ISSN: 0394-6169
ISNN: 1592-1573

ADVANCES IN HORTICULTURAL SCIENCE

n. 4 **2013**

formerly
«Rivista dell'Ortoflorofrutticoltura Italiana»
founded in 1876

FIRENZE UNIVERSITY PRESS

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

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

ADVANCES IN HORTICULTURAL SCIENCE

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

supported by ENTE CASSA DI RISPARMIO DI FIRENZE

Advances in Horticultural Science is covered in the following indexing and abstracting services:

ACNP Catalogo Italiano dei Periodici - AGRICOLA - AGRICULTURE Journals - AGRIS - APE Journal - BASE Bielefeld Academic Search Engine - BIOBASE - Biological Abstracts - Biological Science Journals PQ - BIOSIS Preview THOMSON REUTERS - CAB Abstracts - EBSCO - EZB–Elektronische Zeitschriftenbibliothek – Universitätsbibliothek Regensburg - Google Scholar - HORTICULTURAL Abstracts - JournalSeek. A Searchable Database of Online Scholarly Journals - JURN - Natural Science Journals PQ - NewJour. Electronic Journals & Newsletters, University of Georgetown - OAISTER oclc - Ornamental Horticulture CABI - Plant Breeding Abstract CABI - Proquest - Scirus ELSEVIER - SciTech Journals - SciVerse SCOPUS ELSEVIER - Searchteam - Ulrich's Periodicals Directory - WoS Web of Science THOMSON REUTERS - WorldCat

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

SUBSCRIPTIONS - *The subscription price of volume 27, 2013 is € 60.00 in Italy and € 70.00 in other countries. Mailing costs: € 3 for Italy, € 6.50 for Europe and € 10.00 for the rest of the world. The subscription price of an issue is € 17.00 in Italy and € 20.00 in other countries.*

CONTENTS

Adam A., Idris I., Ayyoubi Z.
In vitro Pseudomonas putida BTP1-induced systemic resistance in grapevine rootstocks against Phylloxera (*Daktulosphaira vitifoliae*) 137

Hosseini H., Chehrazi M., Dehkourdi E.H., Hosseini M.
Application of anatase nanoparticles (TiO_2) on strawberry seed germination (*Fragaria ananassa* L.) 143

Wani R.A., Sheema S., Malik T.H., Geelani S., Bashir S., Dar N.A., Prasad V.M.
Impact of integrated nutrient management on growth, yield and quality of strawberry (*Fragaria x annanassa* Duch.) cultivation in India 147

Ahmad M.S., Thakur K.S., Siddiqui M.W.
Postharvest treatments for preserving quality of 'Kinnow' fruit under different storage conditions 152

Corbino G.B., Sánchez G., González J., Murray R.E., Gabilondo J., Valentini G.H., Arroyo L.E.
Pre and post-harvest management affects functional quality of peach (*Prunus persica* L.) cv. Flavorcrest 159

Vazquez A., Sanchez E., Van Baren C., Frezza D.
Agronomic performance and essential oil composition of *Ocimum basilicum* L.: Effect of genotype and date of harvest 166

Javanmardi J., Rahemi M., Nasirzadeh M.
Post-storage quality and physiological responses of tomato fruits treated with polyamines 173

Book Reviews 183

Reviewers of Manuscript for 2013 185

Author index 187

Subject index 191

Adv. Hort. Sci., 2013 27(4): 137-142

In vitro Pseudomonas putida BTP1-induced systemic resistance in grapevine rootstocks against Phylloxera (*Daktulosphaira vitifoliae*)

A. Adam [(1)], **I. Idris, Z. Ayyoubi**
Department of Biotechnology, Atomic Energy Commission of Syria, P.O. Box 6091, Damascus, Syria.

Key words: B41, grapevine phylloxera, ISR, PGPR, *Pseudomonas putida* BTP1, Ru140.

Abstract: This study investigates the systemic resistance induced by *Pseudomonas putida* strain BTP1 against phylloxera using an *in vitro* model in Ruggeri (Ru140) and B41 roostocks. Significant differences were found with regard to matured females, fecundity and oviposition period between untreated and bacteria-treated plants in both rootstocks. Treated Ru140 rootstocks were more resistant than treated B41 ones. BTP1 impacted negatively on the ability of phylloxera to develop, indicating an increase in grapevine resistance and tolerance toward this pest in bacteria-treated plants. This is the first known study of biocontrol of phylloxera in grapevine rootstocks by non-pathogenic *P. putida* strain BTP1 *in vitro*.

1. Introduction

Grapevine phylloxera (*Daktulosphaira vitifoliae*) is a tiny aphid-like insect that feeds on grapevine (*Vitis vinifera* L.) roots and leaves, leading to stunted growth or death. It is considered the most destructive grapevine pest (Vidart *et al.*, 2013). In Syria, there are more than 70,000 ha of grapevine with an estimated 540,000 ton annual production (Statistics of Syrian Agriculture Ministry, 2011). However, phylloxera causes millions of dollars in losses in grapevine production annually. Grapevine phylloxera forms damaging root galls which are metabolically active organs suited to meet the nutritional requirements of phylloxera and support its generation with high reproductive rates, making this pest capable of destroying the root system of *V. vinifera* vines. Root injuries reduce the vines' ability to absorb nutrients and water, causing a decline in vigor and productivity. As a consequence, weakened plants probably become more susceptible to secondary infections by fungal diseases and other insects and are also vulnerable to environmental stresses (Granett *et al.*, 2001).

The use of resistant rootstocks is considered the most common and effective means to control phylloxera in the field. The vast majority of these rootstocks have been durably resistant for a long period. In Syria, the widely used resistant rootstocks are Ru140 (*V. rupestris* x *V. Berlandieri*), R99 (*V. rupestris* x *V. Berlandieri*), and 3309C (*V. riparia Michaux* x *V. rupestris*) and B41 (*V. vinifera* x *V. Berlandieri*) (Makee *et al.*, 2003). It is important to note that some rootstocks are more resistant than others to grapevine phylloxera. However, for yet unknown reasons, some rootstocks may lose their resistance to phylloxera. For example, AXR#1 (*Vitis vinifera* X *V. rupestris* Scheele hybrid) has failed to resist phylloxera in several parts of the world after many years of use (Granett *et al.*, 1983). Likewise, rootstock B41 has remained resistant in France while it is not resistant in Californian vineyards, therefore farmers have to replant their vineyards with the appropriate resistant rootstocks (Song and Granett, 1990; De Benedictis and Granett, 1993).

Plants have active defense mechanisms against pathogen attacks. A group of microorganisms referred to as plant growth-promoting rhizobacteria (PGPR) are able to reduce disease through the induction of systemic resistance (ISR) that renders the host plant more resistant to further pathogen ingress (Pieterse *et al.*, 2002). This phenomenon can occur in many plant species and was demonstrated to be effective against a broad spectrum of fungal, bacterial and viral diseases beside its effect on insect and nematode pests (Van Loon *et al.*, 1998; Ramamoorthy *et al.*, 2001; Durrant and Dong, 2004; Verhagen *et al.*, 2010; Weller *et al.*, 2012). In addition to eliciting ISR against pathogens, protective effects of PGPR against insects have been noted (Zehnder *et al.*, 1997 a, b; Zehnder *et al.*, 2001; Kloepper *et al.*, 2004; Vijayasamundeeswari *et al.*, 2009; Valenzuela-Soto *et al.*, 2010). However, to our knowledge no studies have been carried out to assess *in vitro* the effects of PGPR on grapevine phylloxera. In this context, a non-pathogenic *Pseudomonas putida* BTP1 strain has shown enhancement of the level of resistance in cucumber, bean and tomato against the fungal pathogens *Pythium aphanidermatum* and *Botrytis*

[(1)] Corresponding author: ascientific@aec.org.sv

Received for publication 16 September 2013
Accepted for publication 10 December 2013

cinerea, respectively (Ongena *et al.*, 1999; Ongena *et al.*, 2004; Adam *et al.*, 2008). In a previous study performed on fresh roots from local grape variety Helwani (*V. vinifera*), we demonstrated the influence of *P. putida* BTP1 on reproduction and development of grapevine phylloxera (Adam *et al.*, 2012).

Implementation of *in vitro* dual culture assay has been used to evaluate the phylloxera/grapevine interaction (Forneck *et al.*, 1996; Makee *et al.*, 2003; Vidart *et al.*, 2013). This method has several advantages for our designed experiments such as providing optimal conditions for phylloxera infestation, conducting experiments in small space, preventing the spreading of phylloxera and rhizobacteria, as well as reliable results in a relatively short period.

The present work aims to demonstrate the ISR-related protective effect triggered by *P. putida* BTP1 *in vitro* in Ru140 and B41 rootstocks against grapevine phylloxera. The percentage of mature females, fecundity and oviposition period of phylloxera were determined.

2. Materials and Methods

Establishment of the phylloxera colony

Grapevine phylloxera was originally collected from field-infested roots of the local grapevine varieties in southern parts of Syria. The phylloxera colony was established following similar procedures to those mentioned by Makee *et al*. (2003). Fresh and healthy pieces of roots (4-7 mm in diameter and 5-7 cm long) of local grapevine cultivar Helwani (*V. vinifera*) were taken and washed with tap water. Each piece was wrapped with moist cotton wool around one end, and then 10 to 15 phylloxera eggs were placed on each piece. The infested root pieces were then placed on a wet filter paper disk inside a plastic Petri dish (12 cm diameter). Each dish had three to four root pieces. For ventilation purposes the Petri dish lid was modified with a 1-1.5 cm cloth-screened hole. The edges of the dishes were sealed with parafilm and they were kept in plastic boxes with tightly fitting lids and incubated at 25±1°C, 70±5% RH and 24 h darkness. The root pieces were replaced when they desiccated, rotted or the phylloxera became crowded.

Microbial strain and inoculum preparation

P. putida strain BTP1, isolated from barley roots, was originally selected for its specific features regarding pyoverdine-mediated iron transport (Jacques *et al.*, 1995; Ongena *et al.*, 2002). It was maintained and prepared for use in the ISR assays as previously described by Ongena *et al*. (2002). For the bioassays, BTP1 strain was grown in Erlenmeyer flasks (250 ml) containing 100 ml of Casamino Acids medium (CAA) for 24 h on a rotary shaker (150 r.p.m.) at 28ºC. Cells were removed by centrifugation at 16500 g for 15 min at 4ºC and washed in sterile NaCl (5 g l^{-1}). The final pellet was resuspended in an adequate volume of sterile distilled water to obtain a bacterial suspension at 10^8 CFU ml^{-1}.

In vitro culture of grapevine plants

For *in vitro* culture of grapevine plants, we used a protocol described by Makee *et al*. (2010). Wood cuttings having four to five nodes of Ru140 and B41 rootstocks were collected from the field while the buds were still dormant. All cuttings were washed in water, and then treated with gentamicine sulphate 160 mg l^{-1}. Thereafter, they were incubated in 0.5 g l^{-1} carbamate fungicide [Methyl-1-(butylcarbamoyl)-2-benzimidazole-Carbamate 50%] (Bell®) for 24 h, and then grown in sterilized water at 25±1°C under 16 h photoperiod (140-150 µmol m^{-2} s^{-1}) from daylight fluorescent tubes (Philips TLD 38/54). Shoots were grown in glass jars (1000 ml) when they became about 8 cm long; buds of 4 mm length were taken from the middle of each stem. These buds were dipped in a solution of 70% ethanol for 3 min, 1.5% commercial bleach for 15 min followed by 0.7% commercial bleach for 5 min (Charbaji and Nabulsi, 1999). After sterilization, they were washed three times with sterile water and planted in tubes containing 20 ml DSD1 medium (Da Silva and Doazan, 1995). The DSD1 media contains 100 mg l^{-1} NH_4NO_3, 1000 mg l^{-1} KNO_3, 180 mg l^{-1} $MgSO_4$. $7H_2O$, 100 mg l^{-1} KH_2PO_4, 500 mg l^{-1} Ca $(NH_3)4H_2O$, 27.5 mg l^{-1} $MnSO_4$.7H2O, 37.5 mg l^{-1} Na_2 EDTA, 0.025 mg l^{-1} $CuSO_4$ $5H_2O$, 0.025 mg l^{-1} $CaCl_2.6H_2O$, 1 mg l^{-1} H_3BO_3, 1 mg l^{-1} $ZnSO_4$ $7H_2O$, 27.5 mg l^{-1} Fe SO_4 7H2O, 10 mg l^{-1} Myoinositol, 1 mg l^{-1} Acid Nicotinic, 1 mg l^{-1} Thiamine, and 1 mg l^{-1} Pyrodoxine. The pH of the medium was adjusted to 6.4 before adding agar and it was then autoclaved at 116°C for 25 min. The tubes were closed using cellophane paper and the edge of the tubes was sealed with parafilm to avoid contamination. All tubes were then incubated as described above.

Experimental design

Six-week-old grapevine plants were used to induced resistance; plantlets with two or three roots were selected. Due to the lack of phylloxera to infest the roots in the medium and to avoid the interaction between phylloxera and BTP_1, one root of each plants was pulled out of the medium but kept within the tube while the other root remained in the medium. The second root was treated with 1 ml of bacterial suspension (10^8 CFU ml^{-1}) of *P. putida* BTP1 on the root surface and inside the medium, or by distilled water for the control plantlets. The tubes were closed again as described above and incubated at 25°C under 16 h photoperiod. Seven days later, the second root was infested with sterile eggs of phylloxera according to Makee *et al*. (2003). Three-day-old eggs were taken from the colony and placed into 1.5 ml Eppendorf tubes for sterilization of the egg cuticle. One ml of formaldehyde (2.5%) was added to the eggs, gently shaken for 10 min, and left for 20 min. The sterilizing solution was then removed with a micro-pipette and the eggs were extracted and placed on sterile filter paper. The sterile eggs were gently transferred and spread on the non-inoculated roots of *in vitro* cultured plants by using a 10 ml sterile loop (Kendall, USA). For each rootstock, five treated and five untreated plantlets were infested with 25 surface-sterile phylloxera eggs. The tubes were resealed with parafilm to

prevent contamination and to avoid the escape of phylloxera crawlers, and were then incubated at 25±1°C under 16 hr photoperiod (140-150 µmol m^{-2} s^{-1}) from daylight fluorescent tubes (Philips TLD 38/54).

Evaluation procedure

Stereo microscope inspection was carried out daily on treated and untreated plantlets maintained in closed tubes to observe distribution of the eggs. The number of eggs hatched, feeding nymphs and adults were recorded to determine the mean developmental time (egg to egg) for each tested plant. Five random of root-feeding phylloxera females in each tube were inspected to determine the mean of oviposition period and the mean of fecundity (total number of eggs) of phylloxera. Thus, 25 females were examined on each plantlet. All eggs laid by each female were observed daily and counted till the female's death. Egg distribution during oviposition period (number of eggs per day), fecundity (total number of eggs) and female longevity were determined.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5 program at 5% level (P= 0.05). Data were subjected to analysis of variance (ANOVA) for the determination of differences in means between tested plants of each treatment. Differences between means were tested for significance using Tukey HSD test.

3. Results

Effect of P. putida BTP1 on grapevine resistance against phylloxera

Percentage of matured females

The result showed significant difference in phylloxera egg numbers that were able to hatch and develop to reach adult stage (matured females) on both rootstocks Ru140 and B41 (F= 79.6; df=3, 16; P<0.001) (Fig. 1). *P. putida* BTP1-treated plants emerged significantly percentage decreased of matured females in both rootstocks comparing to control plants. However, there was no significant difference in the percentage of emerged matured females between treated plants of B41 rootstock and plants of rootstock Ru140 no treated (Fig. 1). The percentage of matured females of phylloxera on treated B41 was significantly greater (33%) than that on treated Ru140 (16%) (Fig. 1).

Fecundity

There was a significant difference in the mean of fecundity between Ru140 and B41 rootstocks (F= 140.8; df=3.96; P<0.001) (Fig. 2), with it resulting greater (19.4 eggs) in Ru140 than in B41 (13.7 eggs). When plants were treated with BTP1, the mean number of eggs laid significantly decreased in both rootstocks, pointing to a significant decrease in the mean of fecundity of phylloxera in both rootstocks. However the decrease in this parameter was greater in treated Ru140 (5.4 eggs) than treated B41 rootstocks (9.9 eggs) (Fig. 2).

Oviposition period

A significant difference was observed in the mean oviposition period of phylloxera between Ru140 and

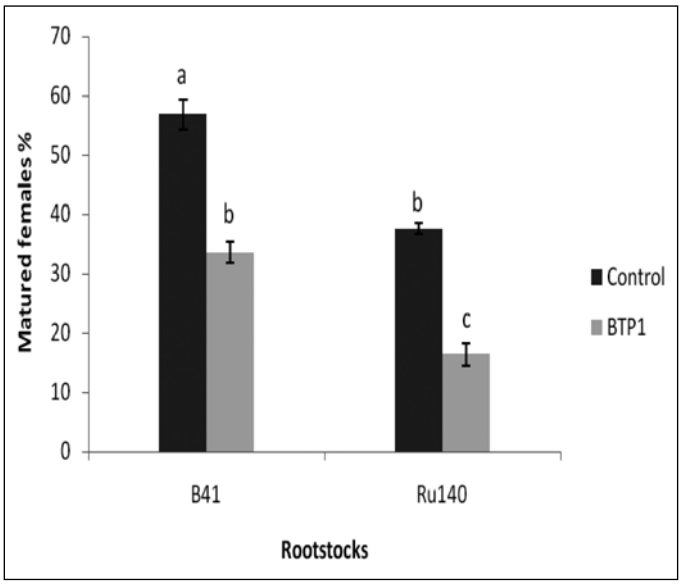

Fig. 1 - Effect of *P. putida* BTP1 on percentage of matured females of phylloxera *in vitro* in B41 and Ru140 rootstocks in comparison with control plants. Each column represents data from 25 samples. Data were subjected to ANOVA analysis and the differences between means were tested for significance using Tukey HSD test (values with different letters are significantly different at P<0.001).

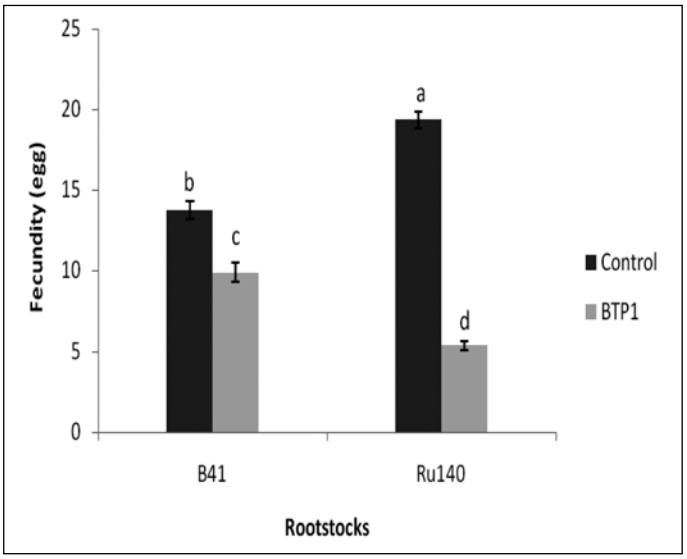

Fig. 2 - Effect of *P. putida* BTP1 on fecundity of phylloxera *in vitro* in B41 and Ru140 rootstocks in comparison with control plants. Each column represent data from 25 samples. Data were subjected to ANOVA analysis and the differences between means were tested for significance using Tukey HSD test (values with different letters are significantly different at P<0.001).

B41 rootstocks (F= 38; df=3.96; P<0.001) (Fig. 3). The oviposition period was 7 and 6 days in Ru140 and B41, respectively. However, when plants were treated with *P. putida* BTP1, the oviposition period decreased in a significant way only in Ru140. No significant differences were observed in the mean oviposition period between treated and untreated B41 plants (Fig. 3). The mean oviposition period of phylloxera on treated B41 was significant: lasting one day longer than treated Ru140 (6 and 5 days respectively) (Fig. 3).

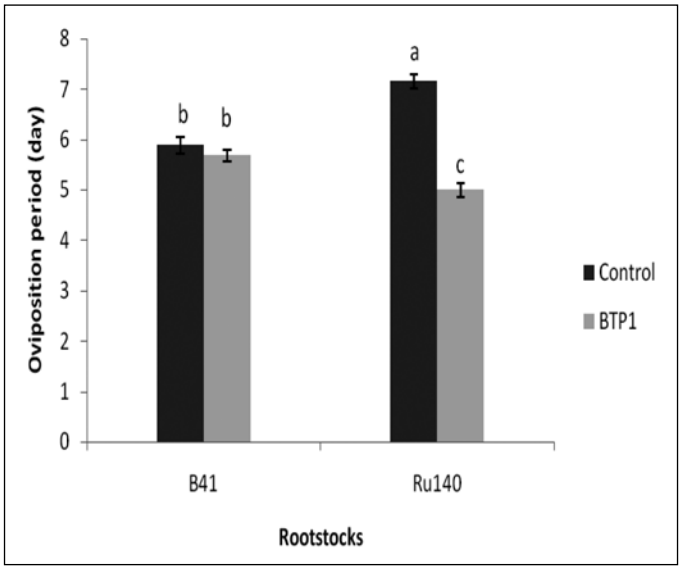

Fig. 3 - Effect of *P. putida* BTP1 on oviposition period of phylloxera *in vitro* in B41 and Ru140 rootstocks in comparison with control plants. Each column represent data from 25 samples. Data were subjected to ANOVA analysis and the differences between means were tested for significance using Tukey HSD test (values with different letters are significantly different at P<0.001).

4. Discussion and Conclusions

A recent study carried out on healthy pieces of roots of local grapevine cultivar Helwani showed the influence of non-pathogenic *P. putida* BTP1 on reproduction and development of grapevine phylloxera (Adam *et al.*, 2012). However, in the present work, the aim was to investigate the ability of this bacteria to induce systemic resistance in two grapevine rootstocks against phylloxera by using *in vitro* cultured plants. For our module this approach provided a strict separation condition between the inducer (bacteria) and the pathogen or pest (phylloxera) to induce systemic resistance (Ongena *et al.*, 2002; Bakker *et al.*, 2007). In agreement with a previous study (Ongena *et al.*, 2002), the bacteria did not migrate through the plants, suggesting the observed decrease in the life cycle of phylloxera was due to induction of systemic resistance in the host plant.

The present study confirmed that *P. putida* BTP1 had a protective effect on Ru140 and B41 rootstocks against phylloxera. The means of fecundity and oviposition period and emerged mature female percentage decreased significantly in both BTP1-treated rootstocks in comparison with control plants. These results are consistent with similar previous studies that demonstrated the ability of some strains of PGPR to induce systemic resistance in tomato against whitefly, where the percentage of matured females decreased in treated plants (Hanafi *et al.*, 2007; Valenzuela-Soto *et al.*, 2010). In addition, similar results were reported when cucumber beetles and American bollworm fed on PGPR-treated cucumber plants and cotton bolls, respectively (Zehnder *et al.*, 1997) (a, b; Vijayasamundeeswari *et al.*, 2009). Other studies also indicated that changes in the feeding behavior of Leaffolder and decreases in the weight of larvae and pupae were observed in rice plants treated with rhizobacteria (Radjacommare, 2002).

On the other hand, our results showed that there was a significant difference in reproduction and development of grapevine phylloxera between BTP1-treated B41 and Ru140 rootstocks. In comparison, the percentage of matured females and the means of fecundity and oviposition period decreased significantly by up to 50%, 45% and 12% respectively in treated Ru140 rootstock versus treated B41 rootstock (Figs. 1, 2 and 3). This is consistent with results of previous studies indicating the presence of a type of gradient from the resistant plant to sensitive plant (Granett *et al.*, 1983; Makee *et al.*, 2010). These results show that phylloxera laid a large number of eggs on sensitive varieties, more than on resistant varieties. It is believed that poor nutrition or the inability to colonize good locations for feeding could directly affect the number of eggs and ultimately the ratio of hatching. Thus, the resistance of grapevine to phylloxera could be a reflection of the strong relationship between poor nutrition and a decline in the productivity of the insect (Granett *et al.*, 1983). In addition, the mechanism of defense in these rootstocks may be due to toxic effects against phylloxera, such as the accumulation of some phenolic compounds in the cells of resistant plants leading to an increase in the death rate (Omer *et al.*, 1999). Other workers illustrated that there is a positive relationship between resistance/susceptibility characteristics against aphids and flavonoid glycoside content (Quercetin and Isorhamentin) of cowpea lines as these compounds possess a good inhibitory rate for aphid reproduction (Lattanzio *et al.*, 2000). Therefore, the resistance of Ru140 and B41 rootstocks to phylloxera may be attributed to an ability to produce such toxic phenolic compounds.

In conclusion, understanding the mechanisms of defense induced by some strains of PGPR in plants is very important to develop systemic resistance in plants. The current study provides evidence that *P. putida* strain BTP1 has the ability to stimulate a systemic resistance in grapevine rootstocks against phylloxera. We suggest that *P. putida* BTP1 treatment leads to an alteration in the plant's metabolic pathway eliciting the induction of plant defense compounds. These substances would have a negative influence on phylloxera feeding and development in treated plants. However, more research in the field must be done before implementing this technique on a large scale.

To our knowledge, this work is the first study interested in biocontrol of phylloxera in grapevine by PGPR strains *in vitro*. Furthermore, this investigation supplies important information about the possibility of implementing this strain to stimulate systemic resistance against plant pests. Moreover, this study illustrates the effectiveness of using *in vitro* dual culture in evaluating the phylloxera/grapevine and grapevine/rhizobacteria interactions. In fact, this testing system could be considered a very promising tool to: I) examine the phylloxera resistance of newly developed rootstocks; II) prevent the spread of phylloxera; III) study phylloxera genetic variation, biology and control method; and IV) study the mechanisms of defense induced in plants by rhizobacteria against pests.

Acknowledgements

The authors thank Prof. I. Othman (Atomic Energy Commission of Syria) and Dr. N. Mirali (Department of Biotechnology) for their help. We thank Prof. P. Thonart and Dr. M. Ongena of the University of Liège, who provided us with the *P. putida* BTP1 strain.

References

ADAM A., MAKEE H., IDRIS I., 2012 - *The influence of a non-pathogenic* Pseudomonas putida *strain BTP1 on reproduction and development of grape phylloxera*. - Adv. Hort. Sci., 26(2): 75-80.

ADAM A., ONGENA M., DUBY F., DOMMES J., THONART P., 2008 - *Systemic resistance and lipoxygenase-related defence response induced in tomato by* Pseudomonas putida *strain BTP1*. - BMC Plant Biology, 8: 113.

BAKKER P.A.H.M., PIETERSE C.M.J., VAN LOON L.C., 2007 - *Induced systemic resistance by fluorescent* Pseudomonas *spp.* - Phytopathology, 97: 239-243.

CHARBAJI T., NABULSI I., 1999 - *Effect of low doses of gamma irradiation on* in vitro *growth gravepine*. - Plant Cell, Tissue and Organ Culture, 57: 129-132.

DA SILVA A.L., DOAZAN J.P., 1995 - *Une méthode d'irrigation aux rayon gamma appliquée a des porte-greffes de vignes* in vitro. - J. Int. Sci. Vigne Vin, 29: 1-9.

DE BENEDICTIS J., GRANETT J., 1993 - *Laboratory evaluation of grape roots as host of California grape phylloxera biotypes*. - Am. J. Enol. Vitic., 44: 285-291.

DURRANT W.E., DONG X., 2004 - *Systemic acquired resistance*. - Annu. Rev. Phytopathol., 42: 185-209.

FORNECK A., WALKER M.A., MERKT N., 1996 - *Aseptic dual culture of grape (*Vitis *spp.) and grape phylloxera (*Daktulosphaira vitifoliae *Fitch).* - Vitis, 35: 95-97.

GRANETT J., BISABRI-ERSHADI B., CAREY J., 1983 - *Life tables of phylloxera on resistant and susceptible grape rootstocks*. - Ent. Exp. & Appl., 34: 13-19.

GRANETT J., WALKER M., KOCSIS L., OMER A., 2001 - *Biology and management of grape phylloxera*. - Annual Review Entomology, 46: 387-412.

HANAFI A., TRAORÉ M., SCHNITZLER W., WOITKE M., 2007 - *Induced resistance of tomato to whiteflies and* Pythium *with the PGPR* Bacillus subtilis *in a soilless crop grown under greenhouse conditions*. - Acta Horticulturae, 747: 315-322.

JACQUES P., ONGENA M., GWOSE I., SEINSCHE D., SCHRODER H., DELFOSSE P., THONART P., TARAZ K., BUDZIKIEWICZ H., 1995 - *Structure and characterization of isopyoverdin from* Pseudomonas putida *BTP1 and its relation to the biogenetic pathway leading to pyoverdines*. - Z. Naturforsch., 50: 622-629.

KLOEPPER J.W., RYU C.M., ZHANG S.A., 2004 - *Induced systemic resistance and promotion of plant growth by* Bacillus *spp.* - Phytopathology, 94: 1259-1266.

LATTANZIO V., ARPAIA S., CARDINALI A., VENERE D.D., LINSALATA V., 2000 - *Role of endogenous flavonoids in resistance mechanism of vigna to Aphids*. - J. Agric. Food Chem., 48: 5316-5320.

MAKEE H., AMMOUNHA H., IDRIS I., 2010 - *Development and reproduction of phylloxera on some local grapevines in Syria*. - Adv. Hort. Sci., 24(3): 169-175.

MAKEE H., CHARBAJI T., AYYOUBI Z., IDRIS I., 2003 - *Evaluating resistance of some rootstocks to grape phylloxera with an* in vitro *and excised root testing systems*. - In vitro Cell. Dev. Biol.-Plant, 40: 225-229.

OMER A.D., GRANETT J., SHEBELUT C.W., 1999 - *Effect of attack intensity on host utilization in grape phylloxera*. - Crop Protection, 18: 341-347.

ONGENA M., DAAYF F., JACQUES P., THONART P., BENHAMOU N., PAULITZ T.C., CORNELIS P., KOEDAM N., BÉLANGER R.R., 1999 - *Protection of cucumber against Pythium root rot by fluorescent pseudomonads: predominant role of induced resistance over siderophores and antibiosis*. - Plant Pathol., 48: 66-76.

ONGENA M., DUBY F., ROSSIGNOL F., FAUCONNIER M.L., DOMMES J., THONART P., 2004 - *Stimulation of the lipoxygenase pathway is associated with systemic resistance induced in bean by a nonpathogenic* Pseudomonas *strain*. - Mol. Plant Microbe Interact., 17: 1009-1018.

ONGENA M., GIGER A., JACQUES P., DOMMES J., THONART P., 2002 - *Study of bacterial determinants involved in the induction of systemic resistance in bean by* Pseudomonas putida *BTP1*. - Eur. J. Plant Pathol., 108: 187-196.

PIETERSE C.M.J., VAN WEES S.C.M., TON J., VAN PELT J.A., VAN LOON L.C., 2002 - *Signaling in rhizobacteria-induced systemic resistance in* Arabidopsis thaliana. - Plant Biol., 4: 535-544.

RADJACOMMARE R., NANDAKUMAR R., KANDAN A., SURESH S., BHARATHI M., RAGUCHANDER T., SAMIYAPPAN R., , 2002 - Pseudomonas *fluorescens based bioformulation for the management of sheath blight and leaffolder in rice*. - Crop Prot., 21: 671-677.

RAMAMOORTHY V., VISWANATHAN R., RAGUCHANDER T., PRAKASAM V., SAMIYAPPAN R., 2001 - *Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases*. - Crop Prot., 20: 1-11.

SONG G.C., GRANETT J., 1990 - *Grape phylloxera (Homoptera: Phylloxeridae) biotypes in France*. - J. Econ. Entomol., 83: 489-493.

STATISTICS OF SYRIAN AGRICULTURE MINISTRY, 2011 - *Area, Production and Number of Grapes Trees by Gover-*

norate for 2011 and their Development at the Country Level during (2002-2011). - Syrian Agricolture Ministry.

VALENZUELA-SOTO J.H., ESTRADA-HERNANDEZ M.G., IBARRA-LACLETTE E., DELANO-FRIER J.P., 2010 - *Inoculation of tomato plants (*Solanum lycopersicum*) with growth-promoting* Bacillus subtilis *retards whitefly* Bemisia tabaci *development*. - Planta, 231: 397-410.

VAN LOON L.C., BAKKER P., PIETERSE C.M.J., 1998 - *Systemic resistance induced by rhizosphere bacteria*. - Annu. Rev. Phytopathol., 36: 453-483.

VERHAGEN B.W.M., TROTEL-AZIZ P., COUDERCHET M., HÖFTE M., AZIZ A., 2010 - Pseudomonas *spp.-induced systemic resistance to* Botrytis cinerea *is associated with induction and priming of defence responses in grapevine*. - Journal of Experimental Botany, 61: 249-260.

VIDART M.V., MUJICA M.V., BAO L., DUARTE F., BENTANCOURT C.M., FRANCO J., SCATONI I.B., 2013 - *Life history and assessment of grapevine phylloxera leaf galling incidence on* Vitis *species in Uruguay*. - SpringerPlus, 2: 181.

VIJAYASAMUNDEESWARI A., LADHALAKSHMI D., SANKARALINGAM A., SAMIYAPPAN R., 2009 - *Plant growth promoting rhizobacteria of cotton affecting the developmental stages of* Helicoverpa armigera. - J. Plant Res., 49: 239-243.

WELLER D.M., MAVRODI D.V., VAN PELT J.A., PIETERSE C.M.J., VAN LOON L.C., BAKKER P.A.H.M., 2012 - *Induced systemic resistance in* arabidopsis thaliana *against* Pseudomonas syringae *pv. tomato by 2,4-diacetylphloroglucinol-producing* Pseudomonas fluorescens. - Phytopathology, 102: 403-412.

ZEHNDER G., KLOEPPER J., TUZUN S., YAO C., WEI G., CHAMBLISS O., SHELBY R., 1997 b - *Insect feeding on cucumber mediated by rhizobacteria-induced plant resistance*. - Entomologia Experimentalis et Applicata, 83: 81-85.

ZEHNDER G., KLOEPPER J., YAO C., WEI G., 1997 a - *Induction of systemic resistance in cucumber against cucumber beetles (Coleoptera: Chrysomelidae) by plant growth-promoting rhizobacteria.* - J. Econ. Entomol., 90: 391-396.

ZEHNDER G.W., MURPHY J.F., SIKORA E.J., KLOEPPER J.W., 2001 - *Application of rhizobacteria for induced resistance*. - Eur. J. Plant Pathol., 107: 39-50.

Adv. Hort. Sci., 2013 27(4): 143-146

Application of anatase nanoparticles (TiO_2) on strawberry seed germination (*Fragaria ananassa* L.)

H.R. Hosseini*, M. Chehrazi*, E.H. Dehkourdi*, M. Hosseini**

* *Department of Horticultural Sciences, Faculty of Agricultural Sciences, University of Shadid Chamran Ahvaz, Khuzestan, Iran.*

** *Department of Management Sciences, University of Vali Asr, Kerman, Iran.*

Key words: Anatase, germination, nano, strawberry,TiO_2.

Abstract: Priming enhances germination, establishment and yields in a range of crops in many diverse environments. This experiment evaluated the effects of soaking strawberry seeds in different concentrations (0, 3.5, 5.5, 7.5 and 9.5 percentage) of nano anatase on germination parameters (germination percentage, germination rate index, radicle and plumule length, fresh weight of seedlings and vigor index) using a factorial design with eight replications. Results showed that an increase in the concentration of nano anatase led to significant differences in the percentage of germination, germination rate index, root and shoot length, fresh weight and vigor index of seedlings. The best nano anatase concentration was found to be 7.5%.

1. Introduction

Strawberry (*Fragaria x ananassa* Duch.), a small fruit crop and a hybrid of two highly variable octoploid species, has adapted to extremely different environmental conditions (Rieger, 2005). Prerequisites for successful strawberry growing are suitable climate, cultivars, soil and nutrition (Almaliotis *et al*., 2002). Seed priming enhances seed performance by rapid and uniform germination in normal and vigorous seedlings, leading to faster and better germination in different crops (Cantliffe, 2003). Priming is responsible for the repair of age-related cellular and sub cellular damage of low vigour seeds that may accumulate during seed development. Priming of seed promotes germination by repairing the damaged proteins, RNA and DNA (Koehler *et al*., 1997).

Several treatments have been used with the aim of improving strawberry achene (seed) germination: humidity (Guttridge and Bright, 1978); exposure to red light (Iyer *et al*., 1975); and osmotic pre-treatment (Hanke, 1993).

With the rapid growth of nanotechnology, there are increasing concerns about the potential adverse impact of engineered nanoparticles (ENPs) in the environment. However, our understanding of how ENPs may affect organisms within natural ecosystems lags far behind our rapidly increasing ability to engineer novel nanomaterials (Bernhardt *et al*., 2010). On the other hand, Lin and Xing (2007) studied the positive effects of suspensions of nanoparticles on seed germination and root growth of six different crop species [radish (*Raphanus sativus*), rape (*Brassica napus*), rye grass (*Lolium perenne*), lettuce (*Lactuca sativa*), corn (*Zea mays*) and cucumber (*Cucumis sativus*)]. Also, the effects of nano-TiO_2 (rutile) and non-nano-TiO_2 on the germination and growth of naturally aged spinach seeds were studied and an increase of these factors was observed at 0.25-4‰ nano-TiO_2 treatment (Zheng *et al*., 2005). Feizi *et al*. (2011) reported that nano-TiO_2 at suitable concentration could promote the seed germination of wheat in comparison to bulk TiO_2 while at high concentrations it had an inhibitory or no effect on the crop.

Limited studies have been done on the effects of nanoparticles on crops and thus, we decided to investigate the phytotoxicity or positive effects of different concentrations of nano-TiO_2 on seed germination and seedling growth of strawberry.

2. Materials and Methods

In order to evaluate the effect of nano priming on the quality of seedling production during germination in strawberry (*Fragaria ananassa* L.), this experiment was conducted in 2013 at Shahid Chamran University Ahvaz in Iran using a factorial design with eight replications. Seeds of strawberry (*Fragaria ananassa* cv. Queen Eliza) were from the Gene Bank of Iran, at Seed and Plant Improvement Institute, Tehran (Karaj). The factors studied included different concentrations of TiO_2 (Control, 3.5, 5.5, 7.5 and 9.55%) and time (24 and 48 h). In order to prepare nano anatase solutions a stock solution with the highest

Received for publication 10 December 2013
Accepted for publication 14 January 2014

concentration (9.5%) was fixed and then with dilution of this stock solution, various concentrations of nano anatase were obtained. Strawberry seeds were sterilized using sodium hypochlorite solution (1%) for 10 min, then washed several times with distilled water, and soaked in nano-TiO_2 solutions at different concentrations. Seeds were placed in disinfected Petri dishes, each dish contained 100 seeds. All of the Petri dishes were irrigated using distilled water. Seeds were allowed to germinate at 25±3°C for 14 days. Every day the number of seeds with visible radicle were counted and recorded as sprouted seeds; the length of seedling shoot and root were also measured.

Germination rate [Eq. (1)] and germination percentage [Eq. (2)] were calculated using the following formula (Hosseini *et al*., 2013):

$$GR = \frac{\sum n}{\sum dn} \quad (1)$$

Where GR is the germination rate, Σn is the number of seeds germinated on the day, and Σdn is the number of days from the start of experiment.

$$GP = \frac{\sum n}{N} \times 100 \quad (2)$$

Where GP is the germination percent, Σn is the number of seeds germinated until the last day of experiments, and N is the total number of seeds.

Seed vigor indices [Eq. (3)] were calculated according to the following formula (Hosseini *et al*., 2013):

$$VI = (RL+PL) \times GP \quad (3)$$

Where VI is the Seed Vigor Index, RLis radicle length, PL is the plume length, and GP the germination percentage.

Statistical analysis

The data were analyzed using SAS 9.1 software. The significant levels of difference for all measured traits were calculated, and the means were compared by the multiple-range Duncan test at 1% level.

3. Results and Discussion

Analysis of variance showed that treatment of nano anatase led to significant differences in germination percentage, germination rate, root and shoot length, allometric index and vigor index of the seedlings. Time treatment was not significant for root, shoot, seedling length and seedling allometric coefficient. Interaction of anatase nanoparticles and time treatment was not significant for root and shoot length and seedling allometric coefficient (Table 1-3).

The interaction of anatase nanoparticles and time showed the lowest percentage of germination in the treat-

Table 1 Analysis of variance of strawberry seed germination indices affected by nano anatase

Sources of variation	df	Percentage of germination	Germination rate	Shoot length	Root length	Seedling length	Allometric	VI
Nano-TiO_2	4	2260.6 *	0.184 *	11.077 *	15.366 *	0.525 *	3.33 *	103028.5 *
Time	1	136.9 *	1.089 *	0.196 ns	0. 016 ns	0.0022 ns	0.049 ns	5712.1 *
Nano*time	4	76.9 *	0.078 *	0.179 ns	0.0053 ns	0.001 ns	0.087 ns	3868.1 *
Error	30	13.7	0.180	0.148	0.145	0.004	0.069	578.23

*= significant at 1% level.
ns= non-significant.

Table 2 - Effect of nano anatase on seed germination of strawberry after 24 hours

Concentration	Germination (%)	Germination rate	Shoot length (cm)	Root length (cm)	Fresh weight (g)
0 %	10.97 e	0.60 d	1.78 e	2.0 d	0.012 d
3.5 %	40.10 d	0.60 c	8.50 d	4.5 c	0.021 c
5.5 %	68.80 ab	0.67 b	9.10 c	4.4 c	0.029 b
7.5 %	92.46 a	1.20 a	12.24 a	6.0 a	0.034 a
9.5 %	74.64 b	1.00 ab	10.00 b	4.8 b	0.030 b

Means with different letters at each column are statistically different at 1% level.

Table 3 - Effect of nano anatase on seed germination of strawberry after 48 hours

Concentration	Germination (%)	Germination rate	Shoot length (cm)	Root length (cm)	Fresh weight (g)
0 %	0 e	0 e	0 e	0 d	0 d
3.5 %	23.2 d	0.17 d	3.5 d	2.5 c	0.011 c
5.5 %	40.6 b	0.26 b	5.3 c	2.9 c	0.017 b
7.5 %	87.6 a	0.56 a	10.3 a	5 a	0.030 a
9.5 %	46.3 bc	0.35ab	7.1 b	3.3 b	0.025 b

Means with different letters at each column are statistically different at 1% level.

ment without nanoparticles and the highest at a concentration of 7.5% of nanoparticles for 48 h (Fig. 1).

Zheng *et al*. (2007) reported that the significant effect of nano-sized TiO_2 on germination is probably due to the small particle size which allows penetration into the seed during the treatment period, exerting its enhancing functions during growth.

Results showed that the lowest germination rate resulted from treatment without nanoparticles while the highest rates were obtained at a concentration of 7.5% of nanoparticles in 24 and 48 h. The key to increase seed geermination is the penetration of nanomaterial into the seed (Hashemi and Mousavi, 2013) (Fig. 2).

Khodakovskaya *et al*. (2009) mentioned that the carbon nanotubes can effectively penetrate through the seed coat, thus influencing seed germination. Exposure of tomato seeds to carbon nanotubes (CNTs) resulted in enhanced seed germination and growth rate.

Among the different nanoparticle TiO_2 concentrations, 7.5% for both times tested (24 and 48 h) showed the maximum seedling vigor index while the control showed the lowest (Fig. 3).

Effects positive of nano anatase are reported that nano-TiO_2 (anatase) improved plant growth by enhanced nitrogen metabolism (Yang, 2006) that promotes the absorption of nitrate in spinach and, accelerating conversion of inorganic nitrogen into organic nitrogen, thereby increasing the fresh weights and dry weights. Studies also showed the effects of nitrogen photoreduction on the improved growth of treated spinach plant (Yang, 2007). Effects of nano-TiO_2 (anatase) on the content of light harvesting complex II (LHC II) on thylakoid membranes of spinach was studied and it showed an increase in LHC II content (Mingyu, 2007 a). These promote energy transfer and oxygen evolution in photosystem II (PS II) of spinach (Hong, 2005).

It has also been found that nano anatase TiO_2 promoted antioxidant stress by decreasing the accumulation of superoxide radicals, hydrogen peroxide, malonyldialdehyde content and enhance the activities of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase and thereby increase the evolution oxygen rate in spinach chloroplasts under UVB radiation (Mingyu, 2007 b).

The results indicated that nano sized TiO_2 in an appropriate concentration could promote the seed germination and seedling growth of strawberry.

The results show that with increasing concentration of nano anatase to 7.5% increased germination parameters. The seedlings grown with nano anatase increased length when compare to the control seedlings. Effect of nano anatase on root, shoot and seedling strawberry may be due to early emergence induced by nano anatase treatment as compared to control seeds. Rapid embryo growth resulted when the obstacle to germination was removed.

Although the concentration of 9.5% caused lower germination parameters toward concentration of 7.5%, showed more desirable effects of the concentration of 5.5% nano anatase and control.

In all treatments, the better result was obtained in time of 48 hour.

In order to understand the possible benefits of applying nanomaterials in agriculture, it is important to analyze penetration and transport of nanoparticles in the plants.

References

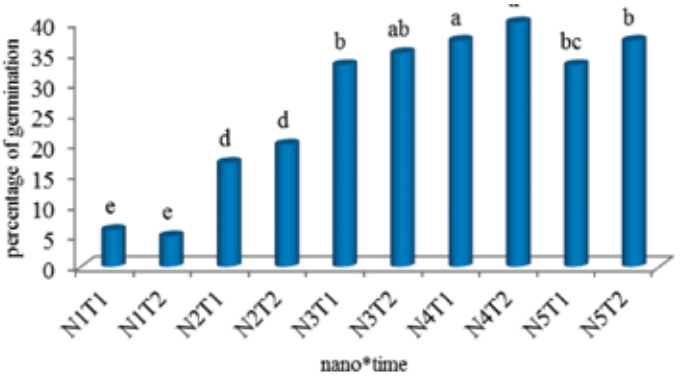

Fig. 1 - Effect of interaction nano anatase and time on germination percentage of strawberry seeds.

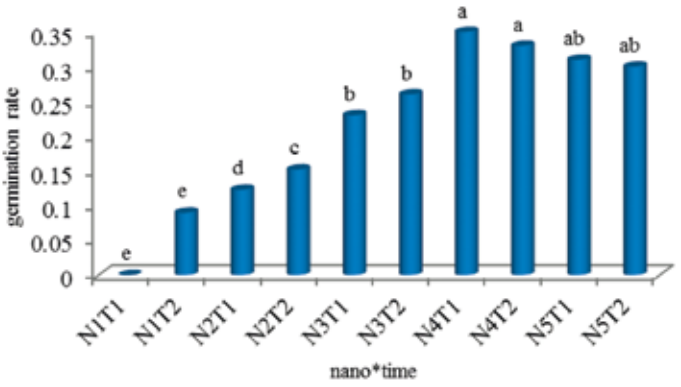

Fig. 2 - Effect of interaction nano anatase and time on germination rate of strawberry seeds.

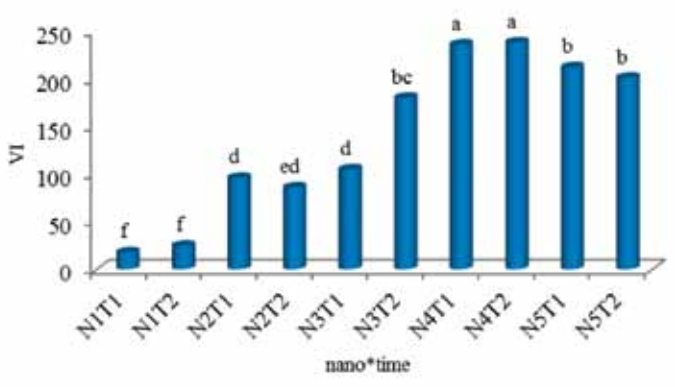

Fig. 3 - Effect of interaction nano anatase and time on vigor index of strawberry seeds.

ALMALIOTIS D., VELMIS D., BLADENOPOULOU S., KARAPETSAS N., 2002 - *Leaf nutrient levels of strawberries (cv. tudla) in relation to crop yield*. - Acta Horticulturae, 567: 447-450.

BERNHARDT E., COLMAN B., HOCHELLA M., CARDINALE B., NISBET R., 2010 - *An ecological perspective on nanomaterial impacts in the environment*. - Journal of Environmental Quality, 39: 1954-1965.

CANTLIFFE D.J., 2003 - *Seed enhancements*. - Acta Horticulturae, 607: 53-59.

FEIZI H., RAZAVI P., SHAHTAHMASEB N., FOTOVAT A., 2011 - *Impact of bulk and nanosized Titanium Dioxide (TiO_2) on wheat seed germination and seedling growth*. - Biol. Trace Elem. Res., 146: 101-106.

GUTTRIDGE C., BRIGHT S., 1978 - *Accelerating and synchronizing germination of strawberry seeds by osmotic pretreatments*. - Euphytica, 3: 843-848.

HANKE V., 1993 - *Untersuchungen zur Keimung von Achänen bei Erdbeere*. - Erwerbsobstbau, 35(4): 105-109.

HASHEMI E., MOUSAVI M., 2013 - *Effect of anatase nanoparticles (TiO_2) on parsely seed germination (*Petroselium crispum*)* in vitro. - Biol. Trace Elem. Res., 155: 283-286.

HONG F., 2005. - *Effect of nano TiO_2 on spectral characterization of photosystem particles from spinach*. - Chem. Res. Chin. Univ., 21: 196-200.

HOSSEINI H.R., CHEHRAZI M., NABATI AHMADI D., MAHMOODI SORESTANI M., 2013 - *Study the effects of colchicine treatment on generation of autopolyploidy in* Catharanthus roseus *Cvs. rosea and alba.* - Msc. Tesis Shahid Chamran University of Ahvaz, Iran.

IYER A., SUBRAMANYAM D., SINGH R., 1975 - *Improving seed germination with mist*. - Curr. Sci., 44: 895-896.

KHODAKOVSKAYA M., DERVISHI E., MOHAMMAD M., XU Y., LI Z., WATANABE F., BIRIS A., 2009 - *Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth*. - Acs Nano, 3: 3221-3227.

KOEHLER K., VOIGET B., SPITLLE H., SCHELENZ M., 1997 - *Biochemical events after priming and priming of seeds*, pp. 531-536. - In: ELLIS R.H., M. BLACK, A.J. MURDOCH, and T.D. HONG (eds.) *Basic and applied aspects of seed biology.* Proc. 5th Int. Workshop on Seeds, Reading, UK.

LIN D., XING B., 2007 - *Phytotoxicity of nanoparticles: inhibition of seed germination and root growth.* - Environ. Pollut., 150: 243-250.

MINGYU S., 2007 a - *Effects of nanoanatase TiO_2 on absorption, distribution of light and photoreduction activities of chloroplast membrane of spinach.* - Biol. Trace Elem. Res., 118: 120-130.

MINGYU S., 2007 b - *Promotion of energy transfer and oxygen evolution in spinach photosystem II by nanoanatase TiO2*. - Biol. Trace Elem. Res., 119: 183-192.

RIEGER M., 2005 - *Strawberry (*Fragaria x ananassa*)*, pp. 383-392. - In: RIEGER M. (eds.) *Introduction to fruit crops.* Haworth Food & Agricultural Products Press, New York, pp. 462.

YANG F., 2006 - *Influences of nanoanatase TiO_2 on the nitrogen metabolism of growing spinach*. - Biol. Trace Elem. Res., 110: 179-190.

YANG F., 2007 - *The improvement of spinach growth by nanoanatase TiO_2 treatment is related to nitrogen photoreduction*. - Biol. Trace Elem. Res., 119: 77-88.

ZHENG L., HONG F., LU S., LIU C., 2005 - *Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach*. - Biology Trace Elemental Reserch, 105: 839-843.

ZHENG l., MINGUY S., XIAO W., CHAO L., CHUNXIANG Q., LIANG C., HUNG H., XIAOQING L., HONG F., 2007 - *Effect of nano anatase on spectral characteristics and distribution of LHCLL on the thylakoid memberance of spinach*. - Biol Trace Elem Res., 120: 273-280.

Adv. Hort. Sci., 2013 27(4): 147-151

Impact of integrated nutrient management on growth, yield and quality of strawberry (*Fragaria* x *annanassa* Duch.) cultivation in India

Rayees A. Wani*[(1)], Sunam Sheema, T.H. Malik**, Seerat Geelani*, Sabiya Bashir*, N.A. Dar*, V. M. Prasad*****

* *Department of Horticulture, Sam Higginbottom Institute of Agriculture Technology and Science, Allahabad, U.P. 211007, India.*

** *Department of Agriculture Jammu, Jammu and Kashmir, India.*

*** *Department of Horticulture SHIATS Allahabad.*

[(1)] *Present address: Dry land Agriculture research station Budgam, SKUAST-K Srinagar, Kashmir India.*

Key words: inorganic fertilizers, integrated nutrient management, organic fertilizers, strawberry.

Abstract: Modern agricultural practices are mostly directed toward high application of commercial fertilizers to achieve high yield. It is widely recognized that application of fertilizer (especially nitrogen) can cause ground water pollution by nitrate leaching through the soil profile. A new approach to farming is often referred to as sustainable agriculture and it seeks to introduce friendlier agricultural practices to the environment and maintains the long term ecological balance of the soil ecosystem. Hence investigations were carried out to develop nutrient management for strawberry cultivar Sweet Charley subjected to various treatment combinations of organic and inorganic fertilizers. Traits such as plant growth characteristics (leaves/plant and plant spread), yield characteristics (flower buds, fruits per plant and fruit yield tons/ha) and quality characteristics (juice content, total sugar content, vitamin C and specific gravity) were observed. The runners of strawberry cv. Sweet Charley were planted in the first week of November with a spacing of 30 x 60 cm. The investigation was laid out in a randomized block design with five treatment combinations replicated thrice. The data regarding the different growth parameters observed at 30, 45, 60, 90, 105, 120, yield parameters at 45, 60, 90, 120, 135, 150 days after planting and their quality parameters clearly indicate that the application of integrated sources of nutrients significantly affect the vegetative, reproductive and yield characteristics of the strawberry plant. However the manure fertilizer combination under treatment T_4 (75% Organic Fertilizers + 25% inorganic Fertilizers) was found to be the best treatment with regard to integrated and combined application of nutrient resources for strawberry cultivation in India.

1. Introduction

The Strawberry (*Fragaria X annanassa* Duch.), a member of the rose family, is not really a berry but a false fruit and consists of many tiny individual fruits embedded in a fleshy scarlet receptacle. The brownish or whitish specks, commonly considered seeds, are the true fruits known as achenes. Strawberries are an excellent source of vitamin C, a good source of folate and potassium, and are relatively low in calories. Strawberry is one of the most widely appreciated fruits and it has attained a premier position in the fresh fruit market and processing industries of the world (Sharma and Sharma, 2003). Initially grown in temperate zones of India, its cultivation has now become possible in the sub-tropical zones as well with the introduction of day neutral cultivars (Asrey and Singh, 2004). Strawberry offers quicker returns on capital outlay than any other fruit crop since under special methods of cultivation a crop can be picked as early as the first summer after planting. The fruit is the first of the home-grown supplies to reach the market. Strawberry is one of the most important high value cash crops around the world. In India, it is cultivated commercially in Hamachal Pradesh, Utter Pradesh, Maharashtra, West Bengal, Nilgiri Hills, Delhi, Haryana, Punjab, Rajasthan and Jammu and Kashmir. Its cultivation can be extended successfully to other suitable areas of the world where irrigation and transportation facilities can be assured. Strawberry is an attractive, luscious, tasty and nutritious fruit with a distinct and pleasant aroma and flavor. It has a unique place among cultivated berry fruits in world. Rich in vitamin C and iron, the principal demand for cultivated strawberry is from the processing and baking industries around the world as suggested by Childs (1937) that the strawberry is a delectable fruit that is highly prized by almost everyone.

Received for publication 16 November 2013
Accepted for publication 30 December 2013

Among the various factors which contribute towards the growth, yield and quality of strawberry, nutrition is the most important and it has direct bearing on crop production (Umar *et al.,* 2008). Integrated nutrient management includes the use of inorganic and organic sources of nutrients to ensure balanced nutrient proportions by enhancing nutrient response efficiency and maximizing crop productivity of desired quality. It also helps to minimize the existing gap between nutrient removal through continuous use of chemical fertilizers and supply through slow release of fertilizers. It is widely reported that the extensive use of chemical fertilizers adversely affects soil health and results in decreased crop productivity and quality (Macit *et al.,* 2007). Even with the application of recommended doses, yield potential of strawberry cropping systems has reduced to a plateau because soil health has deteriorated drastically and especially its organic matter content has depleted significantly. As intensive agriculture is becoming more and more necessary to meet the needs of the population, the soil nutrient balance is becoming increasingly negative and thus requiring appropriate supplement through integrated nutrient management. The use of organic and inorganic nutrient sources not only helps to increase crop yields but also helps to storehouse nutrients for successive crops in addition to improving the physical condition of the soil. Bio-organic nutrition also improves the yield and quality of product. Organically produced fruit fetches higher prices compared to products grown using inorganic fertilization. The practical approach will be organic farming to the most responsive fruit crops and to encourage integrated nutrient supply for tapping the potential yield of crops. As reported by Hennion *et al.* (1999), "strawberry cultivation is highly nutrition responsive" and therefore the present experiment was undertaken to manage the growth, yield and quality of strawberry through an integrated approach.

2. Materials and Methods

The present investigation was carried out at experimental fields of the horticulture department SHIATS, Allahabad during spring season 2009-10. The experimental field is located at an elevation of 78 m above sea level and latitude 81.15° E and longitude 28.87 ° N. The soil of the experimental field was sandy loam in texture, poor in nitrogen (0.24%), comparatively richer in potassium (0.57%) and phosphorus (0.62%) and slightly acidic (pH=6.60) in nature.

The strawberry (cv. Sweet Charley) was procured from Wimco seedling Ltd, Baghwale Rudrapur (U.S.N) Uttar Pradesh, India. The experiment was comprised of the following five treatment combinations:

1. 100% recommended dose of inorganic fertilizers +0% recommended dose of manures (T_1);
2. 5% recommended dose of inorganic fertilizers + 25% recommended dose of manures (T_2);
3. 50% recommended dose of inorganic fertilizers + 50% recommended dose of manures (T_3);
4. 25% recommended dose of inorganic fertilizers +75% recommended dose of manures (T_4);
5. 0% Recommended dose of inorganic fertilizers + 100% recommended dose of manures (T_5).

The manures used in this experiment were farmyard manure and forest litter. The experiment was laid out in a Randomized Block Design with four replications. The observations recorded were on growth parameters (leaves/plant and plant spread), yield characteristics (flower buds, fruits per plant and fruit yield tons/ha) and quality characteristics (juice content, total sugar content, total soluble solids, vitamin C, pH and specific gravity).

3. Results and Discussion

All the treatments in the present investigation had significant impact for all observed traits. However, treatments differed significantly from one another at various time intervals (Tables 1 and 2; figures 1-8). The highest vegetative growth (15.25 mean leaves/plant and 21.50 cm mean plant spread) was recorded for treatment T_4. Observations revealed that plant spread and leaves/plant coincided with peak flower buds/plant, which within one month produced the highest fruits/plant. Afterwards production declined drastically and fewer fruits/plant were produced towards the lag end of growth (i.e. mid April).

Overall, treatment T_4 which contained a greater amount of organic manures and less inorganic fertilizers was judged superior to the other treatments. This treatment showed 45.61% superiority over treatments T_5 and T_1 (control) in average leaves/plant and 61.53% superiority over T_1 in average plant spread/plant. Recently it was revealed that available NPK and micronutrients increased significantly with organic sources of nutrients either alone or in combination with inorganic fertilizers over inorganic fertilizers used alone, thus improving vegetative growth. Similar results were also reported by Klaas (2000), Funt and Blerman (2000), Prasad *et al.* (2002), Gajbhiye *et al*. (2003), Arancon *et al*. (2004) and Singh and Dwivedi (2011). Hence, taking these findings as reference, strawberry cv. Sweet Charley responded positively to the manure and fertilizer combination applied in treatment T_4.

Eight pickings were carried out starting from 60 days after planting to 150 days after planting (i.e. from end of December to mid April). Treatment T_4 produced 2.5 mean flower buds/plant more than T_1 (control) whereas in the number of fruits/plant, treatment T_4 produced 5.75 average number of fruits more than treatment T_1 with greater length/diameter ratio of 1.43, compared to 1.25 for treatment T_1. Thus, treatment T_4 showed 61.53%, 80.95% and 14.4% superiority over treatment T_1 in average flower buds/plant, average number of fruits per plant and length/diameter ratio of fruits, respectively. Also fruit yield per plant was highest (482.6 g/plant=26.81 tons/ha) with treatment T_4 and lowest with treatment T_5 (351.5 g/plant=19.55

Table 1 - Impact of integrated nutrient management on growth and development of strawberry cv. Sweet Charley in India

Treatments	Average leaf size/plant					Average plant spread (cm)					Average flower buds per plant					Average fruits per plant				
	Days after transplanting					Days after transplanting					Days after transplanting					Days after transplanting				
	30	45	60	90	120	30	45	60	90	105	45	60	90	120	135	60	90	120	135	150
T_1	3.25	3.50	4.25	6.75	10.75	9.25	11.25	11.75	14.75	14.25	1.0	2.00	2.25	3.25	3.25	1.25	3.00	3.75	4.5	5.25
T_2	3.75	4.25	4.50	7.25	11.25	9.5	11.25	12.00	15.25	15.00	0.75	2.00	2.50	4.00	4.0	1.00	3.50	4.50	5.25	5.75
T_3	4.25	4.50	5.00	7.50	11.75	10.0	11.75	12.50	15.75	15.50	1.0	2.00	2.00	4.50	4.5	1.00	3.75	5.25	5.75	6.50
T_4	5.75	6.00	7.75	11.25	15.25	11.0	13.0	16.25	21.50	20.75	1.25	3.00	3.75	5.25	5.25	1.75	4.00	8.00	9.0	9.50
T_5	3	3.50	4.00	6.50	10.75	9.0	11.25	11.50	14.75	14.50	1.0	2.25	1.75	3.75	3.75	0.50	3.25	3.75	4.0	6.25
SE±	0.97	0.91	1.36	1.73	1.69	0.70	0.67	1.89	2.57	2.41	0.37	0.40	0.69	0.55	0.68	0.63	0.35	1.57	1.75	1.48
C.D at 5%	0.66	0.39	0.78	0.55	0.55	0.48	0.77	0.50	0.57	0.57	0.55	0.55	0.10	1.52	0.71	0.67	0.98	1.14	2.43	2.15

Table 2 - Impact of integrated nutrient management on yield and quality characteristics of strawberry cv. Sweet Charley in India

Treatments	Length/ diameter ratio of fruits	Total fruits/ plant	Fruit yield tonnes /ha	Juice content (%)	Acidity content (%)	TSS ^{0}B	Total sugar (%)	Ascorbic acid content (mg/100 g)	pH	Specific gravity
T_1	1.25	23.75	21.13	82.10	0.16	9.00	13.50	35.55	3.01	0.80
T_2	1.30	25.25	22.53	84.50	0.15	10.00	14.25	38.56	2.96	1.00
T_3	1.35	27.75	24.93	89.10	0.14	11.00	14.95	42.74	2.91	1.10
T_4	1.43	29.50	26.81	93.20	0.12	13.00	15.00	49.78	2.87	1.30
T_5	1.26	24.50	19.55	91.40	0.11	14.00	15.25	53.60	2.81	0.90
SE±	0.06	2.14	2.60	4.16	0.019	1.85	0.63	6.75	0.06	0.17
C.D at 5%	1.17	5.55	9.08	19.54	0.48	7.40	4.09	7.32	1.01	0.84

tons/ha); treatment T_4 showed 37.14% superiority over treatment T_5 in fruit yield tons/ha. Kopanski and Kawecki (1994), Gajbhiye *et al*. (2003), Asrey and Singh (2004), Umar *et al*. (2008), Singh and Singh (2009), Singh *et al*. (2010) and Yadav *et al*. (2010) all reported the superiority of organic manures compared to NPK inorganic fertilizers in producing higher yields. The superiority of treatment T_4 in producing a maximum number of fruits/plant may be due to a greater growth and reproductive capacity of plants as influenced by a mixture of manures and inorganic fertilizers applied in treatment. As far as fruit quality parameters are concerned, treatment T_4 showed overall significant superiority to treatments T_1 and T_5. The juice content of fruits increased with increasing manure content and decreasing NPK inorganic fertilizer content although reduced slightly when manures were used alone (Table 2;

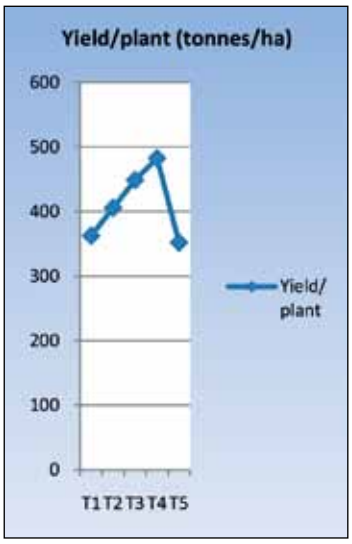

Fig. 1 - Effect of different integrated nutrient management on yield (tons/ha) of strawberry cv. Sweet Charley in India.

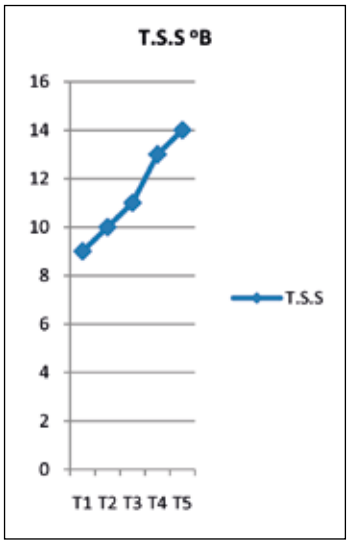

Fig. 2 - Effect of different integrated nutrient management on T.S.S. (°B) of strawberry cv. Sweet Charley in India.

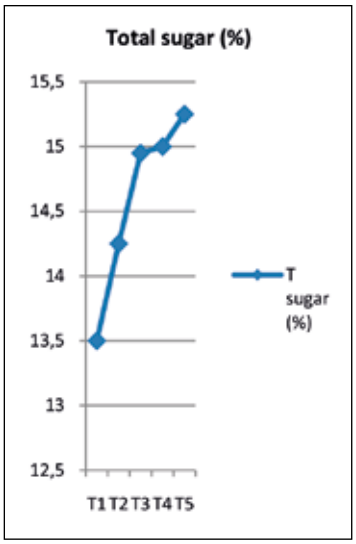

Fig. 3 - Effect of different integrated nutrient management on total sugar (%) of strawberry cv. Sweet Charley in India.

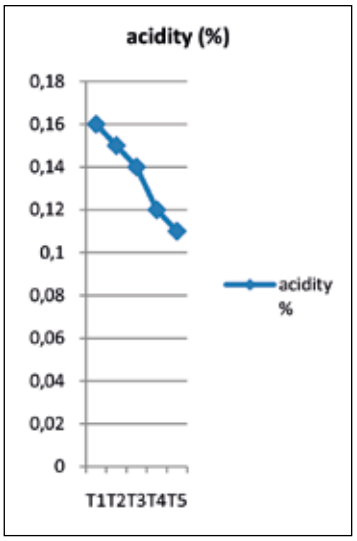

Fig. 6 - Effect of different integrated nutrient management on acidity (%) of strawberry cv. Sweet Charley in India.

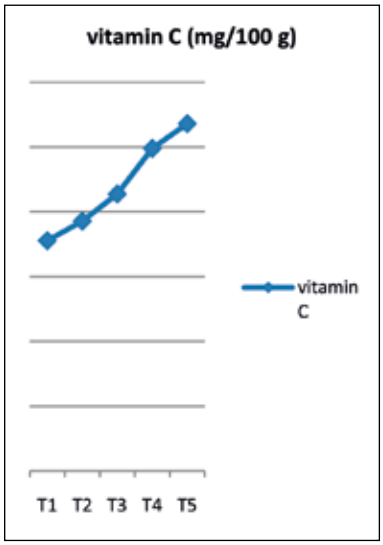

Fig. 4 - Effect of different integrated nutrient management on vitamin C (mg/100 g) of strawberry cv. Sweet Charley in India.

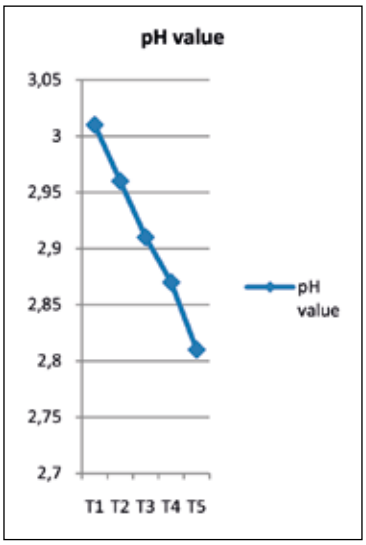

Fig. 7 - Effect of different integrated nutrient management on pH value of strawberry cv. Sweet Charley in India.

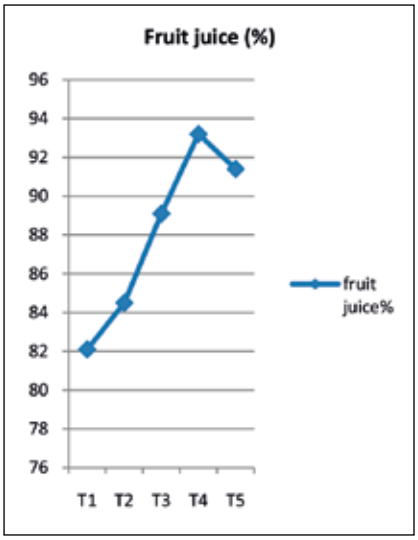

Fig. 5 - Effect of different integrated nutrient management on fruit juice (%) of strawberry cv. Sweet Charley in India.

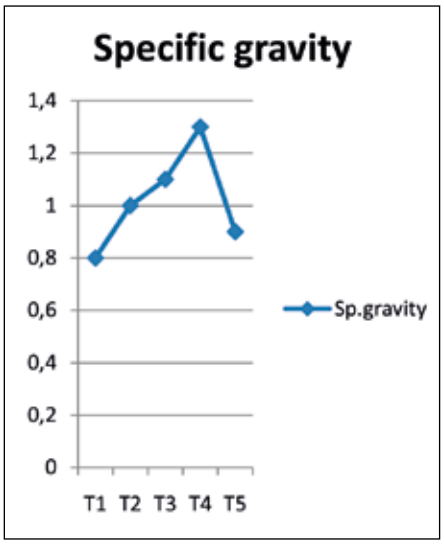

Fig. 8 - Effect of different integrated nutrient management on specific gravity of strawberry cv. Sweet Charley in India.

Figs. 1 and 5). The maximum juice content (93.2%) was recorded for treatment T_4 and the minimum (82.10%) for treatment T_1. The increase in juice content may be due to an interactional effect of mixture of manures and inorganic NPK applied through treatment T_4 which might have improved cell elongation, cell thickening and fruit development, resulting in better ripening with more succulent fruits. Several reports have indicated that manure and fertilizer combinations in strawberry have led to the production of softer fruits (Neuweiler, 1997; Ghaderi and Talaie, 2008; Singh and Singh, 2009).

The maximum TSS (14%) was found in treatment T_5 and the minimum (9%) in treatment T_1, likewise total sugar and vitamin C content was highest in treatment T_5 and lowest in treatment T_1 (Table 2 and figure 2, 3, 4). On the other hand, both acidity (%) and pH were highest in treatment T_1 and lowest in treatment T_5 while treatment T_4 exhibited the highest specific gravity of fruits and treatment T_1 the lowest, as shown in Table 2 and figures 6, 7 and 8. Thus strawberry cv. Sweet Charley responded negatively to NPK inorganic fertilizers and positively to higher manure levels under the experimental conditions of this study. Present findings are in close conformity with the results presented by Dradi and Faedi (1995), Bergamaschi *et al*. (1995), Prasad *et al.* (2002), Nazir *et al.* (2006) and Singh and Dwivedi (2011).

4. Conclusions

It can be concluded from the present study that an integrated nutrient management combination such as treatment T_4 (25% recommended dose of inorganic fertilizers +75% recommended dose of manures) is advisable for maximum returns from strawberry.

Acknowledgements

The authors are thankful to the Head of the department of horticulture and Vice Chancellor Sam Higginbottom Institute of Agriculture Technology and Science for their continuous support during this research work.

References

ARANCON N.Q., EDWARDS C.A., BIERMAN P., WELCH C., METZGER J.D., 2004 - *Influences of vermicompost on field strawberries: 1. Effects on growth and yields*. - Bioresource Technology, 93: 145-153.

ASREY R., SINGH R., 2004 - *Evaluation of strawberry varieties under semi-arid irrigated region of Punjab*. - Indian Journal of Horticulture, 61(2): 122-124.

BERGAMASCHI M., FAEDI W., LAVARONE E., LELLI A., LONGONI F., LOVATI F., LUCCHI P., PIANEZZOLA A., TESTONI A., 1995 - *Aspetti qualitativi e nutrizionali di diverse varietà di fragola*. - L'Informatore Agrario, LI(44): 29-36.

CHILDS W.H., 1937 - *Seasonal distribution of yield and size variation for ever bearing strawberry varieties*. - Proc. Amer. Soc. for Hortic. Sci., 33: 364-367.

DRADI R., FAEDI W., 1995 - *Qualitative variation of strawberry fruits during harvesting*. - South Indian Horticulture, 23: 37-39.

FUNT R.C., BLERMAN P., 2000 - *Composted yard waste improves strawberry soil quality and soil water relation*. - Acta Horticulture, 517: 37-39.

GAJBHIYE R.P., SHARMA R.R., TEWARI R.N., 2003 - *Effect of biofertlizer on growth and yield parameters of tomato*. - Indian Journal of Horticulture, 60(4):368-371.

GHADERI N., TALAIE A.R., 2008 - *Influence of manure and urea on yield and some other fruit characteristics in strawberry cv. Kurdistan*. - Iranian J. Hortic. Sci., 39(1): 99-107.

HENNION B., VAYSSE P., VERPONT F., 1999 - *Fraisier. Fertillisation et qualité du fruit.* - Infos-Ctifl, 154: 36-39.

KLAAS L., 2000 - *Effect of manure to the growth and forming runners in strawberry*. - Proceedings of the International Conference on fruit Production and fruit breeding, Tatru, Estonia, 12-13 September.

KOPANSKI K., KAWECKI Z., 1994 - *Wplyw nawozenia azotem I obornikiem na wzrost I plonowanie dwoch odmian truskawek w warunkach Zulaw Wislanych*. - Roczniki Gleboznawcze, 45(1/2): 66-75. Abstract in English.

MACIT I., KOC A., GULER S., DELIGOZ I., 2007 - *Yield, quality and nutritional status of organically and conventionally grown strawberry cultivars*. - Asian Journal of Plant Sciences, 6(7): 1131-1136.

NAZIR N., SINGH S.R., AROOSA K., MASARAT J., SHABEENA M., 2006 - *Yield and growth of strawberry cultivar Sena-Sengana as influenced by integrated organic nutrient management system*. - Environment and Ecology, 243(3): 651-654.

NEUWEILER R., 1997 - *Fertilizer combinations produce softer fruits in strawberry*. - Punjab Journal of Horticulture, 12: 17-19.

PRASAD V.M., DAS K.S., DASHRATH Y., 2002 - *Effect of biofertilizers on growth and quality of tomato*. - Bioved, 13(1/2): 125-127.

SHARMA V.P., SHARMA R.R., 2003 - *The Strawberry*. - Indian Council of Agricultural Research, New Delhi, pp. 166.

SINGH A., SINGH J.N., 2009 - *Effect of biofertilizer and Bioregulators on growth, yield and nutrient status of strawberry cv. Sweet Charlie*. - Indian Journal of Horticulture, 66(2): 220-224.

SINGH N., DWIVEDI H., 2011 - *Studies on the different mulches on vegetative growth of strawberry (*Fragaria x ananassa *Duch.) cv. Chandler*. - Progressive Horticulture, 43(1): 134-136.

SINGH S.R., ZARGAR M.Y., SINGH U., ISHAQ M., 2010 - *Influence of bio-inoculants and inorganic fertilizers of yield, nutrient balance, microbial dynamics and quality of strawberry (*Fragaria x annanassa*) under rain fed conditions of Kashmir valley*. - Indian Journal of Agricultural Sciences, 80(4): 275-281.

UMAR I., WALI V.K., KHER R., SHARMA A., 2008 - *Impact of integrated nutrient management on strawberry yield and soil nutrient status.* - Applied Biological Research, 10: 22-25.

YADAV S.K., KHOKHAR U.U., YADAV R.P., 2010 - *Integrated nutrient management for strawberry cultivation*. - Indian Journal of Horticulture, 67(4): 445-449.

Adv. Hort. Sci., 2013 27(4): 152-158

Postharvest treatments for preserving quality of 'Kinnow' fruit under different storage conditions

M.S. Ahmad*[(1)], K.S. Thakur, M.W. Siddiqui***

* *Department of Food Science and Technology, Bihar Agricultural University, Sabour, Bhagalpur (Bihar), India.*

** *Department of Food Science and Technology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP), India.*

Key words: edible coating, Kinnow, quality, shelf-life, zero energy cool chamber.

Abstract: 'Kinnow' mandarin is an attractive and nutritious fruit available only for a short period due to its poor shelf life. The effect of different postharvest treatments and storage conditions on the postharvest quality of 'Kinnow' up to 60 days was examined. With progression of the storage period, TSS and total sugars tended to increase whereas acidity, ascorbic acid, juice content, and overall acceptability decreased. Fruits stored at low temperature (4±1°C, RH 85-95%) and Zero Energy Cool Chamber (ZECC) (12-22°C, RH 85-95%) showed a slower rate of physico-chemical changes compared to ambient conditions (18-32°C, RH 45-65%). Both waxing and PE-packaging maintained the external appearance of fruits irrespective of storage systems. However, off-flavour development was noticed in PE-packed fruits after 15 days at room temperature and 40 days in cold storage and ZECC. Waxing of 'Kinnow' mandarin with undiluted Sta-fresh 960 along with low temperature and low cost storage (ZECC) may be recommended to extend the availability of fruits.

1. Introduction

'Kinnow' mandarin occupies a prime position amongst the citrus fruits grown in India. It can be used for processing into a variety of beverages, as well as industrial and medicinal uses due to its attractive colour, distinctive flavour and rich source of vitamin 'C', vitamin 'B', β-carotene, calcium and phosphorous (Sogi and Singh, 2001). Despite its attributes and commercial importance, 'Kinnow' cannot be enjoyed for long periods due to its poor shelf life. The aggregate post-harvest losses from orchards to consumers in 'Kinnow' range from 15 to 22% (Gangwar *et al.,* 2007). Storage at low temperature is one of the potential options to extend the avaibility of many fruits and vegetables (Lei *et al*., 2012). However refrigeration facilities are not generally within the reach of a majority of growers and pathological disease occurrence in 'Kinnow' is very high in cold storage (Singh and Jain, 2004). Edible coatings are promising postharvest treatments to extend the self-life of many fruits as reported in mango (Abbasi *et al.,* 2011; Singh *et al.,* 2012), strawberry (Del Valle *et al*., 2005), custards apple (El-Monem and El-Mayeed, 2003) and sweet orange (Shahid and Abbasi, 2011). Both Semperfresh ™ and Sta-Fresh 960 are commercial edible coating materials, the former is a sucrose-fatty acid ester-based wax while the later is a paraffin polyethylene-based wax. Another important technology used for extending shelf-life of fresh fruits and vegetables is Modified Atmosphere Packaging (MAP) (Ladaniya, 2001; Wasker and Gaikward, 2005; Sharma *et al.,* 2012). Also in 'kinnow' various postharvest treatments such as waxing (Ahmad *et al.,* 2005), MAP packaging (Ahmad *et al.,* 2005; Jawandha *et al.,* 2012), Bavistin dip (Sonkar *et al.,* 2008) and a combination of these treatments are reported to extend the shelf-life during storage and transportation. However, there are few works on edible coating, MAP packaging and low cost storage systems such as Zero Energy Cool Chamber (ZECC) for 'kinnow' fruits and the subject calls for further investigation. Other workers have reported that ZECC may be an alternative low cost storage system (Roy and Khurdiya, 1986; Pal *et al*., 1997) however the treatments must be hazard free and eco-friendly (Siddiqui and Dhua, 2010). Considering these factors, in the present study 'kinnow' fruits were treated with different coating materials to evaluate their performance under ambient, ZECC and cold storage conditions.

2. Materials and Methods

Raw material and treatments

Mature 'kinnow' fruits were procured from the Regional Horticulture Research Station, Dhaulakuan (HP) and brought to the Postharvest Technology Laboratory, UHF, Nauni, Solan immediately after harvest. Sound and unblemished fruits were treated with different waxing

[1] Corresponding author: shamsher73@gmail.com

Received for publication 15 August 2013
Accepted for publication 25 November 2013

materials as follows: T_1= Semperfresh (0.5%), T_2= Semperfresh (1.0%), T_3= Semperfresh (1.5%), T_4= Sta Fresh 960 (100%), T_5= Sta Fresh 960 (50%), T_6= Rice Starch (3%)+Bavistin (0.05%), T_7= Rice Starch (6%)+Bavistin (0.05%), T_8= Rice Starch (3%)+Bavistin (0.05%)+Guar gum (2%), T_9= Rice Starch (6%)+Bavistin (0.05%)+Guar gum (2%), T_{10}= Bavistin (0.05%)+ packing of four fruits in a 150 gauze Polyethylene film, T_{11}= Control.

Storage conditions

Treated and air-dried fruits from all treatments with their replications were divided into three lots and stored in plastic crates with paper moulded trays under ambient (18-32°C, RH 45-65%), Zero Energy Cool Chamber (12-22°C, 80-95% RH) and Cold store (CS, 4±1°C, 80-90% RH) conditions.

Chemical analysis

Different biochemical parameters of the juice were analyzed at fortnightly intervals. Total soluble solids (TSS) were estimated by hand refractometer (0-32°B). The readings obtained were calibrated against a standard temperature at 20°C as per the International Temperature Correction Table and expressed as °Brix. Acidity and ascorbic acid were determined by standard method (AOAC, 1990) and results were expressed as percentage citric acid and mg/100 ml of juice respectively. Total sugars were estimated by the Lane and Eynon volumetric methods (AOAC, 1990).

Physical analysis

The juice was extracted with the help of an electrically operated citrus juice extractor. The fruits were first weighed and then cut into halves and the cut portion of each half was placed on the revolving ridge knob of the extractor till only the skin part remained and all the segments were crushed and pressed; the juice was collected in the bottom of the juice extractor.

Sensory evaluation

Sensory evaluation of samples was conducted by a panel of judges (consisting of teachers, students, and staff) at periodic intervals of storage. The judges were given coded samples consisting of whole and cut fruits for evaluation regarding overall acceptability of the fruits on the basis of appearance, color, taste and defects if any. The evaluation consisted of a 9-point hedonic scale for each attribute (Wills *et al.*, 1980).

Statistical analysis

Interactions among treatments, storage conditions and biochemical attributes were assessed by Completely Randomized Design whereas, sensory attributes were assessed by the Randomized Block Designed using the STATISTICA v. 8.0 (Stat Soft, Tulsa, OK, USA) package.

3. Results and Discussion

Total soluble solids (TSS)

It was observed that TSS in general increased as the storage period progressed under all treatments and storage conditions (Table 1). Among the fruits kept at ambient temperature, the highest mean TSS contents (14.04°B) were recorded in T_{11} (control), whereas the lowest mean TSS contents (12.84°B) were found in treatment T_4 (100% Sta-Fresh 960), which was closely followed by T_{10} and T_5, respectively. The control fruits also exhibited the maximum increase of TSS under ZECC and cold storage conditions, whereas it was usually minimum in response to T_4 followed by T_{10}.

Table 1 - Effect of postharvest treatments on Total soluble solids* (B) of 'Kinnow' fruits under different storage systems during 60 days of storage

Storage systems (S)	Treatments (T)	Storage intervals (days)				
		15	30	45	60	Mean
Room temperature (18-32°C, RH 45-65%)	T_1	11.90	12.59	13.82	14.69	13.25
	T_2	11.84	12.59	13.57	14.10	13.02
	T_3	11.84	12.36	13.41	14.45	13.01
	T_4	11.76	12.31	13.22	14.09	12.84
	T_5	11.78	12.35	13.29	14.49	12.98
	T_6	12.04	12.87	14.18	15.81	13.72
	T_7	12.02	12.84	14.09	15.37	13.58
	T_8	12.00	12.79	14.05	15.50	13.58
	T_9	11.98	12.73	14.92	15.33	13.49
	T_{10}	11.77	12.31	13.16	14.15	12.85
	T_{11}	11.12	13.46	14.45	16.15	14.04
	Mean	11.91	12.65	13.74	14.92	13.03
Zero energy cool chamber (12-22°C, RH 80-95%)	T_1	11.53	11.82	12.12	12.43	11.98
	T_2	11.50	11.80	13.23	13.24	12.44
	T_3	11.49	11.84	12.62	13.25	12.30
	T_4	11.45	11.65	12.06	12.88	12.01
	T_5	11.48	11.87	12.61	13.15	12.28
	T_6	11.57	12.10	12.95	13.40	12.50
	T_7	11.56	11.88	12.20	12.53	12.04
	T_8	11.55	11.86	12.18	12.92	12.13
	T_9	11.54	11.95	12.13	12.83	12.20
	T_{10}	11.46	11.68	11.91	12.84	11.97
	T_{11}	11.59	12.94	13.30	14.68	13.12
	Mean	11.52	11.98	12.48	13.10	12.31
Cold storage (4±1°C, RH 85-95%)	T_1	11.49	11.73	12.00	12.30	11.88
	T_2	11.48	11.72	12.23	12.23	11.82
	T_3	11.46	11.67	12.15	12.15	11.80
	T_4	11.40	11.56	11.91	11.91	11.65
	T_5	11.47	11.67	11.94	11.94	12.04
	T_6	11.54	11.89	12.47	12.47	12.01
	T_7	11.53	11.80	12.41	12.41	11.96
	T_8	11.51	11.78	12.35	12.35	11.92
	T_9	11.51	11.77	12.32	12.32	11.91
	T_{10}	11.42	11.58	11.93	11.93	11.67
	T_{11}	11.65	11.96	12.62	12.62	12.13
	Mean	11.50	11.73	11.98	12.23	

$CD_{0.05}$ Storage systems (S)- 0.10, SxT- 0.33, SxI- 0.19, SxTxI- 0.66.
*Initial Total Soluble Solids (TSS) of 'Kinnow' = 11.35°B.

The TSS content increased due to hydrolysis of insoluble polysaccharides into sugars at a faster rate at high temperature (ambient) and at a slower rate at lower temperatures, i.e. in cold storage and in ZECC (Siddiqui, 2008; Siddiqui *et al.,* 2011; Jawandha *et al.,* 2012). The higher value of TSS in control fruit might be due to a higher concentration of sugars because of higher transpiration losses as these fruits were not covered, which could impede the movement of water out of the fruits. On the other hand, waxing and PE-packing might have reduced moisture losses to a maximum extent as the combination offers excellent moisture barrier properties (Ben-Yehoshua, 1985). Waxing treatments can act as an additional barrier to moisture loss but are less effective because waxes are more permeable to moisture and gases. However, it is a well established fact that wax materials are capable of delaying ripening process by maintaining slow degradation of polysaccharides as observed in mango (Abbasi *et al.,* 2011) and Kinnow mandarin (Chaudhary *et al.,* 2004). Shahid and Abbasi (2011) also reported less change compared to control in TSS in stored sweet orange fruits treated with bee's wax and paraffin wax coatings throughout the storage period. Manzano and Diaz (2001) and Hayat *et al.* (2005) found the similar results in apple after PE-packing and waxing treatments.

Titratable acidity

A gradual decline in titratable acidity contents (Table 2) was observed with an increase in storage duration under all three storage conditions during the entire 60-day storage period. 100% Sta-Fresh 960 (T_4) retained the highest mean TA (0.93%) under ambient conditions whereas T_4 and T_3 presented the maximum values under ZECC (1.02%) and CS followed by T_{10}. At the same time, the control treatment (T_{11}) had the lowest mean titratable acidity (0.64, 0.91, and 0.93%) under ambient, ZECC and CS conditions, respectively.

The faster rate of decline in acidity at room temperature could be due to faster metabolic reactions leading to earlier senescence at higher temperature. Among metabolic reactions in fruits, respiration is an important process which may utilize organic acids as substrate for the production of energy resulting in a decrease in acidity during prolonged storage (Sharma *et al.,* 2012). The organic acids involved in the respiratory process are not oxidized at a faster rate at lower temperature, and therefore their levels remained high. Furthermore, polyethylene and wax materials slow down the metabolism of fruits and vegetables as these have been reported to maintain higher Co_2 and lower O_2 inside the coated/PE-packed fruits (Kader *et al.,* 1989): this might explain the higher acid levels in waxed and PE-packed fruits. These findings are further supported by the findings of Bisen and Pandey (2008) and Siddiqui (2008) in guava and mango, respectively.

Total sugars

Total sugar increased significantly (Table 3) throughout the storage period with the increase being faster under ambient storage and slower under cold storage. Under ambient conditions, T_4 (100% Sta-Fresh 960) proved to be the most effective in delaying the increase in total sugars up to 60 days, whereas, under the other two storage conditions T_{10} proved to be the best during the same period. At the end of the study period, the mean maximum total sugar contents (7.74%) was found in treatment T_{11} (control) whereas the minimum average sugar contents were recorded for treatments T_4 and T_{10} (6.93% and 6.96%, respectively).

Table 2 - Effect of postharvest treatments on titratable acidity* (as % citric acid) of 'Kinnow' fruits under different storage systems during 60 days of storage

Storage systems (S)	Treatments (T)	Storage intervals (days)				
		15	30	45	60	Mean
Room temperature (18-32°C, RH 45-65%)	T_1	1.00	0.86	0.79	0.63	0.82
	T_2	1.00	0.96	0.84	0.68	0.84
	T_3	1.01	0.95	0.88	0.67	0.88
	T_4	1.03	0.98	0.93	0.76	0.93
	T_5	1.00	0.95	0.90	0.70	0.89
	T_6	0.96	0.87	0.74	0.74	0.83
	T_7	0.98	0.88	0.53	0.53	0.78
	T_8	1.00	0.87	0.54	0.54	0.79
	T_9	1.00	0.89	0.54	0.54	0.81
	T_{10}	1.01	0.96	0.72	0.72	0.89
	T_{11}	0.80	0.64	0.60	0.52	0.64
	Mean	0.98	0.89	0.80	0.64	0.82
Zero energy cool chamber (12-22°C, RH 80-95%)	T_1	1.04	1.02	0.99	0.96	1.01
	T_2	1.04	1.02	1.00	0.97	1.01
	T_3	1.04	1.03	1.01	0.98	1.02
	T_4	1.04	1.03	1.01	0.96	1.02
	T_5	1.02	1.02	1.01	0.98	1.00
	T_6	1.02	0.96	0.91	0.85	0.94
	T_7	1.02	0.99	0.95	0.90	0.92
	T_8	1.03	1.00	0.97	0.93	0.98
	T_9	1.03	1.00	0.98	0.94	0.98
	T_{10}	1.04	1.02	1.01	0.98	1.01
	T_{11}	1.00	0.94	0.87	0.82	0.91
	Mean	1.03	1.00	0.95	0.93	0.98
Cold storage (4±1°C, RH 85-95%)	T1	1.04	1.02	1.00	0.97	1.01
	T2	1.04	1.02	1.01	0.99	1.02
	T3	1.04	1.03	1.02	1.00	1.03
	T4	1.04	1.03	1.02	1.01	1.03
	T5	1.03	1.03	1.02	1.00	1.02
	T6	1.02	0.98	0.95	0.91	0.97
	T7	1.02	1.00	0.97	0.93	0.98
	T8	1.03	1.01	0.99	0.96	0.99
	T9	1.04	1.02	1.00	0.97	1.00
	T10	1.04	1.03	1.02	1.01	1.02
	T11	1.01	0.96	0.91	0.85	0.93
	Mean	1.03	1.01	0.99	0.96	1.00

$CD_{0.05}$, Storage systems (S) 0.007, SxT- 0.02, SxI- 0.01, SxTxI- 0.05.
*Initial Titratable acidity (%) of 'Kinnow' = 1.05%.

Table 3 - Effect of postharvest treatments on Total sugar* (%) of 'Kinnow' fruits under different storage systems during 60 days of storage

Storage systems (S)	Treatments (T)	Storage intervals (days)				
		15	30	45	60	Mean
Room temperature (18-32°C, RH 45-65%)	T_1	6.81	7.37	8.27	9.87	8.08
	T_2	7.10	7.25	7.95	9.25	7.88
	T_3	6.71	7.11	7.71	8.70	7.55
	T_4	6.76	7.07	7.49	8.19	7.37
	T_5	6.69	7.17	7.84	8.85	7.63
	T_6	6.90	7.81	9.07	10.65	8.60
	T_7	6.87	7.80	8.91	10.35	8.48
	T_8	6.84	7.75	8.75	10.35	8.42
	T_9	6.81	7.71	8.75	10.45	8.43
	T_{10}	6.72	7.17	7.67	8.40	7.49
	T_{11}	7.00	8.30	10.49	11.60	9.34
	Mean	6.83	7.50	8.44	9.69	8.11
Zero energy Cool Chamber (12-22°C, RH 80-95%)	T_1	6.68	6.75	6.82	6.97	6.80
	T_2	6.67	6.72	6.80	6.93	6.78
	T_3	6.66	6.71	6.78	6.86	6.75
	T_4	6.66	6.72	6.75	6.82	6.73
	T_5	6.65	6.68	6.76	6.84	6.73
	T_6	6.69	6.85	6.95	7.09	6.89
	T_7	6.71	6.81	6.93	7.06	6.87
	T_8	6.70	6.80	6.92	7.04	6.86
	T_9	6.68	6.76	6.90	7.02	6.84
	T_{10}	6.64	6.68	6.74	6.81	6.71
	T_{11}	6.73	6.86	7.03	7.21	6.95
	Mean	6.67	6.76	6.81	6.90	6.77
Cold storage (4±1°C, RH 85-95%)	T_1	6.66	6.73	6.80	6.90	6.77
	T_2	6.64	6.70	6.78	6.86	6.74
	T_3	6.65	6.69	6.74	6.80	6.72
	T_4	6.64	6.67	6.71	6.75	6.69
	T_5	6.64	6.68	6.74	6.80	6.71
	T_6	6.70	6.80	6.91	7.03	6.86
	T_7	6.70	6.79	6.85	7.00	6.83
	T_8	6.46	6.68	6.88	6.98	6.75
	T_9	6.68	6.76	6.86	6.96	6.81
	T_{10}	6.63	6.66	6.70	6.75	6.68
	T_{11}	6.72	6.84	6.99	7.15	6.92
	Mean	6.64	6.72	6.81	6.90	6.77

$CD_{0.05}$, Storage systems (S)- 0.01, SxT- 0.06, SxI- 0.03, SxTxI- 0.12.
*initial Total sugar content of the fruit = 6.51%.

The greater increase in sugar contents under ambient conditions may be due to rapid hydrolysis of insoluble polysaccharides into sugars (Siddiqui *et al.,* 2011; Jawandha *et al.,* 2012). The great content of sugars in control fruit might be due to greater transpiration losses. PE-packing and waxing have been reported as excellent moisture barriers which reduced moisture losses in fruits. Moreover, both PE-packing and waxing can produce modified atmosphere by increasing CO_2 and decreasing O_2 concentration.

Ascorbic acid

The ascorbic acid content showed a general declining trend in all treatments and storage conditions. However, the decrease was more pronounced under ambient conditions as compared to the other two storage systems (Table 4). The slow degradation rate and consequently higher retention of ascorbic acid under cold storage condition

Table 4 - Effect of postharvest treatments on Ascorbic acid contents (mg/100 ml juice) of 'Kinnow' fruits under different storage systems during 60 days of storage

Storage systems (S)	Treatments (T)	Storage Intervals (days)				
		15	30	45	60	Mean
Room temperature (18-32^{0}C, RH 45-65%)	T_1	24.78	24.31	23.84	23.35	24.07
	T_2	24.79	24.33	23.84	23.35	24.08
	T_3	24.81	24.37	23.93	23.49	24.15
	T_4	24.84	24.43	24.02	23.61	24.23
	T_5	24.80	24.35	23.90	23.45	24.12
	T_6	24.65	24.05	23.45	22.82	23.74
	T_7	24.69	24.13	23.57	23.04	23.86
	T_8	24.66	24.07	23.48	22.88	23.77
	T_9	24.65	24.05	23.45	22.85	23.75
	T_{10}	24.85	24.45	23.72	23.65	24.17
	T_{11}	24.64	24.03	23.42	22.78	23.72
	Mean	24.74	24.23	23.69	23.21	23.96
Zero energy cool chamber (12-22°C, RH 80-95%)	T_1	25.08	24.78	24.53	24.27	24.66
	T_2	25.10	24.79	24.55	24.30	24.68
	T_3	25.10	24.82	24.59	24.35	24.71
	T_4	25.15	24.91	24.74	24.55	24.84
	T_5	25.11	24.84	24.62	24.39	24.74
	T_6	25.01	24.66	24.35	24.03	24.51
	T_7	25.05	24.72	24.44	24.15	24.59
	T_8	25.03	24.71	24.38	24.07	24.55
	T_9	25.03	24.69	24.40	24.11	24.56
	T_{10}	25.16	24.94	24.76	24.56	24.85
	T_{11}	25.02	24.64	24.29	24.94	24.47
	Mean	25.08	24.77	24.51	24.25	24.52
Cold storage (4±1°C, RH 85-95%)	T_1	25.12	24.86	24.65	24.43	24.76
	T_2	25.12	24.90	24.71	24.51	24.81
	T_3	25.14	24.94	24.77	24.54	24.86
	T_4	25.16	24.98	24.83	24.67	24.91
	T_5	25.18	24.90	24.71	24.51	24.82
	T_6	25.16	24.82	24.59	24.35	24.71
	T_7	25.10	24.86	24.65	24.43	24.76
	T_8	25.12	24.84	24.62	24.39	24.74
	T_9	25.11	24.80	24.56	24.31	24.69
	T_{10}	25.09	25.00	24.86	24.71	24.94
	T_{11}	25.19	24.76	24.49	24.21	24.63
	Mean	25.07	24.88	24.68	24.46	24.78

$CD_{0.05}$, Storage systems (S)- 0.01, SxT- 0.06, SxI- 0.03, SxTxI- 0.12.
*initial Ascorbic acid content of the fruit = 25.25 mg/100 ml.

and in cool chamber might be due to a reduced metabolic rate at lower temperature. Greater ascorbic acid content under low temperature might be due to a reduced rate of fruit metabolic activities, mainly respiration. These results are in accordance with the findings of Wills *et al.* (2007) and Worawaran *et al.* (2013). Among the treatments, PE-packed fruits (T_{10}) and undiluted Sta-Fresh 960 (T_4) had the highest average ascorbic acid during the 60-day storage. The better retention of ascorbic acid in fruits of both the treatments might be due to modifications in the atmosphere immediately surrounding the fruits. PE packing and waxing have been reported to retain higher ascorbic acid (Bayindirli *et al.,* 1995; Kaushal and Thakur, 1996).

Juice content

The juice content of 'Kinnow' fruit was highest in ZECC (Table 5) followed by CS; the lowest juice content was found in fruits kept under ambient conditions. In the present study it was also observed that the juice content (initially 40.18%) increased under all treatments and storage conditions at the early sampling dates and then declined as the storage period progressed. Maximum juice contents (43.51%) were recorded in treatment T_{10} followed by T_5 and T_4, in comparison to the control fruits which yielded only 40.05 percent at 60 days storage.

These findings might be due to a greater moisture loss at higher temperature coupled with the lower humidity conditions under ambient conditions than ZECC and CS. Among the treatments, higher juice recovery was recorded in PE-packed fruits (T_{10}) followed by the fruits with 100% Sta-Fresh 960 (T_4). This might be due to less water loss in PE-packaging and waxing treatments as the combination acts as a barrier to moisture loss. Similar results were also obtained by Chaudhary *et al. (*2004) in Kinnow mandarin and Bisen and Pandey (2008) in Kagzi lime.

Sensory quality

A perusal of data in Table 6 indicates that the storage temperature had a profound influence on the overall acceptability of the fruits. Cold-stored fruits were the most acceptable after 60 days storage, followed by fruits from ZECC while those stored at ambient conditions were the least acceptable. Up to 60 days storage, fruits from T_4 outscored all other treatments under all three storage conditions, followed by 50% Sta-Fresh 960 (T_5) fruits. At the end of the storage period (60-days), the maximum acceptability (8.01, 7.90 and 6.70) was observed in response to T_4 followed by T_5 (7.85, 7.65 and 6.50), T_3 (7.85, 7.65 and 6.05), T_2 (7.70, 7.45 and 5.90) and T_{10} (7.67, 7.35 and 5.82) in cold store, ZECC and under room temperature respectively.

Better acceptability of cold-stored fruits is understandable as low temperature storage of 'Kinnow' fruit helps maintain storage quality, thereby increasing acceptability. PE packing and waxing creates beneficial effects and these conditions are more effective in retaining fruit quality at higher temperature (Kader *et al.,* 1989; Ladaniya and Sonkar, 1997; Ladaniya, 2007). The fruits treated with Sta-Fresh 960 (T_4) registered overall good acceptability at the end of 60 days. The present results show similarity with the findings of Ladaniya (2001) who demonstrated that taste scores were highest in 'Musambi' sweet orange (*Citrus sinensis*) fruits treated with Sta-fresh 451 wax, and Wang *et al.* (2004) who revealed that due to the waxing, eating quality was good without an unpleasant taste in fruits of Jincheng orange variety. Similar results were also reported by Mahajan *et al. (*2005) in 'Kinnow' fruits.

Table 5 - Effect of postharvest treatments on juice content* (%) of 'Kinnow' fruits under different storage systems during 60 days of storage

Storage systems (S)	Treatments (T)	Storage intervals (days)				
		15	30	45	60	Mean
Room temperature (18-32°C, RH 45-65%)	T_1	44.68	46.02	39.54	35.59	41.47
	T_2	43.01	43.01	40.17	36.17	40.59
	T_3	43.78	41.76	40.11	36.11	40.44
	T_4	40.44	44.20	44.30	43.01	42.98
	T_5	42.88	44.47	42.82	38.82	42.24
	T_6	44.45	45.24	37.12	33.12	39.98
	T_7	42.95	42.17	39.75	35.72	40.14
	T_8	40.41	45.43	36.77	32.94	38.89
	T_9	43.24	43.17	35.78	32.94	38.78
	T_{10}	40.92	42.16	46.70	42.70	43.12
	T_{11}	40.73	39.16	35.43	29.76	36.27
	Mean	42.68	43.34	38.96	36.08	40.44
Zero energy cool chamber (12-22°C, RH 80-95%)	T_1	41.45	43.76	44.02	44.79	43.50
	T_2	41.38	41.78	43.69	44.14	42.74
	T_3	40.98	40.07	44.07	48.11	43.31
	T_4	40.35	42.00	44.50	44.45	42.85
	T_5	40.75	43.20	45.00	48.82	44.44
	T_6	42.08	47.12	46.05	43.12	44.59
	T_7	40.18	42.15	42.17	43.75	42.06
	T_8	42.90	41.75	43.75	40.94	42.33
	T_9	40.05	43.75	45.55	41.76	42.78
	T_{10}	40.28	43.15	45.15	46.68	43.82
	T_{11}	40.30	44.75	42.15	40.76	41.99
	Mean	40.97	43.04	43.39	44.30	43.08
Cold storage (4±1°C, RH 85-95%)	T_1	41.75	43.22	40.75	44.75	42.62
	T_2	42.48	41.78	41.78	49.70	43.93
	T_3	40.35	41.20	40.80	49.43	42.94
	T_4	41.78	41.83	42.65	44.45	42.68
	T_5	41.08	41.35	43.33	48.30	43.52
	T_6	40.47	42.35	41.25	44.10	42.04
	T_7	40.70	40.80	42.80	44.80	42.27
	T_8	41.25	42.15	43.25	48.55	43.80
	T_9	40.25	41.19	42.25	45.50	42.45
	T_{10}	41.20	42.45	44.35	46.48	43.63
	T_{11}	40.75	40.35	42.25	44.25	41.90
	Mean	41.09	41.69	42.12	46.14	42.88

$CD_{0.05}$, Storage systems (S)- 0.37, SxT-1.23, SxI- 0.74, SxTxI- 2.47.
* Initial juice content of fruit = 40.18%.

Table 6 - Effect of postharvest treatments on overall acceptability* of 'Kinnow' fruits under different storage systems during 60 days of storage

Storage systems (S)	Treatments (T)	Storage intervals (days)				
		15	30	45	60	Mean
Room temperature (18-32°C, RH 45-65%)	T_1	6.80	5.80	5.40	3.80	5.45
	T_2	7.20	6.20	5.60	4.60	5.90
	T_3	7.20	6.40	6.00	4.60	6.05
	T_4	7.60	7.20	6.80	5.20	6.70
	T_5	7.40	6.80	6.40	5.40	6.50
	T_6	7.80	5.00	3.80	2.80	4.60
	T_7	7.20	5.60	4.60	4.00	5.35
	T_8	7.00	5.40	4.20	3.40	5.00
	T_9	6.80	5.00	3.80	2.40	4.50
	T_{10}	8.00	6.40	5.70	3.20	5.82
	T_{11}	6.00	4.80	3.00	1.80	3.90
	Mean	7.18	5.87	5.03	3.75	5.43
Zero energy cool chamber (12-22°C, RH 80-95%)	T_1	8.20	7.60	7.20	6.60	7.40
	T_2	7.80	7.80	7.40	6.80	7.45
	T_3	8.00	7.80	7.60	7.20	7.65
	T_4	8.20	8.00	7.80	7.60	7.90
	T_5	8.00	7.80	7.60	7.20	7.65
	T_6	7.80	7.60	7.00	6.40	7.20
	T_7	7.80	7.80	7.20	6.60	7.35
	T_8	7.80	7.60	7.00	6.60	7.25
	T_9	7.60	7.00	5.80	5.60	6.50
	T_{10}	8.60	8.40	6.80	5.60	7.35
	T_{11}	7.60	6.40	5.80	5.40	6.30
	Mean	7.95	7.62	7.02	6.51	7.27
Cold storage (4±1°C, RH 85-95%)	T_1	8.20	8.00	7.80	7.20	7.80
	T_2	8.20	7.80	7.60	7.20	7.70
	T_3	8.20	8.00	7.80	7.40	7.85
	T_4	8.40	8.20	8.00	7.80	8.10
	T_5	8.20	8.00	7.80	7.40	7.85
	T_6	8.00	8.00	7.60	7.00	7.65
	T_7	8.00	7.80	7.40	6.80	7.50
	T_8	7.80	7.80	7.20	7.00	7.45
	T_9	7.80	7.40	6.80	6.40	7.10
	T_{10}	8.80	8.60	7.10	6.20	7.67
	T_{11}	7.80	7.40	7.00	6.40	7.15
	Mean	8.12	7.90	7.46	6.98	7.62

$CD_{0.05}$, Storage systems (S)- 0.09, SxT- 0.31, SxI- 0.18, SxTxI- 0.62.
*Initial score for overall acceptability = 8.60.

4. Conclusions

The result of this investigation showed that using eco-friendly edible coating along with Zero Energy Cool Chamber, the shelf life of 'Kinnow' fruits can be increased substantially. Among the treatments, waxing with undiluted Sta-fresh 960 along with low temperature storage and ZECC has been found the best and may be recommended to extend the availability of fruits.

References

ABBASI K.S., ANJUM N., SAMIM S., MASUD T., ALI S., 2011 - *Effect of coatings and packaging material on the keeping quality of mangoes (*Mangifera indica *L.) stored at low temperature.* - Pak. J. Nutri., 10: 129-138.

AHMAD M.S., THAKUR K.S., KAUSHAL B.B.L., 2005 - *Post-harvest treatments to retain '*Kinnow*' storage quality.* - Indian J. Hort., 62: 63-67.

AOAC, 1990 - *Official methods of analysis*. - 13th ed. Association of Official Analytical Chemist Benjamin Franklin Station, Washington D.C., USA.

BAYINDIRLI L., SUMNU G., KAMADAN K., 1995 - *Effect of Semperfresh and Jonafresh fruit coatings on postharvest quality of Satsuma mandarins*. - J. Food Process. Preser., 19: 339-407.

BEN-YEHOSHUA S., 1985 - *Individual seal packaging of fruit and vegetable in plastic films - a New postharvest technique.* - HortScience, 20: 32-37.

BISEN A., PANDEY S.K., 2008 - *Effect of post harvest treatment on biochemical composition and organoleptic quality in Kagzi lime fruit during storage.* - J. Hort. Sci., 3: 53-56.

CHAUDHARY M.R., DHAKA R.S., FAGERIA M.S., 2004 - *Effect of wax emulsion and gibberellic acid on shelf life and quality of '*Kinnow*' mandarin fruit during storage.* - J. Udyanika Hort. Sci., 10: 6-9.

DEL-VALLE V., HERNÁNDEZ-MUÑOZ P., GUARDA A., GALOTTO M.J., 2005 - *Development of a cactus-mucilage edible coating (*Opuntia ficus *indica) and its application to extend strawberry (*Fragaria ananassa*) shelf-life.* - Food Chem., 91: 751-756.

EL-MONEM A.M., EL-MAJEED M.A., 2003 - *Effect of some post harvest treatments on the Storage quality of annona on its volatile components*. - Annals Agric. Sci., 48: 757-775.

GANGWAR L.S., SINGH D., SINGH D.B., 2007 - *Estimation of post-harvest losses in 'Kinnow' mandarin in Punjab using a modified formula*. - Agric. Eco. Res. Rev., 20: 315-331.

HAYAT I., MASUD T., RATHORE H.A., 2005 - *Effect of coating and wrapping materials on the shelf Life of apple (*Malus domestica *cv. Borkh).* - Int. J. Food Safety, 5: 24-34.

JAWANDHA S.K., TIWANA P.S., RANDHAWA J.S., 2012 - *Effect of low density polyethylene packaging and chemicals on ambient storage of Kinnow.* - Asian J. Food Agric. Indus., 5: 112-118.

KADER A.A., ZAGORY D., KERBEL E.L., 1989 - *Modified atmosphere packaging of fruits and Vegetables.* - Critical Reviews in Food Sci. Nutr., 28: 1-30.

KAUSHAL B.B.L., THAKUR K.S., 1996 - *Influence of ambient and evaporative cool chamber conditions on the quality of polyethylene packed Kinnow fruit*. - Adv. Hort. Sci., 10(4): 179-184.

LADANIYA M.S., 2001 - *Response of 'Musambi' sweet orange (*Citrus sinensis *L.) to degreening, mechanical waxing, packaging and ambient storage conditions*. - Indian J. Agric. Sci., 71: 234-239.

LADANIYA M.S., SONKAR R.K., 1997 - *Effect of curing, wax*

application and packaging on quality of stored Nagpur mandarins. - Indian J. Agric. Sci., 67: 500-503.

LADANIYA M.S., 2007 - *Quality and Carbendazim residues of Nagpur mandarin fruit in modified atmosphere package*. - J. Food Sci. Technol., 44: 85-89.

LEI J., PANG J., LI S., XIONG B., CAI L.G., 2012 - *Application of new physical storage technology in Fruit and vegetable industry*. - Afr. J. Biotechnol., 11: 6718-6722.

MANZANO J.E., DIAZ A., 2001 - *Effect of storage time, temperature and wax coating on the quality of fruits of 'Valencia' orange (*Citrus sinensis *L.).* - Proceedings International Society for Tropical Horticulture, 44: 24-29.

MAHAJAN B.Y.C., DHATT A.S., SANDHU K.S., 2005 - *Effect of different post harvest treatments on the storage life of 'Kinnow'*. - J. Food Sci. Technol., 42: 296-299.

PAL R.K., ROY S.K., SRIVASTAVA S., 1997 - *Storage performance of 'Kinnow' mandarin in evaporative cool chamber and ambient condition*. - J. Food Sci. Technol., 34: 200-203.

ROY S.K., KHURDIYA D.S., 1986 - *Studies on evaporative cooled zero-energy input cool chamber for the storage of horticultural produce*. - Indian Food Packer, 40: 26-31.

SHAHID M.N., ABBASI N.A., 2011 - *Effect of bee wax coating on physiological changes in fruits of Sweet orange cv. Blood red*. - Sarhad J. Agric., 27(3): 385-394.

SHARMA R.R., PAL R.K., RANA V., 2012 - *Effect of heat shrinkable films on storability of 'Kinnow' fruits under ambient condition*. - Indian J. Hort., 69: 404-408.

SIDDIQUI M.W., 2008 - *Studies on some aspects of mango ripening*. - M.Sc. Thesis, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India.

SIDDIQUI M.W., BHATTACHARJYA A., CHAKRABORTY I., DHUA R.S., 2011 - *6-benzylaminopurine improves shelf life, organoleptic quality, and health-promoting compounds of fresh-cut broccoli florets*. - J. Sci. Indus. Res., 70: 461-465.

SIDDIQUI M.W., DHUA R.S., 2010 - *Eating artificially ripened fruits is harmful*. - Curr. Sci., 99: 1664-1668.

SINGH D., JAIN R.K., 2004 - *Post harvest losses in distance marketing of Kinnow*. - Plant Dis. Res., 19: 36-39.

SINGH A.K., SINGH C.P., KUSHWAHA P.S., CHAKRABORTY B., 2012 - *Effect of postharvest treatments on Fruit marketability and physico-chemical characteristics of Dashehri mango*. - Prog. Hort., 44: 215-219.

SOGI D.S., SINGH S., 2001 - *Studies on bitterness development in Kinnow juice ready-to-serve beverage, squash, jam and candy*. - J. Food Sci. Technol., 38: 433-438.

SONKAR R.K., SARNAIK D.A., DIKSHIT S.N., SAXENA R.R., SINGH V.K., 2008 - *Wrapping of Kinnow mandarin with LDPE film under ambient storage*. - 11th International Citrus Congress (ISC Congress) Wuhan China, pp. 333.

WANG R.K., SHAO P.F., ZHOU L., ZHU R.G., 2004 - *Effect of fruit waxing Agent A on the commodity quality of Jincheng orange variety*. - South China Fruits, 29: 13-15.

WASKAR D.P., GAIKWARD R.S., 2005 - *Effect of various postharvest treatments on extension of shelf-life of Kesar mango fruits*. - Indian J. Agric. Res., 39: 95-102.

WORAWARAN R., NITHIYA R., NOPPOL L., DANAI B., 2013 - *Influence of storage conditions on physico-chemical and biochemical of two tangerine cultivars.* - J. Agric. Sci., 5: 70-84.

WILLS R., MCGLASSON B., GRAHAM D., JOYCE D., 2007 - *Postharvest: An Introduction to the physiology and handling of fruit, vegetables and ornamentals.* - Second edition, University of New South Wales Press, Sydney, Australia.

WILLS R.B.H., BAM B.P.A., SCOTT K.J., 1980 - *Use of flesh firmness and their objective tests to determine consumer acceptability of Delicious apple*. - Austral. J. Expt. Agric. Anim. Husb., 20: 252-256.

Adv. Hort. Sci., 2013 27(4): 159-165

Pre and post-harvest management affects functional quality of peach (*Prunus persica* L.) cv. Flavorcrest

G.B. Corbino, G. Sánchez, J. González, R.E. Murray, J. Gabilondo, G.H. Valentini, L.E. Arroyo
Estación Experimental Agropecuaria San Pedro, Instituto Nacional de Tecnologia Agropecuaria, INTA, Ruta 9, km 170, San Pedro, Buenos Aires, Argentina.

Key words: Antioxidants, DPPH, fertilization, heat shock, peach, rootstock.

Abstract: The effect of rootstock, fertilization and post-harvest heat treatments on the antioxidant capacity and total phenolic content in fruits of peach cultivar Flavorcrest was studied. `Flavorcrest´ grafted on `MrS. 2/5´ and `Flordaguard´ rootstocks produced fruits with the highest antioxidant capacity while the activity in the fruit skin was around ten times higher than in the flesh. Treatment without fertilization produced the highest antioxidant capacity in fruit flesh while the fruit skin showed no significant differences between treatments. A moderate heat shock (34 and 42°C), evaluated at 24 h post-harvest, improved the antioxidant capacity of fruits but after keep them for 72 h at 20°C, the values were similar to those observed in untreated fruit. Pre-harvest (rootstocks and fertilization) and post-harvest (heat shock) treatments influenced the functional quality of 'Flavorcrest' peach cultivar fruits.

1. Introduction

In recent years, consumers have paid increasing attention to the health and nutritional aspects (vitamins contents, mineral elements and antioxidants) of horticultural products (Scalzo *et al.*, 2005). And fruits are generally beneficial to human health, conferring not only nutritive value but also physiological and biochemical benefits. They are also excellent functional foods, contributing to the prevention of degenerative diseases. These beneficial properties have been associated to the presence of bioactive compounds such as phenolics, carotenoids, tocopherols and ascorbic acid (Soobrattee *et al*., 2005).

Fruit phenolic compounds are relevant in terms of quality, as they have a role in visual appearance (pigmentation and browning), taste (astringency), and health-promoting properties (free-radical scavengers) (Tomás-Barberán and Robins, 1997). The flavonoids are a large group of phenolic compounds ubiquitously distributed in the plant kingdom, and they exhibit diverse biological activities (Erlund, 2004; Spencer *et al.*, 2004). Many of these biological functions have been attributed to their radical scavenging and antioxidant activity (Soobrattee *et al*., 2005) because they are highly reactive as hydrogen or electron donors (Amić *et al.*, 2003).

The phenolic content in plants varies among genotypes (Tomás-Barberán *et al.*, 2001), environmental conditions, nutrient availability, agricultural practices, and postharvest conditions (Giorgi *et al*., 2005; Chludil *et al*., 2008). In fruits, the phenolic composition varies greatly among cultivars and, generally, skin fruit tissues contain larger amounts of phenolics, anthocyanins and flavonols than flesh tissue (Wang *et al*., 1996). Due to chemical structure, these compounds are able to react with many active substances in the human body, showing high antioxidant activity (Amić *et al.*, 2003).

Peach is one of the most popular fruits in the world due to its high nutrient level and pleasant flavor. In addition to vitamins and carotenoids (Gil *et al*., 2002) peach contains important phytonutrients such as phenolic acids and flavonoids (Prior and Cao, 2000; Tomás-Barberán *et al.*, 2001; Remorini *et al*., 2008).

It is known that the antioxidant activity of peach fruit is dependent on rootstock/genotype combination, ripening time and post-harvest preservation (Di Vaio *et al*., 2001; Scalzo *et al.*, 2005). Worldwide, peaches are still principally produced by grafting selected varieties onto rootstocks. In addition to conferring resistance to diseases and tolerance to stressed soil conditions, rootstocks differentially influence tree physiology resulting in differences in growth and vigor (Layne, 1994). Moreover, the effects of rootstock type on the mineral composition and sugar and organic acid content of the fruit have been reported (Di Vaio *et al.*, 2001). Nevertheless, present knowledge of rootstock effects on peach fruit quality, and particularly

Received for publication 9 January 2014
Accepted for publication 20 February 2014

on nutritional attributes of the fruit, is generally limited (Giorgi *et al*., 2005).

Stresses such as drought, extreme temperatures, low soil quality, nutrient levels and/or the presence of herbicides and pathogens have direct consequences in the proportion of secondary metabolites produced by plants. Often, plants growing in poor nutrient habitats or under stressful soil conditions contain a greater proportion of secondary metabolites (Tang *et al*., 1995) but there is scarce information about the effects of fertilizers on the production of phenolic compounds by plants.

Peaches ripen and deteriorate quickly at ambient temperature. Cold storage has always been used as the main method to slow these processes as well as the development of decay (Wang *et al*., 2006). On the other hand, heat treatments have been used in postharvest fruit technology for insect disinfestations, decay control, ripening delay and modification of fruit responses to other stresses (Lurie, 1998; Paull and Chen, 2000). High-temperature stress induces biosynthesis of phenolic compounds such as flavonoids and phenylpropanoids (Wahid *et al*., 2007).

'Flavorcrest' is a common yellow-flesh mid season peach variety, widely cultivated in Argentina. The aim of this study was to determine the effect of rootstock, fertilization and post-harvest heat treatments on the antioxidant capacity and total phenolic contents of fruits of this cultivar.

2. Materials and Methods

Chemicals

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteu reactive and anhydrous sodium carbonate was obtained from Sigma-Aldrich (Argentina); chlorogenic acid from Fluka (Argentina).

Fruits

Samples (fruit) were obtained from plants grown in controlled experimental plots at the INTA San Pedro Agricultural Experimental Station (San Pedro, Buenos Aires, 33° 44´ 34.7´ S, 59° 47´ 34.4´´ W). The number of replicates is described in each different experiment. After harvest, fruits were immediately transported to the laboratory.

Pre-harvest assays

Cultivar/Rootstock assay. The influence of genotype/rootstock combination was evaluated on fruits of the 'Flavorcrest' cultivar grafted on a) 'Mr. S 2/5' (natural hybrid of *Prunus cerasifera*); b) 'Flordaguard' (a sixth generation descendant from the cross 'Chico 11' x *Prunus davidiana* (Carr.) Franch, C-26712. 'Chico 11' was a seedling of 'Shau Thai', PI 65821) (Sherman *et al*., 1991); and c) 'Cuaresmillo' (a selection of *Prunus persica* (L.) Batsch, from seedlings of a population grown in mountainous regions of western Argentina) (Valentini *et al*., 2003).

A randomized block design was used for the experiment, with five replications per treatment and three trees per replication.

Twenty fruits per cultivar/rootstock combination were harvested at commercial maturity stage. Ten fruits were selected for chemical evaluation.

Fertilization assay. Evaluation of the fertilization effects was carried out on fruit harvested from trees growing on soils belonging to the order of Mollisols, great group *Argiudoles*, sub-group *Vertico* (Ramallo series). Soils of this series are fertile, lightly acidic in the surface, with a good content of organic matter and silty clay loam texture. The transition to the B2t horizon is gradual. The study was carried out on trees planted in June of 2005.

The experiment was comprised of a randomized block design with 12 plants, three plants per block. The assay consisted of four treatments: N, NK, NP, and NPK. Peach trees without fertilizer were used as control (C). Phosphorus, as calcium triple superphosphate (46-48% P_2O_5), 60 g/plant, and potassium, as potassium chloride (60% K_2O), 100 g/plant were applied after planting. Nitrogen, as calcium nitrate (15.5% N), 20 g/plant, was applied after planting in four different moments: November and December 2005, and September and October 2006. The data regarding tree vigor (trunk diameter and cumulative weight of pruned wood) have been published previously by González and Del Pardo (2011).

Post-harvest assay

Heat shock assay. Heat treatments were applied inside an adapted walk-in cooler (Frutitec, Río Negro, Argentina) provided with refrigeration, heating and humidification systems. The fruit was heated to 20, 34, and 42°C (±1°C), 90% RH, and kept at these conditions for 24 h. Another batch of fruit was cooled to 0°C±0.5°C and kept in cold storage for 24 h. A pool of fruit without treatment was used as control (C). The fruit was evaluated after 24 h and then kept at 20°C for 72 h.

Fruit quality parameters

Flesh firmness (FF) was measured on two opposite sides in the equatorial zone of individual fruits with an Effegi 327 Fruit Pressure Tester (Milano, Italy) and expressed as kg/cm^2. Total soluble solids (TSS) were determined in juice from the longitudinal side opposite the suture, with an N1 Atago hand refractometer (Osaka, Japan) and reported as °Brix. Color determination was performed with a Minolta Chroma Meter CR-300 (Osaka, Japan). Results were expressed as L^*, C^* $[(a^2 + b^2)^{1/2}]$ and h^o $(\tan^{-1} b/a)$ color units calculated from a^* (green chromaticity) and b^* (yellow chromaticity).

Sample extractions

Extractions were carried out using 3 g of fresh fruit skin or flesh homogenized in 15 ml of 7% acetic acid in methanol. Tubes were stored for 24 h at 4°C. They were then centrifuged (10 min at 2000 g), filtered and stored at 4°C in darkness until use. Ten fruits per treatment were processed for chemical analyses.

Fruit functional quality

Assay of DPPH radical scavenging activity. The DPPH method was adapted from Brand-Williams *et al*. (1995). A total of 20 µl of peel extract or 200 µl of flesh extract were diluted to 1 ml with methanol. The diluted sample reacted with 2 ml of $DPPH^{\bullet+}$ (150 µM in methanol) at 30°C. Decrease in absorbance was measured at 517 nm after 30 min. Results were expressed as µmols of ascorbic acid equivalents/g of fresh weight (µmol AEAC/g FW).

Total phenolic content. Total phenolic content (TPC) was determined by the Folin-Ciocalteu method (Swain and Hillis, 1959) using chlorogenic acid as standard for the calibration curve. Results were expressed as µmols of chlorogenic acid equivalent/g of fresh weight (CAE/g FW). The sample (20 µl of peel extract or 200 µl of flesh extract) or standard (0, 50, 100, 200, 300, 400, 500 µl of 0.4 g/l chlorogenic acid) were diluted with water to a final volume of 4.45 ml. 50 µl of Folin-Ciocalteu reagent (2 N) were added. After 3 min sodium carbonate (0.1 N) was added. Results were read at 725 nm after 1 h.

Statistical analyses

Data were submitted to analysis of variance and Duncan tests were conducted to identify differences among means. Pearson Correlation test was used to determine the correlations among means. Statistical significance was declared at $p<0.05$.

3. Results and Discussion

Fruit quality parameters

Cultivar/Rootstock assay. Rootstock influence was found to not be significant for firmness (6.8-7.5 kg/cm^2) and soluble solid content (10.9-11.3°Brix). While fruit skin lightness (L*) and hue angle (h^o) values were significantly higher in 'Flavorcrest' fruits grafted on 'Mr.S 2/5' and 'Flordaguard' rootstock, while chroma values (C*) were not significantly affected by rootstock (Table 1).

Fertilization assay. Fruit from NP and NPK treatments presented the greatest firmness with no significant differences in comparison to control. Although fertilizer affected soluble solid content, it did not have pronounced effects. Fruit skin color characteristics, lightness (L*), hue angle (h^o) and chroma (C*) values showed no significant differences with fertilizer treatments (Table 1).

Heat shock assay. In general, fruit firmness decreased after storage at 20°C for 72 h. Flesh lightness (L*) and hue angle (h^o) were not modified by treatments. Chroma (C*) values decreased with 34 °C treatments (24 h) with respect to control (Table 1).

Table 1 - Firmness (FF), total soluble solids (TSS) and color (LCH system) of 'Flavorcrest' peach fruit grafted on 'Mr.S 2/5', 'Flordaguard' and 'Cuaresmillo' rootstocks included in fertilization and heat shock assays

Treatment	FF	TSS	L	C	H
				Peel color characteristics	
Rootstock					
Mr. S 2/5	6.81 a	11.31 a	69.03 a	46.64 a	85.63 a
Flordaguard	7.41 a	11.20 a	66.86 a	45.85 a	79.44 a
Cuaresmillo	7.21 a	10.88 a	62.34 b	44.86 a	71.18 b
Fertilization					
C	5.95 a	12.16 a	55.67 a	43.55 a	61.48 a
N	4.77 b	11.13 b	59.38 a	45.44 a	63.95 a
NP	5.77 a	11.96 ab	57.81 a	45.58 a	61.14 a
NK	3.93 c	11.40 ab	56.84 a	45.17 a	59.95 a
NPK	5.16 ab	11.97 b	55.15 a	45.23 a	58.04 a
Heat Shock				*Flesh color characteristics*	
Control	7.87 a	11.02 ab	73.94 a	49.64 a	99.80 a
0°C	7.84 a	10.96 ab	74.16 a	46.88 ab	98.92 a
20°C	7.42 a	10.70 b	73.63 a	46.35 ab	98.43 a
34°C	7.70 a	11.20 ab	74.57 a	44.13 b	99.49 a
42°C	8.12 a	12.45 a	75.15 a	45.94 ab	97.55 a
Control + 3D	5.92 a	11.44 a	73.83 a	48.72 a	96.49 a
0°C + 3D	5.88 a	11.20 a	71.44 a	46.67 a	96.87 a
20°C + 3D	2.79 b	11.28 a	72.99 a	46.56 a	95.99 a
34°C + 3D	4.47 ab	11.58 a	72.33 a	45.25 a	94.86 a
42°C + 3D	2.01 b	12.05 a	70.96 a	47.45 a	92.72 a

Values are the mean of 30 replications. Means followed by the same letters are not significantly different (p= 0.05).

Fruit functional quality

Cultivar/Rootstock assay. Total antioxidant capacity was determined in fruit flesh and skin. The fruit flesh of 'Flavorcrest' grafted on 'Flordaguard' and 'Mr. S 2/5', both middle vigor rootstocks, presented the highest AEAC/g FW values, with 1.77 and 1.65 µmols of ascorbic acid equivalents/g of fresh weight, respectively (Fig. 1). Previous studies have shown that total antioxidant capacity changes as a function of the rootstock. Remorini *et al*. (2008) demonstrated that 'Mr.S 2/5' produced fruits with the highest total antioxidant capacity, attributing this to low-vigor properties. Despite these results, they did not find a link between rootstock vigor and total antioxidant capacity. On the other hand, Scalzo *et al*. (2005) observed higher antioxidant capacity values with vigorous rootstock. Light has been reported to be one of the major environmental factors that affect phenolic production (Par and Bolwell, 2000). Fruits of dense foliage trees receive less light and this could affect the phenolic content. Phenolics are the major antioxidant compounds in peach fruits (Tomás-Barberan *et al*., 2001).

Fruit skin total antioxidant capacity was approximately five times higher (8.8-10.6 µmols AEAC/g FW) than that of flesh and showed no significant differences between rootstocks (Fig. 1).

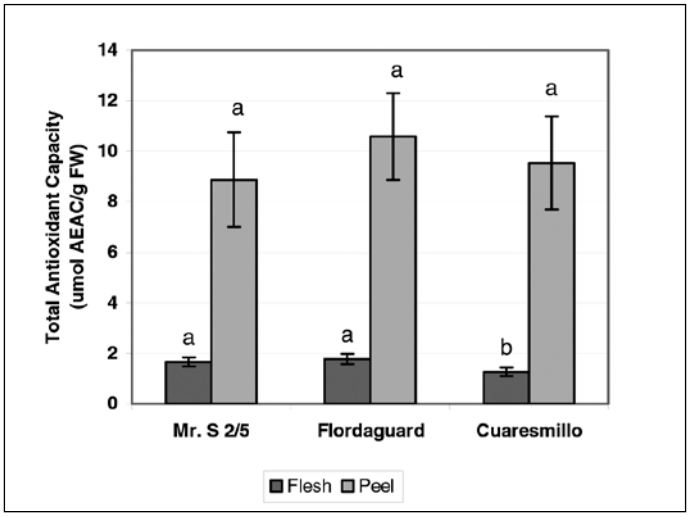

Fig. 1 - Total antioxidant capacity determined by DPPH assay in flesh and peel of fruits of Flavorcrest cultivar grafted on 'Mr.S 2/5', 'Flordaguard' and 'Cuaresmillo' rootstocks. Values are means (± S.E.) of 10 replicates. Inside each group (flesh or peel), means followed by the same letters are not significantly different (p= 0.05).

The effect of rootstock on flesh total phenolic content was significantly different. 'Flavorcrest' grafted on 'Flordaguard' (1.14 µmol CAE/g FW) and 'Mr.S 2/5' (1.02 CAE/g FW) showed the highest values. Fruit skin TPC was higher (ten times) than that of flesh and no differences were observed between rootstocks. Other authors also found a higher phenolic content in fruit skin compared to flesh (Tomás-Barberán *et al*., 2001; Remorini *et al*., 2008), reporting values two to four times higher.

Total antioxidant capacity and total phenolic content were positively correlated in flesh (r= 0.8052) and peel (r= 0.8190), which suggests that phenolic compounds greatly contribute to the total antioxidant capacity (Fig. 2).

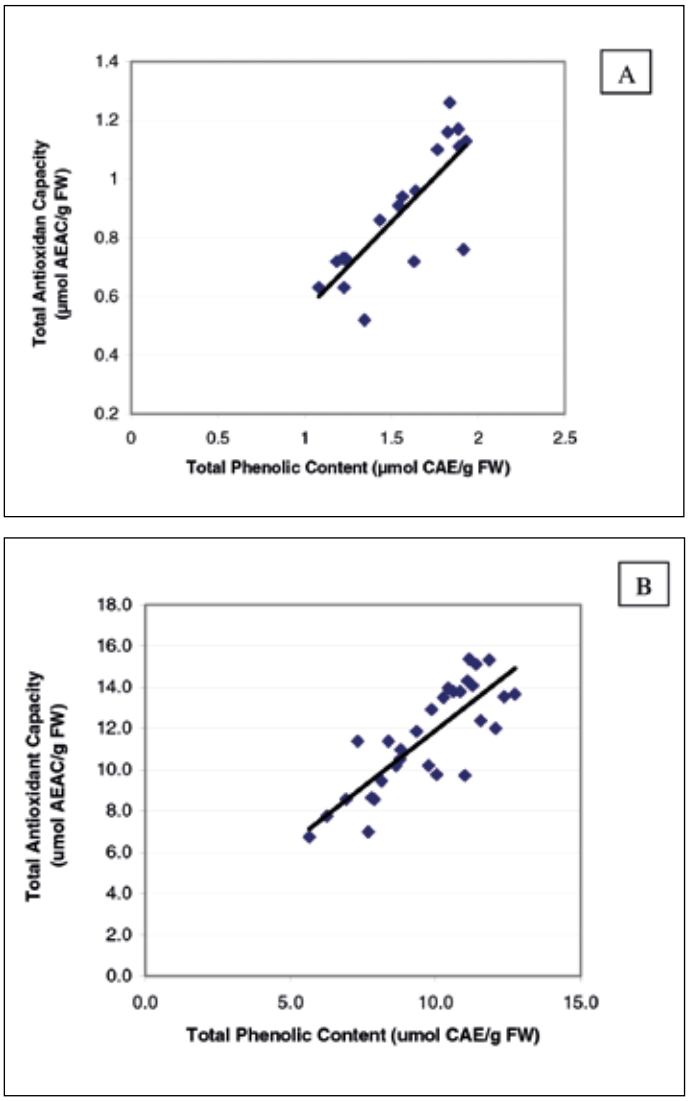

Fig. 2 - Correlation between total phenolic content (µmol CA/g FW) and total antioxidant capacity (µmol AEAC/g FW) of flesh A) and B) fruit skin of Flavorcrest cultivar, grafted on 'Mr.S 2/5', 'Flordaguard' and, 'Cuaresmillo' rootstocks.

Fertilization

Flesh from control fruits (without fertilization) had the highest antioxidant capacity (2.2 µmols AEAC/g FW), whereas the antioxidant capacity decreased (with respect to control) when N (1.55 µmols AEAC/g FW), NP (1.75 µmols AEAC/g FW) and NPK (1.79 µmols AEAC/g FW) treatments were applied, and even more so with NK (1.32 µmols AEAC/g FW). Fruit skin showed no significant differences (Fig. 3).

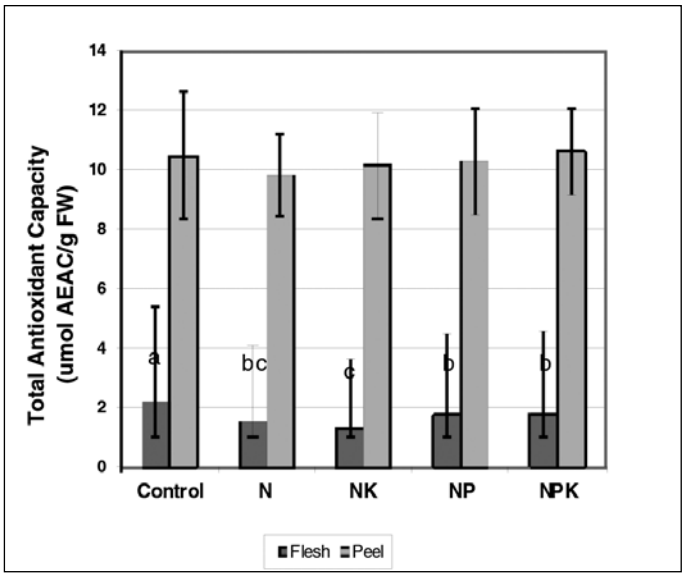

Fig. 3 - Total antioxidant capacity determined by DPPH assay in flesh and fruit skin of Flavorcrest cultivar under fertilizer N, NK, NP and NPK. Values are means (±S.E.) of 10 replicates. In bars corresponding to flesh values, means followed by the same letters are not significantly different (p= 0.05).

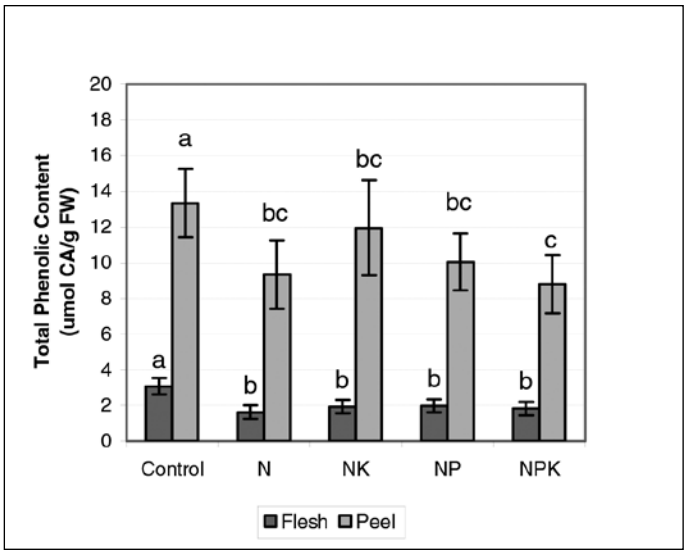

Fig. 4 - Total phenolic content determined by Folin-Ciocalteau assay in flesh and fruit skin of Flavorcrest cultivar under fertilizer N, NK, NP and NPK. Values are means (±S.E.) of 10 replicates.

According to the 'C/N balance theory', when N is readily available, plants will primarily synthesize compounds with high N content (e.g. protein to growth). Instead when N availability is limited, metabolism changes towards carbon-containing compounds such as starch, cellulose, and non N-containing secondary metabolites such as phenolics and terpenoides (Haukioja *et al*., 1998). In plants, it has been shown that competition between protein and phenolic synthesis exists for the common precursor L-phenylalanine (Riipi *et al*., 2002). The relative differences in the release of nutrients from various fertilizers could lead to different C/N ratios in plants and this in turn leads to a difference in the production of secondary metabolites (Brandt and Molgaard, 2001).

Fertilization treatments were found to not significantly affect the vegetative variables (trunk cross sectional area and pruned wood) (González and Del Pardo, 2011). Environmental stresses including nutrient deficiency are known to activate the biosynthesis of phenylpropanoid compounds (Dixon and Paiva, 1995), which could explain why the highest antioxidant activity was found without fertilizer treatment.

The highest total phenolic content in flesh (3.37 µmol CAE/g FW) and fruit skin (13.34 µmol CAE/g FW) was obtained in plants without fertilization (C), which differed significantly from the rest of the treatments (Fig. 4). Flesh total phenolic content showed a low correlation with antioxidant capacity (r= 0.48).

Heat shock treatments

In this assay, the effect of post-harvest temperature on the functional quality of fruit flesh was evaluated. Total antioxidant capacity was significantly different between fruit flesh evaluated at 24 h and fruit held at 20°C for 72 h. The moderate heat shock treatments (34°C and 42°C) at 24 h improved the antioxidant capacity (0.76 µmols and 0.84 µmols AEAC/g FW, respectively) in comparison to control (0.48 µmols AEAC/g FW), 0°C (0.49 µmols AEAC/g FW) and 20°C (0.52 µmols AEAC/g FW). AEAC (µmol/g FW) showed no significant differences between treatments after keeping fruits for 72 h at 20°C. Comparing fruits evaluated at 24 and 72 h, the total antioxidant capacity was significantly increased in control, 0°C and 20°C treatments and significantly decreased in 34°C and 42°C treatments (Fig. 5).

Total phenolic content in the flesh, evaluated 24 h after treatment applications, was significantly higher at 34°C (0.41 CAE/g FW) and 42°C (0.49 CAE/g FW) than control

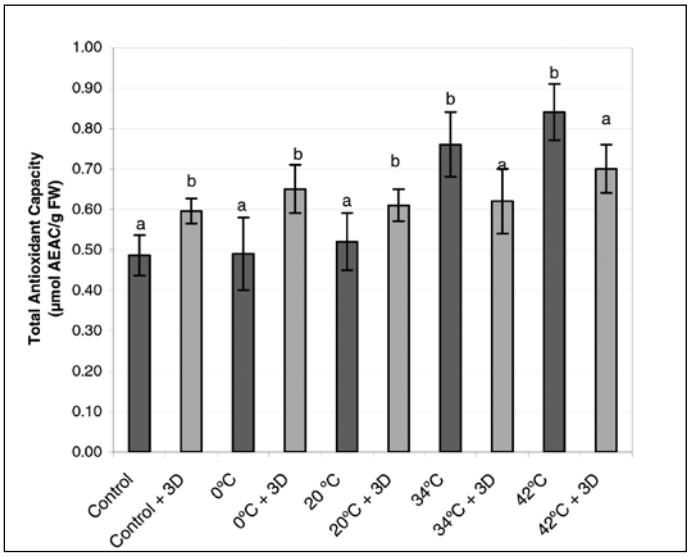

Fig. 5 Total antioxidant capacity determined by DPPH assay in flesh of Flavorcrest cultivar submitted to different temperature treatments: 0, 20, 34, and 42°C. Fruit was evaluated at 24 h (dark grey bars) and after 72 h (light grey bars) from treatment application. Each bar indicates the mean (±S.E.) of 5 replications.

(0.30 CAE/g FW), 0°C (0.30 CAE/g FW) and 20°C (0.34 CAE/g FW), following the same behavior as antioxidant capacity. After 72 h at 20°C, all treatments differenced of control. When treatments for the two evaluation periods (24 and 72 h) were compared, the only heat treatment that showed a significant difference was 42°C (Fig. 6). There was a positive correlation between total antioxidant capacity and total phenolic content (r=0.67) (Fig. 7).

Heat treatment affects several aspects of fruit ripening such as ethylene production and cell wall degradation (Lurie, 1998). Thermal stress enhances activities of oxidative stress enzymes and induces the accumulation of phenolic compounds like flavonoids and phenylpropanoids (Wahid *et al*., 2007). A previous study on peach cultivars showed that heat treatments promoted the development of red color in the fruit flesh (Budde *et al*., 2002) which could link these phenomena to an increased synthesis of phenolic compounds.

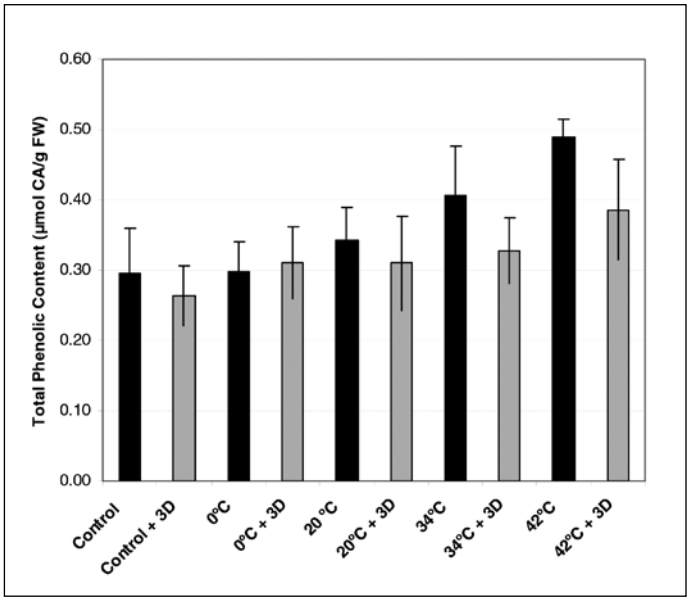

Fig. 6 - Total phenolic content determined by Folin-Ciocalteau assay in flesh of Flavorcrest cultivar subjected to different temperature treatments: 0, 20, 34, and 42°C (± 1°C). Fruit was evaluated at 24 h (Black bars) and after 72 h (gray bars) from treatment application. Each bar indicates the mean (± S.E.) of 5 replications.

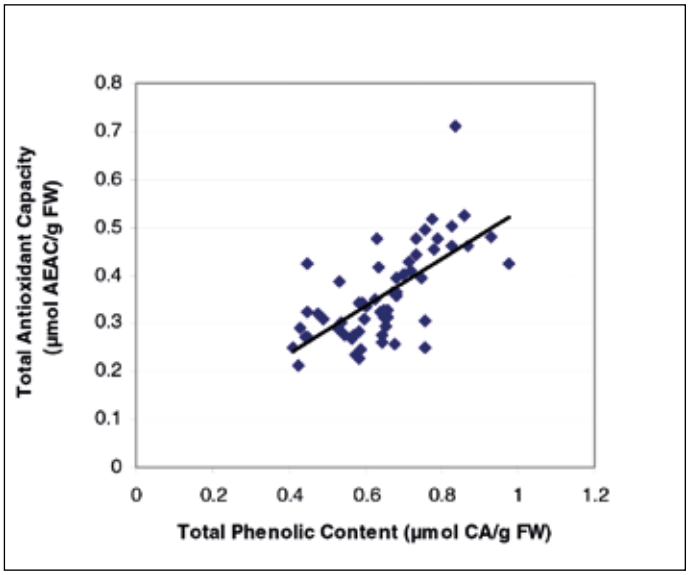

Fig. 7 - Correlation between total phenolic content (µmol CA/g FW) and total antioxidant capacity (µmol AEAC/g FW) of fruit flesh in the heat treatment assay.

4. Conclusions

The results of this study show that pre-harvest (rootstocks and fertilization) and post-harvest (heat shock) treatments influence the functional quality of 'Flavorcrest' peach fruits. 'MrS. 2/5' and 'Flordaguard' rootstocks produced fruits with the highest antioxidant capacity and phenolic content, whereas 'Cuaresmillo', the most commonly used peach rootstock in our peach growing area, showed the lowest. Although these results could be attributed to vigor it is not possible to determine a general behavior; assays with other rootstocks could be useful.

It has been reported that soluble phenolics are the principal contributors to the total antioxidant capacity. The accelerated plant growth induced by fertilization may cause a reduction in concentrations of phenylpropanoids (Haukioja *et al*., 1998), resulting in the lowest antioxidant capacity observed in fertilized treatments.

Heat stress causes accumulation of secondary metabolites of a multifarious nature in plants (Wahid *et al.*, 2007). While higher antioxidant capacity was observed in heat-treated fruit at 24 h, the total antioxidant capacity values were similar to those observed in non heat-treated fruit after they were held for 72 h at 20°C.

Acknowledgements

The authors thank the Instituto Nacional de Tecnología Agropecuaria (INTA), PNFRU 3191 and AETA 2682 projects by financial support.

References

AMIĆ D., DAVIDOVIÉ-AMIĆ D., BESLO D., TRINAJSTIĆ N., 2003 - *Structure-radical scavenging activity relationships of flavonoids*. - Croatica Chemica Acta, 76(1): 55-61.

BRANDT K., MOLGAARD P., 2001 - *Organic agriculture: does it enhance or reduce the nutritional value of plants foods?* - J. Sci. Food and Agric., 81: 924-931.

BRAND-WILLIAMS W., CUVELIER M., BERSET C., 1995 - *Use of a free radical method to evaluate antioxidant activity*. - Food Science Technology, 28: 25-30.

BUDDE C., LUCANGELI C., POLENTA G., MURRAY, R., 2002 - *Golpe de altas temperaturas aplicado en poscosecha afectó la calidad de melocotón*. - ITEA, 98: 95-107.

CHLUDIL H.D., CORBINO G.B., LEICACH S.R., 2008 - *Soil quality effects on* Chenopodium album *flavonoids content and antioxidant potential.* - J. Agric. Food Chem., 56(13): 5050-5056.

DI VAIO C., BUCCHERI M., GRAZIANI G., RITIENI A., SCALFI L., 2001 - *Attività antiossidante di frutti di pesco (cv. Maycrest).* - Frutticoltura, 63: 83-86.

DIXON A., PAIVA N., 1995 - *Stress-induced phenylpropanoid metabolism*. - The Plant Cell, 7: 1085-1097.

ERLUND I., 2004 - *Review of the flavonoids quercetina, hesperetin, and naringenin. - Dietary sources, bioactivities, bioavailability, and epidemiology.* - Nutrition Research, 24: 851-874.

GIL M.I., TOMÁS-BARBERÁN F.A., HESS-PIERCE B., KADER A.A., 2002 - *Antioxidants capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California*. - J. of Agric. and Food Chem., 50: 4976-4982.

GIORGI M., CAPOCASA F., SCALZO J., MURRI G., BATTINO M., MEZZETTI, B., 2005 - *The rootstock effects on plant adaptability, production, fruit quality, and nutrition in peach (cv. 'Suncrest').* - Scientia Horticulturae, 107: 36-42.

GONZÁLEZ J., DEL PARDO K., 2011- *Fertilización en duraznero Flavorcrest.* - IV Jornada del grupo de fertilización de la SECH, CastelldeFells, Barcelona, España.

HAUKIOJA E., OSSIPOV V., KORICHEVA J., HONKANEN T., LARSSON S., LEMPA K., 1998 - *Byosynthetic origin of carbon-based secondary compounds: cause a variable responses of woody plants to fertilization?* - Chemoecology, 8: 133-139.

LAYNE R.E.C., 1994 - Prunus *rootstocks affect long-term orchard performance of Redhaven peach on Brookston clay loam*. - HortScience, 29: 167-171.

LURIE S., 1998 - *Postharvest heat treatments.* - Postharvest Biology and Technology, 14: 257-269.

PAR A.J., BOLWELL G.P., 2000 - *Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile.* - J. of Sci. of Food and Agric., 80: 985-1012.

PAULL R.E., CHEN N.J., 2000 - *Heat treatment and fruit ripening*. - Postharvest Biology and Technology, 21: 21-37.

PRIOR R.L., CAO C., 2000 - *Antioxidant phytochemicals in fruits and vegetables: diet and health implications.* - HortScience, 35(4): 588-592.

REMORINI D., TAVARINI S., DEGL'INNOCENTI E., LORETI F., MASSAI R., GUIDI L., 2008 - *Effect of rootstocks and harvesting time on the nutritional quality of peel and flesh fruits.* - Food Chemistry, 110(2): 361-367.

RIIPI M., OSSIPOV V., LEMPA K., HAUKIOJA J., KORICHEVA J., OSSIPOVA S., PIHLAJA K., 2002 - *Seasonal changes in birch leaf chemistry: are the tradeoffs between leaf growth and accumulation of phenolics?* - Oecologica, 130: 380-390.

SCALZO J., POLITI A., PELLEGRINI N., MEZZETTI B., BATTINO M., 2005 - *Plant genotype affects total antioxidant capacity and phenolic contents in fruit.* - Nutrition, 21: 207-213.

SHERMAN W.B., LYRENE P.M., SHARPE R.H., 1991 - *Flordaguard peach rootstock.* - HortScience, 26(4): 427-428.

SOOBRATTEE M.A., NEERGHEEN V.S., LUXIMON-RAMMA A., ARUOMA O.I., BAHORUM T., 2005 - *Phenolics as potential antioxidant therapeutic agents: Mechanism and actions.* - Mutation Research, 579(1-2): 200-213.

SPENCER J.P.E., ABD EL MOHSEN M.M., RICE-EVANS C., 2004 - *Cellular uptake and metabolism flavonoids and their metabolites: implications for their bioactivity.* - Archives of Biochemistry and Biophysics, 423: 148-161.

SWAIN T., HILLIS W.E., 1959 - *The phenolic constituents of* Prunus domestica*. I. The quantitative analysis of phenolic constituents.* - J. of the Sci. of Food and Agric., 10: 63-68.

TANG C.S., CAI W.-F., KOLH K., NISHIMOTI R.K., 1995 - *Plant stress and allelopathy*, pp. 142-157. - In: INDERJIT DAKSHINI K.M.M., and F.A. EINHELLIG (eds.). *Allelopathy organisms, processes and applications*. American Chemical Society, ACS Symposium Series 582, W.D.C., USA.

TOMÁS-BARBERÁN F.A., GIL M.I., CREMIN P., WATERHOUSE A.L., HESS-PIERCE B., KADER A.A., 2001 - *HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums*. - J. of Agric. Food Chem., 49: 4748-4760.

TOMÁS-BARBERÁN F.A., ROBINS R.J., 1997 - *Phytochemistry of fruit and vegetables.* - Clarendon Press, Oxford, UK.

VALENTINI G.H., MURRAY R.E., ARROYO L.E., 2003 - *Evaluación de los efectos de distintos portainjertos sobre características productivas de dos variedades de melocotón.* - ITEA, 99(3): 234-248.

WAHID A., GELANI S., ASHRAL M., FOOLAD M.R., 2007 - *Heat tolerance in plants: An overview.* - Environ. & Exp. Bot., 61: 199-223.

WANG H., CAO G., PRIOR R.L., 1996 - *Total antioxidants capacity of fruits.* - J. of Agric. and Food Chem., 44: 701-705.

WANG L., CHEN S., KONG W., LI S., ARCHBOLD D.D., 2006 - *Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage.* - Postharvest Biol. and Tech., 41(3): 244-251.

Adv. Hort. Sci., 2013 27(4): 166-172

Agronomic performance and essential oil composition of *Ocimum basilicum* L.: Effect of genotype and date of harvest

A. Vazquez[(1)], E. Sanchez*, C. van Baren, D. Frezza***

* *Cátedra de Horticultura, Facultad de Agronomía de la Universidad de Buenos Aires, Av. San Martin 4453 C1417DSE, Buenos Aires, Argentina.*

** *Cátedra de Farmacognosia, Facultad de Farmacia y Bioquímica de la Universidad de Buenos Aires, Junin 954, 2° piso, C1113AAD, Buenos Aires, Argentina.*

Key words: green basil, purple basil, soilless culture, volatile oils, yield.

Abstract: An experiment was conducted to assess the agronomic performance and essential oil composition of *Ocimum basilicum* L. (basil) with two genotypes during autumn-winter cycle, in a hydroponic system in greenhouse. Genotypes (i.e. green and purple) were provided to study productive parameters. Two harvest dates and both genotypes formed treatments to investigate oil composition and its stability. One pruning was made before the last harvests. Results of fresh and dry weight (g. plant^{-1}), absolute growth rate (g. day^{-1}) and relative growth (g. g^{-1}.day^{-1}) and yield (g. plant^{-1}) showed great differences in comparison with the optimal growing season values. Although pruning encourages new growth, it was strongly reduced in purple basil. Essential oil composition varied for both genotypes and between harvest dates. Linalool prevailed at the first harvest date whereas methyl-eugenol increased towards the second harvest date, and significantly in purple basil. Radiation and temperature data showed a downward trend during the cycle which influenced biomass production and essential oil composition. Green basil had better productive behavior than the purple variety. Essential oil stability between harvest dates varied for both genotypes. Pruning strongly affected purple basil growth which altered essential oil composition. The findings presented in this study confirm that it is possible to grow basil in autumn-winter season in greenhouse. Although yield slightly decreases in comparison with optimal growing season, high quality aromatic plants can be obtained.

1. Introduction

Sweet Basil (*Ocimum basilicum* L.) is an annual herbaceous crop cultivated mainly for culinary purposes. There is a significant demand by consumers seeking fresh and high quality herbs all year round. The volatile oils in basil are responsible for its characteristic aroma (Fischer *et al.,* 2011) and flavor as a condiment, which along with color and freshness determine its commercial value. Essential oil aromatic compounds and productive behavior are affected by the environment, genotype and agronomic techniques. Some chemotypes from different geographic origins have been classified based on the aroma profiles of essential oil (Suppakul *et al.,* 2003). Another classification of *O. basilicum* cultivars has been made according to morphology, height, leaf color, dimension and flower color (Darrah, 1980). Yield and essential oil composition are remarkably variable between purple and green genotypes (Marotti *et al.,* 1996; Sajjadi, 2006) which also differ in biomass yield (Hochmuth and Leon, 1999). Basil is cultivated under a range of conditions but temperate climates are the most suitable for the crop. Chang *et al.* (2005) stated that the maximum dry matter content was obtained with temperatures of 30°C. Putievsky (1983) reported that increasing daytime temperatures between 21°C and 30°C enhanced plant height. Light influences essential oil composition and productive behavior. When the irradiance level decreases the methyl-eugenol content increases, plants are smaller, have thinner leaves, less dry and fresh weight, sprouts and foliar area, whereas with high irradiance levels, linalool, eugenol and the total content of essential oil rise and photosynthesis and growth rate increase. Under high irradiance conditions, more photosynthates are biosynthesized and a greater amount of secondary metabolites accumulate (Chang *et al*., 2008). Although this aromatic crop is grown in open field and greenhouse conditions, hydroponic basil cultivation in a protected environment is an efficient commercial alternative, most importantly for those areas with limited agricultural soils and dependant on irrigation (Hassanpouraghdam *et al*., 2010). The benefits of this system include high quality plants, rapid growth, off-season and

[(1)] Corresponding author: avazquez@agro.uba.ar

Received for publication 13 September 2013
Accepted for publication 21 January 2014

all-year-round production, maximizing the benefits for producers. On the other hand, in a pure hydroponic system the nutritive solution is recycled, reducing the environmental impact and with minimal groundwater contamination (Resh, 2001). Nutritive solution management is important to obtain plants with high yield and good quality.

Studies regarding electrical conductivity demonstrated that the highest fresh weight (g.plant^{-1}) was obtained with 1.5 ds.m^{-1} and it did not affect essential oil concentration (Carrasco and Izquierdo, 1996), while values above 3 ds.m^{-1} affected plant growth. Considering environmental conditions, the production system and the different varieties of *Ocimum basilicum* L., the aim of this work was to evaluate the agronomic performance and essential oil composition of green and purple genotypes during the autumn-winter cycle.

2. Materials and Methods

The trials were carried out in the experimental fields of the Horticultural Department of Agriculture College of the University of Buenos Aires, in a polyethylene-metallic greenhouse. Seeds of two varieties of basil, purple and green (*Ocimum basilicum* var. Violeto and *Ocimum basilicum* var. Genovese) were obtained from Zorzi, di Hortus sementi SRL. Seeds were sown, at the beginning of autumn to finish the crop cycle in winter, in expanded polystyrene growing trays on a soilless media mix (vermiculite, peat moss, perlite and fertilizer NPK with micro elements 1.3 g l^{-1}, pH 5.5-6.5, with fine structure).The trays were located in a hydroponic floating system until the seedlings had two to three pairs of unfolded leaves. Plants were transplanted into a closed hydroponic NFT (Nutrient Film Technique) system. A low polyamide tunnel was built to avoid frost damage and it was used from late afternoon to early morning each day. The nutrient solution was composed of Ammonium Nitrate 5.625 g, Potassium Nitrate 75 g, Calcium Nitrate 93.75 g, Mono Potassium Phosphate 28.13 g, Magnesium Sulphate 33.75 g and micro elements 18.75 c.c. Crop density was 25 pl. m^{-2}. Electrical conductivity and pH of the nutrient solution were measured three times a week. Environmental temperature (°C), radiation (W.m^{-2}), relative humidity and nutrient solution temperature (°C) were measured using a data logger (Hobo). Thermal time was calculated using base temperature for basil (T_{base}=10.9°C),

A. Plant growth

Fresh and dry, aerial and root plant weight (g.plant^{-1}), number of leaves, root density (g.cm^{-3}), plant height (cm), absolute growth rate (AGR g.d^{-1}), relative growth rate (RGR g.g^{-1}.d^{-1}) and yield (g. m^{-2}) were measured throughout the cycle.

B. Identification and quantification of volatile oils

Oil extraction. Essential oil analysis was carried out with leaves harvested on two harvest dates with an interval of 37 days between them for both genotypes. Basil samples were collected during a period of 92 days in the autumn-winter season and two harvests were made with a pruning between them: sample 1 (5 days after transplant), sample 2 (13 days after transplant), sample 3 (21 days after transplant), sample 4 (29 days after transplant), sample 5 (first harvest and 50 days after transplant), pruning, sample 6 (second harvest and 92 days after transplant).

Fresh leaf material (250 g per sample) was subjected to a 2-h water distillation using a Clevenger type apparatus where material and distilled water where located. A refrigerant attached to the distillation balloon allowed accumulation and separation of the essential oil from the condensed mixture. The oils obtained were dried over anhydrous sodium sulfate.

Identification of volatile oils. The essential oils were analyzed by CG-FID-MS, with Perkin Elmer GC equipment model Clarus 500. Chromatograph operating conditions with CG-FID-MS were: Helium as a carrier gas at a constant flow rate of 1.87 ml/min, and an auto sampler connected to an injector split (Split rate: 1:100) in turn connected to a flux divisor of two fused silica capillary column (polar and no polar). The temperature parameters were T. initial: 90°C; ramp (3°C /min); T. final: 225°C (15 min); T. injector: 255°C; T. detector: 275°C; final run time 70 min; mass range scanned 40-400 m/z. The injected samples consisted of 0.2 µl in a dilution of 10% ethanol.

The components of the essential oil were identified by comparing their retention times obtained from the two columns of different polarity with those of authentic samples and/or data in the literature, and comparison with the mass spectra in the database of the Pharmacognosy Department of the University of Buenos Aires and other commercial sources. The relative percentage amounts of the volatile oil constituents were evaluated from total peak area (TIC).

Statistical analysis. The experiment was conducted in a complete randomized block design repeated over time, with three replications. The treatments for growth stage were green genotype and purple genotype. The treatments for essential oil analysis were according to harvest date (first and second) and genotype: green genotype, 92 days after transplant (DAT); purple genotype, 92 DAT; green genotype, 50 DAT; purple genotype, 50 DAT. Data was analyzed by ANOVA and means were compared by Tukey test at the 0.05 probability level.

3. Results and Discussion

Plant growth

Fresh and dry aerial weight. The aerial fresh weight was significantly different between genotypes ($p<0.0001$) and harvest dates ($p<0.0001$). On the third sample date, the differences between genotypes began to be greater. On the fifth sample date the difference for green genotype climbed up to a 47%, a result that coincided with other authors who obtained 91% more biomass for green genotypes than purple (Neikin and Schuch, 2010).

The data from the present study contrast with a crop

grown in optimum season, as reported by some authors for green basil with values between 64.44 and 110.33 g.plant^{-1} (Benito and Chiesa, 2000; Carrasco *et al.,* 2007) and fresh weights of 57.84 g.plant^{-1} and 41.50 g.plant^{-1} for purple basil (Krizaj, 2010). Differences in aerial dry weight between genotypes ($p<0.0001$) and date of harvest ($p<0.0001$) were significant. Aerial dry weight followed the same trend as aerial fresh weight (Fig. 1). The values recorded for 50 DAT (Table 1) were lower than those registered in the same productive system in optimum season, with 10% fewer days of cycle; green and purple genotype with 6.72 g.plant^{-1} and 4.26 g. plant^{-1} (Krizaj, 2010) respectively. An increase of 92% and 184% compared to values reported in this experiment.

Fresh and dry root weight. There were statistical differences in fresh and dry root weight (Table 1) between genotypes (p_{fresh}= 0.0007 and p_{dry} = 0.0024) and date of harvest ($p_{fresh}<0.0001$ and $p_{dry}<0.0001$).

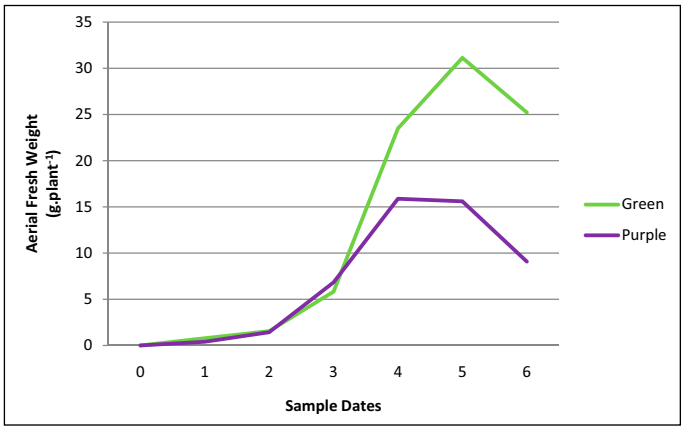

Fig. 1 - Fresh aerial weight (g.plant^{-1}) over the whole growth cycle (5th sample date = first harvest; 6th sample date = second harvest) for both genotypes.

Table 1 - Growth parameters for both genotypes 50 days after transplant. Average values and standard error

Growth parameters (g.plant^{-1})	Genotype	
	Green	Purple
Aerial fresh weight	31.14±0.23	16.5±2.18
Root fresh weight	18.13±0.23	6.5±1.80
Aerial dry weight	3.5±0.01	1.5±0.10
Root dry weight	0.7±0.02	0.43±0.12

Aerial and root dry matter

Aerial dry matter differed significantly between genotypes ($p=0.0339$) and sample dates ($p=0.0169$). The difference increased greatly for green basil at the third sample date. At the first harvest (50 DAT), dry matter percentage for purple basil was 19% lower than the green basil value (Table 2). The data obtained was similar to other authors' results, in protected environments and soilless systems, for green (11.67%) and purple basil (9.85%) (Cenóz and Burgos, 2010). These authors also concluded that dry matter (%) was effectively reduced in protected environment systems compared with other systems (Cenóz and Burgos, 2010). However, other experiments did not show significant differences in dry matter between genotypes in optimum season for NFT system (Krizaj, 2010). The present study was carried out in autumn-winter and both genotypes performed differently which would suggest a noticeably genotypic effect when the season is not optimal. Root dry weight was not significantly different between dates of harvest and genotypes.

Table 2 - Aerial and root dry matter values 50 days after transplant for both genotypes. Mean values and standard error

Genotype	Areal dry matter (%)	Root dry matter (%)
Green	11.24±0.1	3.86±0.05
Purple	9.26±1.92	7.33±3.68

Root density, plant height, leaf number and leaf apparition rate

Root density was significantly different between genotypes ($p<0.0001$) and sample dates ($p<0.0001$). Root density was markedly higher in purple basil at all sample dates except sample date 2, however root weight (dry and fresh) were not higher due to the high content of water and the lower percentage of dry matter. Significant differences were detected for plant height values between sample dates ($p<0.0001$) and genotypes ($p<0.0115$). From the third sample date, plant height was markedly higher for green basil until the end of the study. In optimal season studies (Benito and Chiesa, 2000) with similar growing cycle duration, taller plants were obtained with 133% more height in 56 days (79.8 cm). Leaf number also showed significant differences between sample dates ($p<0.0001$), however this difference was not significant between genotypes. In a shorter cycle (10% fewer days) in optimum season, 124% more leaves were obtained (120 leaves.plant^{-1})(Krizaj, 2010); 208% more leaves.plant^{-1} in NFT in greenhouse (Carrasco *et al.,* 2007). Parameter values are presented in Table 3. Leaf apparition rate 50 DAT was 1.074 leaves.day^{-1} for green and 21% lower for purple basil (0.84 leaves.day^{-1}).

Absolute and relative growth rates

Green basil maintained a higher absolute growth rate (AGR) over almost all the cycle period, and reached the maximum (1.86 g.day^{-1}) on the fourth sample date (29 DAT) as did purple basil (0.74 g.day^{-1}). On the contrary, the highest relative growth rate (RGR) for both genotypes was reached with the first sample date (0.2 g.g^{-1}.d^{-1}). Both genotypes presented a downward trend over the study pe-

Table 3 - Growth parameters at each sample date for both genotypes. Mean values and standard error

Parameter	Genotype	1	2	3	4	5
Root density (g.cm^{-3})	Green	3.7±0.6	3.1±1.7	1.1±1	1.1±5	1.2±1.1
	Purple	4.4±0.0	2.9±1.3	1.2±4.3	1.3±0.01	1.2±1
Height (cm)	Green	10.6±0.4	7.7±1.5	13.7±3.5	25.6±3.8	34.3±1.2
	Purple	10.1±1.6	9.8±1	15.7±2.5	22.5±0.5	23.3±0.5
Leaves Number	Green	4±0.5	11±3.6	25±4.7	44±10.9	54±1.5
	Purple	5±1.1	10±3.7	21±11.9	43±1.5	42±1.7

riod (Figs. 2 and 3). Results obtained 50 DAT were notably low, supporting another author's findings (Krizaj, 2010). As expected, plant response to decreasing radiation and temperature was translated into lower parameter values in comparison with those of an optimal season. In general, until the fourth sample date, temperature and radiation allowed moderate photosynthesis and growth. From the beginning of the study, radiation declined 50% (from 406 W.m^{-2} to 197 W.m^{-2}).

Pruning between the first and second harvest stressed plants, however it caused a different effect in purple basil, which had a slower regrowth compared with the green variety. Purple genotype showed a lower leaf apparition rate

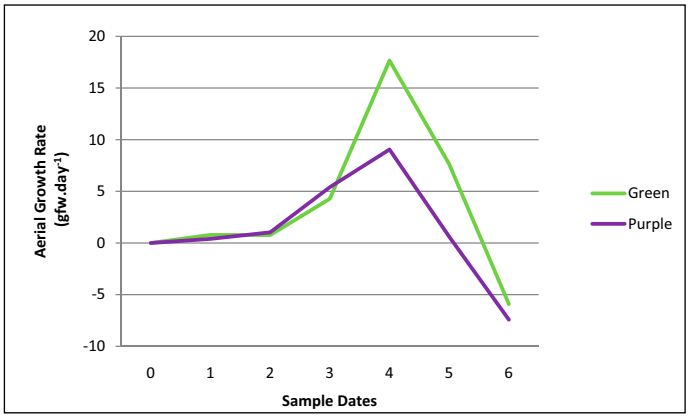

Fig. 2 - Absolute growth rate (AGR) for aerial fresh weight (g fw.day^{-1}) for both genotypes during the cycle. Values represent the mean.

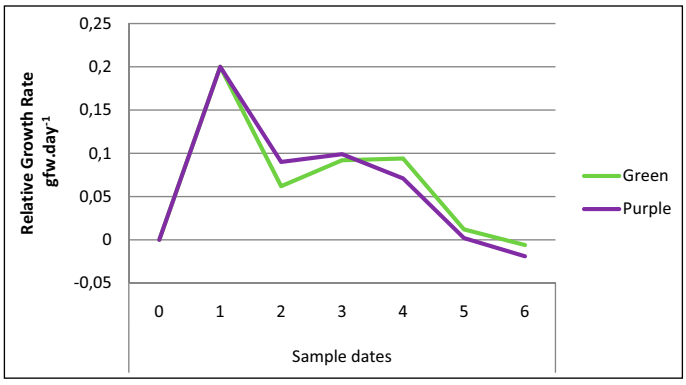

Fig. 3 - Relative growth rate (RGR) for aerial fresh weight (g fw.g^{-1}. day^{-1}) for both genotypes during the cycle. Values represent the mean.

than the green genotype during the cycle. This was added to the adverse environmental conditions after pruning, which led to a slower recovery. Average temperature during the study was inferior to optimal for the species and showed a downward trend, furthermore it was outside the range for maximum dry matter accumulation. The data revealed a genotypic effect in growth response in the autumn-winter season that does not occur in an optimal season (Krizaj, 2010). The contrast in behavior between genotypes was noted when environmental conditions began to be adverse. Relative humidity, nutritive solution temperature, electrical conductivity and nutritive solution pH did not show great variations that could influence growth parameters. Although thermal time 50 DAT was 50% lower than thermal time achieved in optimal season for the same duration and crop conditions (Krizaj, 2010), it was possible to grow basil under protection in the autumn-winter season.

Biomass yield: descriptive analyses

Biomass yield for green basil, 50 DAT, was 778.5 g.m^{-2} and 412.5 g.m^{-2} for purple basil. In contrast, greater values were obtained in an optimal season in open field, with yields between 1000 and 1500 g.m^{-2} for green genotype (Gill and Randhawa, 1996), 29% and 92% more respectively than found in the present investigation. Also, in greenhouse and NFT system and in optimal season, 86% and 151% more biomass yield was obtained for green and purple basil respectively (Krizaj, 2010). At the fifth sample date (first harvest) fresh weight yield per square meter was 778.5 g.m^{-2} for green basil and 47% lower (412.5 g.m^{-2}) for purple basil. At the second harvest date, after pruning, green and purple genotype yielded 630.5 g.m^{-2} and 227 g.m^{-2} respectively.

Volatile oil analysis

Essential oil composition and genotype effect. GC-MS analyses identified 32 aromatic compounds in green and 30 aromatic compounds in purple basil. In both cases the identified compounds account for 94% of the total. The composition is expressed relative to 100%, as each peak has an area and the total of areas is 100%. The essential oils from *O. basilicum* show significant differences between genotypes: linalool (p= 0.0001), eugenol (p=0.0457), methyl- eugenol (p=0.0001), alpha transbergamotene (p= 0.0251), 1.8 cineol (p= 0.0113) and tau cadinol (p= 0.0253). The other components were not consid-

ered in the statistical analyses as they were only detected in some samples and with extremely low values. Values for the main compounds are shown in Figure 4.

Date of harvest effect

The mean percentages of linalool (p=0.0001) and methyl-eugenol present significant differences between dates of harvest (p=0.0019) whereas eugenol, alpha trans-bergamotene, 1.8 cineol and tau cadinol values did not show significant variation between dates.

The mean contents for linalool and methyl eugenol for both genotypes varied as follows: linalool from 41.8% at the first harvest date to 25.7% at the second; methyl-eugenol from 5.78% at the first harvest date to 18.78% at the second. Linalool significantly decreased at the second harvest while methyl eugenol increased, however the content was lower than linalool as seen in figures 5 and 6.

Different radiation led to essential oil variations. It could be that the highest radiation level before the first harvest led to higher rates of linalool. Radiation decreased over the period of the experiment: at the second harvest date lower levels of methyl- eugenol were found. This might be explained by the fact that in this moment radiation was lower. Unlike other authors' findings (Chang *et al.,* 2008), in this study no differences in eugenol values between dates of harvest were found. Temperature influences aromatic compounds and metabolic activity of plants. The effect of temperature at 25°C, in which the highest contents of linalool, 1.8 cineol and eugenol are obtained, was reported (Chang *et al.,* 2005). Although is known that geranial pyrophosphate is precursor of both linalool and 1.8 cineol, and the enzymes linalool synthetase and 1.8 cineol synthetase were identified, the environmental effects on the enzymes activity is not clear at the moment. (Chang *et al.,* 2005). In this study, linalool percentages in the two successive harvests (36.6% and 22.7%) were higher than values reported by other authors in hydroponic systems (Fernandes *et al.,* 2004). Higher radiation and moderate temperatures before the first harvest date might explain the higher content of linalool for both genotypes.

Eugenol is metilated to methyl- eugenol by the enzyme eugenol-O-methyl-transferase (Lewinsohn *et al.,* 2000;

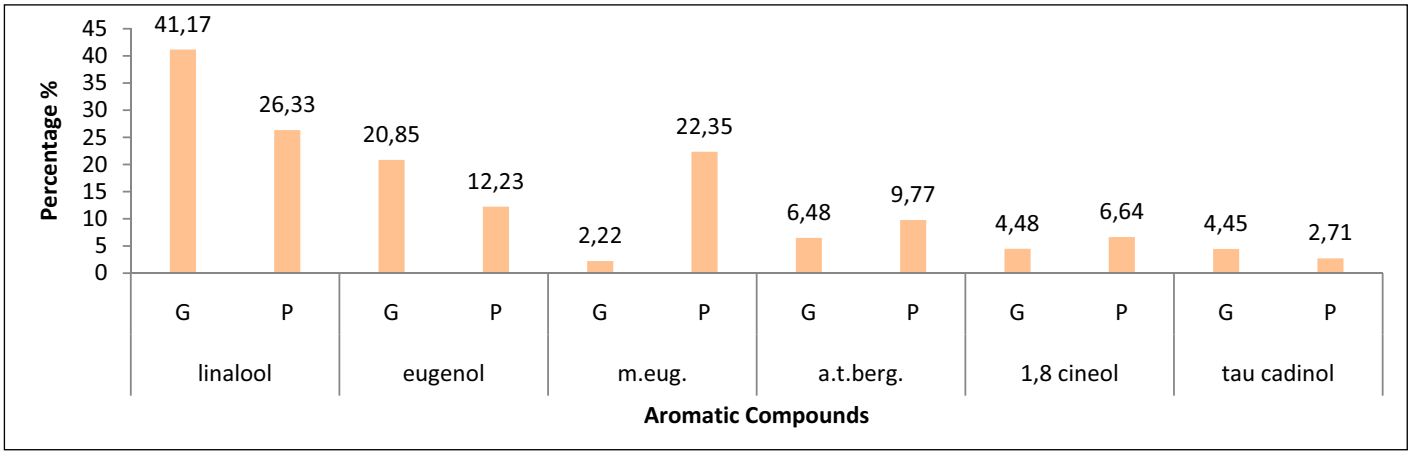

Fig. 4 - Percentage composition of the main aromatic compounds of basil essential oil for both genotypes (G= green, P= purple). Mean value of the two harvest dates (Methyl eugenol= m.eug, Alpha-trans-bergamotene= a.t.berg.).

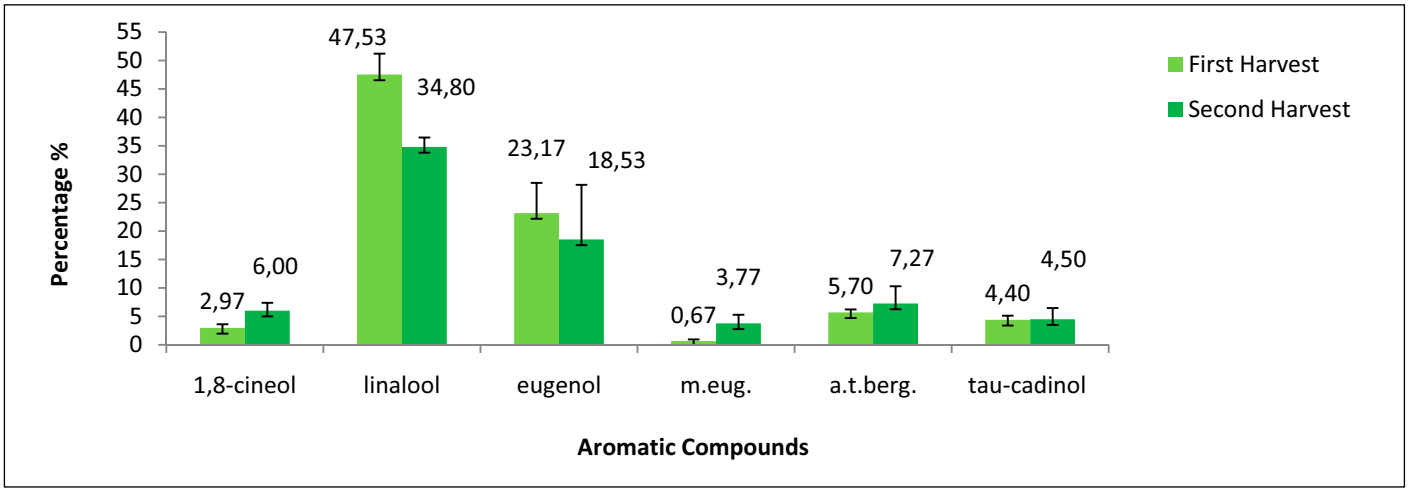

Fig. 5 - Percentages (relative to total in essential oil= 100%) of the main aromatic compounds of basil essential oil for green genotype at first and second harvest. Mean values ± standard error (Methyl eugenol= m.eug, Alpha-trans-bergamotene= a.t.berg.).

Robison and Barr, 2006). Methyl- eugenol is in the group of the alikobenzenes together with iso-eugenol, eugenol, estragol and safrol, which are considered carcinogenic following the tested effects on rats and mice after intake of high doses. The effect in human beings generates certain concern, however the doses at which humans are exposed through diet (mainly via intake) are very low (Robison and Barr, 2006).

Methyl- eugenol content is related to vegetal tissue age. Greater enzyme activity in young leaves was reported (Lewinsohn *et al.,* 2000). This supports other authors who state that enzyme activity is significantly greater in young and developmental leaves than in mature leaves, because as the leaf develops it produces glandular trichomes with high levels of this enzyme (Gang *et al.,* 2002). In young leaves there are more trichomes per unit area before cell expansion (Gang *et al.,* 2001) but when glands reach maturity, enzyme levels decrease. In totally mature plants of *O. basilicum* var. Genovese methyl- eugenol was not found (Marotti *et al.,* 1996). In mature hydroponic plants in greenhouse a percentage similar to this study was found for *O. basilicum* var. Genovese (0, 6% methyl-eugenol in leaves) (Hassangpouraghdam *et al.,* 2010).

In the present investigation, agronomic techniques (pruning) and environmental conditions may have influenced the content of this compound. Pruning after the first harvest decreased sinks, caused regrowth and the development of abundant young tissue, which might have influenced the rise in glandular trichome density, and as a consequence, the higher amount and activity of the eugenol-O-methyl-transferase

At the sixth sample date (second harvest), methyl-eugenol content increased in the essential oil of both genotypes. The increase was remarkably higher in purple basil (33.80%). This result could be due to the slower regrowth of this variety, with a lower foliar apparition rate throughout the cycle (at sixth sample date foliar apparition rate was 1.01 leaves.day^{-1} in purple basil and 1.52 leaves.day^{-1} in green basil) which led to a greater amount of young and developmental leaves with elevated density of trichomes and eugenol-O-methyl-transferase enzyme activity.

4. Conclusions

The agronomic performance of green basil was greater throughout the growing cycle. This genotype showed greater fresh and dry weight (aerial and root), dry matter percentage, number of leaves, plant height and total biomass yield than purple basil. The environment could have influenced agronomic performance and volatile oils composition. Hence, it can be concluded that variations in aromatic compounds and the productive behavior were affected by temperature and radiation.

Differences in volatile oils between dates of harvest and genotypes was clear. At the fifth sample date (first harvest) linalool prevailed in green and purple basil, but in green basil it was significantly higher. At the sixth sample date (second harvest) methyl- eugenol content increased in both genotypes but the increase was markedly higher in purple basil. Pruning after the first harvest might have promoted sprouts, but in purple basil it also might have decreased yield because regrowth after the cut is more difficult for this variety. This difficulty was also enhanced by the environmental conditions (sub-optimal radiation and temperatures). It is also concluded that this agronomic technique could have altered the methyl- eugenol content.

References

BENITO A.P., CHIESA A., 2000 - *Parámetros fisiológicos y productivos en cultivares de albahaca (*Ocimum basilicum *L.).* - FAVE, 14(1): 19-28.

CARRASCO G., IZQUIERDO J., 1996 - *La empresa hidropónica de mediana escala: la técnica de la solución nutritiva recirculante ("NFT").* - Ed. Univ. de Talca, Talca, Chile, pp. 62.

CARRASCO G., RAMÍREZ P., VOGEL H., 2007 - *Efecto de la conductividad eléctrica de la solución nutritiva sobre el*

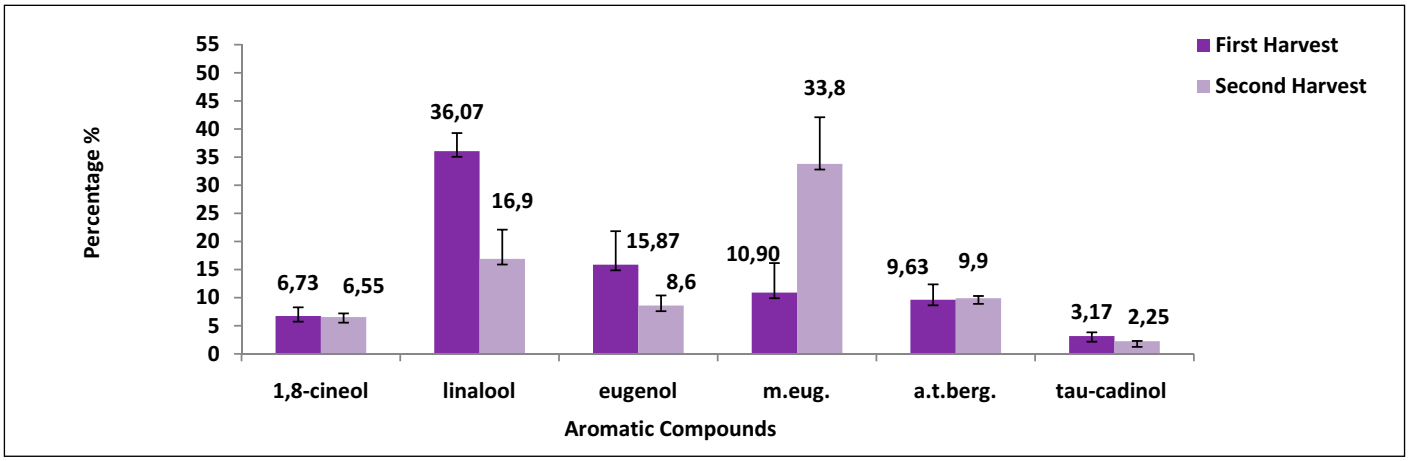

Fig. 6 - Percentages (relative to total in essential oil= 100%) of the main aromatic compounds of basil essential oil for purple genotype at first and second harvest. Mean values ± standard error (Methyl eugenol= m.eug, Alpha-trans-bergamotene= a.t.berg.).

rendimiento y contenido de aceite esencial en albahaca cultivada en NFT. - Idesia, Arica, 25(2): 59-62.

CENÓZ P., BURGOS A., 2010 - *Influencia de la fertilización nitrogenada en el rendimiento de la albahaca (*Ocimum basilicum *L.).* - Boletín Hortícola, Fac. CS. Agr. Y Forestales, Univ. Nac. de La Plata, 8(27): 4-7.

CHANG X., ALDERSON P.G., WRIGHT C., 2005 - *Effect of temperature integration on the growth and volatile oil content of basil (*Ocimum basilicum *L.).* - J. Hort. Sc. Bio., 80(5): 593-598.

CHANG X., ALDERSON P.G., WRIGHT C., 2008 - *Solar irradiance alters the growth of basil (*Ocimum basilicum *L.) and its content of volatile oils*. - Env. Exp. Bot., 63(1-3): 216-223.

DARRAH H.H., 1980 - *The cultivated basils*. - Buckeye Printing, Independence.

FERNANDES P.C., FACANALI F., TEIXEIRA J.P.F., FURLANI P.R., MARQUES M.O.M., 2004 - *Cultivo de manjericão em hidroponia e em diferentes substratos sob ambiente protegido*. - Hort. Bras., Brasília, 22(2): 260-264.

FISCHER R., NITZAN N., CHAIMOVITSH D., RUBIN B., DUDAI N., 2011 - *Variation in essential oil composition within individual leaves of sweet basil* (Ocimum basilicum *L.) is more affected by leaf position than by leaf age*. - J. Agric. Food Chem., 59(9): 4913-4922.

GANG D.R., LAVID N., ZUBIETA C., CHEN F., BEUERLE T., LEWINSOHN E., JOSEPH N., PICHERSKY E., 2002 - *Characterization of phenylpropene O-methyltransferases from sweet basil.* - Plant Cell, 14(2): 505-519.

GANG D.R., WANG J., DUDAREVA N., NAM H.K., SIMON J.E., LEWINSOHN E., PICHERSKY E., 2001 - *An investigation of the storage and biosynthesis of phenylpropenes in sweet basil.* - Plant Phys., 125(2): 539-555.

GILL B., RANDHAWA G., 1996 - *Effect of different transplanting dates and harvesting stages on the quality of French basil oil.* - J. Herbs, Spices Med. Plants, 4(3): 35-42.

HASSANPOURAGHDAM M.B., GOHARI R.G., TABATABAEI J.S., DADPOUR R.M., 2010 - *Inflorescence and leaves essential oil composition of hydroponically grown* Ocimum basilicum *L.* - J. Serbian Chem. Soc., 75(10): 1361-1368.

HOCHMUTH R.C., LEON L.L., 1999 - *Evaluation of six basil cultivars grown in a vertical hydroponic production system inside a greenhouse.* - Univ. of Florida, FLO, NFREC-SV Research Report, 99-06, p. 2.

KRIZAJ C., 2010 - *Comportamiento agronómico-productivo de albahaca (*Ocimum basilicum *L.) verde y morada para consumo en fresco*. - Facultad de Agronomía Universidad de Buenos Aires, Buenos Aires, Trabajo de intensificación para acceder al título de Ingeniero Agrónomo, p. 25.

LEWINSOHN E., ZIV-RAZ I., DUDAI N., TADMOR Y., LASTOCHKIN E., LARKOV O., 2000 - *Biosynthesis of estragole and methyl-eugenol in sweet basil (*Ocimum basilicum *L.): developmental and chemotypic association of allylphenol O-methyltransferase activities.* - Plant Science, 160(1): 27-35.

MAROTTI M., PICCAGLIA R., GIOVANELLI E., 1996 - *Differences in essential oil composition of basil (*Ocimum basilicum *L.) Italian cultivars related to morphological characteristics.* - J. Agric. Food Chem., 44(12): 3926-3929.

NEIKIN J.B., SCHUCH U.K., 2010 - *Retractable roof greenhouse production of basil (*Ocimum basilicum *and Lemon Grass (*Cymbopogon citrates*) in a Semi-Arid Climate).* - Acta Horticulturae, 659: 113-120.

PUTIEVSKY E., 1983 - *Temperature and day length influences on the growth and germination of sweet basil and oregano.* - J. Hort. Sc., 58(4): 583-587.

RESH H.M., 2001 - *Cultivos hidropónicos. Nuevas técnicas de producción.* - Mundi-Prensa, Madrid, España.

ROBISON S.H., BARR D., 2006 - *Use of biomonitoring data to evaluate methyl eugenol exposure.* - Env. Health Persp., 114(11): 1797-1801.

SAJJADI E.S., 2006 - *Analysis of the essential oils of two cultivated basil (*Ocimum basilicum *L.) from Iran*. - DARU, 14(3): 128-130.

SUPPAKUL P., MILTZ J., SONNEVELD K., BIGGER S.W., 2003 - *Antimicrobial properties of basil and its possible application in food packaging.* - J. Agric. Food Chem., 51(11): 3197-3207.

Adv. Hort. Sci., 2013 27(4): 173-181

Post-storage quality and physiological responses of tomato fruits treated with polyamines

J. Javanmardi[(1)], M. Rahemi, M. Nasirzadeh
Department of Horticultural Sciences, College of Agriculture, Shiraz University, Shiraz, Iran.

Key words: chilling injury, electrolyte leakage, fruit quality, vitamin C.

Abstract: Two greenhouse F1 tomato cultivars, M19 and M79, were grown hydroponically and the mature green fruits were harvested and subjected to eight polyamine (PA) treatments including 1 and 2 mM putrescine (Put), spermidine (Spd) and their combination before being placed at 3°C for 15 and 25 days. Electrolyte leakage, weight loss, fruit firmness, decay percentage, chilling injury index, titratable acidity, total soluble solid content and ascorbic acid content were then measured after keeping at 20°C for 3 days and compared to control. The Put:Spd (2:2 mM) treatment decreased electrolyte leakage (over 50%), chilling injury index and fruit decay percentage. Combinations of PAs caused greater total soluble solids and greater effect on decreasing weight loss during storage when compared to their sole PA application. PAs caused a net increase in fruit firmness during post-harvest life. Titratable acidity increased with increasing duration of low temperature storage for all treatments. Ascorbic acid in fruits stored at low temperature for 25 days was greater than those stored for 15 days. The effects of exogenous PAs on reducing chilling-related disorders decreased with time. Correlations among weight loss, electrolyte leakage, chilling injury, decay percentage and fruit firmness during low temperature storage were positive and significant, but they were non-significant or significantly negative when compared against ascorbic acid, titratable acidity and TSS.

1. Introduction

Tomato as a plant indigenous to tropical regions is susceptible to chilling injury when subjected to low temperature storage (Saltveit, 2001). Chilling injury limits tomato storage life and leads to significant degradation of fruit quality and decreases the market value. It can increase membrane permeability, and a resultant increase in leakage of ions from cell membrane, surface pitting, susceptibility to decay and diseases, weight losses, abnormal ripening, change in respiration, ethylene production and senescence. Chilling injury symptoms mainly develop during shelf life following cold storage (Candan *et al.*, 2007).

Numerous attempts, such as breeding for increased chilling tolerance, genetic engineering, modifying crop management practices and application of chemicals, have been made to increase chilling tolerance and avoid chilling injury (Baninasab, 2009). Chilling alleviation in fruits and vegetables has been attributed to several factors including accumulation of polyamines, nitric oxide and proline (Aghdam and Bodbodak, 2013).

Over the years, studies have shown the involvement of polyamines (PAs) in a wide array of processes in plants, ranging from triggering organogenesis to protecting against stress (Walden *et al*., 1997). PA accumulation occurs under abiotic stresses including drought, salinity, extreme temperatures, UV-B, heavy metals, mechanical wounding and herbicide treatment (Hussain *et al*., 2011). Amongst different kind of PAs, the diamine putrescine (Put), triamine spermidine (Spd), and tetramine spermine (Spm) are the most common PAs in plant cells, while others are of more limited occurrence (Galston and Sawhney, 1990; Valero *et al*., 2002). Distribution of these biogenic amines differs between species with Put and Spd being particularly abundant and Spm the least abundant in plant cells. These amines are important for cell viability and their intracellular levels are tightly regulated, making it difficult to characterize individual effects of Put, Spd and Spm on plant growth and developmental processes (Mattoo *et al.*, 2010).

It has been reported that exogenous PA application leads to an inhibition of ethylene emission rate in the climacteric fruits (Valero *et al*., 2002) and delays ripening and fruit abscission (Paksasorn *et al*., 1995). On the other hand, PAs are precursors of many important secondary metabolites and changes in PA biosynthesis appear to have a reciproca1 effect on ethylene biosynthesis (Walden *et al.*, 1997). Accumulation of Put in tissues seems to be a general response of plants to chilling temperatures (Faust and Wang, 1992). Accordingly, we hypothesized that exogenous application of PAs may have effects on the postharvest physiology of fruits, especially on chilling tolerance. An experiment was arranged to study the effects of exogenous PA application

[(1)] Corresponding author: javanm@shirazu.ac.ir

Received for publication 4 January 2014
Accepted for publication 7 March 2014

on the level of chilling injury, ripening, shelf life and some quality factors of tomato fruit (as a model plant) after a period of low temperature storage. Another goal of the study was to determine which polyamine, concentration and/or their combination is more effective. Two greenhouse tomato cultivars were selected to evaluate whether PAs play the same role in different cultivars or not.

2. Materials and Methods

Plant material and polyamine treatments

F1 hybrid seeds of tomato (*Solanum lycopersicom* L.) cultivars M19 and M79 (Tropica Seeds, IndoSem Ltd. India) were sown in 30 × 30 × 60 mm/cell plastic plug trays filled with peat and perlite (1:1 v:v). Seedlings were grown for five weeks in a polycarbonate greenhouse (25/21°C and 60-70% relative humidity). Plugs were then transplanted into 12-l pots filled with a peat:perlite (60:40 v/v) mixture. Pots were kept in the same greenhouse with an average 60% relative humidity and 25±5°C temperature until the end of the experiment. Cultural practices consisting of standard recommendations for growing tomato seedlings and plants in a hydroponic system were according to Papadopoulos (1991). Fruits for study were harvested at mature green stage based on the "Color Classification Requirement in United States Standards for Grades of Fresh Tomatoes" chart, published by the USDA.

Eight polyamine treatments consisted of immersing same-sized, selected mature green fruits in 1 and 2 mM putrescine (Put), spermidine (Spd) and their combination for 4 min as indicated by Mirdehghan *et al.* (2007 a). Immersion in distilled water for the same period was taken as control (Table 3). Fruits were then held at 3°C in a temperature-controlled chamber in darkness with relative humidity of 90%. After 15 and 25 days, five fruits from each treatment replicate were sampled and stored at 20°C for 3 days. Electrolyte leakage, weight loss, fruit firmness, decay percentage, chilling injury index, titratable acidity, total soluble solid content (TSS) and ascorbic acid content of fruits were measured as described below.

Electrolyte leakage

Electrolyte leakage was used to assess membrane permeability. The procedure was based on Lutts *et al*. (1996) with slight modification. Briefly, five tomato fruit pericarp discs (10 mm diameter) per replicate from randomly chosen fruits were taken and placed in test tubes containing 10 mL of distilled water followed by three washes with distilled water to remove surface contamination. Samples were incubated at room temperature on a shaker for 24 h. Electrical conductivity (EC) of the bathing solution (EC1) was read after incubation. The samples were then placed in a boiling water bath for 20 min and the second reading (EC 2) was determined after cooling the solution to room temperature. The electrolyte leakage was calculated using EC1/EC2 and expressed as a percentage. All leakage data were expressed as a percentage of the total electrolyte readings.

Weight loss

Weight loss during postharvest storage of individual replicates was determined by subtracting sample weights on sampling dates (day 15 or 25 of low temperature storage) from their initial weight on day 0 and presented as percent of weight loss compared to initial weight.

Fruit firmness

Fruit firmness was determined according to Ben-Yehoshua *et al*. (1983). A compression tester using a 1 kg weight centered over a locule on the equatorial region of each tomato fruit was used. Full deformation was measured 30 s after exerting the force on the fruit, then the weight was removed and residual deformation was measured 15 s later. Lower readings denoted firmer fruit. Five fruits were measured for each treatment replicate. Firmness was expressed as mm deformation.

Chilling injury index

Chilling injury (CI) was evaluated according to Ding *et al*. (2002) at 20°C for 3 days, following 15- and 25-day low temperature storage period. Briefly, tomato fruit surface pitting was considered as CI symptom. The severity of CI symptoms was assessed visually according to a four-stage scale: 0= no pitting; 1= a few scattered pits; 2= pitting covering up to 5% of the fruit surface; 3= extensive pitting covering 5-25% of the fruit surface, and 4= extensive pitting covering more than 25% of the fruit surface. The average extent of CI damage was expressed as a CI index and calculated using the following formula:

$$CI\ index = \frac{\sum(\text{injury classification level} \times \text{number of fruit at that level})}{\text{total number of friut at the treatment}}$$

Decay percentage

Extent of decay was assessed according to González-Aguilar *et al*. (2000), based on the area of decay and the surface area with microorganisms growing on it. Decay was rated for each replicate of treatments at the end of the 15- and 25-day cold storage periods after an additional 3 days at 20°C. The values were expressed as decay percentage.

Total soluble solids, titratable acidity and ascorbic acid content

Total soluble solids concentrations (TSS) of fruit juice were determined using a digital refractometer (Pal-3, ATAGO Co., Ltd. Tokyo, Japan) at 20°C and presented as °Brix. Fruit juice titratable acidity (TA) was determined by titration of 1 mL juice in 25 mL distilled water with 0.1N NaOH until the pH reached 8.1, according to El Ghaouth *et al*. (1992). Results were expressed as gram of citric acid equivalent per 100 g fresh weight (g CAE/100 g fw).

Fruit ascorbic acid content was determined according to the method described by the AOAC (1984).

Statistical analysis

The experiment was arranged in a completely random-

ized design. Each polyamine treatment consisted of three replicates; each replicate consisted of 10 plants. Data were analyzed separately for tomato cultivars and days of low temperature incubation using one-way analysis of variance. Means for each low temperature storage duration and cultivar were compared separately using the least significant differences (LSD) test at p≤0.01. All data analyses including correlation analysis were performed using SPSS21 (SPSS Inc., Chicago, IL) computer software for Windows.

3. Result and Discussions

One-way analysis of variance showed significant differences for all measured characteristics in both cultivars except for ascorbic acid content in cv. M79 after 15 days of low temperature storage (Table 1 a, b, and Table 2 a, b).

Electrolyte leakage

Application of PAs decreased electrolyte leakage of low-temperature incubated tomato fruits. The highest

Table 1 a - One way analysis of variance for characteristics of polyamine-treated tomato cultivar M19 after 15 and 25 days storage at 3°C

SOV	df	Mean Squares							
		Electrolyte leakage		Weight loss		Fruit firmness		Chilling injury	
		15	25	15	25	15	25	15	25
Polyamines	8	88.692**	163.37**	5.193**	3.055**	0.755**	0.87**	114.593**	65.167**
Error	18	3.751	3.731	0.161	0.283	0.051	0.047	3.625	16.759
Total	26	29.887	52.848	1.709	1.136	0.268	0.3	37.767	31.654

ns, *, ** non-significant and significant at 0.05 and 0.01, respectively.

Table 1 b - One-way analysis of variance for characteristics of polyamine-treated tomato cultivar M19 after 15 and 25 days storage at 3°C

SOV	df	Mean Squares							
		Decay percentage		TSS		Titratable acidity		Ascorbic acid	
		15	25	15	25	15	25	15	25
Polyamines	8	370.37 **	902.759 **	0.832 **	0.592 **	0.028 **	0.033 **	1.697 **	15.319 **
Error	18	37.037	65.852	0.167	0.079	0.002	0.003	0.185	0.999
Total	26	139.601	323.362	0.369	0.236	0.01	0.012	0.650	5.406

ns, *, ** non-significant and significant at 0.05 and 0.01, respectively.

Table 2 a - One-way analysis of variance for characteristics of polyamine-treated tomato cultivar M79 after 15 and 25 days storage at 3°C

SOV	df	Mean Squares							
		Electrolyte leakage		Weight loss		Fruit firmness		Chilling injury	
		15	25	15	25	15	25	15	25
Polyamines	8	48.792**	101.07**	4.593**	4.708**	0.564**	0.749**	80.624**	135.995**
Error	18	0.948	0.673	0.034	0.087	0.017	0.046	5.701	10.417
Total	26	15.669	31.564	1.437	1.509	0.185	0.262	28.754	49.056

ns, *, ** non-significant and significant at 0.05 and 0.01, respectively.

Table 2 b - One-way analysis of variance for characteristics of polyamine-treated tomato cultivar M79 after 15 and 25 days storage at 3°C

SOV	df	Mean Squares							
		Decay percentage		TSS		Titratable acidity		Ascorbic acid	
		15	25	15	25	15	25	15	25
Polyamines	8	193.667**	424.833**	0.431**	0.951**	0.021**	0.029**	1.043ns	3.987**
Error	18	13.852	29.667	0.026	0.029	0.003	0.002	0.989	0.822
Total	26	69.179	151.256	0.150	0.313	0.009	0.01	1.005	1.795

ns, *, ** non-significant and significant at 0.05 and 0.01, respectively.

electrolyte leakage was observed in control treatments as almost 100% greater than those treated with the highest PA concentrations in combined treatments on both cultivars (Table 3). Pretreatment of cucumber plants with PAs diminished the increased electrolyte leakage caused by chilling in the leaves (Gill and Tuteja, 2010). The exogenous application of polyamines on pomegranate (*Punica granatum* L.) protected the membrane lipid from being converted from liquid crystalline to a solid-gel state (induced by chilling) through preventing lipid peroxidation (Mirdehghan *et al*., 2007 b). Previously, Put has been reported to act as protective toward cold stress in tomato plants, since reduced cold-induced electrolyte leakage in leaves due to its application was observed (Kim *et al*., 2002). In addition, PA application induced cold acclimation through maintenance of membrane fluidity at low temperatures and reduced electrolyte leakage, skin browning, and thus the severity of CI symptoms (Mirdehghan *et al*., 2007 a).

In general, the effect of Spd on decreasing electrolyte leakage was significantly greater than Put but the differences were not significant when they were applied in combinations (Table 3). In agreement with our results, Gill and Tuteja (2010) found different patterns of Put and Spd action in different cucumber cultivars. Accordingly, it seems the mode of action of PAs may differ within species.

A large amount of evidence showed that exogenous application of PAs plays a role in stabilizing plant cell membranes and protecting them from damage under stress conditions (Liu *et al*., 2007; He *et al*., 2008; Gill and Tuteja, 2010). PAs in their free forms have been described as anti-senescence agents (Valero *et al*., 2002) due to their capacity to preserve membrane stability, which is crucial in plant adaptation to temperature stresses (Oufir *et al*., 2008). Their attachment to membranes by way of phospholipids results in altered patterns of solute permeation through those membranes and decreased fluidity of membrane components (Galston and Sawhney, 1990). They are involved in the regulation of many basic cellular processes, including cellular cation-anion balance and membrane stability (Gill and Tuteja, 2010). In the present experiment, tomato cv. M19 showed greater electrolyte leakage for all PA and low temperature storage treatments compared to cv. M79 (Table 3). Possibly the PAs pattern of action in the studied tomato cultivars was at different rates, as previously found for cucumber cultivars (Gill and Tuteja, 2010).

Increasing the duration of low temperature storage from 15 to 25 days increased electrolyte leakage in both cultivars (Table 3). This could be an indicator that the effects of exogenous PAs on lowering electrolyte leakage decrease with time.

Weight loss

It has been reported that tomatoes at room temperature showed greater weight loss than those stored in cold storage (Javanmardi and Kubota, 2006). At least 50% greater weight loss was found in control treatments compared to those with PA applied in both cultivars and low temperature durations (Table 3). Transpiration has been considered the main cause of weight loss during tomato storage (Javanmardi and Kubota, 2006). Reduction in weight loss and respiration rate due to Put and Spd application in mango has been reported (Malik and Singh, 2005).

The differences between combined PA treatments were not significant in the studied cultivars for the two low temperature durations, however they showed less weight loss when PAs were applied singularly (Table 3).

It has been reported that chilled fruits had a greater weight loss rate than non-chilled fruits after transfer to non-chilling conditions. This is due to the development of microscopic cracks in peel tissue (Cohen *et al*., 1994), cellular breakdown and loss of membrane integrity which have an important role in water exchange through the rind (González-Aguilar *et al*., 2000). Storage conditions or treatments that reduce fruit water loss have been shown

Table 3 - Effect of polyamine application and duration (15 and 25 days) of low-temperature (3°C) storage on electrolyte leakage and fruit weight loss in tomato cultivars M19 and M79

Treatment	Electrolyte leakage				Weight loss			
	M 19		M 79		M 19		M 79	
	15	25	15	25	15	25	15	25
Control	30.06 a	42.43 a	24.40 a	35.64 a	5.48 a	5.22 a	5.27 a	5.87 a
Put 1 mM	19.55 b	24.96 b	15.43 b	20.13 b	3.13 b	3.27 b	2.52 b	3.22 b
Put 2 mM	18.50 b	22.77 bc	14.20 b	19.78 b	2.54 bc	2.90 bcd	2.40 b	2.86 bc
Spd 1 mM	16.43 bc	22.98 bc	14.20 b	19.47 bc	2.40 cd	3.17 bc	2.14 bc	2.96 b
Spd 2 mM	14.93 bc	21.83 bc	13.91 b	17.73 bc	1.92 cde	2.48 bcd	1.90 cd	2.40 cd
Put 1 mM + Spd 1 mM	15.56 bc	21.31 bc	13.13 bc	18.81 bc	1.69 de	2.23 cd	1.59 de	2.20 de
Put 1 mM + Spd 2 mM	14.98 bc	21.99 bc	12.97 bc	17.74 bc	1.61 de	2.13 d	1.61 de	2.00 de
Put 2 mM + Spd 1 mM	11.45 c	18.19 c	10.32 c	17.31 c	1.19 e	2.17 cd	1.19 e	1.93 de
Put 2 mM + Spd 2 mM	13.01 c	18.05 c	11.41 c	17.30 c	1.40 e	1.97 d	1.27 e	1.79 e
LSD value 0.01	4.55	4.54	2.29	1.92	0.94	1.25	0.43	0.69

Means in columns followed by the same letter are not significantly different, $P \leq 0.05$, LSD test. Means for each column were compared separately.

to alleviate CI (Wang, 1993). In our experiment, PA application resulted in less membrane permeability (less electrolyte leakage) and therefore less water loss than control fruits. Also storage duration affected weight loss: the longer fruits remained in low-temperature storage, the greater their weight loss.

Fruit firmness

Fruit firmness was affected by PA application (Table 4). All PA-treated fruits (except for Put 1 mM after 15 days of storage in M19) showed firmer fruit (less compression) than control fruits (Table 4). The differences between combined PA treatments in each low temperature storage duration were not significant. However, when compared to the control they showed at least 43 and 69% greater firmness in M19 tomato, and 80 and 45% in M79 tomato for 15 and 25 days storage, respectively. It has been shown that fruits and vegetables infiltrated with PAs had a net increase in firmness during post-harvest life. This effect of PAs on fruit firmness has been attributed to the cross linking to the COO^- group of the pectic substance and changes in polygalacturonic acids in the cell wall (Valero *et al*., 2002). Retarded fruit softening due to Put and Spd application in mango has been reported (Malik and Singh, 2005). Although it is believed that the overall softening process results from a number of changes in turgor pressure, cell wall and membrane composition and degradation, but cell wall modifications have been implicated to be the major determinant of fruit softening (Smith *et al.*, 2002). Put and Spd have anti-senescence properties (Saftner and Baldi, 1990), and are able to retard the maturation process (including softening) in a wide range of climacteric and non-climacteric fruits (Valero *et al*., 2002). The ethylene production in tomato fruits, enhances softening, but its effect may decrease due to increased polyamine level (Tiecher *et al*., 2013). Increased duration of low temperature storage decreased fruit firmness for all treatments. Fruit firmness of control plants M19 and M79 showed 52 and 56% decrease, respectively, when 15 days of low temperature storage values were compared width 25 days. The values obtained from 2.2 mM, Put:Spd tratment were 108 and 148% for M19 and M79, respectively (Table 4). PAs with higher number of available cations have greater effect on fruit firmness, as Spm+4>Spd+3>Put+2 (Valero *et al*., 2002). According to our results, the PA concentration would also affect the fruit firmness.

Chilling injury index

Chilling injury index drastically increased with time of low temperature storage in both cultivars (Table 4). The increased percentages of 25 days compared to 15 days of low temperature storage for the control treatment were 64 and 102% in M19 and M79, respectively. The combined PA treatments resulted in greater CI index (but less than control) after 25 days of low temperature storage than 15 days, especially when 2:2 mM Put:Spd was used (Table 4). The effects of PA on 15 days low temperature stored tomatoes showed significant decrease in CI index, but its impact for a longer period (25 days) was not significant, except for 1 mM Put (Table 4). The lowest CI index after 15 days of low temperature storage was found in Put:Spm (2:2 mM) treated tomatoes in both cultivars (Table 4). It is possible that the impact of PAs on lowering CI index in tomato are time as well as species dependent. Pre-storage application of PAs improved shelf-life of pomegranate (*Punica granatum* L.) stored at chilling temperature by increasing endogenous polyamine levels (Mirdehghan *et al*., 2007 a). Although increased endogenous Put in tomato due to low temperature storage is considered to act as protective toward cold stress, the reported mechanism is unclear (Gonzalez-Aguilar *et al.*, 1998). The involvement of polyamines in reducing chilling injury has been related to reducing oxidative damage via increases of antioxidant or reducing the activity of oxidative enzyme (Oufir *et al*., 2008).

Table 4 - Effect of polyamine application and duration (15 and 25 days) of low-temperature (3°C) storage on fruit firmness and chilling injury index in tomato cultivars M19 and M79

Treatment	Fruit firmness (mm deformation)				Chilling injury index (%)			
	M 19		M 79		M 19		M 79	
	15	25	15	25	15	25	15	25
Control	2.41 a	3.67 a	2.19 a	3.43 a	19.17 a	31.50 a	16.42 a	33.33 a
Put 1 mM	2.04 ab	2.95 b	1.69 b	2.85 b	16.67 ab	25.67 ab	13.33 ab	20.00 b
Put 2 mM	1.82 b	2.48 c	1.45 c	2.52 bc	15.50 b	24.17 abc	9.17 bc	16.67 bcd
Spd 1 mM	1.88 b	2.49 c	1.54 bc	2.79 b	9.17 c	21.67 bc	10.00 bc	17.50 bcd
Spd 2 mM	1.68bc	2.24 cd	1.40 c	2.37 cd	5.83 c	19.18 bc	7.67 cd	14.17 bcd
Put 1 mM + Spd 1 mM	1.68bc	2.16 cd	1.21 d	2.35 cd	6.67 c	22.50 bc	4.17 def	15.00 bcd
Put 1 mM + Spd 2 mM	1.16 c	1.94 d	1.03 d	2.04 de	1.75 d	15.83 c	2.50 ef	11.67 cd
Put 2 mM + Spd 1 mM	1.18c	1.99 d	1.04 d	2.19 cde	0.75 d	21.33 bc	6.67 cde	14.17 bcd
Put 2 mM + Spd 2 mM	0.92 c	1.92 d	0.78 d	1.94 e	0.42 d	17.67 bc	0.0f	10.83 d
LSD value 0.01	0.53	0.49	0.31	0.37	4.47	9.62	5.61	7.85

Means in columns followed by the same letter are not significantly different, $P \leq 0.05$, LSD test. Means for each column were compared separately

Decay percentage

Decay incidence in all control treatments was significantly greater than in PA-treated fruits (Table 5). Prolonging low temperature storage from 15 to 25 days resulted in greater decay incidence. Although the differences between PA-treated fruits were not significant, the combined PA treatments could be considered more effective since 0% decay decreases possible further contamination and decay. As a susceptible crop to chilling injury, tomato shows increased susceptibility to decay when stored at low temperatures after harvest (Ding *et al*., 2002). In the present study, treatment with Put:Spd (2:2 mM) was very effective in alleviating chilling injury and decreasing the incidence of decay in tomato fruits (Tables 4, 5). This result suggest that PA application enhances the natural resistance of the fruits to chilling injury and decaying agents. Taken together, results obtained in this study indicate that the higher levels of PAs reduce CI and decay of tomato fruit. The reduction in CI symptoms by PAs has been related to their capacity to preserve membrane integrity, both by lowering the membrane phase-transition temperature fluidity and by retarding lipid peroxidation, resulting in increased cell viability (González-Aguilar *et al*., 2000).

Total soluble solids and titratable acidity

In all cases the highest values for TSS were observed in Put:Spd (2:2 mM), however the differences among other PA combinations were also not significant (Table 5). The differences in TSS between 15 and 25 days of low temperature storage were not significant in the two cultivars (data not shown). The earlier experiment showed no significant changes in TSS between room temperature and low temperature stored tomatoes (Javanmardi and Kubota, 2006). It has been reported that TSS remains unchanged after chilling and reconditioning (Luengwilai and Beckles, 2010), however it is possible that individual sugars and the sugar-acid balance may be adversely affected (Beckles, 2012). Harvesting riper fruit (i.e. those with already well-developed sugar profiles) would reduce the harm caused by chilling injury due to lower temperature (Beckles, 2012).

Titratable acidity in combined PA treatments for fruits stored 15 days at low temperature were significantly greater than other treatments in both cultivars. Treated fruits with Put:Spd (2:2 mM) showed the highest titratable acidity after 25 days of storage (Table 6). Titratable acidity increased with increasing duration of low temperature storage for all treatments (Table 6).

It is reported that increased endogenous Pas, spermine and spermidine, through transgenic manipulation of tomato showed similar levels of juice TSS, pH and titratable acidity in transgenic, azygous, and wild-type fruits (Mehta *et al*., 2002). Application of Put and Spd on apricot did not affect TSS and TA (Koushesh Saba *et al.*, 2012).

Ascorbic acid content

All treated fruits showed greater ascorbic acid in fruits stored for 25 days than 15-day low temperature storage group (Table 6). The pattern of changing ascorbic acid level in tomato fruit varies with the physiological ripening stages as it increases slowly reaching a maximum and then declines slowly coinciding with the initiation of ripening, as indicated by color change, and an increase in the activity of ascorbate oxidase (Yahia *et al*., 2001). In this experiment the mature green fruits treated and stored at low temperature showed the same increasing pattern until 25 days of storage. At that time the color had not started to change. Most likely PA application does not change the ascorbic acid pathway at least until ripening symptoms (color change) appear. It is reported that during the ripening process, the levels of Spd and Spm decline

Table 5 - Effect of polyamine application and duration (15 and 25 days) of low-temperature (3°C) storage on fruit decay percentage and total soluble solid content in tomato cultivars M19 and M79

Treatment	Decay percentage				Total soluble solid content (°Brix)			
	M 19		M 79		M 19		M 79	
	15	25	15	25	15	25	15	25
Control	33.33 a	60.00 a	25.00 a	40.00 a	4.01 b	4.28 b	4.80 b	4.13 c
Put 1 mM	13.33 b	16.33 b	9.67 b	9.67 b	4.50 b	4.85 ab	5.18 ab	4.73 bc
Put 2 mM	6.67 b	11.37 b	3.33 b	4.00 b	5.43 ab	5.13 ab	5.43 ab	5.20 b
Spd 1 mM	3.33 b	12.33 b	3.33 b	6.33 b	5.35 ab	5.08 ab	5.25 ab	5.00 bc
Spd 2 mM	0.00 b	10.00 b	2.33 b	5.00 b	5.55 a	5.25 ab	5.33 ab	5.55 ab
Put 1 mM + Spd 1 mM	0.00 b	6.00 b	0.00 b	3.33 b	5.35 ab	5.30 a	5.35 ab	5.50 ab
Put 1 mM + Spd 2 mM	0.00 b	5.00 b	0.00 b	1.67 b	5.60 a	5.50 a	5.73 a	5.63 ab
Put 2 mM + Spd 1 mM	0.00 b	6.67 b	0.00 b	6.67 b	5.28 ab	5.48 a	5.68 a	5.60 ab
Put 2 mM + Spd 2 mM	0.00 b	5.67 b	0.00 b	3.33 b	5.75 a	5.68 a	5.88 a	5.85 a
LSD value 0.01	14.3	19.07	8.74	12.78	0.96	0.66	0.38	0.40

Means in columns followed by the same letter are not significantly different, P≤ 0.05, LSD test. Means for each column were compared separately.

in fruits (Mattoo and Handa, 2008). The ripening process has been shown to be delayed in tomato fruits by infusing them with Put (Saftner and Baldi, 1990). Polyamines and ethylene are known to have opposite effects in relation to fruit ripening and senescence (Saftner and Baldi, 1990). Free polyamines inhibit ethylene production in a variety of tissues (Suttle, 1981) and the elevated level of free polyamines may be responsible for the reduction in both ethylene production and ripening processes of tomato fruits (Saftner and Baldi, 1990). Elevated levels of polyamines help maintain cellular vitality and longer life of ripening tomato (Mattoo *et al*., 2010).

Correlations among fruit characteristics

The correlations among weight loss, electrolyte leakage, chilling injury, decay percentage and fruit firmness for 15 and 25 days of low temperature storage in both cultivars were positive and significant, but they were either non-significant or significantly negative when analyzed against ascorbic acid, titratable acidity and TSS (Tables 7-10). The greatest impact of electrolyte leakage was found on weight loss and decay percentage for the studied

Table 6 - Effect of polyamine application and duration (15 and 25 days) of low-temperature (3°C) storage on fruit juice acidity and ascorbic acid content in tomato cultivars M19 and M79

Treatment	Titratable acidity (g CAE/100 g fw)				Ascorbic acid (mg/100ml)			
	M 19		M 79		M 19		M 79	
	15	25	15	25	15	25	15	25
Control	0.34 b	0.43 c	0.37 bc	0.47 b	9.09 b	11.01 c	11.88 a	12.07 c
Put 1 mM	0.35 b	0.41 c	0.31 c	0.37 c	9.74 b	11.97 bc	10.53 a	12.12 c
Put 2 mM	0.41 b	0.44 c	0.31 c	0.42 c	9.81 b	11.02 c	11.35 a	13.19 bc
Spd 1 mM	0.37 b	0.43 c	0.36 bc	0.39 c	9.60 b	11.97 bc	11.25 a	12.25 c
Spd 2 mM	0.39 b	0.50 b	0.40 bc	0.44 bc	11.01 a	12.44 bc	10.63 a	13.07 bc
Put 1 mM + Spd 1 mM	0.52 a	0.56 b	0.48 ab	0.53 b	10.40 a	13.47 b	11.39 a	15.00 a
Put 1 mM + Spd 2 mM	0.57 a	0.63 a	0.43 abc	0.69 a	11.32 a	17.91 a	12.40 a	15.60 a
Put 2 mM + Spd 1 mM	0.54 a	0.60 a	0.46 ab	0.54 b	11.05 a	12.06 bc	11.88 a	13.62 abc
Put 2 mM + Spd 2 mM	0.58 a	0.70 a	0.56 a	0.65 a	11.80 a	13.60 b	12.15 a	14.03 ab
LSD value 0.01	0.11	0.13	0.13	0.10	1.01	2.35	2.29	2.13

Means in columns followed by the same letter are not significantly different, P ≤ 0.05, LSD test. Means for each column were compared separately.

Table 7 - Correlation analysis between fruit characteristics of tomato cv. M19 treated with different polyamines after 15 days storage at 3°C

	Weight loss	Electrolyte leakage	Chilling injury	Decay percentage	Fruit firmness	Ascorbic acid	Titratable acidity
Electrolyte leakage	0.927 **						
Chilling injury	0.745 **	0.623 **					
Decay percentage	0.769 **	0.803 **	0.694 **				
Firmness	0.753 **	0.761 **	0.741 **	0.699 **			
Ascorbic acid	-0.332 NS	-0.367 NS	-0.258 NS	-0.340 NS	-0.380 NS		
Titratable acidity	-0.679 **	-0.629 **	-0.680 **	-0.555 **	-0.726 **	0.377 NS	
TSS	-0.701 **	-0.719 **	-0.642 **	-0.701 **	-0.618 **	0.083 NS	0.421 **

NS, *, ** non-significant, significant at 0.05 and 0.01, respectively.

Table 8 - Correlation analysis between fruit characteristics of tomato cv. M79 treated with different polyamines after 15 days storage at 3°C

	Weight loss	Electrolyte leakage	Chilling injury	Decay percentage	Fruit firmness	Ascorbic acid	Titratable acidity
Electrolyte leakage	0.950 **						
Chilling injury	0.716 **	0.737 **					
Decay percentage	0.894 **	0.909 **	0.735 **				
Firmness	0.861 **	0.825 **	0.806 **	0.815 **			
Ascorbic acid	-0.018 NS	0.023 NS	-0.162 NS	0.003 NS	-0.166 NS		
Titratable acidity	-0.353 NS	-0.378 NS	-0.664 **	-0.314 NS	-0.617 **	0.291 NS	
TSS	-0.642 **	-0.633 **	-0.632 **	-0.624 **	-0.806 **	0.415 *	0.548 **

NS, *, ** non-significant, significant at 0.05 and 0.01, respectively.

Table 9 - Correlation analysis between fruit characteristics of tomato cv. M19 treated with different polyamines after 25 days storage at 3°C

	Weight loss	Electrolyte leakage	Chilling injury	Decay percentage	Fruit firmness	Ascorbic acid	Titratable acidity
Electrolyte leakage	0.954 **						
Chilling injury	0.625 **	0.714 **					
Decay percentage	0.912 **	0.859 **	0.536 **				
Firmness	0.849 **	0.845 **	0.721 **	0.731 **			
Ascorbic acid	-0.346 NS	-0.287 NS	-0.539 **	-0.400 *	-0.576 **		
Titratable acidity	-0.485 *	-0.532 **	-0.600 **	-0.464 *	-0.664 **	0.545 **	
TSS	-0.837 **	-0.806 **	-0.624 **	-0.710 **	-0.821 **	0.428 *	0.583 **

NS, *, ** non-significant, significant at 0.05 and 0.01, respectively.

Table 10 - Correlation analysis between fruit characteristics of tomato cv. M79 treated with different polyamines after 25 days storage at 3°C

	Weight loss	Electrolyte leakage	Chilling injury	Decay percentage	Fruit firmness	Ascorbic acid	Titratable acidity
Electrolyte leakage	0.946 **						
Chilling injury	0.884 **	0.884 **					
Decay percentage	0.860 **	0.912 **	0.764 **				
Firmness	0.839 **	0.787 **	0.793 **	0.752 **			
Ascorbic acid	-0.429 *	-0.433 *	-0.518 **	-0.415 *	-0.636 **		
Titratable acidity	-0.331 NS	-0.232 NS	-0.344 NS	-0.237 NS	-0.604 **	-0.549 **	
TSS	-0.846 **	-0.795 **	-0.807 **	-0.717 **	-0.862 **	0.651 **	0.605 **

NS, *, ** non-significant, significant at 0.05 and 0.01, respectively.

cultivars and low temperature durations.

4. Conclusions

The results of this experiment indicate that exogenous Put and Spd application on tomato fruit could maintain or even improve fruit quality during low temperature storage. The combined application of Put with Spd (2:2 mM) could be recommended for low temperature and long duration storage of tomato fruits.

References

AOAC, 1984 - *Official methods of analysis*. - Association of Official Agricultural Chemists, *14th ed.,* Washington, DC, USA.

AGHDAM M.S., BODBODAK S., 2013 - *Physiological and biochemical mechanisms regulating chilling tolerance in fruits and vegetables under postharvest salicylates and jasmonates treatments*. - Scientia Horticulturae*,* 156: 73-85.

BANINASAB B., 2009 - *Amelioration of chilling stress by paclobutrazol in watermelon seedlings*. - Scientia Horticulturae, 121: 144-148.

BECKLES D.M., 2012 - *Factors affecting the postharvest soluble solids and sugar content of tomato (*Solanum lycopersicum *L.) fruit*. - Postharvest Biology and Technology*,* 63: 129-140.

BEN-YEHOSHUA S., SHAPIRO B., CHEN Z.E., LURIE S., 1983 - *Mode of action of plastic film in extending life of lemon and bell pepper fruits by alleviation of water stress*. - Plant Physiology*,* 73: 87-93.

CANDAN A.P., GRAELL J., LARRIGAUDIÈRE C., 2007 - *Chilling injury as related to climacteric behaviour in plums*, pp. 431-436. - In: RAMINA A., C. CHANG, J. GIOVANNONI, H. KLEE, P. PERATA, and E. WOLTERING (eds.) *Advances in plant ethylene research.* Springer, The Netherlands.

COHEN E., SHAPIRO B., SHALOM Y., KLEIN J., 1994 - *Water loss: a nondestructive indicator of enhanced cell membrane permeability of chilling-injured citrus fruit*. - J. Amer. Soc. for Hortic. Sci.*,* 119: 983-986.

DING C.-K., WANG C., GROSS K., SMITH D., 2002 - *Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit*. - Planta*,* 214: 895-901.

EL GHAOUTH A., PONNAMPALAM R., CASTAIGNE F., ARUL J., 1992 - *Chitosan coating to extend the storage life of tomatoes*. - HortScience*,* 27: 1016-1018.

FAUST M., WANG S.Y., 1992 - *Polyamines in horticulturally important plants*. - Horticultural Reviews*,* 14: 333-356.

GALSTON A.W., SAWHNEY R.K., 1990 - *Polyamines in plant physiology*. - Plant Physiology*,* 94: 406-410.

GILL S.S., TUTEJA N., 2010 - *Polyamines and abiotic stress tolerance in plants*. - Plant Signaling & Behavior*,* 5: 26-33.

GONZÁLEZ-AGUILAR G., ZACARIAS L., LAFUENTE M., 1998 - *Ripening affects high-temperature-induced polyamines and their changes during cold storage of hybrid Fortune mandarins*. - J. of Agric. and Food Chem.*,* 46: 3503-3508.

GONZÁLEZ-AGUILAR G.A., GAYOSSO L., CRUZ R., FORTIZ J., BÁEZ R., WANG C.Y., 2000 - *Polyamines induced*

by hot water treatments reduce chilling injury and decay in pepper fruit. - Postharvest Biol. and Techn., 18: 19-26.

HE L., BAN Y., INOUE H., MATSUDA N., LIU J., MORIGUCHI T., 2008 - *Enhancement of spermidine content and antioxidant capacity in transgenic pear shoots overexpressing apple spermidine synthase in response to salinity and hyperosmosis*. - Phytochemistry, 69: 2133-2141.

HUSSAIN S.S., ALI M., AHMAD M., SIDDIQUE K.H., 2011 - *Polyamines: natural and engineered abiotic and biotic stress tolerance in plants*. - Biotechnology Advances, 29: 300-311.

JAVANMARDI J., KUBOTA C., 2006 - *Variation of lycopene, antioxidant activity, total soluble solids and weight loss of tomato during postharvest storage*. - Postharvest Biol. and Techn., 41: 151-155.

KIM T.E., KIM S.-K., HAN T.J., LEE J.S., CHANG S.C., 2002 - *ABA and polyamines act independently in primary leaves of cold-stressed tomato (*Lycopersicon esculentum*)*. - Physiologia Plantarum, 115: 370-376.

KOUSHESH SABA M., ARZANI K., BARZEGAR M., 2012 - *Postharvest polyamine application alleviates chilling injury and affects apricot storage ability*. - J. of Agric. and Food Chem., 60: 8947-8953.

LIU J.-H., KITASHIBA H., WANG J., BAN Y., MORIGUCHI T., 2007 - *Polyamines and their ability to provide environmental stress tolerance to plants*. - Plant Biotechnology, 24: 117-126.

LUENGWILAI K., BECKLES D.M., 2010 - *Climacteric ethylene is not essential for initiating chilling injury in tomato (*Solanum lycopersicum*) cv. Ailsa Craig*. - J. of Stored Products and Postharvest Res., 1: 1-8.

LUTTS S., KINET J., BOUHARMONT J., 1996 - *NaCl-induced senescence in leaves of rice (*Oryza sativa *L.) cultivars differing in salinity resistance*. - Annals of Botany, 78: 389-398.

MALIK A., SINGH Z., 2005 - *Pre-storage application of polyamines improves shelf-life and fruit quality of mango*. - J. of Hortic. Sci. & Biotech., 80: 363-369.

MATTOO A.K., HANDA A.K., 2008 - *Higher polyamines restore and enhance metabolic memory in ripening fruit*. - Plant Science, 174: 386-393.

MATTOO A.K., MINOCHA S.C., MINOCHA R., HANDA A.K., 2010 - *Polyamines and cellular metabolism in plants: transgenic approaches reveal different responses to diamine putrescine versus higher polyamines spermidine and spermine*. - Amino Acids, 38: 405-413.

MEHTA R.A., CASSOL T., LI N., ALI N., HANDA A.K., MATTOO A.K., 2002 - *Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality, and vine life*. - Nature Biotechnology, 20: 613-618.

MIRDEHGHAN S., RAHEMI M., CASTILLO S., MARTÍNEZ-ROMERO D., SERRANO M., VALERO D., 2007 a - *Pre-storage application of polyamines by pressure or immersion improves shelf-life of pomegranate stored at chilling temperature by increasing endogenous polyamine levels*. - Postharvest Biol. and Techn., 44: 26-33.

MIRDEHGHAN S.H., RAHEMI M., SERRANO M., GUILLÉN F., MARTÍNEZ-ROMERO D., VALERO D., 2007 b - *The application of polyamines by pressure or immersion as a tool to maintain functional properties in stored pomegranate arils*. - J. of Agric. and Food Chem., 55: 760.

OUFIR M., LEGAY S., NICOT N., VAN MOER K., HOFFMANN L., RENAUT J., HAUSMAN J.-F., EVERS D., 2008 - *Gene expression in potato during cold exposure: changes in carbohydrate and polyamine metabolisms*. - Plant Science, 175: 839-852.

PAKSASORN A., HAYASAKA T., MATSUI H., OHARA H., HIRATA N., 1995 - *Relationship of polyamine content to ACC content and ethylene evolution in Japanese apricot (*Prunus mume*) fruit*. - J. of the Japan. Soc. for Hortic. Sci., 63: 761-766.

PAPADOPOULOS A.P., 1991 - *Growing greenhouse tomatoes in soil and in soilless media.* - Agriculture Canada Publication, Ottawa, Canada.

SAFTNER R.A., BALDI B.G., 1990 - *Polyamine levels and tomato fruit development: possible interaction with ethylene*. - Plant Physiology, 92: 547-550.

SALTVEIT M.E., 2001 - *Chilling injury is reduced in cucumber and rice seedlings and in tomato pericarp discs by heat-shocks applied after chilling*. - Postharvest Biol. and Techn., 21: 169-177.

SMITH D.L., ABBOTT J.A., GROSS K.C., 2002 - *Down-regulation of tomato* β*-galactosidase 4 results in decreased fruit softening*. - Plant Physiology, 129: 1755-1762.

SUTTLE J.C., 1981 - *Effect of polyamines on ethylene production*. - Phytochemistry, 20: 1477-1480.

TIECHER A., DE PAULA L.A., CHAVES F.C., ROMBALDI C.V., 2013 - *UV-C effect on ethylene, polyamines and the regulation of tomato fruit ripening*. - Postharvest Biol. and Techn., 86: 230-239.

VALERO D., MARTÍNEZ-ROMERO D., SERRANO M.A., 2002 - *The role of polyamines in the improvement of the shelf life of fruit*. - Trends in Food Sci. & Techn., 13: 228-234.

WALDEN R., CORDEIRO A., TIBURCIO A.F., 1997 - *Polyamines: small molecules triggering pathways in plant growth and development*. - Plant Physiology, 113: 1009.

WANG C.Y., 1993 - *Approaches to reduce chilling injury of fruits and vegetables*. - Horticultural Reviews, 15: 63-95.

YAHIA E.M., CONTRERAS-PADILLA M., GONZÁLEZ-AGUILAR G., 2001 - *Ascorbic acid content in relation to ascorbic acid oxidase activity and polyamine content in tomato and bell pepper fruits during development, maturation and senescence*. - LWT. Food Science and Technology, 34: 452-457.

BOOK REVIEWS

LA CURA DEI GIARDINI STORICI. TEORIA E PRASSI. *Michael Rohde*. Edizione italiana a cura di Massimo de Vico Fallani. Giardini e paesaggio, vol. 31. Leo S. Olschki, Firenze (Italy), 2012. pp. XIII + 569. ISBN 978-88-222-6149-6. € 58.00.

The author of this interesting volume has, for some time, worked with historic gardens in Germany and has asked the important question of how to define professional, sustainable and lasting care of historic gardens and how to put it into practice. Michael Rohde, director of the garden of the Prussion Castles and Parks of Berlin-Brandenburg Foundation, together with a team of four other authors, has created an important base not only for understanding historic gardens but also for future research about caring for and preserving them. This 570-page book is the result of two research projects undertaken at the Department of Landscape Architecture at the University of Hannover.

The first part of the book is dedicated to the past: the theory of the art and design, and the care for trees, flowers, paths and waterworks are documented in detail, crossing over all stylistic periods. In the first part, the various members of the working group describe the techniques adopted in the various periods considered. Barbara Vogt reports about studies on flowers, flowerbed ornamentation and provides a list of flowers used and certification of their origin from Renaissance up to the XX century. The chapter by Andreas von Hoeren documents changes in the use of irrigation and hydraulic systems from the Renaissance to the 20th century.

The second part of the volume is dedicated to the care and restoration of historic gardens. Some well-known parks are taken as example and interventions undertaken and recommended for trees, shrubs, blossoms, paths and waterworks are described, with precise instructions for their care. There are numerous color illustrations of historic plans, engravings, drawings and inventories, adding valuing to the theoretical and practical parts of the work.

The volume concludes with results and recommendations to care for historic gardens properly.

As Massimo De Vico Fallani points out in the Presentation, “reading this book will, for some, be a surprise because it reveals a well-structured reality toward the mastery of curative nature which is not always known, at least not in present times, by those working in Italy, and draws attention to maintanence, which is often overlooked.”

This book enriches the considerable literature available on historic gardens from historic and cultural and scientific and technical points of view, offering the reader meaning and knowledge about his topic which plays a leading role in the image of our country.

Francesco Ferrini

REVIEWERS OF MANUSCRIPT FOR 2013

The Editorial Management wishes to thank the following referees for their assistance and evaluation of manuscripts submitted

ANDRENELLI L. - Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50144 Florence, Italy.

ARGENTI G. - Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50144 Florence, Italy.

ARIVAZHAGAN G. - Plant Biotechnology Laboratory, School of Biotechnology and Genetic Engineering, Bharathiar University, Coimbator, Tamilnadu, India.

ARORA R. - Department of Horticulture, Iowa State University, 50011-1100 Ames, Iowa, USA.

BAZIHIZINA N. - Dipartimento di Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50019 Sesto Fiorentino (FI), Italy.

BE TA BARNAB S. - Agricultural Research Institute, Hungarian Academy of Sciences, H 2462 Martonv, Hungary.

BIRICOLTI S. - Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50019 Sesto Fiorentino (FI), Italy.

FABBRI A. - Dipartimento di Biologia Evolutiva e Funzionale, University of Parma, 43100 Parma, Italy.

FERGUSON L. - Department of Plant Sciences, University of California at Davis, 95616-8683 Davis, California, USA.

FERRANTE A. - Dipartimento di Produzione Vegetale, University of Milan, 20133 Milan, Italy.

FINI A. - Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50019 Sesto Fiorentino (FI), Italy.

FIORINO P. - Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50019 Sesto Fiorentino (FI), Italy.

FOTOPOULOS V. - Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 3603 Limassol, Cyprus.

GIORDANI E. - Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50019 Sesto Fiorentino (FI), Italy.

GUIDI NISSIM W. - Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50019 Sesto Fiorentino (FI), Italy.

LAZZARO L. - Dipartimento di Agronomia Ambientale Produzioni Vegetali, University of Padua, 35020 Legnaro (PD), Italy.

LENZI A. - Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Pisa, 50144 Firenze, Italy.

LOVATI F. - CRA - Unità di ricerca per i processi dell'industria agroalimentare, 20133 Milan, Italy.

LUVISI A. - Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, Sezione di Patologia Vegetale, University of Pisa, 56127 Pisa, Italy.

MANGANARIS G. - Department of Agricultural Science, Biotechnology and Food Science, Cyprus University of Technology, Lemesos, Cyprus.

MATTII G.B. - Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50019 Sesto Fiorentino (FI), Italy.

MIGNANI I. - Dipartimento di Scienze Agrarie ed Ambientali, University of Milan, 20133 Milan, Italy.

MORINI S. - Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, University of Pisa, 56127 Pisa, Italy.

MUGNAI S. - Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50019 Sesto Fiorentino (FI), Italy.

NATIV D. - Unit of Medicinal and Aromatic Plants, Newe Ya'ar Research Center, ARO - Volcani Center, 30095 Ramat Yishay, Israel.

NENCETTI V. - Dipartimento Scienze Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50019 Sesto Fiorentino (FI), Italy.

OTTO S. - Istituto di Biologia Agroambientale e Forestale, Sezione di Malerbologia, 35020 Legnaro, Italy.

PACINI C.G. - Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50144 Florence, Italy.

PANDOLFI C. - Dipartimento Scienze Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50019 Sesto Fiorentino (FI), Italy.

PARDOSSI A. - Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, University of Pisa, 56124 Pisa.

PECCHIONI N. - Dipartimento di Scienze Agrarie, University of Modena e Reggio, 42100 Reggio Emilia, Italy.

RADICE S. - Centro de Estudios Farmacologicos y Botanico CEFYBO-CONICET-UBA, C1121ABG Buenos Aires, Argentina.

RUGGERI R. - DAFNE, Tuscia University, 01100 Viterbo, Italy.

RUÍZ D. - CABAS-CSIC, Murcia, Spain.

SCALABRELLI G. - Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, University of Pisa, 56127 Pisa, Italy.

SCHNEIDER A. - Dipartimento di Scienze Agrarie, Forestali e Alimentari, University of Turin, 10095 Grugliasco (TO), Italy.

STORCHI P. - CRA- VIV, Istituto Sperimentale per la Viticoltura, Sezione di Arezzo, 52020 Pratantico (AR), Italy.

TAITI C. - Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, 50019 Sesto Fiorentino (FI), Italy.

ZANIN G. - Dipartimento di Agronomia Ambientale e Produzioni Vegetali, University of Padua, 35020 Legnaro (PD) Italy.

INDEX
VOLUME 27, 2013

AUTHOR INDEX

Adam A. - *In vitro Pseudomonas putida* BTP1-induced systemic resistance in grapevine rootstocks against Phylloxera (*Daktulosphaira vitifoliae*), 137

Adekpe D.I. - Screening of herbicides for weed control, growth and yield of irrigated onion (*Allium cepa* L.) in tropical Savanna climate, 67

Ahmad M. - Trace and minor elements in bee honeys produced in Syria, 55

Ahmad M.S. - Postharvest treatments for preserving quality of 'Kinnow' fruit under different storage conditions, 152

Aliyu L. - Screening of herbicides for weed control, growth and yield of irrigated onion (*Allium cepa* L.) in tropical Savanna climate, 67

Anaclerio F. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

Andreini L. - Apricot flower bud dormancy: main morphological, anatomical and physiological features related to winter climate influence, 5

Angelini E. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

Angelini E. - Prevention and control strategies against *Agrobacterium vitis* in grapevine multiplication material, 109

Angelini E. - Sanitary and agronomic selection of Tuscan germplasm to improve wine production, 98

Antonelli M.G. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

Arroyo L.E. - Pre and post-harvest management affects functional quality of peach (*Prunus persica* L.) cv. Flavorcrest, 159

Ayyoubi Z. - *In vitro Pseudomonas putida* BTP1-induced systemic resistance in grapevine rootstocks against Phylloxera (*Daktulosphaira vitifoliae*), 137

Bandinelli R. - The evolution of clonal heritage registry available at TOS.CO.VIT., 96

Barba M. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

Bartolini S. - Apricot flower bud dormancy: main morphological, anatomical and physiological features related to winter climate influence, 5

Bashir S. - Impact of integrated nutrient mangenement on growth, yield and quality of strawberry (*Fragaria x ananassa* Duch.) cultivation in India, 147

Bazzo I. - Sanitary and agronomic selection of Tuscan germplasm to improve wine production, 98

Bertazzon N. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

Bevilacqua G. - Prevention and control strategies against *Agrobacterium vitis* in grapevine multiplication material, 109

Bianchedi P. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

Bianchi G. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

Bianco P.A. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

Bonhomme M. - Evidence for non-occurrence of node-to-node or stem-to-bud transfer of chilling temperature signal for dormancy release, 33

Bornice M. - Updated knowledge of the vine rootstocks, 104

Borrelli C. - Effect of mulching and plant density on out-of-season organic potato growth, yield and quality, 115

Botti S. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

Bragagna P. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

Cabia Cardoso N. - Effect of active modified atmospheres on the quality of non-astringent persimmons (Caqui Giomo) *D. Kaki* Thunb. when stored under refrigerated conditions, 73

Caramia D. - Ground cover management strategies in an Apulian oil-producing olive grove: agronomic and ecological assessment proposals, 44

Cardoni M. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

Carputo D. - Effect of mulching and plant density on out-of-season organic potato growth, yield and quality, 115

Caruso G. - Effect of mulching and plant density on out-of-season organic potato growth, yield and quality, 115

Casati P. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

Chehrazi M. - Application of anatase nanoparticles (TiO_2) on strawberry seed germination (*Fragaria ananassa* L.), 143

Colla C. - Prevention and control strategies against *Agrobacterium vitis* in grapevine multiplication material, 109

Conti S. - Effect of mulching and plant density on out-of-season organic potato growth, yield and quality, 115

Corbino G.B. - Pre and post-harvest management affects functional quality of peach (*Prunus persica* L.) cv. Flavorcrest, 159

Credi R. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107

D'Onofrio C. - State of the art in grapevine variety and clone identification through polymorphism in DNA molecular markers, 106

Daiuto É.R. - Effect of active modified atmospheres on the quality of non-astringent persimmons (Caqui Giomo) *D. Kaki* Thunb. when stored under refrigerated conditions, 73
Dallavalle E. - Propagation of endangered grapevine cultivars: some reasons to recover and protect this patrimony, 101
Dar N.A. - Impact of integrated nutrient management on growth, yield and quality of strawberry (*Fragaria x ananassa* Duch.) cultivation in India, 147
De Luca E. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
De Moraes M.R. - Effect of active modified atmospheres on the quality of non-astringent persimmons (Caqui Giomo) *D. Kaki* Thunb. when stored under refrigerated conditions, 73
Dehkourdi E.H. - Application of anatase nanoparticles (TiO_2) on strawberry seed germination (*Fragaria ananassa* L.), 143
Di Collalto G. - Characteristics of recent released clones selected by DISAAA-A in Tuscan Coast line premultiplied by TOS.CO.VIT., 102
Di Marco S. - Prevention and control strategies against *Agrobacterium vitis* in grapevine multiplication material, 109
Durante G. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Emami S. - Application of sucrose on tomato seedlings improves transplant quality, crop establishment, cold and dark hardiness, 122
Faggioli F. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Falconi R. - Prevention and control strategies against *Agrobacterium vitis* in grapevine multiplication material, 109
Ferroni G. - Characteristics of recent released clones selected by DISAAA-A in Tuscan Coast line premultiplied by TOS. CO.VIT., 102
Ferroni G. - Updated knowledge of the vine rootstocks, 104
Fracchiolla M. - Ground cover management strategies in an Apulian oil-producing olive grove: agronomic and ecological assessment proposals, 44
Frausin C. - Prevention and control strategies against *Agrobacterium vitis* in grapevine multiplication material, 109
Frezza D. - Agronomic performance and essential oil composition of *Ocimum basilicum* L.: Effect of genotype and date of harvest, 166
Frusciante L. - Effect of mulching and plant density on out-of-season organic potato growth, yield and quality, 115
Gabilondo J. - Pre and post-harvest management affects functional quality of peach (*Prunus persica* L.) cv. Flavorcrest, 159
Gambino G. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Geelani S. - Impact of integrated nutrient management on growth, yield and quality of strawberry (*Fragaria* x *ananassa* Duch.) cultivation in India, 147
Gianinazzi C. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Giannetti F. - Sanitary and agronomic selection of Tuscan germplasm to improve wine production, 98
González J. - Pre and post-harvest management affects functional quality of peach (*Prunus persica* L.) cv. Flavorcrest, 159
Gretter L. - Preservation and premultiplication of selected grape material in Trentino: collaboration between FEM-S. Michele all'Adige and Trentino Grape-Nurseries AVIT-Consortium, 100
Gribaudo I. - RFID microchips as a tool for traceability in grapevine nurseries: pre- and post-grafting implants, 112
Gualandri V. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Guglielminetti L. - Physiological responses of C_4 grasses to prolonged heat stress, 127
Hasan R. - Trace and minor elements in bee honeys produced in Syria, 55
Hosseini H. - Application of anatase nanoparticles (TiO_2) on strawberry seed germination (*Fragaria ananassa* L.), 143
Hosseini M. - Application of anatase nanoparticles (TiO_2) on strawberry seed germination (*Fragaria ananassa* L.), 143
Idris I. - *In vitro Pseudomonas putida* BTP1-induced systemic resistance in grapevine rootstocks against Phylloxera (*Daktulosphaira vitifoliae*), 137
Javanmardi J. - Application of sucrose on tomato seedlings improves transplant quality, crop establishment, cold and dark hardiness, 122
Javanmardi J. - Post-storage quality and physiological responses of tomato fruits treated with polyamines, 173
Khuder A. - Trace and minor elements in bee honeys produced in Syria, 55
Kumar A. - Protandrous-protogynous dimorphism in indigenous selections from North Western India and some exotic cultivars of Persian walnut (*Juglans regia* L.), 61
Lacointe A. - Evidence for non-occurrence of node-to-node or stem-to-bud transfer of chilling temperature signal for dormancy release, 33
Lasorella C. - Ground cover management strategies in an Apulian oil-producing olive grove: agronomic and ecological assessment proposals, 44
Lucchetta G. - Prevention and control strategies against *Agrobacterium vitis* in grapevine multiplication material, 109
Luison D. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Luvisi A. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Luvisi A. - How information technology can support regulations and best practices for the management of health status of grapevine and product safety, 110
Maddaluno P. - Effect of mulching and plant density on out-of-season organic potato growth, yield and quality, 115
Malik T.H. - Impact of integrated nutrient management on growth, yield and quality of strawberry (*Fragaria* x *ananassa* Duch.) cultivation in India, 147
Malossini U. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Malossini U. - Preservation and premultiplication of selected grape material in Trentino: collaboration between FEM-S. Michele all'Adige and Trentino Grape-Nurseries AVIT-Consortium, 100

Mannini F. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Mannini F. - Innovative techniques for pre-multiplication of grapevine clones: the CE.PRE.MA.VI. experience, 99
Mannini F. - RFID microchips as a tool for traceability in grapevine nurseries: pre- and post-grafting implants, 112
Mirandola R. - Prevention and control strategies against *Agrobacterium vitis* in grapevine multiplication material, 109
Montemurro P. - Ground cover management strategies in an Apulian oil-producing olive grove: agronomic and ecological assessment proposals, 44
Mordenti G. - Prevention and control strategies against *Agrobacterium vitis* in grapevine multiplication material, 109
Murray R.E. - Pre and post-harvest management affects functional quality of peach (*Prunus persica* L.) cv. Flavorcrest, 159
Nasirzadeh M. - Post-storage quality and physiological responses of tomato fruits treated with polyamines, 173
Navacchi O. - Micropropagation in viticulture: twenty years of experience, 105
Nervo L. - Innovative techniques for pre-multiplication of grapevine clones: the CE.PRE.MA.VI. experience, 99
Pagano M. - The evolution of clonal heritage registry available at TOS.CO.VIT., 96
Pompeiano A. - Physiological responses of C_4 grasses to prolonged heat stress, 127
Prasad V.M. - Impact of integrated nutrient management on growth, yield and quality of strawberry (*Fragaria* x *ananassa* Duch.) cultivation in India, 147
Qrunfleh I.M. - Delaying bud break in 'Eldelweiss' grapevines to avoid spring frost injury by NAA and vegetable oil applications, 18
Rageau R. - Evidence for non-occurrence of node-to-node or stem-to-bud transfer of chilling temperature signal for dormancy release, 33
Rahemi M. - Post-storage quality and physiological responses of tomato fruits treated with polyamines, 173
Read P.E. - Delaying bud break in 'Eldelweiss' grapevines to avoid spring frost injury by NAA and vegetable oil applications, 18
Saldarelli P. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Salehi H. - Comparison of tall fescue (*Festuca arundinacea* Schreb.) and common bermudagrass (*Cynodon dactylon* [L.] Pers.) turfgrasses and their seed mixtures, 81
Salehi M.R. - Comparison of tall fescue (*Festuca arundinacea* Schreb.) and common bermudagrass (*Cynodon dactylon* [L.] Pers.) turfgrasses and their seed mixtures, 81
Sanchez E. - Agronomic performance and essential oil composition of *Ocimum basilicum* L.: Effect of genotype and date of harvest, 166
Sánchez G. - Pre and post-harvest management affects functional quality of peach (*Prunus persica* L.) cv. Flavorcrest, 159
Saour G. - Trace and minor elements in bee honeys produced in Syria, 55
Sartori E. - Prevention and control strategies against *Agrobacterium vitis* in grapevine multiplication material, 109
Scalabrelli G. - Characteristics of recent released clones selected by DISAAA-A in Tuscan Coast line premultiplied by TOS.CO.VIT., 102
Scalabrelli G. - Competitiveness of the wine sector: considerations on future scenarios, 113.
Scalabrelli G. - Updated knowledge of the vine rootstocks, 104
Sharma N. - Protandrous-protogynous dimorphism in indigenous selections from North Western India and some exotic cultivars of Persian walnut (*Juglans regia* L.), 61
Shebayan J.A.Y. - Screening of herbicides for weed control, growth and yield of irrigated onion (*Allium cepa* L.) in tropical Savanna climate, 67
Sheema S. - Impact of integrated nutrient mangenment on growth, yield and quality of strawberry (*Fragaria* x *ananassa* Duch.) cultivation in India, 147
Shinggu C.P. - Screening of herbicides for weed control, growth and yield of irrigated onion (*Allium cepa* L.) in tropical Savanna climate, 67
Siddiqui M.W. - Postharvest treatments for preserving quality of 'Kinnow' fruit under different storage conditions, 152
Smith R.E. - Effect of active modified atmospheres on the quality of non-astringent persimmons (Caqui Giomo) *D. Kaki* Thunb. when stored under refrigerated conditions, 73
Storchi P. - Sanitary and agronomic selection of Tuscan germplasm to improve wine production, 98
Takemura Y. - Bud dormancy in Japanese pear, 25
Tamura F. - Bud dormancy in Japanese pear, 25
Tempesta G. - System application and the availability of vine germplasm, 95
Terlizzi F. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Thakur K.S. - Postharvest treatments for preserving quality of 'Kinnow' fruit under different storage conditions, 152
Toffanin A. - Competitiveness of the wine sector: considerations on future scenarios, 113.
Triolo E. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Triolo E. - Propagation of endangered grapevine cultivars: some reasons to recover and protect this patrimony, 101
Trisciuzzi N. - Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules, 107
Valentini G.H. - Pre and post-harvest management affects functional quality of peach (*Prunus persica* L.) cv. Flavorcrest, 159
Valentini P. - Sanitary and agronomic selection of Tuscan germplasm to improve wine production, 98
Van Baren C. - Agronomic performance and essential oil composition of *Ocimum basilicum* L.: Effect of genotype and date of harvest, 166
Vazquez A. - Agronomic performance and essential oil composition of *Ocimum basilicum* L.: Effect of genotype and date of harvest, 166
Vieites R.L. - Effect of active modified atmospheres on the quality of non-astringent persimmons (Caqui Giomo) *D. Kaki* Thunb. when stored under refrigerated conditions, 73
Viti R. - Apricot flower bud dormancy: main morphological, anatomical and physiological features related to winter cli-

mate influence, 5
Volterrani M. - Physiological responses of C_4 grasses to prolonged heat stress, 127
Wani R.A. - Impact of integrated nutrient management on growth, yield and quality of strawberry (*Fragaria x ananassa* Duch.) cultivation in India, 147
Zuccherelli G. - Micropropagation in viticulture: twenty years of experience, 105

Agrobacterium vitis
Grapevine, 109

***Allium cepa* L.** see Onion

Ampelography
Grapevine, 106

Anatase nanoparticles
Strawberry, 143

Ancient cultivar
Grapevine, 101

Antioxidants
Peach, 159

Apricot
Budbreak, 5
Bud dormancy, 5
Climatic change, 5
Endodormancy process, 5
Global warming, 5
Review paper, 5

Arabis mosaic virus
Grapevine, 107

Ascorbic acid
Tomato, 173

Autochthounous vines
Grapevine, 101

Basil
Genotype, 166
Green basil, 166
Harvest, 166
Purple basil, 166
Soilless culture, 166
Volatile oils, 166
Yield, 166

Bermudagrass
Heat shock proteins, 127
Heat stress, 127
Lawn, 81
Management practices, 81
Organ senescence, 127
Protein content, 127
Seed mixtures, 81
Total soluble sugars, 127
Visual quality, 81

Biodegradable mulch
Potato, 115

Budbreak
Apricot, 5
Grapevine, 18
Japanese pear, 25

Bud dormancy
Apricot, 5
Grapevine, 18
Japanese pear, 25
Peach, 33

Chill units
Japanese pear, 25

Chilling injury
Tomato, 173

Chilling perception
Peach, 33

Chilling requirements
Japanese pear, 25

Chilling tolerance
Tomato, 122

Chopping
Olive, 44
Olive grove, 44

Climatic change
Apricot, 5
Japanese pear, 25

Climatic characteristics
Grapevine, 104

Clonal heritage registry
Grapevine, 96

Clonal selection
Grapevine, 96, 98, 99, 100, 102, 104, 105

Cluster analysis
Honey, 55

Control strategies
Grapevine, 109

Consumers
Grapevine, 113

Cover crop
Olive, 44
Olive grove, 44

Crown gall disease
Grapevine, 109

Cultivation techniques
Grapevine, 104

***Cynodon dactylon* L.** see Bermudagrass

Daktulosphaira vitifoliae see Phylloxera

Diagnostic protocols
Grapevine, 107

Dichogamy
Walnut, 61

***Diospyros kaki* L.** see Persimmon

DNA molecular markers
Grapevine, 106

Dry matter
Potato, 115

Edible coating
Kinnow mandarin, 152

Electrolyte leakage
Tomato, 173

Endodormancy process
Apricot, 5
Peach, 33

Fanleaf virus
Grapevine, 107

Fertilization
Peach, 159

***Festuca arundinacea* Schreb** see Tall fescue

Firmness
Persimmon, 73

Fleck virus
Grapevine, 107

***Fragaria x ananassa* Duch.** see Strawberry

Frost damage
Grapevine, 18

Genetic analysis
Grapevine, 101

Genotype
Basil, 166
Green basil, 166
Purple basil, 166

Genotypic identification
Grapevine, 106

Germination
Strawberry, 143

Germination rate index
Strawberry, 143

Germplasm
Grapevine, 95

Global warming
Apricot, 5
Japanese pear, 25

Grapevine
Agrobacterium vitis, 109
Ampelography, 106
Ancient cultivar, 101
Arabis mosaic virus, 107
Autochthonous vines, 101
Budbreak, 18
Bud dormancy, 18
Climatic characteristics, 104
Clonal heritage registry, 96
Clonal selection, 96, 98, 99, 100, 102, 104, 105
Control strategies, 109
Consumers, 113
Crown gall disease, 109
Cultivation techniques, 104
Diagnostic protocols, 107
DNA molecular markers, 106
Fanleaf virus, 107
Fleck virus, 107
Frost damage, 18
Genetic analysis, 101
Genotypic identification, 106
Germplasm, 95
Growers, 113
Healthy material, 105, 110
In vitro assessment, 137
In vitro culture, 105
Leafroll virus, 107
Microchips, 110, 112
Micropropagation, 105
Microsatellites, 106
NAA, 18
New clones, 95
New clones released, 102
Nursery, 105, 106, 112, 113
Nursery demand, 95
Pathogens, 110
Phylloxera, 137
Plant pathogens, 107
Pre-multiplication, 99, 100
Preservation, 99, 100, 101, 106
Prevention strategies, 109
Producers, 113
Propagation, 101
Pseudomonas putida, 137
Quality, 98, 110
RFID systems, 110, 112
Rootstocks, 104, 105, 137
Sanitary selection, 98
Soil conditions, 104
Tuscan coast, 102
Vegetable oil application, 18
Viruses, 107
Wine production, 98

Green basil
Genotype, 156
Harvest, 156
Soilless culture, 166
Volatile oils, 166
Yield, 166

Growers
Grapevine, 113

Growth
Onion, 67
Strawberry, 147

Harvest
Basil, 166
Green basil, 166
Purple basil, 166

Healthy material
Grapevine, 105, 110

Heat shock
Peach, 159

Heat shock proteins
Bermudagrass, 127
Japanese lawn grass, 127

Heat stress
Bermudagrass, 127
Japanese lawn grass, 127

Herbicides
Olive, 44
Olive grove, 44
Onion, 67

Hoe weeding
Onion, 67

Honey
Cluster analysis, 55
Minerals, 55
X-ray fluorescence, 55

***In vitro* assessment**
Grapevine, 137
Phylloxera, 137

***In vitro* culture**
Grapevine, 105

Inorganic fertilizers
Strawberry, 147

Integrated nutrient management
Strawberry, 147

Japanese lawn grass
Heat shock proteins, 127
Heat stress, 127
Organ senescence, 127
Protein content, 127
Total soluble sugars, 127

Japanese pear
Budbreak, 25
Bud dormancy, 25
Chill units, 25
Chilling requirements, 25
Climatic change, 25
Global warming, 25
Phytohormone, 25

***Juglans regia* L.** see Walnut

Kinnow mandarin
Edible coating, 152
Postharvest, 152
Quality, 152
Shelf life, 152
Storage conditions, 152

Lawn
Bermudagrass, 81
Tall fescue, 81

Leafroll virus
Grapevine, 107

Low productivity
Walnut, 61

Management practices
Bermudagrass, 81
Tall fescue, 81

Microchips
Grapevine, 110, 112

Micropropagation
Grapevine, 105

Microsatellites
Grapevine, 106

Minerals
Honey, 55

NAA
Grapevine, 18

New clones
Grapevine, 95

New clones release
Grapevine, 102

Non-astringent
Persimmon, 73

Nursery
Grapevine, 105, 106, 112, 113

Nursery demand
Grapevine, 95

***Ocimum basilicum* L**. see Basil

***Olea europaea* L.** see Olive

Olive
Chopping, 44
Cover crop, 44
Herbicides, 44
Soil management, 44
Weeds, 44

Olive grove
Chopping, 44
Cover crop, 44
Herbicides, 44
Soil management, 44
Weeds, 44

Onion
Growth, 67
Herbicides, 67
Hoe weeding, 67
Yield, 67
Weed control, 67

Organ senescence
Bermudagrass, 127
Japanese lawn grass, 127

Organic fertilizers
Strawberry, 147

Pathogens
Grapevine, 110

Peach
Antioxidants, 159
Bud dormancy, 33
Chilling perception, 33
Endodormancy process, 33
Fertilization, 159
Heat shock, 159
Rootstocks, 159
Temperature sensing, 33

Persimmon
Firmness, 73
Non-astringent, 73
Postharvest, 73
Quality, 73
Refrigerated conditions, 73

Phylloxera
Grapevine, 137
In vitro assessment, 137
Pseudomonas putida, 137
Rootstocks, 137

Phytohormone
Japanese pear, 25

Plant leaf area
Potato, 115

Plant pathogens
Grapevine, 107

Pollen shedding
Walnut, 61

Postharvest
Kinnow mandarin, 152
Persimmon, 73
Tomato, 173

Potato
Biodegradable mulch, 115
Dry matter, 115
Plant leaf area, 115
Quality, 115
Weed control, 115
Yield, 115

Pre-multiplication
Grapevine, 99, 100

Preservation
Grapevine, 99, 100, 101, 106

Prevention strategies
Grapevine, 109

Producers
Grapevine, 113

Propagation
Grapevine, 101

Protein content
Bermudagrass, 127
Japanese lawn grass, 127

***Prunus armeniaca* L.** see Apricot

***Prunus persica* L.** see Peach

Pseudomonas putida
Grapevine, 137
Phylloxera, 137

Purple basil
Genotype, 166
Harvest, 166
Soilless culture, 166
Volatile oils, 166
Yield, 166

***Pyrus pyrifolia* L.** see Japanese pear

Quality
Grapevine, 98, 110
Kinnow mandarin, 152
Persimmon, 73
Potato, 115
Strawberry, 147
Tomato, 173

Refrigerated conditions
Persimmon, 73

Review
Book review, 88, 133, 183
Review paper Apricot, 5

RFID systems
Grapevine, 110, 112

Rootstocks
Grapevine, 104, 105, 137
Peach, 159
Phylloxera, 137

Sanitary selection
Grapevine, 98

Seed mixtures
Bermudagrass, 81
Tall fescue, 81

Shelf life
Kinnow mandarin, 152

Soil conditions
Grapevine, 104

Soil management
Olive, 44
Olive grove, 44

Soilless culture
Basil, 166
Green basil, 166
Purple basil, 166

***Solanum lycopersicum* L.** see Tomato

***Solanum tuberosum* L.** see Potato

Stigma receptivity
Walnut, 61

Storage conditions
Kinnow mandarin, 152

Strawberry
Anatase nanoparticles, 143
Germination, 143
Germination rate index, 143
Growth, 147
Inorganic fertilizers, 147
Integrated nutrient management, 147
Organic fertilizers, 147
Quality, 147
Yield, 147

Sucrose application
Tomato, 122

Survival
Tomato, 122

Tall fescue
Lawn, 81
Management practices, 81
Seed mixtures, 81
Visual quality, 81

Temperature sensing
Peach, 33

Tomato
Ascorbic acid, 173
Chilling injury, 173
Chilling tolerance, 122
Electrolyte leakage, 173
Postharvest, 173
Quality, 173
Sucrose application, 122
Survival, 122
Transplant, 122

Total soluble sugars
Bermudagrass, 127
Japanese lawn grass, 127

Transplant
Tomato, 122

Tuscan coast
Grapevine, 102

Vegetable oil application
Grapevine, 18

Viruses
Grapevine, 107

Visual quality
Bermudagrass, 81
Tall fescue, 81

***Vitis vinifera* L.** see Grapevine

Volatile oils
Basil, 166
Green basil, 166
Purple basil, 166

Walnut
Dichogamy, 61
Low productivity, 61
Pollen shedding, 61
Stigma receptivity, 61

Weed control
Onion, 67
Potato, 115

Weeds
Olive, 44
Olive grove, 44

Wine production
Grapevine, 98

X-ray fluorescence
Honey, 55

Yield
Basil, 166
Green basil, 166
Onion, 67
Potato, 115
Purple basil, 166
Strawberry, 147

***Zoysia japonica* Steud** see Japanese lawn grass