Fe^2= siderophore production, HCN production, AIA and PO= phosphate solubilisation.

bacteria that showed a clear halo zone around the colonies on NBRIP's agar plates amended with bromophenol blue. 20 isolates out of total rhizospheric flora had the capacity to produce were IAA in the presence of L-Tryptophane.

Molecular nitrogen fixation. The ability of the isolates to grow on N-free medium indicated positive results for nitrogen fixation. In fact 70% of the isolates were able to grow even being transferred ten times in this medium which indicated their capacity to fix molecular nitrogen (Fig. 2).

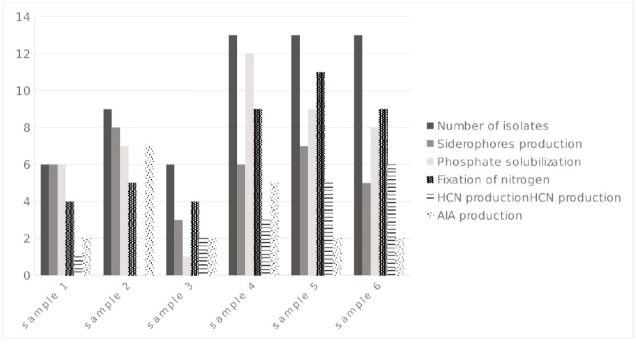

Fig. 2 - Siderophores production, phosphate solubilization, fixation of nitrogen, HCN and AIA productions of the Rhizobacteria isolates of ucria (Ahaggar).

Siderophore and HCN production. Siderophore production was registered at 58.33% of the strains based on the appearance of a halo zone of yellow orange color around the colony inoculated on CAS-agar plates. Seventeen isolates were positive for HCN production (Fig. 2).

Physiological properties of PGPR isolates under abiotic stresses. The isolated bacteria were tested for their ability to tolerate abnormal growth conditions after incubation in a wide range of salt, pH and temperature stress condition, heavy metal toxicity and antibiotics.

Influence of salinity. According to the figure 3, the most percentages of strains which can grow in the 1% and 5% salinity are respectively 96.66% and 81.66%. In addition, only 16.66% of strains can tolerate % of salinity.

Influence of pH and temperature. The rhizobacterial isolates seems to tolerate the alkalinity better than the acidity with 66.66% and 46.66% respectively. However, these isolates could grow up to higher temperature of 50°C with 85%, which means that the temperature hadn't a remarkable effect on bacterial growth (Fig. 3).

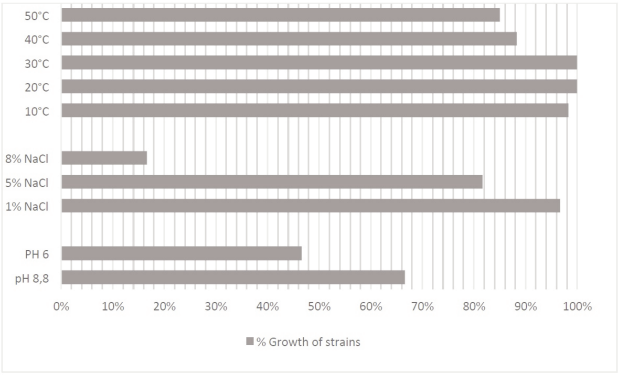

Fig. 3 - Estimation of tolerance of PGPR strains to alkalinity, acidity, salinity and temperature.

Tolerance to heavy metal. Depending on the metal tested and the species, the tolerance to heavy metals is different. In fact, we recorded a very good tolerance to silicon and bromine with respectively 80% and 78.33%. 55% of our strains have well tolerated fluoride followed by 30% for cyanide. The lowest percentages recorded were for copper and zinc with 13.33% and 3.33%, respectively. These results showed that heavy metals affected the bacterial growth (Fig. 4).

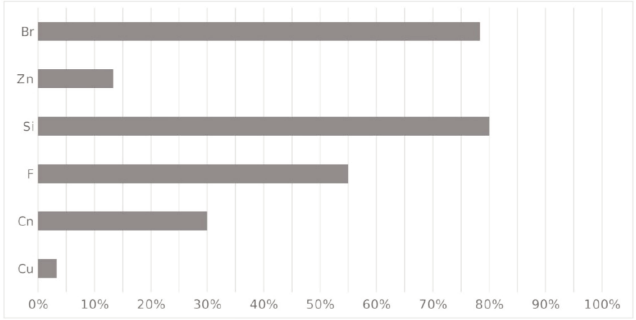

Fig. 4 - Estimation of tolerance of PGPR strains to heavy metals toxicity. Br= Bromine, Zn= Zinc, Si= Silicon, F= Fluorine, Cn= Cyanide, Cu= Copper.

Antibiotics resistance. The collection of PGPR isolates was tested for susceptibility to 10 antimicrobials. High frequency of resistance was observed for Métronidazole (MT) followed by Amoxyclav (AMC) with respectively 93.33% and 91.66% of strains. The minimum resistance was recorded for Fosfomycin (FOS) with 16.66% (Fig. 5).

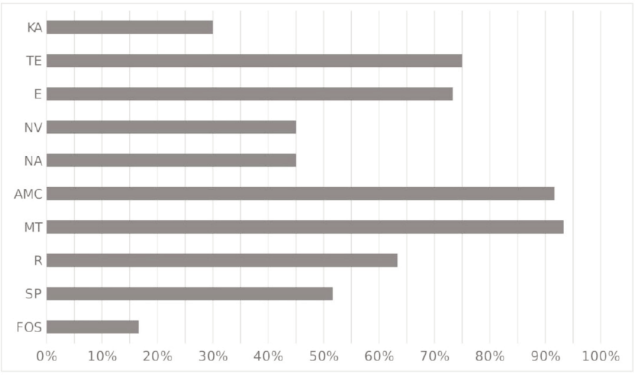

Fig. 5 - Estimation of resistance of PGPR strains to antibiotics.

4. Discussion and Conclusions

The diversity of native bacterial species in the southern Algerian soils remains largely unknown, especially for the rhizosphere of *Rhus tripartitus*. Most of the rhizobacteria isolated from ucria's rhizospheric soils have been dominated by Gram-negative (54.19%) and all were catalase positif. These results are in-line with studies realized on rhizosphere of *Brassica campestris* (Poonguzhali *et al.*, 2006) and *Eragrostis tef Zucc. Trotter* (Woyessa and Assefa, 2011).

Plant growth promoting rhizobacteria (PGPR) represent a diverse range of soil bacteria that stimulate the growth of their host when grown in association. Such rhizosphere microbes benefit by utilization of metabolites secreted by plant roots as a nutrient for their growth and promote plant growth through more than one mechanism, including production of phytohormones and biocontrol of plant pathogens (Rana *et al.*, 2011).

This present study showed a high diversity among the isolates of these species under different combination of PGP effects. The most often genera identified was *Bacillus* (35%) with a high diversity of species, such as *Bacillus megaterium, B. licheniformis, B. subtilis, B. circulans* and *B. non-reactiv*. PGPR of the genera *Bacillus* have been reported in many studies (Trivedi and Pandey 2008; Zou *et al.*, 2010; Liang *et al.*, 2011; Woyessa and Assefa, 2011; Nadeem *et al.*, 2012; Mishra *et al.*, 2014; Susilowati *et al.*, 2015). The second PGPR group found throughout this study was belonging to species *Chryseomonas luteola* (11.6%) and *Ewingella americana* (8.3%). The latter was able to increase the growth of pipper and spinach (Hou and Oluranti, 2013). The third and last PGPR group (45.1%) belonged to the genera *Cedecea, Providencia, Yersinia, Heomophilus, Aeromonas, Acinetobacter, Micrococcus, Alcaligenes, chryseobacterium, Shigella, Staphylococcus* and *Pseudomonas.* Earliers studies showed that *Pseudomonas* PGPR is a producer of HCN (Castric, 1975), improving the availability of necessary nutrient (Islam *et al.*, 2014) and an agent of biocontrol (Weller and Thomashow, 1993). The genera *Acinetobacter* promotes production of wheat, pea, chickpea, maize and barley through nitrogen fixation, siderophore production and mineral solubilization (Gulati *et al.,* 2009; Sachdev *et al.*, 2010). In constrat, *Cedecea* have never shown in the litterature as PGPR potential and molecular analysis is necessary to confirm the identification.

The industrialization of chemical fertilizers such phosphate and nitrogen has increased in the agricultural sector. These minerals are considered important limiting factors for many crops (Ahmad *et al.*, 2008). However, the aim of this study is also to evaluate the ability of rhizobacteria to promote the biodisponibility of N and P, in order to reduce of industrial fertilizers. In fact, most of ucria's rhizobacteria could fix atmospheric nitrogen (70%) and 71.66% were able to solubilize inorganic phosphate in NBRIP's medium. Similary, many studies shown that phosphorus-solubilizing microorganisms are ubiquitous in soils (Chandra *et al.*, 2007; Tsavkelova *et al.*, 2007; Banerjee *et al.*, 2010). PGPR can produce auxin-like compounds that increase the development of root system thus improving nutrient uptake by plants (Voisard *et al.*, 1989); However, out of total isolated rhizobacteria, 31.66% exhibited as producers of IAA in medium supplemented with l-tryptophan. The number of PGPR producers of HCN represent 28.33% of total isolates strains. These PGP traits have the capacity to enhance indirectly plant growth and protect them from phytopathogens (Lugtenberg and Dekker, 1999; Shahat *et al.*, 2016). Rhizobacteria producing siderophores are of great importance for the plant because they make bioavaible iron. The siderophores decreases the metal bond and formation of free radicals in the roots zone, which prevented the degradation of IAA (Yang *et al.*, 2009). Among the isolates of PGPR obtained in this study, 58.33%

are producers of siderophores. The production of siderophores in the rhizosphere increases the bacterial competition as well the root colonization (Abrol *et al.*, 1988).

The rhizosphere is characterized by large environmental fluctuations, which may promote high diversity in the rhizosphere microbial community by maintaining high niche diversity. Thus, microbial community diversity may be important especially in extreme condition, like high temperature, salinity or pH changes. Under stress conditions, bacterial rhizosphere may promote the plant growth (Cheikh and Jones, 1994). In the present research, these PGPR isolates were traited under several environmental conditions. They have showed a good potential of tolerance for the previuosly conditions, which is a value, added to their beneficial effect. Therefore, the aim of this work is not only to deal with the diversity of PGPR but also the selection of resistant strains at the most extreme conditions.

The ucria's rhizobacteria, were isolated from an arid area of Ahaggar (Algeria) and salinity is a natural feature of ecosystems in arid and semi-arid regions (Curl and Truelove, 1986). The 81.66% of PGPR studied exhibited as tolerant to the presence of 5% NaCl and 85% could growth until 50°C. The variation on temperature is an important factor that can affect the hormonal balance of the plant (Lovley, 1995). Then, certain beneficial microorganisms can influence plants response to abiotic stresses like drought and high temperature (Grover *et al.*, 2011). Therefore, the necessity of discovering species able to grow under salt stress conditions and in a high temperature, are important to include them in revegetation system in arid area. The pH is one the obvious influencing factors of microbial activity and populations in soil (Woyessa and Assefa, 2011). Most of these isolates (66.66%) can growth over alkaline pH and more than 46% of growth recorded in acidity condition, it suggests that there is a good potential to inoculate them over a range of wide pH. The growth of rhizobacteria at acidic pH values could be explained by their adaptation at arid soil. Effectively, low rainfall can probably cause an increase in acidity of soil. On the other hand, an acidic environment of roots due to CO_2 and organic acid can be included in soil acidity (Gururani *et al.*, 2012).

Moreover, the PGPR isolates of this work, demonstrated a good tolerance *in-vitro* for heavy metals toxicity, with an average of 43.33% for chemicals forms of six metals. The accumulation in soil of heavy metals can perturbate the growth and the diversity of bacterial communities. Many studies are shown the potential of application of PGPR in resistance and uptake of heavy metal by certain plants (Lovley, 1995; Yang *et al.*, 2009; Gururani *et al.*, 2012). The ability to colonize roots and antibiotic resistance are other parameters needed to detect effective PGPR strains (Siddiqui, 2005). Effectively, the study of the sensitivity of rhizobacteria to antibiotics adds PGP potential. Indeed, our strains have showed some resistance to the majority of antibiotics tested which can be involve high microbial competition in the rhizosphere. This resistance increased the chances of survival and colonization of the rhizospheric soil.

The present study reflects the preliminary work done on the rhizosphere of *Rhus tripartitus* in arid soils of Algeria. Indeed, the selection of PGPR strains, which can effectively grow under abiotics stresses conditions, can be used as promising biofertilizers and biocontroling of plants and useful in revegetation system of arid area. Before that, others investigations should be done like a bioassay *in vitro* and *in vivo* of these strains on crops according to the inoculation treatments. The study of mechanisms of toleration to toxic substances or hard environment is an interesting step to support their utilities.

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Effect of a novel hydrogel amendment and seedling plugs volume on the quality of ornamental/miniature tomato

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Key words: copolymer, pot plant, *Solanum lycopersicum* L., transplants.

Abstract: The market for ornamental/miniature plants values aesthetic morphological characteristics, which give harmony to the potted plant. These traits depend on the growing media capacity to maintain quality and plant longevity. The use of hydrogels has increased recently in order to achieve visually attractive and long-lasting plants. Thus, this study aimed to evaluate the effects of a novel hydrogel (H) amendment and seedling plugs volume (SPV) on the quality of ornamental/miniature tomato. Seedlings of tomato cv. BRS Finestra were produced in 200 and 162 plugs plastic trays - with 18 cm³ trapezium-shaped plugs and 50 cm³ conically shaped plugs, respectively. 18 cm³ plugs and H amendment presented several significant responses for plant characters - height, soluble solids, number of leaves, stem diameter, shoot dry matter and fruits weight per plant; making evident the advantages of using a growing medium of smaller volume and this copolymer amendement. Although consistent results were obtained, a combination of both these factors in terms of an optimal aesthetic value and considering all the morphological traits could not be accomplished. Therefore it's necessary to study other elements such as plant nutrition and the use of plant growth regulators to complement them, aiming to promote better quality.

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1. Introduction

Miniature tomatoes can be grown singly in small pots, or more plants in larger hanging pots. They are also ideal for window boxes or garden borders because their plant canopy diameter is little (Scott and Harbaugh, 1995). These plants combine ornamental aspects of a well-proportioned, diminutive, tomato plant with tasting fruits that can be eaten. Small plant sizes are ideal for commercial growing, shipping, and retail selling (Scott and Harbaugh, 1995).

The commercial success of the cultivation of miniature/ornamental potted plants such as tomatoes and peppers depends on consumer appeal conferred by the plant beauty, quality, vigor, color, shape, and size of leaves and fruits. In addition to that, the cultivars must present canopy harmony and be able to develop in relatively small pots (Costa *et al.*, 2015).

Non-miniature tomato cultivars tend to overgrow small containers, and their plant size is restricted by the container size (Scott and Harbaugh, 1995), producing commercially unviable plants, requiring the use of plant growth regulators (PGR) to achieve attractive compact potted grown plants (Moraes *et al.*, 2005). Another important feature of ornamental plants is the maintenance of interior quality and longevity, a never-ending effort by producers (Wang, 1992).

Growing medium ability to prevent drying out is desirable, especially in peat-based substrates. Some growers report a benefit when plants are watered with a wetting agent prior to shipment, thus making it easier for retail clerks and consumers to thoroughly rewet the medium. Interest has increased recently among growers and mass market buyers in using water-absorbing gels (Nell, 1991). However, studies investigating the effects of hydrogel (H) application on ornamental plants are scarce and limited (Ljubojević *et al.*, 2017). Particularly to ornamental/miniature vegetables, this scenario can be considered negligible.

In regard to this matter, a promising nanocomposite hydrogel developed by an innovative technique using calcium montmorillonite showed great swelling degree, higher than 2000 times in water. The formulated H with high calcium montmorillonite content (approximately 50.0% wt) as well as featuring high loading capacity and individual and simultaneous release, denotes an interesting material for agricultural applications (Bortolin *et al.*, 2016). Thus, the present study has the objective of evaluating the effects of a novel hydrogel amendment and seedling plugs volume (SPV) on the quality of ornamental/miniature tomato.

2. Materials and Methods

Plant material and growth conditions

An experiment was conducted from July 18 (sowing) to October 25 (harvest), 2017 at Embrapa Vegetables - 996 m altitude, 15° 56' S, and 48° 08' W - Brasília-DF, Brazil, in a glass-glazed greenhouse. A photoselective shading net (Aluminet® IC 50 - Ginegar Polysack®) was installed 2 m above the benches. The greenhouse presented an air temperature of 15°C minimum and 44°C maximum, average DLI (daily light integral) of ≈ 11 $mol.m^{-2}.d^{-1}$ considering a 12h period of sunlight and 81% maximum and 11% minimum of humidity. Measures were taking after seedlings transplantation 15 minutes apart by sensors connected to a Datalogger (Watchdog 1000 Series Micro Station - Spectrum Technologies®).

Seedlings were produced in plastic trays with 200 and 162 plugs (JKS® - 18 cm^3 trapezium shaped plugs and 50 cm^3 conically shaped plugs, respectively) using a peat moss based substrate (Carolina Soil®) (Fig. 1 A). *Solanum lycopersicum* L. cv. BRS Finestra was selected, being the first Brazilian ornamental/miniature tomato cultivar released by Embrapa as a product for a very specific and demanding market (Giordano *et al.*, 2001).

They were transplanted at 36 DAS (days after sowing) to 1 dm^3 pots (Nutriplan® NP14) filled with a pine bark-based substrate (Rohbacher®) with the following characteristics: EC - 0.4; pH - 6.0; Water holding capacity - 50%; Humidity - 60%; Density - 185 kg m^3. Filling were complemented with 5 g of Bokashi compost per pot (Korin® - Garden Bokashi).

Nutrients were supplied weekly during the experiment, with a solution developed for ornamental peppers containing 14.4, 1.95, 12.92, 2.5, 1.0, 2.44 mmol/L of N, P, K, Ca, Mg, S and 30, 5, 50, 40, 2 and

Fig. 1 - (A) Seedling plugs volume and visual comparison - 50 cm^3 (left) and 18 cm^3 (right); (B) plants from 50 cm^3 plugs with hydrogel amendment (left) and without (right); (C) plants from 18 cm^3 plugs with hydrogel amendment (left) and without (right).

0.1 mol/L of B, Cu, Fe, Mn, Zn and Mo, respectively, according to Costa *et al.* (2015).

Watering from seeding to the transplantation was performed twice daily with enough water to start the run off at the bottom of the trays. All other cultural practices were performed using technical recommendations for controlled environment miniature tomato cultivation (Schwarz *et al.*, 2014).

Plant morphological characteristics and fruit quality analysis

All plant morphological characteristics were analyzed when a commercial stage was reached, meaning that when 50% of the plant population of each treatment had at least 30% of fully ripe fruits or visually marketable fruits with the maximum size and shape typical of growth for miniature/ornamental tomato. These agronomic characteristics are based on the morphological descriptors suggested by IPGRI (1995) and were validated by Costa *et al.* (2015) for ornamental peppers:

(P) - Precocity - expressed by the number of days between transplantation and commercial stage;

Dry matter content of shoot (SDM) - parts were dried in oven at 70°C until constant weight and values were obtained by equation:

SDM% = {dry weight (g)/fresh weight (g) x 100};

(PH) Plant height (cm) - measured using a ruler, from the stem bottom until the last fully expanded leaf;

(SD) Stem diameter (mm) - measured above the cotyledon leaves using a digital caliper;

(CR) Plant canopy ratio - obtained from between the longitudinal (LD) and transverse diameters (TD), where the closer the value to 1, more circular is the canopy.

(NL) Number of leaves - expressed by counting the number of leaves per plant;

Number of fruits per plant (NFP);

Number of fully ripe (NR);

(FW) Fruit weight per plant (g) - expressed by the sum of different fruits ripening stages per plant;

(FD) Ripe fruits diameter (cm) - obtained from the longitudinal diameter of 4 fruits from each treatment.

The following fruit quality basic parameters were analyzed by AOAC (2010) and McGuire (1992) methodologies, utilizing 4 fruits from each treatment:

(SS) Soluble solids (°brix);

(AC) Acidity;

SS/AC ratio;

Color (C) - evaluated by means of ripe fruits with the measurement of the colorimetric parameters L*, a*, b* C* and angle Hue (h°) in fruits. The L * coordinate expresses the degree of clarity of the measured color (L = 100 = white; L = 0 = black), C* the intensity of the color and h ° the saturation of the color.

Statistical design and analysis

The trial was conducted in a 2x2 factorial design in a complete randomized scheme, with six replications. The presence (amendment) and absence of H represented the first factor. SPV- 18 cm³ plugs and 50 cm³ plugs - represented the second factor. Each replication was composed of 10 plants. The rate of H per substrate consisted of 2.0% (on volume/volume basis) and followed previous studies recommendations (Bortolin *et al.*, 2016). Data were subjected to an analysis of variance (ANOVA). All computations were performed with ASSISTAT® software (Silva and Azevedo, 2016). Preliminary analysis indicated that PH, and NPP presented a skew and overdispersed distribution, and it was required a transformation to normalize data. Thus, their means were evaluated after square root transformation. Normality of residuals was tested using Shapiro-Wilk test (alpha 5%) and the distribution presented as normal subsequently.

3. Results

Plants grown utilizing 50 cm³ plugs required a shorter period of time to reach the ideal commercial stage - precocity (P). When 18 cm³ plugs were used, this stage was reached at the same time, independently of H amendment (Table 1). This differentiates the 50 cm³ plugs in 5 days (earlier) when compared to 18 cm³ plugs. A difference of 9 or 14 days when 50 cm³ plugs were amended or not with H, respectively, was also observed when comparing to 18 cm³ plugs. 50 cm³ plugs use resulted in plants with a very long stem (Fig. 1 B).

Table 1 - Ornamental/miniature tomato precocity (P) stage reached according to hydrogel amendment and seedlings plugs volume

Plug volume	P (days after transplantation)	
	With hydrogel	Without hydrogel
18 cm³	57	57
50 cm³	43	48

P= when 50% of the plant population had at least 30% of fully ripe fruits or visually marketable fruits with the maximum size and shape typical of growth for miniature/ornamental tomato.

PH values were significant for both factors and their interaction (Tables 2 and 3), with 18 cm³ plugs and H combination reaching 19.63 cm. The general mean height value achieved in this trial (17.1 cm) is typical of the cv. BRS Finestra and is within the values quantified by Scott and Harbaugh (1995) evaluating different miniature tomatoes, ranging from 9 cm with cv. Micro-Tom to 25 cm with cv. Micro-Gold.

Characters of aesthetic significance, such as plant architecture, number, position, and color of fruits, leaves shape and density are some of the reasons that ornamental species of the *Solanaceae* family are admired, being strictly related to plant longevity and to facilitate cultural handlings (Neitzke *et al.*, 2016). One of these traits, NL, is consistent with the cultivar and growing conditions, presenting a significant response for all the studied factors. The interaction of 18 cm³ plugs and H enhanced the NL to 51.6 (Table 2) which can be perceived by the observation of leaves density in Fig. 2 D, but this response was not converted into a greater NFP.

CR represents the aspect of the aerial parts of the plant, where the closer the value to 1, more circular it is the canopy. Therefore, when 50 cm³ plugs were used, a more circular shape was attained (LD/TD = 1.16) (Table 2). This format has a greater visual appeal, which is often decisive in the choice of the consumer to become more attractive and makes it easier to handle (Costa *et al.*, 2015), although with plants being long-stemmed, a visual aspect uncommon for miniature tomatoes was observed, resembling a palm tree shape (Fig. 1 B).

Table 2 - Interaction between hydrogel and seedling plugs volume for plant ornamental/miniature tomato characters

Plug volume	50 cm³	18 cm³
Plant height (cm)		
With hydrogel	16.49 aB	19.63 aA
Without hydrogel	16.30 aA	16.02 bA
Soluble solids (°Brix)		
With hydrogel	4.65 aB	5.35 aA
Without hydrogel	4.50 aB	5.20 aA
Number of leaves (per plant)		
With hydrogel	37.41 aB	51.60 aA
Without hydrogel	37.45 aB	43.88 bA
Canopy ratio		
With hydrogel	1.16 aB	1.32 aA
Without hydrogel	1.23 aA	1.26 aA

Means followed by the same lowercase letters in the columns and capital letters in the lines do not differ by Tukey test at 5% probability.

Table 3 - Hydrogel amendment and seedlings plug volume effect on ornamental/miniature tomato plant characters

Plant characters	Hydrogel		Plug volume	
	With	Without	50 cm³	18 cm³
Stem diameter (mm)	5.53 a	5.31 b	5.29 b	5.55 a
Shoot dry matter (%)	19.49 a	18.37 b	18.31 b	19.55 a
Fruit weight per plant (g)	68.39 a	67.17 b	77.39 a	58.17 b
Number of fully ripe fruits			3.08 a	2.43 b
Number of fruits per plant			9.90 a	6.01 b

Means followed by the same lowercase letters in the columns and capital letters in the lines do not differ by Tukey test at 5% probability.

Fig. 2 - Ornamental/miniature tomato plants visual aspect when precocity stage was reached. (A) from 50 cm³ plugs with hydrogel amendment; (B) from 50 cm³ plugs without hydrogel amendment; (C) from 18 cm³ plugs without hydrogel amendment; (D) from 18 cm³ plugs with hydrogel amendment.

SDM and SD values were significant for both treatments (Table 3). Costa *et al.* (2015) evaluated the quality of ornamental pepper using two substrates and genotypes, founding distinct SDM content responses, with values ranging from 20.58% to 26.54%, corroborating with the best results here found. SD results presented in this study (5.53 mm with H amendment and 5.55 mm from 18 cm³ plugs)

(Table 3) are inferior but consistent with the ones found in the work of Backes *et al.* (2007) with ornamental pepper. They obtained an SD of 6.42 mm as the best result using controlled release fertilizer mixed to a commercial substrate. Even though SD values in this study were significant to H and SPV, only a slight increase was observed amongst treatments.

NFP, NR, and FW values were superior with the use of 50 cm³ plugs (Table 3). With a compact canopy and well-distributed leaves, the photosynthetic process that depends on the interception of light energy most likely was converted into chemical energy in an efficient way, resulting in this positive response.

SS and AC values were significant for SPV and also for the interaction of 18 cm³ plugs with H. The values of 5.35 and 0.61 achieved for SS and AC, respectively, are comparable to 5.37 and 0.65 from cv. Micro-Gold bred by Scott and Harbaugh (1995), ensuring that cv. BRS Finestra produces mild tasting fruits that can be appreciated/consumed.

NI (general mean value of 1.52), FD (general mean value of 3.1 cm), SS/SC ratio (general mean value of 8.38) and C (general mean value of L* 40.67; C* 51.05 and h° 48.51) were not significant (NS) to both treatments. For C values, although being NS, ripe fruits exhibited an intense red coloration (Fig. 1B). This color is favored by consumers, a point which is believed to possess the highest carotenoids content such as β-carotene and lycopene (Kader *et al.*, 1977).

4. Discussion and Conclusions

Plants grown with 50 cm³ plugs, in the presence or absence of H amendment, demanded a shorter period of time to reach the ideal commercial stage - precocity (P). Precocity is a very important attribute, as it would allow the grower to commercialize plants earlier and to reutilize the spaces emptied in the greenhouse benches. But for its recommendation, the fact that PH in this plug volume resulted in an undesired visual aspect displaying a very long stem, needs to be taking in to account.

The 50 cm³ plugs were significant for NFP, NR, NG, and FW characters as well. An efficient interception of light with plants produced using this plugs made most likely converted chemical energy into fruits, being a response of a compact canopy with well-distributed leaves. 18 cm³ plugs were significant for SD, SDM and FW, making evident the advantages of using a smaller volume of substrate, which can be fully employed for the growing of ornamental/miniature tomatoes in 1 dm³ pots.

The use of H resulted in higher SD, SDM and FW values, considered fundamental in the aspect of the plant canopy and its longevity. H and SPV interaction responded differently to the analyzed plant characteristics, with 18 cm³ plugs and H amendment together showing significant responses only for PH and NL.

In conclusion, although several consistent results for plant characters were obtained when 18 cm³ plugs and H were used solely, a combination of both in terms of an optimal aesthetic value and considering all the morphological traits could not be accomplished. Therefore it's necessary to study other elements such as plant nutrition and the use of PGR to complement them, aiming to promote better quality.

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Yield, quality, antioxidants and mineral nutrients of *Physalis angulata* L. and *Physalis pubescens* L. fruits as affected by genotype under organic management

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Abstract: Introduction and selection of unconventional plants with high concentration of biologically active compounds is one of the worthy ways for producing functional food, which is beneficial to human health. Research was carried out in northern Europe (Russia) with the purpose to assess yield, quality and biologically active compounds concentration in *Physalis angulata* and *Physalis pubescens* fruits. *P. angulata* cultivars Konditer and Konditer 2 gave the highest yield (11.3 and 11.0 t·ha^{-1} respectively), due to the highest mean fruit weight (80 and 70 g respectively); *P. pubescens* variety Zolotaya rossip had the worst outcome due to the very small berries (3 g), in spite of their highest number per plant (165). 'Zolotaya rossip' fruits overall attained higher values of quality indicators compared to all *P. angulata* cultivars. Positive correlations were recorded between dry matter and polyphenols as well as between total sugars and polyphenols. *Physalis* fruits showed to be a good source of antioxidants, K, Mg and P for human beings. Taste index turned out highly reliable in evaluating fruit quality and it was dependant on dry matter, total sugars, polyphenols and Ca.

1. Introduction

The availability of new functional food has been becoming more and more important for human health regulation and protection against the major diseases of the millennium.

Physalis fruit is mostly an exotic product, imported from tropical and subtropical regions, particularly from Central America, where this genus is characterized by the highest biodiversity (Medina-Medrano *et al.*, 2015). The popularity of this genus plants has been increasing in several world

Countries, due not only to its high nutritional value and exclusive taste (Puente *et al.*, 2011), but also to its effectiveness as a medical plant. Indeed, all plant parts are useful in modern medicine for their properties: antiasthma, antihepatitis, antimalaria and antidermatitis (Chang *et al.*, 2008), antiallergenic, anticarcinogenic, anti-inflammatory, antihyperglycemic, antimicrobial, antiseptic, antiviral, cardio-protective, diuretic, expectorant, febrifuge (Bastos *et al.*, 2006; Sharma *et al.*, 2015). In this respect, a negative correlation has been shown between the level of *Physalis* consumption and the risk of cardiovascular, pulmonary and gastrointestinal diseases (Leenders *et al.*, 2014; Hjartaker *et al.*, 2015). Moreover, the high antioxidant activity of water and alcoholic extracts of *Physalis* is reportedly beneficial to patients suffering from Alzheimer's disease as well as from memory and concentration deficit (Susanti *et al.*, 2015). Among the tens of *Physalis* species, *P. angulata* and *P. pubescens* are the most suitable for production in the northern Europe, due to their frost resistance and tolerance to fungal and bacterial diseases (Mamedov *et al.*, 2017). Notably, the genetic selection carried out in Russia allowed to create interesting varieties, suitable for cultivation both in central Russia (Kondratieva and Engalichev, 2013; Mamedov *et al.*, 2017) and in Siberia (Makarov, 2002), mainly belonging to *P. angulata*. However, a few results concerning the variability of interspecies quality characteristics and even no cultivar comparisons of biologically active compounds accumulation in *Physalis* fruits have been published so far.

Taking into account the scarcity of literature reports, the present research was aimed at evaluating the varietal differences in yield, quality and antioxidant content of *P. angulata and P. pubescens* fruits grown in northern Europe.

2. Materials and Methods

Growth conditions

Six *P. angulata L.* cultivars (Violet, Lakomka, Konditer, Konditer 2, Lezhky, Korolek) and one *P. pubescens L.* cultivar (Zolotaya rossip) were compared in a research carried out, under the organic farming management, in the experimental fields of the Federal Scientific Center of Vegetable Production (Moscow region, 55° 39' 23'' N, 37° 12' 43'' E) in 2015 and 2016. The trial was conducted on a clay-loam soil, with pH 6.8, 2.1% organic matter, 108 mg·kg^{-1} N, 450 mg·kg^{-1} P_2O_5, 357 mg·kg^{-1} K_2O, exchangeable bases sum as much as 95.2%. The air temperature values, recorded at plant level, were: 14.2°C and 13.5°C in May, 16.9°C and 17.1°C in June, 21.2°C and 20.4°C in July, 19.3°C and 18.0°C in August, 12.1°C and 12.2°C in September, in 2015 and 2016 respectively.

A randomized complete blocks design, with three replicates, was used for treatments distribution in the field; each treatment had a 19.25 m^2 (5.5 × 3.5 m) surface area, including 50 plants.

Physalis seeds were sown in peat boxes on 14 April and the seedlings were transplanted in the field on 23 May, spaced 55 cm along the rows, the latter being 70 cm apart. As for *P. angulata*, the six cultivars chosen for the trial are the most spread in Russia, whereas within *P. pubescens* only the cultivar Zolotaya rossip tested in our research is cultivated in this Country.

The organic farming practice complied with EC Regulation 834/2007. *Physalis* crops were preceded by pea and each year the fertilization supplied the crops with 56 kg ha^{-1} of N, 16 of P_2O_5 and 98 of K_2O. Half of the fertilizers dose was given just before transplanting and the remaining 50% on dressing at two week intervals. Drip irrigation was practiced for watering the crops when needed. Plant protection from fungal diseases and insects was achieved by adopting Trichoderma suspensions, copper, sulphur, azadirachtin.

Harvests were carried out from mid-August to the end of September.

General analytical methods

Ripe, undamaged and regularly shaped fruits were classified as "marketable". At each harvest, the weight and number of marketable fruits in each plot was recorded and the mean weight was assessed on random samples of 50 fruits per plot. Cumulative plant biomass was calculated as the sum of the above-ground plant biomass at the end of the experiment plus the total fruit production from the beginning of the harvest period. Dry weight was assessed after dehydration of the fresh samples in an oven at 70°C until they reached constant weight. In each plot, a sample of twenty-five fruits was collected and transferred to the laboratory for analysis.

Samples preparation

Prior to analyses, *Physalis* fruits were extracted from papery husk and homogenized with a stainless steel blender for 1 min. The resulting homogenates

were immediately subjected to the analysis.

Total soluble solids (TSS) and sugars

Determination of total soluble solids was carried out by a refractometer (IRF-22, Russia). The results were reported as °Brix at 20°C.

Mono- and disaccharides were determined using cyanide method (Kidin, 2008).

Titratable acidity (TA)

TA was measured using 20 ml of the water *Physalis* extract (1:1), titrated to pH 8.1 using 0.1 N NaOH (GOST, 1996). The following formula was used for calculation:

$$TA = V_1 \times 0.1 \times 0.064 \times 100 : 20$$

where V_{-1} is the volume of NaOH used; 0.1 is the NaOH normality; 0.064 is the weight of a citric acid milliequivalent in g; 20 is the volume of the *Physalis* extract used.

Mineral nutrients

K, Mg, Mn, Ca, Na and P contents in dried homogenized fruit samples were assessed using ICP-MS on quadruple mass-spectrometer Nexon 300D (Golubkina *et al.*, 2017). Nitrate content in fresh fruits of *Physalis* species was determined using ion-selective electrode on ionomer Expert-001 (Russia), as previously described (Golubkina *et al.*, 2017).

Antioxidants

Total polyphenols were assessed using Folin-Ciocalteu colorimetric method (Sagdic *et al.*, 2011). The ascorbic acid content was determined by visual titration of fruit extracts in 6% trichloracetic acid with Tillmans reagent (AOAC, 2012).

Taste

The assessment of *Physalis* taste was performed using two methods: chemical and organoleptic. Taste index was calculated starting from the Brix degree and acidity values, using the equation proposed by Navez *et al.* (1999) and Nielsen (2003) for tomato fruits:

$$TI = (Brix/20\ TA) + TA$$

where TI is the taste index and TA is the titratable acidity calculated referring to citric acid.

Though organoleptic perception of taste depends on the cultural background of judges and cannot be considered universally objective, *Physalis* fruit taste was evaluated additionally by 10 experts, via sensory analysis using 5 balls scales (Krueger and Casey, 2000).

Statistical analysis

Data were processed by analysis of variance and mean separations were performed through the Duncan multiple range test, with reference to 0.05 probability level, using SPSS software version 21. Data expressed as percentage were subjected to angular transformation before processing. Notably, the factor "year of research" had no significant effects on the variables examined, both in terms of main effects and of interactions with the other experimental factor. Therefore, we have reported the results obtained from the data statistical processing as means of the two years of research.

3. Results and Discussion

Plant growth and yield

As it can be seen in Table 1, a correspondence between plant height and dry matter was recorded in *P. pubescens* variety Zolotaya rossip, which had the smallest plants and the lowest dry matter, whereas within *P. angulata* the cultivar Korolek showed the

Table 1 - Biometrical, growth and yield parameters of *P. angulata* and *P. pubescens* cultivars

Cultivar	Plant height (cm)	Plant dry weight ($g \cdot m^{-2}$)	Planting to harvest beginning (days)	Fruits per plant (no.)	Yield ($t \cdot ha^{-1}$)	Mean fruit weight (g)
Violet (*P. angulata*)	90 d	296.6 c	105 bc	58 cd	9.0 b	60 c
Korolek (*P. angulata*)	130 a	320.3 c	108 b	64 b	9.3 b	56 cd
Lakomka (*P. angulata*)	105 c	200.7 d	90 d	47 f	7.3 c	60 c
Konditer (*P. angulata*)	125 ab	402.5 a	115 a	62 bc	11.3 a	70 b
Konditer 2 (*P. angulata*)	120 b	368.3 b	102 c	53 e	11.0 a	80 a
Lezhky (*P. angulata*)	110 c	223.6 d	118 a	55 de	7.7 c	54 d
Zolotaya rossip (*P. pubescens*)	80 e	133.3 e	85 e	165 a	1.3 d	3 e

Within each column, means followed by different letters are significantly different according to the Duncan multiple range test at $p \leq 0.05$.

tallest plants but 'Konditer' and 'Konditer 2' produced the highest dry matter amounts. The latter cultivar was characterized by the longest crop cycle, similarly to Lezhky, and *P. pubescens* Zolotaya rossip resulted in the earliest fruit ripening; among *P. angulatum* varieties, only 'Lakomka' showed early ripeness comparable to 'Zolotaya rossip' one. Correspondently to plant dry matter production, *P. angulata* cultivars Konditer and Konditer 2 also gave the highest yield (11.3 and 11.0 t·ha^{-1} respectively), due to the highest mean berry weight (80 and 70 g respectively), whereas *P. pubescens* variety Zolotaya rossip had the worst outcome, in spite of the huge number of fruits per plant which were, however, very small (3 g). Among *P. angulatum* varieties, Lakomka and Lezhky showed the lowest values (7.3 and 7.7 t·ha^{-1} respectively), due to the lowest prolificity and the smallest fruits respectively.

Quality indicators and mineral nutrient content

As reported in Table 2, the fruits of *P. pubescens* cultivar Zolotaya rossip overall attained higher values of quality indicators than the six *P. angulata* varieties examined; compared to the average of the latter six cultivars, *P. pubescens* fruits showed 1.6 and 1.95 times higher content of total sugars and monosaccharides respectively as well as 1.27 times higher titratable acidity. Among *P. angulata* cultivars, in 'Violet' fruits the highest levels of dry residue, soluble solids and total sugars were recorded and in 'Lezhky' the lowest. Otherwise, 'Lakomka' showed the highest monosaccharide content (32.2% out of total sugars) and cultivar Violet the lowest (18.8%); the variation coefficient relevant to monosaccharide content in *P. angulata* species attained 18.3%. As for quality indicators, the *Physalis* cultivars tested showed high variability in the content of dry matter (15.0%) and sugars (28.2%) as well as in juice acidity (16.7%), whereas the juice pH variability was low (2.1%).

In our research, dry matter and sugar content in *P. pubescens* are similar to those detected in *Physalis* berries grown in tropical and subtropical countries (Yildiz *et al.*, 2015). Notably, soluble sugars highly affect flavour quality of tomato fruits (Doras *et al.*, 2001) and, according to Olivares-Tenorio *et al.* (2016) reports, the main carbohydrates of *Physalis* fruits are sucrose and glucose, whereas fructose content is neglectable.

The analysis of mineral nutrient content performed in our research showed the close element concentrations of *P. pubescens* and *P. angulata* fruits grown in the same environmental conditions (Table 3). As for varietal differences in mineral nutrient

Table 2 - Quality indicators of *Physalis angulata* and *P. pubescens* cultivars fruits

Cultivar	Dry matter (%)	Soluble solids (°Brix)	Total sugars (%)	Reducing sugars (%)	Titratable acidity (%)	pH
Violet	10.5 b	8.1 b	7.8 b	1.8 d	0.79 c	4.82 ab
Korolek	9.3 d	5.9 c	5.6 d	1.8 d	0.70 d	4.65 bc
Lakomka	8.7 e	6.1 c	6.0 c	2.9 b	0.45 f	4.94 a
Konditer	10.0 bc	6.0 c	5.9 cd	1.6 d	0.88 b	4.62 bc
Konditer 2	9.7 cd	6.1 d	5.9 c	2.2 c	0.79 c	4.51 c
Lezhky	8.5 e	4.9 d	4.7 e	1.7 d	0.63 e	4.70 ac
Zolotaya rossip	15.5 a	9.7 a	9.6 a	3.9 a	0.90 a	4.72 ac
Mean	9.5	6.2	6.0	2.0	0.71	4.71

Within each column, means followed by different letters are significantly different according to the Duncan multiple range test at p≤0.05.

Table 3 - Mineral nutrient concentrations in *Physalis angulata* and *P. pubescens* cultivars fruits (mg kg^{-1} d.w.)

Cultivar	Ca	K	Mg	Na	P	NO_3^-
Violet	415 b	2465 ab	1449 c	51 b	3483 c	2390 a
Korolek	722 a	2013 d	1740 ab	48 b	3825 b	2108 b
Lakomka	763 a	1284 f	1845 a	53 b	4345 a	2365 a
Konditer	720 a	2300 bc	1809 a	34 b	4277 a	1990 bc
Konditer 2	739 a	1723 e	1614 b	6 c	3503 c	1876 c
Lezhky	797 a	2152 cd	1286 d	61 b	3509 c	2287 a
Zolotaya rossip	414 b	2561 a	1708 ab	293 a	3572 c	1181 d

Within each column, means followed by different letters are significantly different according to the Duncan multiple range test at p≤0.05.

accumulation, in *P. angulata* fruits the variability was high in Na concentration (33%) and low in Mg, P and nitrates (11.6, 8.5 and 6.9%).

Recently, the element composition of vegetable edible parts has been drawing attention as one of the most important factors affecting human being ingestion of mineral nutrients. Unfortunately, the few literature reports available do not give the opportunity to perform correct interspecific or varietal comparisons, due to research carried out under different environmental conditions and species (El-Sheikha *et al.*, 2010; Eken *et al.*, 2014).

Referring to recommended dietary allowance values (Institute of Medicine, 2001), the consumption of 300 g fresh *Physalis* fruits per day results in the ingestion of 24.8% potassium, 14.4% phosphorous and 12.3% magnesium needed by human organism. The latter benefits from these mineral nutrients in terms of optimization of carbohydrates, protein and lipid metabolism, bone integrity and brain activity, protection against cancer as well as cardiovascular diseases, obesity and diabetes. In this respect, *P. angulata* and *P. pubescens* fruits as well as the *P. peruviana* ones (Zhang *et al.*, 2013) may be considered as good sources of several elements.

Antioxidants

Polyphenols. In the present research, *P. pubescens* and *P. angulata* cultivars growing in the same geochemical environment (central Russia) resulted in fruit polyphenols accumulation ranging between 18.7 and 25.1 mg GAE/g d.w. (Fig. 1). Notably, the top concentration detected in the cultivar Violet was 34.2% higher than the lowest level recorded in 'Korolek', both belonging to *P. angulata* species; *P. pubescens* cultivar Zolotaya rossip ranked third (20.5 mg GAE/g d.w.). Compared to our findings, in previous research (Medina-Medrano *et al.*, 2015) higher phenolic values were detected in the fruits of five *Physalis* wild species grown in Mexico (32 to 86 mg AGE/g d.w.), with the lowest concentrations recorded in *P. angulata* berries. Moreover, *P. peruviana* fruits produced in Colombia accumulated 400 to 600 mg GAE/g f.w. of polyphenols (Narvaez-Cuenca *et al.*, 2014), but unfortunately this species is not suitable for cultivation in northern Europe (Kondratieva and Engalichev, 2013). Indeed, among natural secondary plant metabolites, polyphenols are considered to be the strongest antioxidants, which are able to inhibit carcinogenesis at initial and development stages, thus suggesting the great importance of their accumulation in agricultural plants (Yang *et al.*, 2001).

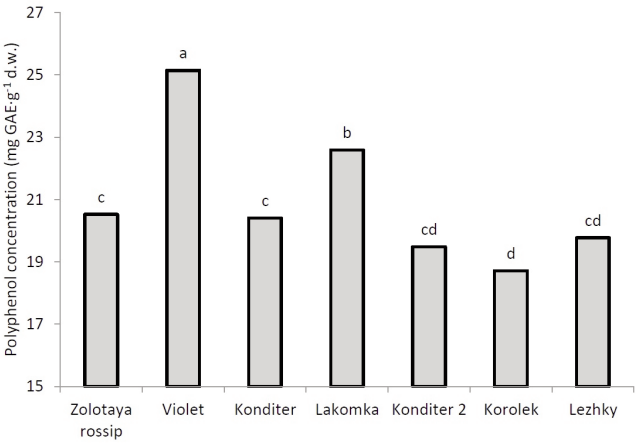

Fig. 1 - Polyphenol concentration as a function of *Physalis* cultivar. Means followed by different letters are significantly different according to the Duncan multiple range test at p≤0.05.

The correlations between polyphenols and total sugars concentrations and between polyphenols and dry matter content were positive and highly significant (r= 0.99 and r= 0.91, respectively, at P≤0.001). The first one is supposed to reflect the existance of phenolic glycosides, identified as the main phenolics in wild *Physalis* species (Medina-Medrano *et al.*, 2015). The second correlation explains the higher dry matter and phenolics contents detected in *Physalis* fruits produced in southern Countries compared to those obtained in central Russia. The high statistical significance of the correlations between the above parameters gives the opportunity to highlight the quality performances of cultivar Violet, which showed the highest concentrations of polyphenols as well as the highest carbohydrates and dry matter content among *P. angulata* varieties and *P. pubescens* cultivar Zolotaya rossip, with the highest content of dry matter and carbohydrates. These parameters may be used in *Physalis* breeding for increasing fruit nutritional quality.

Ascorbic acid. In our research, vitamin C concentration in *P. angulata* cultivars ranged from 0.96 mg/g d.w. (cultivar Violet) to 1.33 mg/g d.w. (cultivar Korolek); however, the lowest value was recorded in the berries of *P. pubescens* cultivar Zolotaya rossip (Fig. 2). These results show that the synthesis of this antioxidant in Russia is much lower than that reported for *P. peruviana* and *P. pubescens* in tropical and subtropical areas: i.e. 10 to 30 *vs* 20 to 50 mg/100 g f.w. (El Sheikha *et al.*, 2008, 2010; Olivares-Tenorio *et al.*, 2016). Indeed, the higher light intensity occurring at lower latitudes enhances ascorbic acid accumulation (Bartoli *et al.*, 2006), whereas in previous investi-

gations carried out in Colombia, Kenia and Southern Africa (Fischer *et al.*, 2000), no relationship of this antioxidant with the altitude was found. Moreover, the low vitamin C variability obtained in the present intestigation prove the predominance of environmental effects on the genetic one in affecting this antioxidant accumulation in *Physalis* fruits (Fig. 1). Notably, the outer husk of *Physalis* fruits is known to prevent ascorbic acid oxidation (Valdenegro *et al.*, 2012) and, despite the relatively low vitamin C content, 100 g of fresh *Physalis* fruits produced in the northern hemisphere can supply human organism with 14 to 21% of the required vitamin C consumption (70 mg per day).

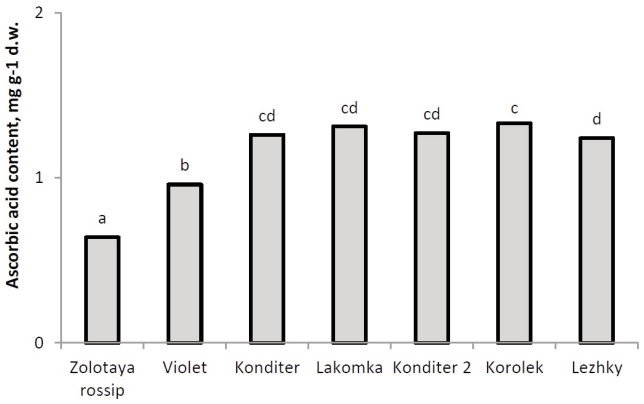

Fig. 2 - Ascorbic acid concentration as a function of *Physalis* cultivar. Means followed by different letters are significantly different according to the Duncan multiple range test at p≤0.05.

Taste

Among *P. angulata* and *pubescens* cultivars examined, the highest taste index (TI) and nutritional values were recorded in 'Zolotaya rossip' (*P. pubescens*) and 'Violet' (*P. angulata*). As far as taste assessment is concerned, it is a critical point in nonconventional plants produce and, indeed, this determination has never been performed on *Physalis* fruits up to date. A general approach based on tomato berry testing suggests several significant factors affecting taste: dry matter, soluble solids, juice electrical conductivity, carbohydrates and organic acids contents (Adams and Ho, 1989; Clement *et al.*, 2008). In our research, we have assessed that the taste index (TI) used for tomato fruits can be successfully used for *Physalis* berries. Indeed, the organoleptic analysis and TI approach in evaluating *Physalis* fruit quality show a good convergence of the results and suggest significant prospects of TI utilization in determining new varieties quality (Fig. 3).

In this respect, several correlations arose between the taste index and the following quality parameters of *Physalis* fruits: total sugar content (r=+0.98; P<0.001), dry matter (r = +0.92; P<0.001), polyphenol content (r= +0.96; P<0.001); calcium (r= -0.91; P<0.001). Notably, the two latter correlations had never been reported previously, neither in *Physalis* nor in tomato.

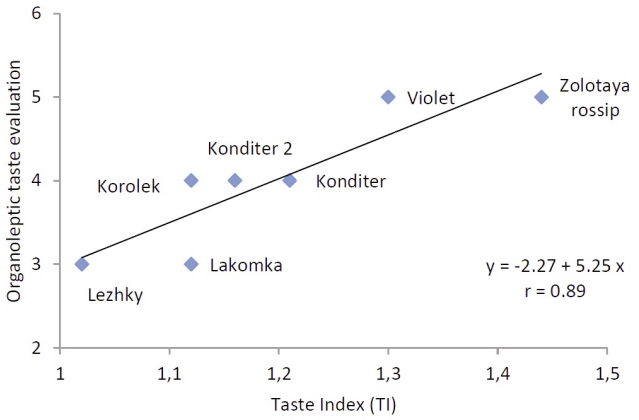

Fig. 3 - Correlation between taste index (TI) and organoleptic evaluation of *Physalis* taste.

4. Conclusions

The present research, carried out in central Russia, allowed to assess interspecies and varietal differences in yield and quality characteristics of *P. angulata* and *P. pubescens* fruits grown under organic management. In this respect, this investigation provided with interesting clues, mainly concerning the nutritional and antioxidant properties of the cultivars tested and their growing prospects by organic farming procedures in northern Europe. The variability of biologically active compounds, macroelement content and Taste Index, as well as their significant correlations may serve as the basis for enhancing the high potential of the *Physalis* varieties examined for functional food production.

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Flower development and pollen vitality of *Moringa oleifera* Lam. grown in a humid temperate climatic condition

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Key words: fertility, flower anatomy, microsporogenesis.

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All relevant data are within the paper and its Supporting Information files.

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Abstract: *Moringa oleifera* is a tropical tree cultivated in many countries. This species has acquired a great importance in human nutrition and it was recently indicated as a "novel food" by the European Commission. Recently, moringa plants have been introduced in humid temperate climatic areas, among which Moreno (Buenos Aires Province - Argentina). In such area, the cultivation is possible for the production of leaves, but plants need protection during winter time in order to overcome damages due to low temperatures and hence to produce capsules and seeds. The main objective of this research was to study flower morphology and anatomy of *M. oleifera*, as well as microsporogenesis and viability of pollen grains of plants cultivated in Moreno in comparison with those produced in a humid sub-tropical climatic area of Argentina (San Miguel de Tucumán). Flowers grown in the temperate environment resulted similar for morphological parameters to those observed in the sub-tropical environment. Nevertheless, pollen grain fertility depended directly on air temperature and it was negatively affected by the lower temperatures registered in the temperate site. According to the observed results, pollen viability increases with mean monthly temperatures above 16°C.

1. Introduction

Moringa oleifera Lam. (moringa) is a multipurpose small to medium-sized, evergreen or deciduous tree, native to northern India, Pakistan and Nepal. It has a spreading open crown with drooping, fragile branches, feathery foliage with tripinnate leaves, and a thick corky whitish bark (Marcu, 2005). *M. oleifera* is utilised as animal fodder and employed in human nutrition due to its healthy properties (Fuglie, 1999; Palada and Chang, 2003; Ganatra *et al.*, 2012; Paula *et al.*, 2017), as well as in the production of fuel (Foidl *et al.*, 2001), water sanitation (Wilson, 1992; Lekgau, 2009; Padilla *et al.*, 2012). Moringa leaves are considered a "novel food" by the European Commission, so confirming their valuable properties in terms of energy, nutrients, proteins and minerals, as reported by several authors (Atawodi *et al.*, 2010; Tende *et al.*, 2011; Yameogo

et al., 2011; Gopalakrishnan *et al*., 2016; Vats and Gupta, 2017). Araujo *et al.* (2016) highlighted also the importance of *M. oleifera* in regions characterized by desertification and water deficit.

Moringa cultivation is expanding all over the world, including in climatic areas, which differ noticeably from those of its tropical origin. Recently some experiments showed the feasibility of cultivating moringa in the humid temperate climatic conditions of Buenos Aires Province (Argentina) for leaf production. Leaf extracts from trees grown in that conditions showed higher phenol content and antioxidant activity than those obtained from plants cultivated in typical tropical climates (Arena and Radice, 2016). Nevertheless, flower differentiation, anthesis and fertility resulted negatively altered and the production of pods and seeds, both of them important source of nutrients, was very low. The main objective of this research was to study the effect of air temperature on flower morphology and anatomy of *M. oleifera*, as well as on the microsporogenesis and pollen grain viability, observed on trees cultivated at Moreno (Buenos Aires Province) in comparison with those grown in San Miguel de Tucumán (Argentina), the first characterized by a humid temperate climate and the latter by a humid subtropical environment.

2. Materials and Methods

Plant material

All plants were obtained from the same seed lot. Homogeneous seedlings (n= 10) were grown in soil and in open air and cultivated in San Miguel de Tucumán (26° 49'59.00" S, 65° 13'00" W, elevation 456 m asl), while another similar set of seedlings (n=10) was planted in Moreno (34° 39' 0" S, 58° 47' 0" W, elevation 14 m asl) in 25 l plastic pots under a glasshouse from April to September. Successively pots were placed in open air. Moreno has a humid temperate climate, with an average temperature of 23.4°C in January and 10.0°C in winter time (June); San Miguel de Tucumán, has humid subtropical climate (19.4°C the average annual temperature) with a hot and long summer and mild and dry winter. The precipitation pattern is monsoonal with an average of 997 mm (climate-data.org). A set of monthly air temperature parameters is reported in figure 1.

Flower morphology

Flowers (*n = 100*) on different phenological stages were observed on both groups of plants, and samples were collected monthly for further observations from September to December. Button flower collected were used fresh and fixed in FAA (formaldehyde, 100 ml; ethyl alcohol, 500 ml; acetic acid, 50 ml; distilled water, 350 ml).

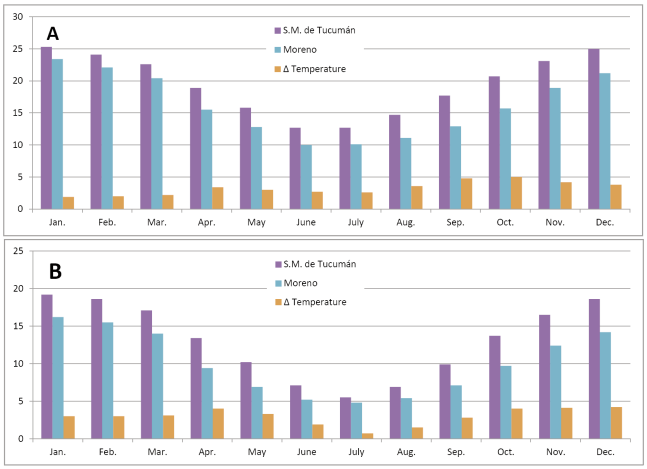

Fig. 1 - Mean (A) and average minimum (B) air monthly temperatures (°C) in San Miguel de Tucumán and Moreno locations.

Light microscopy

Button flowers (*n= 10*) were immediately frozen to -25°C and embedded in a medium consisting of polyethylene glycol and polyvinyl alcohol. Successively they were cut frozen by microtome inside the cryostat. Histologic slices were cut at 5 to 10 µm.

A set of ten button flowers were dehydrated in an ethanol series and embedded in Spurr's resin. Thin sections (75-90 nm thick) were stained with uranyl acetate and lead citrate. Sections were observed with a Leica DM 2500 microscope.

Fluorescent microscopy

Flowers in anthesis phase (*n= 50*) fixed in FAA were shaved with distilled water and softened with NaOH (8N) as described by Martin (1959). Then, they were stained with aniline blue to study pollen tube growth. Squash material was observed by a Leica microscope (DM 2500) using fluorescence with excitation filter BP: 450-490.

Scanning electron microscopy (SEM)

Button flowers fixed in FAA (*n= 10*) were dehydrated in an ethanol series and critical point-dried with liquid CO_2 was employed. Then it was sputter-coated with gold-palladium (40% gold and 60% palladium) for 3 minutes. Samples were observed with Philips XL30 SEM.

Pollen viability

Pollen viability was performed with fluorescent microscopy according to Radice and Arena (2016) on fresh anthers taken from button flowers of two localities (Moreno and San Miguel de Tucumán). Pollen evaluation was expressed in percentage. To determine the statistical significance of the hypothesis the chi-squared test (χ^2) was used.

3. Results

Flower development

Flower development starts with the appearance of the flower on a clustered inflorescence (Fig. 2). These first buds are green reddish and about 1mm long (Fig. 2A). They grow up to about 10mm and turn to white greenish (Fig. 2A). Anthesis takes place sequentially among the flowers of the inflorescence (Fig. 2B). At anthesis, flower shows a zygomorphy symmetry. The larger transversal petal is bent upwards, while the others are reflexed downwards together with the sepals (Fig. 2C). Anthers are yellow and no dehiscent; flowers have odour and nectar in this phase.

As the flower develops, anthers change colour to dark yellow (Fig. 2C) and finally to brown (Fig. 2D). It was observed that 1-3 anthers were not developed in flowers collected from Moreno field. Pistil, which at the time of the anthesis is below the anthers, continues to grow until it protrudes several millimetres above the androecium (Fig. 2C). Finally, petals wither and fall, while the ovary enlarges and turns to reddish colour regardless its fertilisation (Fig. 2E).

Flower structure

Moringa oleifera plants grown in Moreno and San Miguel de Tucumán experimental fields developed flowers with average values of ≈5 petals, ≈6 sepals, ≈6 stamens, ≈5 staminoides and 22 ovules (data not showed). In the observed flowers, the unique largest petal (referred as “primordium petal”) stands right; the others are folded (Fig. 2C). Female part of the flower shows the complete pistil with the style and a hairy ovary (Fig. 3A). Ovary is tricarpelar and ovules are located in parietal placentation (Fig. 3B). Stigma is just a hole (Fig. 3C). Some nectarostomata surround the gynophore (Fig. 4). Glandular hairs, with the function of expelling the nectar, are present on the nectarostomata surface (Fig. 4A). Nectarostomata produces nectar in sub epidermal cells, that accumulate it and transfer it through the intercellular spaces (Fig. 4B).

Frozen section of a button flower just before the anthesis phase allows to appreciate the state of the

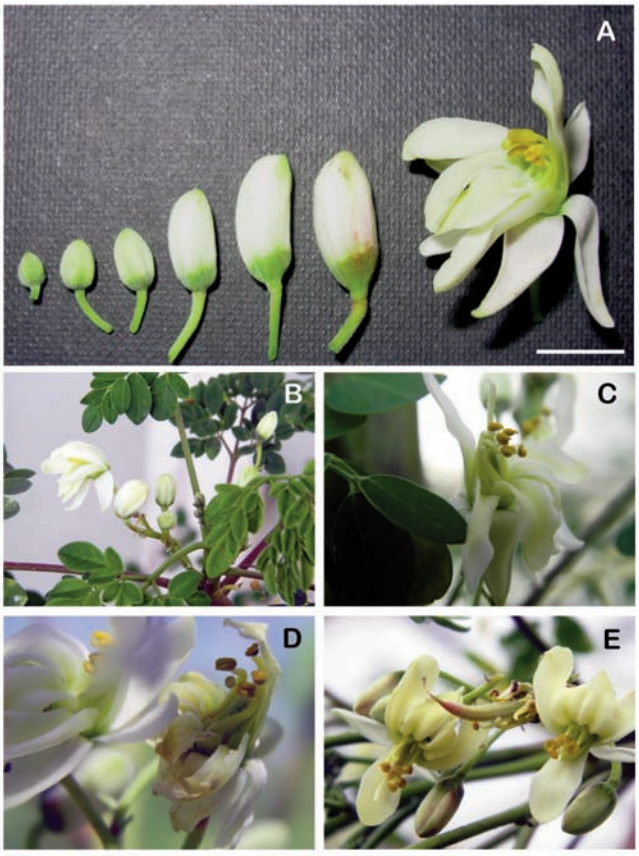

Fig. 2 - Flower development of *Moringa oleifera* Lam. A, from button flower to anthesis. B, beginning of anthesis. C, anthesis; D, flower senescence; E, pod formation. Bar = 1 cm.

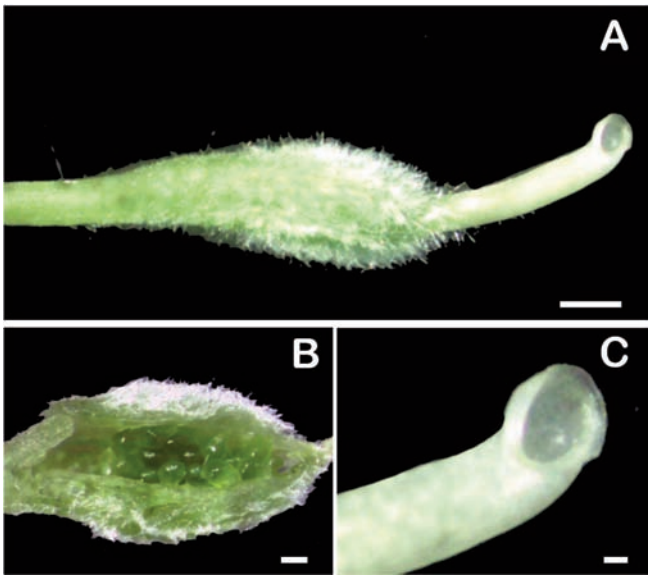

Fig. 3 - Pistil of *Moringa oleifera* Lam. A, external view of the pistil; B, internal view of the ovary with ovules; C, detail of the stigma. Bars = A-C, 1mm.

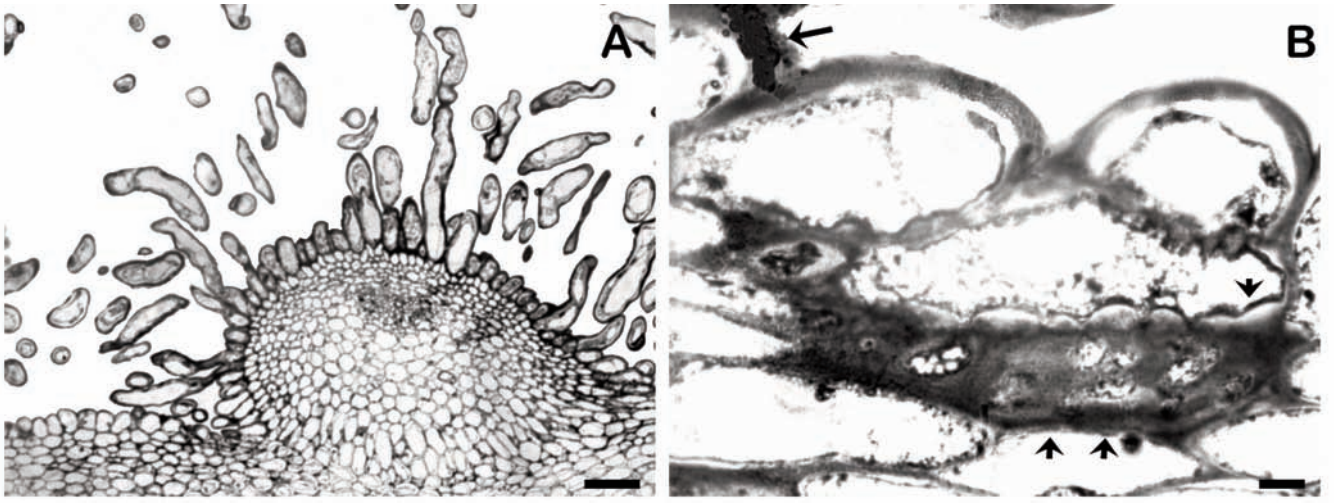

Fig. 4 - Nectary of *Moringa oleifera* Lam. (light micrographs). A, longitudinal section of the nectary; B, detail of the nectar cumulated in the intercellular spaces (arrows). Bars = A, 100 µm; B, 10 µm.

structures and their normal coloration (Fig. 5). In fact, it is possible to see anthers with pollen grains already formed wrapped in a yellow substance similar to sporopollenin (Fig. 5B). Pistil shows developing ovules attached to the carpelar wall (Fig. 5A). Finally, all internal organs are enveloped by sepals and petals (Fig. 5A).

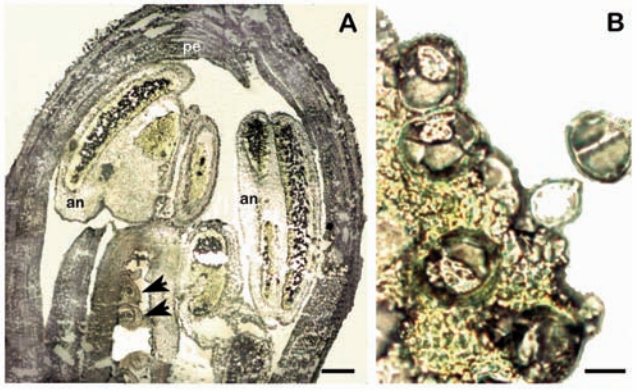

Fig. 5 - Flower in pre anthesis phase of *Moringa oleifera* Lam. (frozen section). A, Longitudinal section of flower with petals (pe), anthers (an) and ovary with ovules (arrows); B, mature pollen grain surrounded by sporopollenin. Bars = A, 10 cm; B, 10 µm.

Flowers studied by SEM showed there is no defined stigmata structure. Pistil is coronate by a smooth cell structure as a continuation of the style (Fig. 6A). The internal cavity of the ovary is covered with hairs (Fig. 6C) and ovules adhere to their walls on the connection of two carpels. Ovules appear to be campylotropous (Fig. 6D).

Microsporogenesis

Button flowers from 1mm to 10 mm (Fig. 2A) have been used to observe different steps of pollen grain formation. Different stages of pollen grain differentiation were observed: microsporocytes mother cells, tetrads, microsporocytes and mature pollen (Fig. 7). Development was very fast and it accomplished in less than one week.

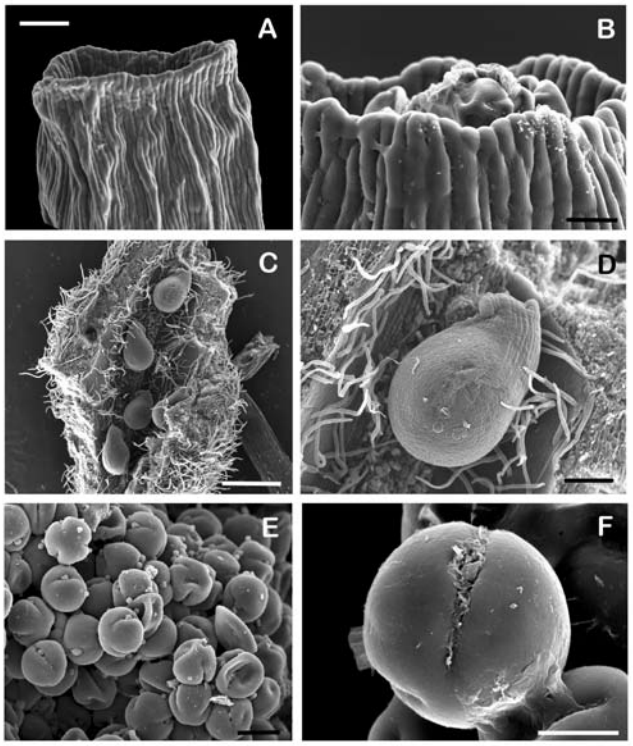

Fig. 6 - SEM micrographs of *Moringa oleifera* Lam pistil. A-B, style and stigma; B, detail of the stigma with pollen grain; C, ovarian cavity with ovules; D, detail of an ovule; E-F, pollen grains. Bars = A, 50 µm; B, E, 20 µm; C, 500 µm; D, 100 µm; F, 10 µm.

Moringa flowers have monothecal anthers. Button flowers shorter than 5 mm contains inside the just formed pollen sac microspore mother cells (Fig. 7A). These cells show a big and visible nucleus and a very dense cytoplasm indicating a high activity.

During this stage, the last inner layer of the anther wall corresponds to tapetal cells that are formed by large and binucleate cells (Fig. 7A).

As the buds lengthen, more advanced stages of microsporogenesis are observed. In fact, it was observed the tetrad (Fig. 7B) and then the release of microsporocites (Fig. 7C). At this point, tapetum degrades (Fig. 7C). The young microspores show a central nucleus and a vacuolated cytoplasm.

The last stage shows mature pollen grains and free orbicules (Fig. 7D). When mature pollen grains are formed, tapetum disappears completely. Mature pollen grains measure about 20 µm. It is possible to observe the exine of the grains well formed and the cytoplasm of the vegetative cell with a lot of amyloplasts (Fig. 7D).

Pollen viability

It was possible to differentiate green, red and orange yellowish pollen grains corresponding to viable, non-viable and sub viable pollen grains respectively.

Pollen viability among flowers at different dates (Table 1) showed great variations on flowers collected in Moreno; on the contrary, no differences were observed between flowers collected from San Miguel de Tucumán throughout the study period (Table 2). Moreno flowers showed a very low percentage of viable pollen grains respect to San Miguel de Tucumán flowers during September to November. Although the number of pollen grains per anther in

Table 1 - Viability of pollen grains collected on different months and different locality

Source	Month	Viable	Non Viable	Sub-viable
Moreno	September	10 c	79 a	11 a
Moreno	October	28 c	53 a	19 a
Moreno	November	48 b	32 b	20 a
Moreno	December	74 a	3 c	23 a
S.M.Tucumán	September	68 a	22 b	10 a
S.M.Tucumán	October	63 a	25 b	12 a
S.M.Tucumán	November	70 a	22 b	8 a
S.M.Tucumán	December	69 a	28 b	13 a

Values with different letters between the same column are significant different. Tukey (p≤0.05).

Table 2 - Viability of pollen grains collected on September from anthers of the same flower

Source	Anther	Viable	Non Viable	Sub-viable
Moreno	1-1	10 a	84 a	6 a
Moreno	1-2	7 a	91 a	2 a
Moreno	1-3	10 a	82 a	8 a

Values are expressed on percentage. Values with different letters between the same column are significant different. Tukey (p≤0.05).

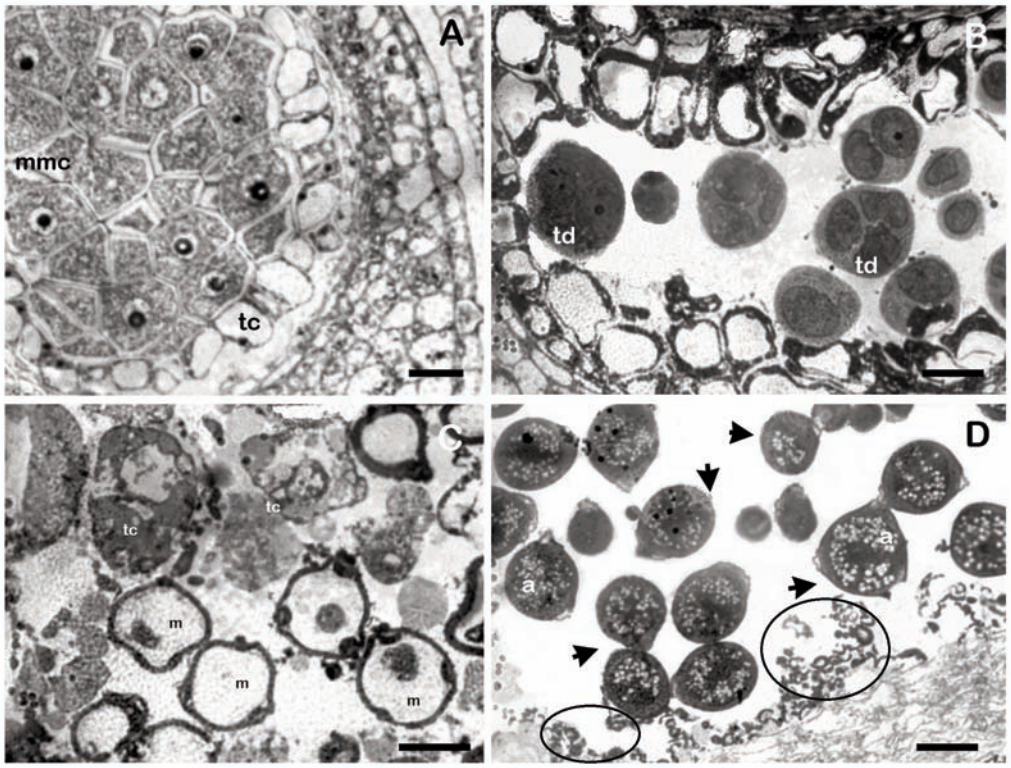

Fig.. 7 - Microsporogenesis of *Moringa oleifera* Lam (light micrographs). microsporangium with conspicuous microspore mother cells (mmc) and tapethal cells (tc); B, tetrads (td); C, microporocytes free (m) and tapetal cells (tc) in metabolization phase; D, mature pollen grains (arrows) with amyloplast (a) in the cytoplasm and orbicules (circles). Bars = A 10 µm; B-D, 20 µm.

the flowers was not evaluated, it was observed that flowers collected from San Miguel de Tucumán had more amount of pollen grain in each anther. Some anthers of Moreno flowers contained immature pollen and a viscous substance. Additionally, according to the harvesting period of the flowers, some anthers developed only dead pollen grains.

According to these results, a more detailed analysis of pollen viability was performed between pollen grains derived from different anthers of the same flower and between anthers from different flowers collected from Moreno trial. Viability of pollen grains of different anthers collected from the same flower was not statistically different (Table 2), while viability of pollen from different flowers of different trees collected on the same date resulted significantly different between flowers (Table 3). Viable pollen grains were contained on great proportion in some flowers but scarce in others, with a random distribution among trees.

Table 3 - Viability of pollen grains from different flowers of the same plant

Source	Flower	Viable	Non viable	Sub-viable
Moreno	1 A	7 b	78 ab	15 a
Moreno	1 B	6 b	82 a	12 a
Moreno	1 C	18 a	67 b	15 a
Moreno	1 D	2 b	95 a	3 b
Moreno	1 E	15 a	72 b	13 a

Values are expressed on percentage. Values with different letters between the same column are significant different. Tukey ($p \leq 0.05$).

Pollination

During anthesis, pistils both with or without germinated pollen were observed. When pollination occurs, pollen grains fall freely into the stigma cavity and then germinate (Fig. 6B). In effect, pistils treated by Martin technique showed that a mass of pollen grains is housed in the cavity and that many pollen tubes germinated (Fig. 8A) and later on reached the ovary and fertilized the ovules (Fig. 8B).

Pollination was rarely observed on flowers collected from Moreno during spring time (September to November) but it was very frequent in summer time. On the contrary, it was observed that flowers collected on San Miguel de Tucumán were profusely pollinated in both periods and fruit production was continuous throughout the year. Fruit production on Moreno plants started in summer until May, while it resulted continuous in San Miguel de Tucumán.

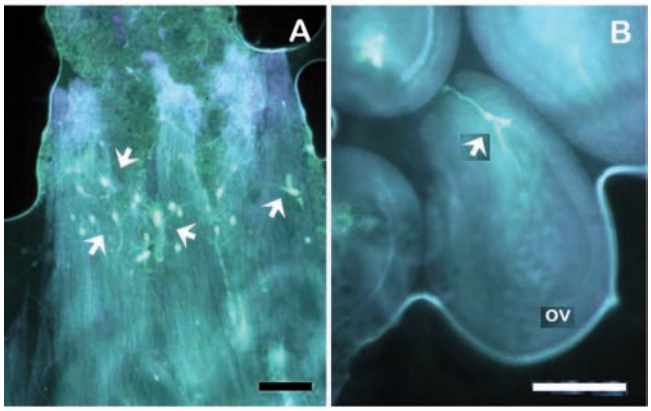

Fig. 8 - Fertilization of *Moringa oleifera* Lam (fluorescent light micrographs). A, pollen tube growing toward the style (arrows); B, ovules (ov) and pollen tube crossing the micropile and entering the embryo sac (arrows). Bars = A-B, 100 μm.

4. Discussion and Conclusions

Flower morphology and anatomical structure observed in flowers collected from Moreno field and San Miguel de Tucumán did not show significant difference with those described in literature (Ramachandran *et al.,* 1980). Furthermore, flowers from the two localities studied in Argentina have a normal external and internal development.

Frozen sections allowed to see the internal anatomy with its natural colorations and to show more clearly the presence of orbicules, that, in the traditional cuts, appear very confused because of the different colorations applied. On the other hand, flowers studied by SEM allowed clarifying some concepts. In fact, it was clearly showed how pollen germination begins even in the absence of any connection to any structure of the stigma or style. Bhattacharya and Mandal (2004) found that some extra proteins and esterases contribute towards the stigmatic receptivity; furthermore, the occurrence of intraovarian trichomes, which is not widespread in the angiosperms, could facilitate the growth of the pollen tubes. This assessment made by Dickison (1993) is based on that the trichomes functionally resemble obturators.

Pollen grain formation seems to be very variable depending on the geographical location and the time of year. In fact, Muhl *et al.* (2011) showed that low temperature regime induces flowering but provokes low pollen viability and this statement would explain the results obtained in Moreno spring flowers, thus confirming that pollen grain viability is affected by air temperature. In fact, the lowest values of pollen

grain viability were observed on September and October when mean temperatures were lower than 16°C. Additionally, the higher percentage of pollen viability observed in San Miguel de Tucuman is consistent with the air temperatures registered in that location, which resulted about 4/5°C higher than those observed in Moreno (Fig. 1).

The difference of the quality of pollen grains collected in spring and summer could be related to the amount of orbicules or Ubisch bodies. Studies on Ubisch body formation in *Brachypodium* support the evidence that they are formed in the tapetum and are involved in exine synthesis (Sharma *et al*., 2014). Actually, during spring time, anthers with mature pollen grains brings many orbicules. This fact suggests that when the exine was not well formed the quantity of dead pollen was important.

On the other hand, pollen viability seems to have an influence on the efficiency of pollination. As directly observed, all plants were very visited by insects during blooming on both experimental locations, but pollination was rarely observed in Moreno flowers, while it was very frequent in flowers collected in San Miguel de Tucumán.

Although tropical climates are those considered ideal for *M. oleifera* according to Muhl *et al.* (2011), good results obtained with San Miguel de Tucumán flowers confirm that sub-tropical climates are also suitable for this species. Taking into account reproductive functions, and namely the microsporogenesis, Moreno environment seems to be just below the threshold of good temperature regime for *M. oleifera* during spring time despite the climatological predictions made by Falasca and Bernabé (2008).

In conclusion, the reported results demonstrate the possibility of moringa cultivation unusual non tropical climates. Flowers of the trees grown in Moreno field were normal from the morphological point of view only in some periods of the year and the quality of their development was related to air temperatures. Plants grown on San Miguel de Tucumán have an un-interrupted production of flowers despite not being in a tropical climate. On the other hand, moringa cultivated in marginal areas, as the locality of Moreno can be considered, could offer important advantages.

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Short note

AHS
Advances in Horticultural Science

In vitro responses of Gerbera (*Gerbera jamesonii*) cultivars multiplied under different photoperiods

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Key words: cost reduction, *in vitro* development, light, micropropagation, shoot multiplication.

Abstract: Light affects several aspects of micropropagation. Five cultivars of gerbera were subjected to photoperiodic regimes of 10, 12 and 16-h of light for the shoot multiplication. The reduction of photoperiod from 16-h to 12-h resulted in increases in 41.8% to 97.2% of shoot multiplication in all gerbera cultivars.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

Gerbera is one of the most important species for cut flower market in the world, together with lilies, tulips and roses (Bhatia *et al*., 2011; University of Kentucky, 2015), owing to several breeding programs that have resulted in a rich diversity of colors (Cardoso and Teixeira da Silva, 2013).

Although gerbera can be propagated *in vivo* using seeds or rhizome division (Son *et al*., 2011), micropropagation using apical shoots or through organogenesis is the only way that can provide realistic large scale clonal propagation with genetic fidelity and disease free plantlets (Kanwar and Kumar, 2008; Bhatia *et al*., 2009) to meet the current demand of highly technological floriculture market.

Multiplication stage in micropropagation is one of the most crucial steps to establish *in vitro* propagation. In this regard, most of the earlier studies on gerbera micropropagation were focused on establishment of different types and concentrations of plant growth regulators.

Several factors affected gerbera *in vitro* shoot multiplication, as culture medium, plant growth regulators (especially citokinins as Benziladenine), growth conditions and the propagation method. However, genotype is the most important factor influencing *in vitro* development, including the rate of multiplication of gerbera (Son *et al*., 2011; Cardoso and Teixeira da Silva, 2013). The basal culture medium MS

(Murashige and Skoog, 1962) added 3% sucrose and Benziladenine (0.5 to 3.0 mg L^{-1}) is the most used for gerbera shoot multiplication (Kanwar and Kumar, 2008; Cardoso and Teixeira da Silva, 2013).

Although the chemical factors have been well studied in relation to the micropropagation for several species, including gerbera, the physical factors such as temperature and light (wavelength, PPFD and photoperiod) conditions are among the less discussed ones, despite their great biological and economic influence on the different development stages of micropropagation.

The electrical energy used in laboratories, especially in growth rooms, is the second most important cost factor of *in vitro* plantlet production, after labor cost (Cardoso and Teixeira da Silva, 2012). Therefore, an optimum light source is significant for this cost factor (Cardoso *et al*., 2013).

In this study, we tested different photoperiodic regimes at multiplication stage of micropropagation of gerbera cultivars to determine its effect on plant development and energy cost reduction in growth room conditions.

2. Materials and Methods

Five cultivars of gerbera were used in this study: 'Basic' and 'Kiserian' with both rose and pink inflorescences, and 'Orange Dino', 'Orca' and 'Onedin' with orange, white and yellow inflorescences, respectively.

The plant material used as donor was cultivated under greenhouse conditions and prepared as described by Cardoso and Teixeira da Silva (2012). Briefly, the plants in reproductive phase were completely defoliated and the rhizomes were maintained in cold chamber at 4°C for seven days, followed by transplanting these rhizomes to plastic pots containing organic substrate consisting of coconut fiber. After 14 days of transplantation, the first shoots were observed, and 21-28 days old shoots (5-10 cm in length and 2-3 leaves) were excised from the mother plants and were taken to the laboratory for surface disinfestations.

The shoots were reduced to 1.0-1.5 cm and washed for 5 min with tap water, followed by immersion in alcohol 75% (*v/v*), then in sodium hypochlorite with 1-1.25% of active chlorine for 20 min. Finally, the explants were washed three times in autoclaved distilled water. Shoot-tips of 2-3 mm length were used as explants to start gerbera micropropagation. The plants were multiplied to obtain the required quantity for the experiment.

The experiment was carried out during the multiplication stage of micropropagation. The culture medium used was Murashige and Skoog (1962) supplemented with 30 g L^{-1} of sucrose, 0.1 g L^{-1} of *myo*-inositol, 0.5 mg L^{-1} of benzyladenine (BA) and 6.0 g L^{-1} of agar-agar (Algagel, Type 900, Barueri, Brazil).

The flasks containing the culture medium and the individual shoots were maintained in growth room at 25±1°C and cultivated under cool white fluorescent lamps (Philips®, Barueri, Brazil) as light source with photon flux density of 20-25 µmol m^{-2} s^{-1}. The plants were subjected to three different photoperiods as follows: 10-h light/14-h dark, 12-h light/12-h dark and 16-h light/8-h dark. The last photoperiod condition was taken as control, as this photoperiod condition is regularly used for many species, including gerbera, for *in vitro* micropropagation (Cardoso and Teixeira da Silva, 2013).

In total, five borosilicate flasks (10 cm height x 5.5 cm diameter) containing four shoots, and covered with transparent polypropylene caps (repetitions), for each treatment were used. The experimental design was 5 × 3 factorial with five cultivars and three photoperiods.

The experiments were conducted for three months with time of transplanting of shoots each time for 30 days, resulting in three cultivation periods. In each pricking time, the multiplication factor was determined by counting the number of shoots obtained for the next subcultivation divided by the number of plantlets used at the start of the experiment. Five homogenized flasks with four plantlets in each turn were separated for the next evaluation, maintaining the plantlets in the same photoperiodic regime used in the previous experiment.

The data were subjected to the analysis of variance (ANOVA) and the Shapiro-Wilk test; if necessary, the data were normalized before comparison of means. The means were compared by the Scott-Knot test at 5% of probability.

3. Results, Discussion and Conclusions

Around 20% of the explants were successfully established *in vitro,* regenerated into plantlets, and transferred to the multiplication medium. Shoot apices with first leaf developed and without signals

of bacterial or fungal contamination in culture medium were transferred to the light condition and used to obtain the first shoots for the experiment. Around 60% of the explants were contaminated and around 20% formed callus with no regeneration into plantlets. The shoots so obtained were successfully *in vitro* multiplicated and used for the experiments of multiplication. These results were similar to those observed in other works at the establishment stage (Cardoso and Teixeira da Silva, 2012, 2013).

Genotype is one of the most important factors that affect the micropropagation, including the rate of multiplication and the responses to the factors affecting *in vitro* development of explants being micropropagated. This was also observed in our work with a positive interaction between the genotypes and photoperiods (Fig. 1). Under 16-h photoperiod, the cultivars 'Onedin', 'Orange Dino' and 'Orca' showed a higher multiplication rate (5.5, 5.0 and 4.8 shoots/explant, respectively) than the rose inflorescences cultivars Basic and Kiserian, which showed a lower multiplication rate of 3.6 and 2.4 shoots/explant, respectively. Interestingly, when the plantlets were subjected to the photoperiod of 12-h (4-h less than control photoperiod), only the cultivar Kiserian showed the multiplication rate less than 7.0 shoots/explant. Thus, it can be concluded that the response to the photoperiods has a direct correlation with the genotype used for micropropagation (Fig. 1). In fact, some cultivars of gerbera with rose and red inflorescences showed recalcitrance characteristics during *in vitro* multiplication, limiting their propagation and requiring protocol modification to accelerate the production of shoots and the efficiency of multiplication stage, compared with other cultivars. As example, a historical mean of multiplication along three years of *in vitro* cultivation showed that gerbera cv. Lamborghini (red inflorescences) was 3.8 shoots/explant compared with 6.8 and 4.6 shoots/explant for 'Suzzane' (orange) and 'Dino' (yellow) (Cardoso, personal observations in commercial lab).

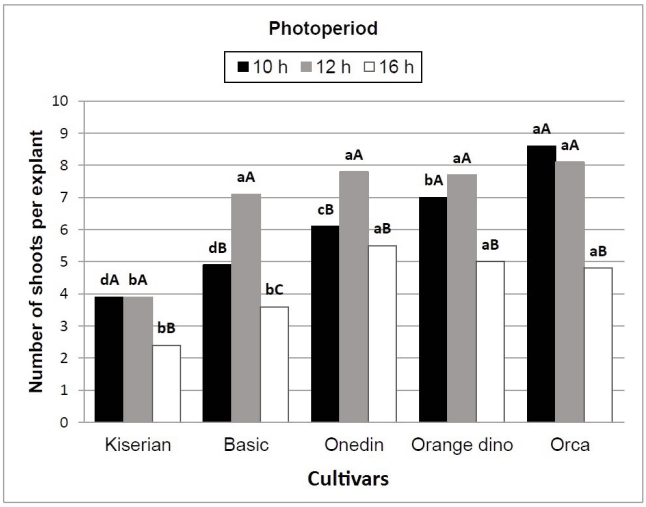

Fig. 1 - *In vitro* multiplication of gerbera (*Gerbera jamesonii*) cultivars under different photoperiodic regimes. The means were obtained from three multiplication times (experiments) considering five replicates (flasks) with four plants. Different small and capital letters show the differences among genotypes and photoperiod regimes, respectively, by the Scott-Knot test at 5% probability. The Coeficient of Variation is 13.52. The data were considered normal by Shapiro-Wilk normality test and the F was significative for all factors, including the interaction genotype x photoperiod.

The influence of genotype toward shoot multiplication was also observed by Hartl *et al*. (1993). They classified gerbera cultivars according to the rate of multiplication into three multiple-shoot induction cultivars: high (8 shoots/inoculum), moderate (6-7 shoots/inoculum) and low (4-5 shoots/inoculum). We also observed all these classes in our cultivars. However, the different photoperiodic conditions had different effects on the position of the cultivars in different classes of multiplication as proposed by Hartl *et al*. (1993). In our work the classification of cultivars using the Scott-Knot test with the best photoperiod for multiplication (12-h photoperiod) showed at least two classes of multiplication: (**a**) easily multiplied with number of shoots/explant >7.0; and (**b**) recalcitrant for multiple shoot induction, with the number of shoots/explant <4.0. An intermediary classes can also be considered as (**ab**), where the multiple shoot induction rates was 4 to 7 shoots/explant, but this was observed only in 10-h and 16-h photoperiod conditions.

The photoperiod had significant effects on gerbera *in vitro* multiplication (Fig. 1 and 2). Normally, the multiplication at 25±2°C associated with 16-h photoperiod is most used for gerbera (Cardoso and Teixeira da Silva, 2013), but in our actual work, we observed that for all five cultivars tested, the use of 12-h photoperiodic regime resulted in significant increase in multiple shoot induction when compared with 16-h standard photoperiod conditions (Fig. 1). The maximum positive effects (97.2%) were obtained in one of the most recalcitrant gerbera cultivar Basic, followed by 'Orca' (68.8%), 'Kiserian' (62.5%), 'Orange Dino' (54.0%) and 'Onedin' (41.8%).

Similar results were observed in micropropagation of ornamental pineapple *Annanas comosus* var. *erec-*

tifoilius, in which the reduction of photoperiod for 12-h resulted in better multiplication rate (6.6 shoots/explants) than 16-h photoperiod (4.2 shoots/explants) (Santos *et al*., 2015). The influence of photoperiod on *in vitro* development was also noted for other species and found affecting other *in vitro* plantlet characteristics of development, such as micro-tuberization in potato cultivars (Seabrook *et al*., 1993). In amaryllis (*Hippeastrum johnsonii*), the authors observed that 16-h photoperiodic regime resulted in better *in vitro* root length and number, bulblet diameter, and leaf length, compared with 14-h and 12-h of photoperiod (Zakizadeh *et al*., 2013).

Several amendments to reduce the cost have been proposed for gerbera micropropagation, such as chemical sterilization in place of autoclaving during all stages of micropropagation (Cardoso and Teixeira da Silva, 2012) and the use of greenhouse rooting and elongation, called PAG culture, in place of laboratory growth conditions (Cardoso *et al*., 2013). The reduction in photoperiodic regime resulted in low energy consumption and so the low cost for the multiplication stage of gerbera. This might be useful for the production of low cost plantlets from micropropagation of this important cut flower. The major conclusion can be drawn from this study is that a reduction in photoperiod by 4-h of light per day along with increase in multiplication rate may increase the efficiency of the propagation system. Chen (2016) concluded that the electrical energy for control environmental conditions on growth room, associated with the low efficiency of multiplication, is the most important factor that leads to increase cost of micropropagated plantlets of *Phalaenopsis*. This author showed that increase in multiplication rate from 1.5 to 2.5 could lead to a 50% cost reduction in micropropagated *Phalaenopsis*.

Photoperiod has a direct effect on the development of greenhouse cultivated gerbera genotypes, affecting plant width, height, shoot dry weight and number of flowers in a condition of 16-20-h of photoperiod (Gangnon and Dansereau, 1990). However, the interaction of photoperiod and the cultivation and development of gerbera could be more complex involving possible role of other environmental factors (Pettersen and Gislerød, 2003), and only few of them have been understood. Our results showed for the first time that the *in vitro* development of gerbera plantlets can be affected by photoperiod regimes (Fig. 1 and 2). Increases in axillary shoot production was observed in reduced photoperiods (12 and 10-h) compared with 16-h.

In addition, the use of white fluorescent lamps in the *in vitro* cultivation of gerbera resulted in a higher multiplication rate compared to different colors of light-emitting diodes (LEDs) lamps (Gök *et al*., 2016).

Our actual experiments showed that reducing the photoperiod to 12-h produced slightly increased shoot height with no visual effect on the size of leaves (Fig. 2). On the other hand, a further reduction of photoperiod to 10-h reduced the size of leaves (diameter and length) and resulted in etiolated development, showing negative effects on quality of gerbera shoots produced for rooting stage (Fig. 2). As example, the fresh weight of gerbera shoots in 10-h photoperiod is 20.0% ('Onedin'), 29.7% ('Orca') and 11.6% ('Orange Dino') less than compared with shoots produced in 12 or 16-h photoperiodic regimes (data not showed). Only gerbera cv. Kiserian showed practically the same fresh weight in 10-h and 12-h photoperiod.

Fig. 2 - Morphological characteristics of *in vitro* cultivation gerbera (*Gerbera jamesonii*) cv. Onedin under different photoperiodic regimes: 10-h and 12-h. Shoots from 10-h photoperiod presented smaller leaves (sl) and smaller and etiolated shoots (ses), compared to 12 and 16-h photoperiod. Bar = 1.0 cm

Plantlets obtained from all treatments were successfully *in vitro* rooted (95-100% of rooting) in ½MS with 30 g L^{-1} sucrose and 0.5 mg L^{-1} of indol-butyric acid and were acclimatized in greenhouse conditions using polypropylene trays with coconut powder as organic substrate. No morphological alterations were observed in acclimatized plantlets obtained, independent to the cultivar or photoperiod used, and the plantlets maintained the main characteristics of the donor plants until the flowering stage.

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Short note

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A preliminary investigation on developmental and biochemical responses of *Amsonia orientalis* to ultraviolet-C irradiation

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Abstract: The present study aims to investigate the developmental and biochemical responses of ornamental *Amsonia orientalis* irradiated with ultraviolet-C (UV-C). Nodal explants of the species were exposed to UV-C irradiation on the first, 15th and the last days of the *in vitro* culture for 15 (3.47 kJ m^{-2}), 30 (6.94 kJ m^{-2}) and 60 min (13.87 kJ m^{-2}). In general, root lengths and numbers were negatively influenced by prolonged UV-C exposure. However, mean shoot numbers and lengths were slightly enhanced after 15 and 30 min of irradiation. High hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels were observed due to the UV-C exposure. Activities of the antioxidant enzymes, POD (peroxidase) and CAT (catalase) were found to be enhanced whereas SOD (superoxide dismutase) was reduced. These results indicated that UV-C irradiation for shorter durations may be carefully used to improve *in vitro* shoot proliferation in *A. orientalis*. However, it should be noted that longer irradiation durations can trigger stress responses and lipid peroxidation-dependent cell membrane damage which will further result in the plant loss.

1. Introduction

Ultraviolet (UV) irradiation is present in sunlight in three different wavelengths which are classified as UV-A (400-315 nm), UV-B (315-280 nm) and UV-C (280-100 nm). UV irradiation may affect growth and metabolic processes in plants due to its high quantum energy (Kobashigawa *et al.,* 2011). Since wavelengths below 280 nm are absorbed by the ozone layer they do not reach to the surface of the Earth (Alexieva *et al.,* 2001). However, reduction of stratospheric ozone and, a decrease in the ozone layer may lead to increase in UV-C irradiation reaching the biosphere.

Beside its germicidal activity, application of UV-C irradiation at 254 nm wavelength is used for several purposes including regulation of fruit ripening in tomato (Tiecher *et al.,* 2013), fruit quality stabilization during storage of fresh-cut watermelon (Artés-Hernández *et al.,* 2010) and retardation of fruit decay in strawberry (Erkan *et al.,* 2008). Its role in growth regulation and modulation of flowering time in ornamental plants was declared since UV-C light application increased branching and the number of flowers in *Salvia splendens* and *Viola tricolor* (Bridgen, 2016). Also, the antioxidative function was found to be enhanced after UV-C application in *Spinacia oleracea* (Kobashigawa *et al.,* 2011). However, the application of UV irradiation can be hazardous in higher doses. Therefore, to better understand the developmental and metabolic responses of horticultural plants to UV-C irradiation, UV-dose-dependent studies in controlled environmental factors should be also conducted on other plant species.

Amsonia orientalis Decne. [syn. *Rhazya orientalis* (Decne.) A. DC.] which is also known as European Blue star or Eastern Rhazya is an ornamental plant with medicinal properties. It is cultivated more commonly by gardeners in the USA than those in Europe since it is deer-resistant due to its slightly toxic latex content (Acemi *et al.,* 2016). However, the natural populations of the species were taken under conservation by the European Council in the frame of Bern Convention since they are quite limited in nature (Acemi *et al.,* 2017). In the current study, the effects of dose-dependent UV-C irradiation applications on *in vitro* development and antioxidative enzymes of *A. orientalis* were investigated to shed light the usability of this treatment in horticultural plant propagation.

2. Materials and Methods

Plant material preparation and in vitro UV-C treatments

Nodal explants were excised from mature individuals of *Amsonia orientalis* growing in the garden of Kocaeli University. The shoots were multiplied by following the protocol described by Acemi *et al.,* (2013 a). Nodal explants excised from *in vitro*-raised shoots were inoculated into the MS (Murashige and Skoog's) medium (1962) supplemented with 30 g L^{-1} sucrose and 7 g L^{-1} of plant agar. The pH of the medium was set to 5.7, and the cultures were incubated for 30 days under the same conditions defined by Acemi *et al.,* (2013 a). The applied UV-C doses were determined from previous reports (López-Rubira *et al.,* 2005; Artés-Hernández *et al.,* 2010; Bridgen 2014; Castronuovo *et al.,* 2014; Bridgen, 2016). The cultures were subjected to UV-C irradiation at the first, 15th and the last days of the culture period. The irradiation was applied from 30 cm distance for 15, 30 and 60 min per application day by using Sylvania G15W UV-C lamp (λ_{max} 253.7 nm). UV-C treatments were applied from the top of the culture vessels in a sterile biosafety cabinet, and lids of the culture vessels were kept opened during treatments to ensure penetration of the irradiation energy. The total accumulated irradiation levels were estimated as 3.47, 6.94 and 13.87 kJ m^{-2} for 15, 30 and 60 min of UV-C irradiation, respectively (Kobashigawa *et al.,* 2011; Severo *et al.,* 2015).

Biochemical assays

The optimized method of Acemi *et al.* (2017) was followed to determine malondialdehyde (MDA) as lipid peroxidation product, and H_2O_2 contents. The crude extract for antioxidant enzyme activities' determination was prepared by homogenizing the tissue samples (shoots and roots together) in extraction buffer of 50 mM sodium phosphate (pH 7.0) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA). After centrifugation of the homogenate at 14,000 *g* for 15 min at 4°C, the resulting supernatants were collected and used for catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activity assays. CAT activity was determined according to the method of Aebi (1974). POD activity was assayed using the pyrogallol oxidation method (Kar and Mishra, 1976) while SOD activity was determined by following the method of Dhindsa *et al.* (1981).

Data collection and statistical analysis

The morphometric evaluation was done using 30 explants in each repeat. All assays were repeated thrice and mean values were compared using Duncan's multiple range test at $p<0.05$ significance level. The enzyme assays and extract preparation were done on the same day to minimize the loss of enzymatic activities.

3. Results

Plant growth and organ development

At the end of the incubation period, the mean shoot length slightly increased in response to 15 and

30 min UV-C exposure while this parameter was negatively affected by 60 min of exposure. However, increments in the mean shoot length were not statistically different than the control group (Fig. 1A). The mean shoot numbers were influenced positively by 15 and 30 min UV-C exposure whereas only the result of 15 min exposure was found to be statistically different than the control group (Fig. 1B). The mean root lengths were found to be decreased due to UV-C application even at the shortest duration. This reduction in root lengths was between 62.5% (15 min) and 95% (60 min) compared to the control (Fig. 1A). In contrast to mean shoot numbers, the mean root numbers reduced gradually in response to the elevated UV-C exposure (Fig. 1B).

Lipid peroxidation and H_2O_2 content

Application of UV-C irradiation significantly changed the lipid peroxidation level of the plant. Fifteen and 30 min of UV-C exposures triggered MDA accumulations without statistical differences between them. The highest accumulation level was observed from the cultures exposed to UV-C for 60 min (Fig. 2A). H_2O_2 content increased following UV-C exposure maintaining similar levels within treatments (Fig. 2B).

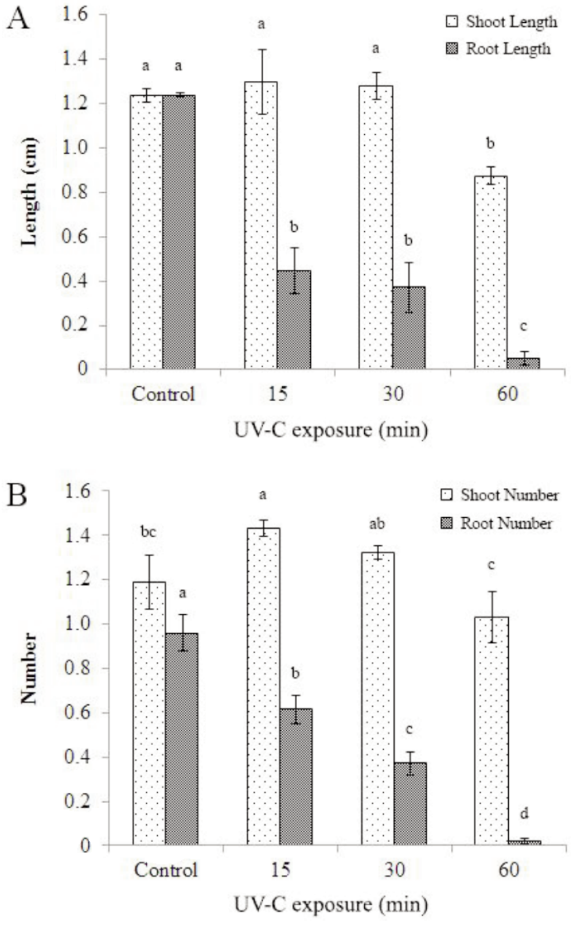

Fig. 1 - Effects of UV-C irradiation on *Amsonia orientalis* growth parameters. A= Length of shoot and root, B= Number of shoot and root. The values are represented as means ± SD, different letters denote significant differences ($p<0.05$).

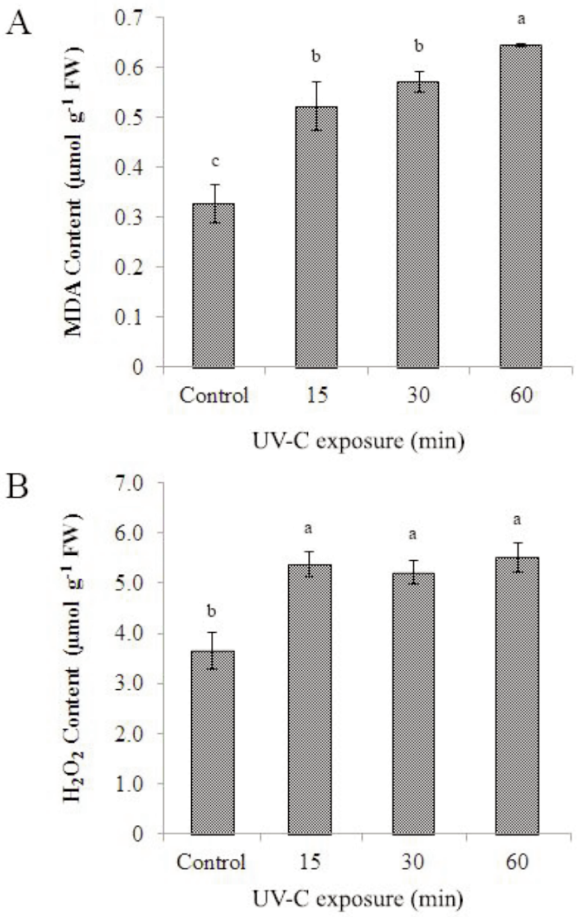

Fig. 2 - Oxidative effects of UV-C irradiation on *Amsonia orientalis*. A= MDA and B= H_2O_2 contents. The values are represented as means ± SD, different letters denote significant differences ($p<0.05$).

Antioxidant enzyme activities

UV-C irradiation caused a decrease in SOD activity without statistical differences between treatments (Fig. 3A). POD activity significantly increased due to UV-C exposures. The highest POD activity was observed in cultures exposed to 15 min of UV-C followed by a significant decrease at 30 and 60 min of UV-C exposures, which were not significantly different (Fig. 3B). CAT activity exhibited a similar trend with POD activity. The shortest UV-C exposure caused the highest increase in the CAT activity while longer exposures induced statistically same results (Fig. 3C).

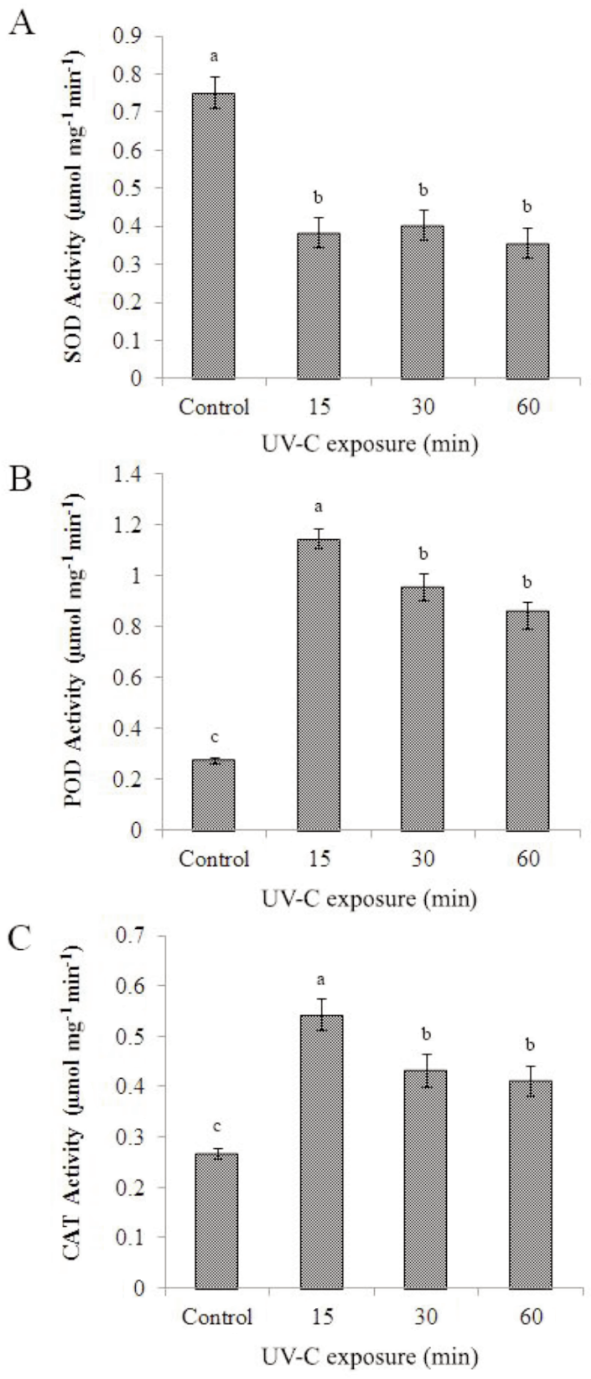

Fig. 3 - Effects of UV-C irradiation on enzymatic antioxidant activities in *Amsonia orientalis*. A= SOD, B= POD and C= CAT activities. The values are represented as means ± SD, different letters denote significant differences ($p<0.05$).

4. Discussion and Conclusions

UV irradiation can damage DNA and affect plant growth through various regulatory and/or stress-mediated processes. As one of these processes, cell cycle modulation sourced by DNA damage may particularly slow down the progress from G_1 to S phase (Jiang *et al.,* 2011). Therefore, in our results the decrease in the mean shoot length after 60 min UV-C irradiations might be due to the UV-induced DNA damage and impaired cell cycle progress. Differently, mean shoot number was found to be increased in *A. orientalis* after 15 min of UV-C exposure, which was in accordance with the previous report that found an increment of axillary branching in *Viola tricolor* (Bridgen, 2016). Our results also showed adverse effects of UV-C on the root growth of *A. orientalis*. This negative effect can be explained by oxidative degradation and/or inhibited-synthesis of indole acetic acid (IAA); a phytohormone responsible for root proliferation (Berli *et al.,* 2013). This possible inhibition of IAA might also be a result of photo-oxidation of the plant growth regulator (Ciurli *et al.,* 2017). The oxidative degradation of IAA starts with a peroxidase-involved decarboxylation process on the side chain or oxidation of the indole ring (Normanly, 2010). In the enzyme assays, elevated peroxidase activity against UV irradiation supports this discussion which is still in need of further experiments to be clearly proven.

MDA content indicates reactive oxygen species (ROS)-mediated cellular damage considering damage to membrane lipids of stress-exposed plants. The observed increase in H_2O_2 levels coincided with enhanced MDA levels in *A. orientalis*. Also, POD and CAT activities were found to be increased to remove ROS to limit MDA production in *A. orientalis*. Degradation of the enzyme proteins and nucleic acids can be started after ROS-induced peroxidation of the cell membrane lipids. At the same time, H_2O_2 could activate mitogen-activated protein kinases (MAPKs) in plants, leading to an enhanced antioxidant defense system (Nie *et al*., 2013). However, excess accumulation of H_2O_2 causes cellular damage (Gong *et al.,* 2001). The increase in both H_2O_2 and MDA levels indicates cellular damage in *A. orientalis*. Excessive ROS in UV-C-exposed plants may be produced because of disruption in metabolic activities or increased activity of membrane-localized NADPH-oxidase (Kalbina and Strid, 2006). The increment in the activities of CAT and POD enzymes did not seem to limit ROS production in *A. orientalis* since H_2O_2 accumulation was consistently found at high levels.

As a defense mechanism against environmental stress factors, plants favor the production of antioxidant enzymes (Berli *et al.,* 2013). Maintenance of the

antioxidant defense system to cope with ROS plays a significant role in keeping the cell membranes stabilized. The antioxidant enzymes SOD, CAT, and POD are widely distributed in all higher plants and involved in decomposition of different forms of ROS (Foyer and Noctor, 2000). Excessive production of O^{2-} triggers SOD enzyme activity which converts superoxide radicals into either O_2 or H_2O_2 while the excess accumulation of H_2O_2 is prevented by catalase and/or the ascorbate-glutathione cycle enzymes (Ma *et al.,* 2014). Although the O^{2-} content was not determined in our study, the inhibition of SOD activity by UV irradiation might be due to increased O^{2-} content. POD is involved in such processes like lignification and tolerance to environmental stresses in higher plants. During UV exposure, elevated POD and CAT activities showed that activities of both enzymes can be triggered in *A. orientalis.* The bulk H_2O_2 removal activity of CAT in the cell is followed by the scavenging action of POD on the H_2O_2 which is not taken by CAT (Willekens *et al.,* 1997). In this report, this cooperation between both enzymes is also shown in *A. orientalis*.

Thanks to *in vitro* plant tissue culture technique, several conservation studies on *A. orientalis* were conducted and a high number of individuals were propagated thereby populations in Turkey were conserved (Acemi *et al.,* 2013 a, b). The present study has revealed that although short-term application of UV-C irradiation can enhance shoot induction, it limits root growth and triggers oxidative stress at extended exposure durations. Therefore, in further detailed studies on *A. orientalis* UV-C radiation should be applied for less than 30 min (6.94 kJ m^{-2}). However, it should be noted that short-term UV-C application can be considered as a promoter factor only in shoot multiplication phase in *A. orientalis*. The lower doses may be carefully applied to enhance branching in the horticultural industry. However, the possible developmental and biochemical responses given against UV-C irradiation would change species to species.

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Short note

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Field evaluation of new Kabuli chickpeas lines for the production of canned seeds

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Key words: canned products, canned seeds, chickpea, *Cicer arietinum* L., seed grade, Tuscany.

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Abstract: There is an increased demand for canned chickpeas worldwide, which has also resulted in increased chickpea cultivation in Italy. The availability of Italian chickpea varieties, suitable for industrial transformation, is limited. The objective of the present study was to evaluate the field production of new Kabuli chickpea accessions and their suitability for industrial transformation. Thirteen accessions, provided by the International Centre for Agricultural Research of Turkey, together with 'Blanco Sinaloa', a commercial Mexican variety, were cultivated in Cesa, Tuscany, in 2016 and 2017. The average seed yield was 2.8 and 3.4 t ha^{-1}, respectively, in 2016 and 2017. Increased yield in 2017 was attributable to a more balanced rainfall pattern, compared to that in 2016 which induced increased vegetative growth. Based on production stability over the two-year trial, the varieties FLIP08-69C, FLIP-160C and FLIP05-157C turned out particularly suitable for cultivation. The climatic conditions in 2017 favored an increased presence of a Grade B seed classification [220-250 seeds per 100 g (caliber 8)] which is preferred by industry. Most varieties showed potential regarding seed dimension classification, however, of the varieties with a good production, FLIP05-157C was also characterized by seed gradation stability.

1. Introduction

In recent years, there has been a change in the style of food consumption by Italian people. This includes the growing interest in legumes as an alternative protein source to meat. The lower fat content of legumes appears to be a more appreciated characteristic. Here, we are referring to "food safety", where consumers are prepared to incur higher costs when purchasing foods for health safety and towards guaranteeing environmentally friendly production techniques (Dixon and Sumner, 2003; Fratianni *et al*., 2014).

The industrial uses of legumes vary, ranging from animal feed (flour

and supplements) to the processing of the seeds for human consumption, especially in the form of preserved (canned) products.

According to 2016 statistics, the Italian market for canned vegetables is valued at approximately 400 million Euros, representing an increase of +1.2% compared to the previous year (Limonta, 2016). The same data showed that, in the face of production decreases in sweet corn, green beans and peas, there is an increase in beans and chickpeas. More specifically, the categories "beans" and "other legumes" (chickpeas and lentils) constitute more than 60% of the market. As regards chickpeas, with a 62% share, an increase of 10.2% was recorded.

As an inevitable consequence, there was an increase in the area dedicated to chickpea cultivation in Italy (Palumbo, 2017), from 5.000 to almost 16.000 ha, from 2008 to 2017, respectively. This notable increase is attributable, not only to the aforementioned food trends, but also to agronomic benefits such as reducing the use of both fertilizers and pesticides. In turn, this serves to improve the structure and fertility of the soil and, above all, a more sustainable return to crop rotation with cereals (Palumbo, 2017). These benefits were already sought after, as early as the beginning of 2000 (Watson *et al*., 2017). Concomitant with Italian scenario for chickpea cultivation, there has been a steady increase in the world production of chickpeas from 63.4 to 77.5 million tons, from 2009 to 2015, respectively (Muehlbauer and Sarker, 2017).

This positive trend in the chickpea market has naturally resulted in the involvement of the Italian canning industry, always attentive to the needs of the market. An increasing number of industries, through their own incentive, are producing the raw material directly in Italy just to meet the needs of "Food safety". In this way, through cultivation contracts, they are certain to direct cultivation towards the production of the raw material, adhering, as closely as possible to both product requirements and processing techniques for the production of canned chickpeas. The Italian consumer prefers Kabuli chickpeas. The characteristics of "Kabuli" include a light color, preferably with a caliber of 8 (220-250 seeds per 100 g), a typical shape with rough surface, and with a thin integument that still adheres to the seeds once cooked (Palumbo, 2017).

The availability of Italian chickpea varieties, suitable for industrial transformation, is rather limited. With regard to Central Italy, more specifically Tuscany, experimental tests conducted in the '80s and '90s (Casini, 1987, 1989), showed that either autumn or end of winter sowing subjected the cultivation to high risks of Anthracnose [*Ascochyta rabiei* (Pass.) Labr. Trot.]. More recent research (Radicetti *et al*., 2012), has highlighted that "Pascià" and "Principe" are the two most widespread Italian varieties, suitable for industrial transformation with yields varying between 2.5 and 2.7 t ha^{-1}. However, industry is continually looking for new varieties either for use in genetic improvement programs or for use towards better production, tolerance to diseases and technological qualities.

The aim of the present study was to evaluate the field production of new accessions of Kabuli chickpeas and their suitability for industrial transformation based on the preferred seed dimensions classification characteristics.

2. Materials and Methods

The field experiments were carried out in Tuscany, Central Italy, in 2016 and 2017 at the "Centro per il Collaudo ed il Trasferimento dell'Innovazione di Cesa (Arezzo)", 43° 18' N; 11° 47' E; 242 m a.s.l. The cultivation environment was characterized of a neutral, loamy-sandy soil. The principle physical and chemical characteristics of the soil were as follows: sand 36%, loam 38%, and clay 26% respectively. The soil pH was 7.0. Total N was 0.11% and P (Olsen) 13 ppm. Exchangeable Ca, Mg and K were 4123, 595 and 141 ppm, respectively.

Thirteen accessions, provided by the International Centre for Agricultural Research (ICARDA) of Ankara (Turkey), were used, in addition to "Blanco Sinaloa", a commercial variety from Mexico. Based on previous experiments carried out in Central Italy (Casini, 1989), the Autumn-Winter sowing period was not taken into consideration due to serious damage caused by Anthracnose blight. As a result, the sowing dates were March 14, 2016 and February 22, 2017, respectively. Plots were arranged according to a complete RCB design, with three replicates. The size of the plots were 2.0 x 4.0 m (four rows wide with 0.5 m row spacing). The sampling area was comprised of the two central rows of 3.0 m long. A seed quantity of 380 per plot was used. In order to obtain the correct planting density of 25 plants m^{-2}, seedlings were thinned soon after complete emergence. Plots were hand-weeded twice (45 and 65 days after emergence

[DAE]) during the growth cycle. The agricultural interventions performed during the two-year experimental period are reported in Table 1. Plant height, number of stems, height of the first pod and number of pods per plant were determined at maturation stage, using a total of 10 plants per sample plot. Yield calculation and the number of seeds amounting to 100 g were performed using seed samples at standard humidity of 12%. Grading of seeds was carried out according to the standard tables of Conserve Italia (2015) for the Italian market as follows: Grade A [<220 seeds per 100 g (caliber 6)]; Grade B [220-250 seeds per 100 g (caliber 8)] and Non Standard or Off-Type (>250 seeds per 100 g). Both Grades A and B are considered suitable for processing, but B is the preferred grade.

Table 1 - Agronomic technique, date of sowing, date of emergence and harvesting of the two field trials

	2016	2017
Previous crop	Wheat	Sunflower
Plowing	September 6, 2015	-
Rippering and rolling	-	October 22, 2016
Harrowing	September 28, 2015	November 2, 2016
Grubbering	-	January 10
Harrowing	March 14	February 22
Pre-sowing fertilization	March 14	February 22
	N 52 and P_2O_5 114 kg ha^{-1}	N 52 and P_2O_5 114 kg ha^{-1}
Sowing	March 15	February 23
Emergence	April 11	March 14
Harvesting	August 22	August 19

Data collected in the experiments were processed utilizing a mixed-model analysis of variance (ANOVA), where accession was considered as a fixed effect factor, and year as random effect factor. Statistical differences were tested at P≤0.05, P≤0.01 or P≤0.001. The Tukey's HSD test was used to stress significant differences between means and homogenous groups.

3. Results, Discussion and Conclusions

The climatic trends over the two-year experimental period were very different, especially with regard to rainfall. In the first year, the crop benefited from 562 mm of rainfall, of which 142 mm were evenly distributed during the period May-mid-June. In the second year, total rainfall over the cultivation cycle was 290 mm. In 2017 the maximum temperatures were particularly high with an average of 24.8°C (31.0°C in the summer).

Principle biometric characteristics of the lines (Table 2) were significantly different at either p≤0.01 or p≤0.001, with the exception of the number of empty pods per plant. As regards the main biometric characteristics, the higher rainfall of 2016, contributed to a greater vegetative growth of the plants, compared to that in 2017. The height of the plants

Table 2 - Principle biometric characteristics of the lines

Source of variation	Plant height (cm)	Stems per plant (n)	Height of first pod (cm)	Filled pods (n)	Empty pods (n)	Seeds per pod (n)
Lines (L)						
FLIP05-69C	58.3 a	5.1 bcd	34.3 a	43.5 bc	3.5 a	1.2 ab
FLIP05-156C	55.1 ab	5.3 a-d	28.2 bcd	45.6 bc	4.9 a	1.1 ab
FLIP05-157C	53.9 ab	6.7 ab	29.2 bc	39.1 cd	3.4 a	1.1 ab
FLIP07-230C	58.7 a	6.9 a	30.7 ab	45.1 bc	5.0 a	1.1 ab
FLIP07-318C	52.7 ab	5.5 a-d	30.0 abc	42.0 cd	3.0 a	1.3 a
FLIP08-69C	55.7 ab	6.6 ab	28.9 bcd	48.5 bc	3.9 a	1.2 ab
FLIP08-160C	52.6 ab	6.2 abc	29.3 bc	47.2 bc	4.4 a	1.1 ab
FLIP08-170C	56.8 a	5.1 bcd	27.5 bcd	58.5 a	4.5 a	1.2 ab
FLIP08-200C	55.4 ab	4.7 cd	25.8 cde	43.7 bc	4.9 a	1.2 ab
W6-12861	59.0 a	6.0 a-d	31.3 ab	63.3 a	4.9 a	1.1 ab
W6-9484	54.9 ab	5.1 bcd	24.3 de	37.2 cd	4.3 a	1.0 b
W6-30	54.0 ab	5.7 a-d	25.4 cde	46.2 bc	4.1 a	1.0 b
W6-25	52.4 ab	4.5 d	28.0 bcd	57.2 ab	4.6 a	1.2 ab
Blanco Sinaloa	49.1 b	5.1 bcd	21.4 e	29.4 d	2.6 a	1.2 ab
f	**	***	***	***	NS	**
Year (Y)						
2016	42.1	6.7	31.8	65.8	5.8	1.2
2017	42.9	4.6	24.5	28.0	2.4	1.2
f	**	**	***	***	***	NS
L x Y	***	**	***	***	***	NS

NS= not significant; ** significant at P≤0.01; ***significant at P≤0.001.
Means followed by the same letter(s) are not different for P≤0.05 according to Tukey test.

was almost 20 cm higher (average of 64.7 cm) with a corresponding increased height of first stage pods (31.8 cm from the ground), more favorable for mechanical harvesting. Increased vegetative growth was also expressed in the greater ramification number of 6.7 stems per plant in 2016 as compared to 4.6 in 2017, respectively. The height of the plants was both positively and significantly correlated to the number of full pods per plant (r^2 = 0.799**), which in the first year was more than double than that observed for the second year: 65.8 vs. 28.0 cm, respectively.

The average seed yield was 2.8 and 3.4 t ha^{-1}, respectively, in 2016 and 2017 (Fig. 1). The yield increases in 2017 were observed for all varieties. Varieties showing a yield increase that exceeded 25% included FLIP 69C, W6 12861, W6 9484 and W6 25, respectively. These increases were attained despite a lower production of full pods per plant in 2017. This yield increase was likely attributable to the more favorable climatic conditions of 2017, in comparison to those of 2016, which induced increased vegetative growth.

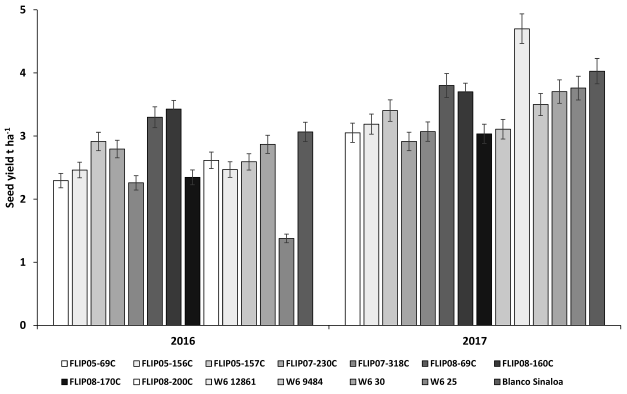

Fig. 1 - Seed production of tested lines in 2016 and 2017

Table 3 shows an increase in the number of seeds per each 100 g weight for almost all varieties, with the exception of W6 25, in which the number seeds (195-196 seeds per 100 g) is maintained. Table 3 also provides the commercial classification values for varieties and their suitability for processing into pre-cooked products. In this regard, all the lines were found to be suitable for both years with a grade classification of either A or B, with the prevalence of the more desired latter classification in 2017.

The results of this experiment highlighted a good production capacity of the majority of the lines tested. Some varieties, such as FLIP08-69C, FLIP-160C and FLIP05-157C, are particularly suitable, as based on production stability over the two-year trial. Moreover, among these lines, FLIP05-157C was also characterized by seed gradation stability, suitable for industrial transformation. Conditions of balanced rainfall, evident in 2017, favored an 8 caliber (B) seed yield, which is preferred by industry.

In conclusion, with some exceptions, all the lines tested are considered suitable for use in selection and/or for breeding programs, aimed at obtaining varieties dedicated to the production of pre-cooked seeds for the Italian market.

Table 3 - Number of seeds per 100 g and the corresponding grade for canned products

Lines	2016		2017	
	Seeds per 100 g (n)	Grade	Seeds per 100 g (n)	Grade
FLIP05-69C	241 ab	B	232 ab	B
FLIP05-156C	236 ab	B	243 ab	B
FLIP05-157C	233 ab	B	238 ab	B
FLIP07-230C	229 bc	B	224 bcd	B
FLIP07-318C	219 cd	A	231 bc	B
FLIP08-69C	202 e	A	240 ab	B
FLIP08-160C	200 ed	A	245 a	B
FLIP08-170C	215 cd	A	238 ab	B
FLIP08-200C	219 cd	A	246 a	B
W6-12861	216 cd	A	231 bc	B
W6-9484	198 ed	A	229 bc	B
W6-30	195 d	A	196 ed	A
W6-25	225 bcd	B	231 bc	B
Blanco Sinaloa	198 ed	A	229 bc	B

Means followed by the same letter(s) are not different for P≤0.05 according to Tukey test.

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