Journal of Applied Horticulture

ISSN 0972-1045
Vol. 8, No. 1, January-June, 2006

Journal of
THE SOCIETY FOR ADVANCEMENT OF HORTICULTURE

JOURNAL OF APPLIED HORTICULTURE

A Journal of

THE SOCIETY FOR THE ADVANCEMENT OF HORTICULTURE

(Registered under Act XXI of 1860) Registration No. 2526-1998-99

ISSN 0972-1045

www.hoticultureworld.net\journalhorticulture.htm or **www.hortresearch.net\journalhorticulture.htm**

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Effect of grafting on growth and yield of tomato (*Lycopersicon esculentum* Mill.) in greenhouse and open-field

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Abstract

Seedlings of tomato (*Lycopersicon esculentum* Mill.) cv. 'Big Red' were used as scion and rootstock (self-grafted) and non-grafted control, while two hybrid tomatoes 'Heman' and 'Primavera' were used as rootstocks. Grafted and non-grafted plants were grown in the greenhouse and in the open-field. Grafted plants (BH and BP) were more vigorous than the non-grafted ones in the greenhouse as well as in the open-field. Plants grafted onto 'Heman' and 'Primavera' produced 32.5, 12.8% and 11.0 and 11.1% more fruit than the control (B) in the greenhouse and the open-field, respectively, whereas self-grafted plants BB had a lower yield in both cultivation conditions. However, the self-rooted plants B presented earliness in their performance, probably due to the lack of stress that followed the grafting operation. Quality and qualitative fruit characteristics were not affected by grafting.

Key words: *Lycopersicon esculentum*, *Lycopersicon hirsutum*, grafting, rootstock, scion, tomato, yield.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a crop of high importance in many countries; according to FAO (1998), in Greece, 1.8 millions MT were produced. In the Mediterranean area, where land use is very intensive and continuous cropping is in common practice, vegetable grafting is considered an innovative technique with an increasing demand by farmers. Viewing recent data concerning the Mediterranean area by Leonardi and Romano (2004) it was reported that Spain is the most important country for the spreading of vegetable grafting with mainly tomato and watermelon, with 40 and 52% of the total of 154 million plants in 2004, respectively. They also indicated that in Italy an increasing dissemination of the grafting technique increased the number of the vegetable grafted plants from 4 million in 1997 to 14 million in 2000.

In Greece, grafting is becoming highly popular, especially in southern areas, where the ratio of the production area using grafted plants to the total production area, amounts to almost 90-100% for early cropping watermelons, 40-50% for melons under low tunnels, 5-10% for cucumbers and 2-3% for tomato and eggplant. In contrast, in northern Greece, the cultivation of grafted fruit-bearing vegetables is rare (Traka-Mavrona *et al.*, 2000).

Although in the beginning, tomato grafting was adopted to limit the effects of Fusarium wilt (Lee, 1994; Scheffer, 1957), the reasons for grafting have increased dramatically over the years. For example, grafts have been used to induce resistance against low (Bulder *et al.,* 1990) and high (Rivero *et al.,* 2003) temperatures; to enhance nutrient uptake (Ruiz *et al.,* 1997); to improve yield when plants are cultivated in infected soils (Bersi, 2002; Kacjan-Marsic and Osvald, 2004); to increase the synthesis of endogenous hormones (Proebsting *et al.* 1992); to improve water use (Cohen and Naor, 2002); to increase flower and seed production (Lardizabal and Thompson, 1990); to enhance vegetable tolerance to drought, salinity and flooding (AVRDC, 2000; Estan *et al.,* 2005). Moreover, many researchers reported that an interaction between rootstocks and scions exists resulting in high vigor of the root system and greater water and mineral uptake leading to increased yield and fruit enhancement (Lee, 1994; Oda, 1995; Bersi, 2002; White, 1963; Leoni *et al.,* 1990; Ioannou, *et al.,* 2002; Kacjan-Marsic and Osvald, 2004). On the contrary, Romano and Paratore (2001) stated that vegetable grafting does not improve the yield when the selection of the rootstock is not suitable, for example the self-grafted plant 'Rita x Rita' had a lower yield than the non-grafted plants. Also there are some contradictory results about the fruit quality traits and how grafting affects them. For example Traka-Mavrona *et al.* (2000) report that the solutes associated with fruit quality are translocated in the scion through the xylem, whereas Lee (1994) states that quality traits *e.g.* fruit shape, skin colour, skin or rind smoothness, flesh texture and colour, soluble solids concentration *etc*. are influenced by the rootstock. However, other researchers showed that grafting did not affect fruit quality (Leoni *et al.,* 1990; Romano and Paratore, 2001).

The aim of this study was to evaluate a popular Greek commercial hybrid tomato, self-grafted and grafted on two new improved tomato rootstocks, for agronomic performance, yield and fruit quality attributes.

Materials and methods

Plant material: The commercial tomato (*L. esculentum* Mill.) hybrid cv. 'Big Red' was used as self-grafted and non-grafted control, while two hybrid tomatoes 'Heman' (*L. hirsutum*) and 'Primavera' (*L. esculentum* Mill.) were used as rootstocks.

'Heman' possesses resistance to *Pyrenochaeta lycopersici* and nematodes, whereas 'Primavera' is resistant to *Verticillium* and nematodes. Grafting combinations were as follows: BB (scion and rootstock 'Big Red'), BP (scion 'Big Red' and rootstock 'Primavera'), BH (scion 'Big Red' and rootstock 'Heman') and B (non-grafted, control).

The seeds of the scion cultivars were sown 5 days earlier than the seeds of the 2 rootstocks to ensure similar stem diameters at the grafting time because of the differences in growth vigour. Seedlings were grafted by hand, applying the splice grafting method when the scion had 2 real leaves and the rootstock 2.5-3 real leaves. Then the grafted plants were kept for 7 days under controlled conditions (90-95% RH, 24-26°C and 45% shading). Plants were transplanted to the soil in a greenhouse on 4/3/2004 and to the open-field on 13/5/2004 at the Velestino Farm (Magnesia, Greece) of the University of Thessaly, at a density of 12800 plants ha^{-1}. Normal cultural practices were followed for irrigation, fertilizer and pesticide application. A randomised complete block design was adopted with 4 replications, each consisting of 8 plants. Plants were cultivated in 4 replicated plots each of which contained 8 plants spaced at 0.6x1.0m. Four plants from each replicate were evaluated for height, flowering and yield, one was used for dry and wet weight measurements, while the others remained as guard plants and were not included in the evaluations.

Measurements: Mean maximum and minimum air temperature, relative humidity and the amount of rainfall were recorded daily throughout the two cultivations. Plant height was recorded between 8-96 DAT (Days After Transplantation) in the greenhouse cultivation and between 34-130 DAT in the open-field cultivation. In order to obtain flowering data, flowers of 5 clusters was considered. The fresh weight was determined for plants that were harvested at ground level and separated into leaves, stem, flowers and fruits. For the dry weight determination the plant tissues were dried in a ventilated oven at 90° C for 48h. Due to the different environmental condition in field and greenhouse, plants from both conditions were harvested almost in the same optical size and assessment was made at 107 DAT and 121 DAT for greenhouse and open-field, respectively. Total leaf area was measured by a Portable Area Meter (model LI3000A, LI-COR). Yield measurements were recorded on ripe fruits, which were hand-harvested, counted and weighed. For the greenhouse cultivation, 16 harvests were carried out between 75-192 DAT, while for the open-field cultivation 8 harvests were carried out between 68-130 DAT.

Finally 6 fruits were randomly harvested from each replication and were used for qualitative measurements *i.e.,* firmness (penetrometer FT327-8mm), soluble solids (refractometer), pH, titratable acidity, lycopene concentration (spectrophotometer at 600 nm) and concentration of Zn, Cu, Mn, Fe and Ca (atomic absorption spectrophotometer).

Data analysis: Statistical analysis was performed using 'SPSS 11.0 for Windows' and the differences between the means were compared using the criterion of the Duncan's multiple range test and LSD (P=0.05).

Results and discussion

Plant height was not significantly affected by grafting under greenhouse conditions, whereas in the open-field cultivation at 130 DAT the height of BH was significantly greater than the control and BP (Table 1). This result agrees with the results of Lee (1994) and Ioannou *et al.* (2002) who found that grafted plants were taller and more vigorous than self-rooted ones and had a larger central stem diameter.

Table 1. Plant height of non-grafted (B) and 3 grafted tomato plants (BH, BP, BB) over different growth periods in greenhouse and open-field conditions

	DAT	Plant height (cm)			
		BH	BP	BB	B
Greenhouse	30	42.70b	48.44c	36.80a	38.00b
	70	83.06a	91.88a	82.75a	80.31a
	96	95.88a	106.38a	100.75a	94.19a
Open-field	34	53.75bc	46.44a	51.06ab	56.81c
	89	67.75b	62.50a	64.38ab	63.13a
	130	75.31b	69.31a	72.00ab	70.32a

Means followed by the same letter are statistically not significant according Duncan's multiple range test (P=0.05). DAT: Days After Transplanting, BH: 'Big Red' x 'Heman', BP: 'Big Red' x'Primavera', BB: 'Big Red' x 'Big Red', B: 'Big Red'.

It was observed that in both greenhouse and open field cultivations flowering began earlier in the self-rooted plant, probably due to the fact that grafting caused stress and delayed flower formation. However, by the 5th cluster, grafted plants generally appeared to have a larger number of flowers but no significant differences between all the treatments with respect to the total number of flowers per plant were found. Also, it is worth mentioning that the number of flowers in the open field were almost 50 % less than in the greenhouse in all the treatments (Table 2).

Table 2. The mean number of flowers per cluster and total number of flowers per plant of non-grafted (B) and 3 grafted tomato plants (BH, BP, BB) at different growth periods under greenhouse and open-field conditions

	Cluster number	DAT	Number of flowers/cluster			
			BH	BP	BB	B
Greenhouse	1st	96	4.31a	4.13a	4.19a	4.56a
	2nd	96	5.19b	4.38a	4.25a	4.81ab
	3rd	96	3.81a	4.81a	5.25a	4.75a
	4th	96	5.13b	4.88ab	3.75a	5.38b
	5th	96	3.69a	4.81a	4.06a	4.94a
Total	5th	96	22.13a	23.01a	21.50a	24.44a
Open-field	1st	68	3.44a	3.25a	3.81a	3.69a
	2nd	68	0.69a	1.19a	1.0a	0.63a
	3rd	89	2.5a	3.06a	2.69a	2.44a
	4th	89	3.31a	3.31a	2.19a	2.38a
	5th	97	2.88a	2.56a	2.63a	2.25a
Total lowers	5th	97	12.82	13.37a	12.32a	11.39a

Means followed by the same letter are statistically not significant according Duncan's multiple range test (P=0.05)

From the data presented in Table 3, it is seen that there were no significant differences between the fresh and dry weights of stems, leaves and fruits both in the greenhouse and in the open-field after 107 and 121 DAT respectively, with the exception of the BH plants, which had a significantly lower fresh and

Table 3. Fresh and dry weight, plant height and total leaf area of non-grafted (B) and 3 grafted tomato plants (BH, BP, BB) at 107 DAT and 121 DAT under greenhouse and open-field conditions, respectively

Characterstics/ part		Greenhouse				Open-field			
		BH	BP	BB	B	BH	BP	BB	B
Stem	FW	204.30a	283.78a	242.38a	226.10a	185.00a	175.00a	208.33a	163.75a
	DW	36.30a	60.69a	45.10a	40.28a	26.73a	25.65a	31.90a	25.70a
Leaves	FW	884.08a	980.28a	775.60a	766,33a	351.25a	300.00a	310.00a	312.50a
	DW	139.84a	153.54a	126.69a	133.48a	33.34a	27.82a	30.27a	31.55a
Flowers	FW	13.35a	26.98b	20.40ab	14.93ab	5.00a	5.00a	5.00a	5.00a
	DW	2.23a	4.70b	3.73ab	3.03ab	0.73a	0.38a	0.73a	0.95a
Fruits*	FW	1776.63a	2787.78a	2241.38a	2531.38a	1955.00a	1873.33a	2840.00a	1740.00a
	DW	59.38a	55.80a	40.23a	71.19a	33.36a	27.09a	39.58a	26.42a
Total DW/FW %		8.86a	9.15a	7.49a	7.68a	4.38a	3.57a	3.81a	3.90a
Total leaf area (cm^2)		10923.10a	8646.20a	7598.10a	8693.20a	4949.0a	4087.80a	3997.0a	4296.50a
Plant height (cm)		127.75a	135.00a	144.50a	139.00a	74.00a	69.25a	71.33a	65.25a

*Ripe and Unripe. Means followed by the same letter are statistically not significant (Duncan's multiple range test, P=0.05)

dry weight of flowers than BP in the greenhouse cultivation. However, the ratio of total dry weight to total fresh weight was not significantly different between grafted plants and the control in both cultivations (Table 3). Moreover, in the greenhouse, grafted plants of BH and BP had a heavier fresh and dry weight than the open field cultivation. Table 3 shows that although the distribution of dry matter in the various parts of the plant was even in greenhouse cultivation, grafted plants had a higher accumulation of dry matter. It is worth mentioning that Romano and Paratore (2001) also reported that the dry weight of the aerial organs of grafted tomato plants ('Rita x Beaufort') was greater than that of the self-rooted plants.

Leaf area measurements at 107 DAT and 121 DAT in the greenhouse and in the open-field, respectively (Table 3) revealed that the plants of BH grafting had a larger leaf area than the other treatments. However, there was no significant difference. Also Pulgar *et al.* (1998) observed increased production of leaves in grafted plants as a result of an increased uptake of water and nutrients.

In the greenhouse as well as in the open-field during the harvest period 0-84 DAT, the self-rooted plants B had a greater yield than the grafted plants. This could be due to the fact that grafted plants were initially subjected to stress following the grafting operation. This early negative effect of grafting has also been reported by other authors (Ginoux, 1974; Tsouvaltzis *et al.,* 2004). However, during the 2nd harvest period the grafted plants BH and BP had a greater yield than the self-rooted B, while during the 3rd harvest period the three types of grafted plants had a greater yield than the self-rooted control (Table 4). It seems that the 4 treatments produced a higher quantity of fruits per plant at the 2nd harvest period when the plants had more favourable environmental conditions for growth. Mean daily temperatures for the first, second and third harvesting periods were 22.3, 27.8, 3 and 33.1°C for the greenhouse and 20.3, 26.8 and 23.5°C for the open field cultivations respectively. Finally, these increases in the total fruit yield of the BH and BP plants of the greenhouse cultivation, at 192 DAT resulted into 32.5% and 10% more fruit weight per plant than the control B, respectively, whereas self-grafted plants gave almost the same yield as the control. Similar results were found for the open-field cultivation where a higher total fruit weight of BH and BP at 130 DAT were obtained (12.8 and 11.1% higher than in the control, respectively) (Table 4).

Regarding fruit qualitative characteristics (Table 5) there were no significant differences between the 4 treatments in pH, Brix (%), concentration of lycopene or firmness. However, fruit acidity in grafted plants of BH cultivated in the open field was higher than in BB and B plants. The above results in general agree with other researchers who found that fruit descriptive and qualitative characteristics were not affected by grafting. (Leoni *et al.,* 1990; Romano and Paratore, 2001).

The fruit Cu, Mn and Fe contents were not significantly different

Table 4. Yield at different harvest periods and total of non-grafted (B) and 3 grafted tomato plant types (BH, BP, BB) under greenhouse and open-field conditions

	DAT	Fruit weight (g) $plant^{-1}$			
		BH	BP	BB	B
		Greenhouse			
1st	0-84	628.76ab	376.40a	738.62ab	786.52b
2nd	85-155	5066.90a	4267.76a	3411.79a	3483.59a
3rd	156-192	1872.50a	1042.31a	844.75a	836.25a
Total		7568.16b	5671.47ab	4995.16a	5106.36ab
		Open-field			
1st	0-84	420.94a	379.06a	388.44a	549.69a
2nd	85-121	1137.81a	1355.63a	1064.69a	1122.81a
3rd	122-130	537.50b	321.25b	318.75ab	154.94a
Total		2096.25a	2055.94a	1771.88a	1827.44a

Means followed by the same letter are statistically not significant according Duncan's multiple range test (P=0.05)

Table 5. Qualitative fruit parameters of non-grafted (B) and 3 grafted tomato plants (BH, BP, BB) under greenhouse and open-field conditions

Cultivars	pH	BRIX (%)	Acidity (% citric acid)	Lycopene (mg/ 100gDW)	Firmness (kg)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)	Ca (ppm)
Greenhouse										
BH	3.42a	4.4a	0.35a	2.83a	2.58a	0.35a	0.52a	0.13a	0.44a	25.25b
BP	3.72a	4.5a	0.25a	3.41a	2.58a	0.27a	0.44a	0.09a	0.45a	19.72ab
BB	3.30a	5.1a	0.31a	3.23a	2.49a	0.33a	0.42a	0.10a	0.56a	16.70a
B	3.48a	4.8a	0.33a	3.87a	3.15a	0.33a	0.40a	0.14a	0.61a	16.83a
Open-field										
BH	4.41a	4.04a	0.35b	6.00a	2.28a	0.36ab	0.31a	0.11a	0.51a	17.24a
BP	4.33a	3.90a	0.28ab	4.86a	2.15a	0.36ab	0.30a	0.11a	1.28a	18.99a
BB	4.30a	3.15a	0.25a	6.63a	2.10a	0.35a	0.32a	0.07a	0.52a	13.68a
B	4.34a	3.68a	0.25a	4.37a	2.37a	0.48b	0.39a	0.10a	0.62a	19.11a

Means followed by the same letter are statistically not significant (Duncan's multiple range test, P=0.05)

between the grafted plants and the control plants, either in the greenhouse or in the open-field. However, analyses showed that the fruit concentration of Ca in grafted plants BH was greater than in the fruits of the grafted plants BB and B in the greenhouse cultivation. The absorption of Ca could be associated strongly with the higher rate of absorption of water and minerals from the soil by roots of the rootstock Heman and therefore this could improve the absorption of Ca. Tsouvaltzis *et al.* (2004) recorded similar results, when tomato cv. 'Sacos F1' was grafted on 'Primavera' rootstock and fruit yield and mineral concentration increased. Also Lee (1994) found an increase in yield which was attributed to the vigour of the rootstock and the higher uptake of water and nutrients. Passam *et al.* (2005) found that eggplants grafted on to two tomato rootstocks gave a higher yield and bigger fruit size than those grafted on to two eggplant rootstocks, but the mineral composition of fruits from grafted plants did not differ from that of non grafted plants.

This study showed that in both the greenhouse and the open-field, tomato cv. 'Big Red' grafted on tomato rootstock 'Heman' gave a higher total yield without having significant effects on the quality of the fruits produced.

The results showed that tomato grafting on suitable rootstocks has positive effects on the cultivation performance, especially in the greenhouse conditions. The use of improved genotypes for rootstocks is required so as to improve yields under a variety of climatic and soil conditions. It is well known that the root system of the plants affects vegetative growth and yield. So, the effects of grafting recorded in most research papers are obviously related to the differences in the root system between grafted and non-grafted plants, *i.e.* to the efficiency of water and nutrient uptake by the roots, or even to the distribution of growth regulators.

In Greece, where the vegetable cultivation is still carried out mostly by traditional methods and modern cultivated techniques are adopted slowly, the grafting technique could help in the solution of many problems. Therefore, we consider the advantages of grafted plants, which offer increased yield and consequently higher profit, to be of value for farmers. Finally, the use of grafting is a simple step for more developed cultivation forms, like hydroponics.

References

AVRDC, 2000. Grafting takes root in Taiwan. *Center point,* the quarterly Newsletter of the Asian Vegetable Research and Development Centre. September 2000: 1-3.

Bersi, M. 2002. Tomato grafting as an alternative to methyl bromide in Marocco. Institut Agronomieque et Veterinaire Hasan II. Marocco.

Bulder, H.A.M., P.R. van Hasselt., P.J.C. Kuiper., E.J. Speek and A.P.M. Den Nijs, 1990. The effect of low root temperature in growth and lipid composition of low temperature tolerant rootstock genotypes for cucumber. *Journal of Plant Physiology,* 138**:** 661–666.

Cohen, S. and A. Naor, 2002. The effect of three rootstocks on water use, canopy conductance and hydraulic parameters of apple trees and predicting canopy from hydraulic conductance. *Plant, Cell and Environment,* 25: 17–28.

Estan, M.T., M.M. Martinez-Rodrigues, F. Perez-Alfoce, T.J. Flowers and M.C. Bolarin, 2005. Grafting raises the salt tolerance of tomato through limiting the transport of sodium and chloride to the shoot. *J. Experimental Botany*, 56(412) : 703-712.

FAO, 1998. *Production yearbook*, Agricultural Statistics Series. FAO, Rome. Vol. 52.

Ginoux, G. 1974. Bilan de quatre année de expérimentation sur le greffage de solanacées dans le Sud-Est. *Pépiniéristes Horticultures Maraîchers*, 152: 35-54.

Ioannou, N., M. Ioannou and K. Hadjiparaskevas, 2002. Evaluation of watermelon rootstocks for off-season production in heated greenhouses. *Acta Horticulturae*, 579: 501-506.

Kacjan-Marsic, N. and J. Osvald, 2004. The influence of grafting on yield of two tomato cultivars (*Lycopersicon esculentum* Mill.) grown in a plastic house. *Acta Agriculturae Slovenica*, 83(2): 243-249.

Lardizabal, R.D. and P.G. Thompson, 1990. Growth regulators combined with grafting increase flower number and seed production in sweet potato. *HortScience*, 25: 79-81.

Lee, J.M. 1994. Cultivation of grafted vegetables I, current status, grafting methods and benefits. *HortScience*, 29: 235-239.

Leonardi, C. and D. Romano, 2004. Recent issues on vegetable grafting. *Acta Horticulturae*, 631: 163-174.

Leoni, S., R. Grudina, M. Cadinu, B. Madeddu and M.C. Garletti, 1990. The influence of four rootstocks on some melon hybrids and a cultivar in greenhouse. *Acta Horticulturae*, 287: 127-134.

Oda, M. 1995. New grafting method for fruit-bearing vegetables in Japan. *Japan Agricultural Research Quarterly*, 29: 187-194.

Passam, H.C., M. Stylianoy and A. Kotsiras, 2005. Performance of Eggplant Grafted on Tomato and Eggplant Rootstocks. *European Journal of Horticultural Science*, 70(30): 130-134.

Proebsting, W.M.P., M.J. Hedden, S.J. Lewis and L.N. Croker-Proebsting, 1992. Gibberellin concentration and transport in genetic lines of pea. *Plant Physiology,* 100: 1354-1360.

Pulgar, G., R.M. Rivero, D.A. Moreno, L.R. Lopez-Lefebre, G. Villora, M. Baghour and L. Romero, 1998. Micronutrientes en hojas de sandía injertadas. In: VII Simposio nacional-III Ibérico sobre Nutrición Mineral de las Plantas. Gárate A. (Ed.), Universidad Autónoma de Madrid, Madrid., 255-260.

Rivero, R.M., J.M. Ruiz and L. Romero, 2003. Role of grafting in horticultural plants under stress conditions. *Food, Agriculture & Environment*, 1(1): 70-74.

Romano, D. and A. Paratore, 2001. Effects of grafting on tomato and eggplant. *Acta Horticulturae*, 559: 149-153.

Ruiz, J.M., L. Belakbir., J.M. Ragala and L. Romero, 1997. Response of plant yield and leaf pigments to saline conditions: effectiveness of different rootstocks in melon plants (*Cucumis melo* L.). *Soil Science Plant Nutrition*, 43: 855–862.

Scheffer, R.P. 1957. Grafting experiments with Fusarium wilt resistant and susceptible tomato plants. *Phytopathology*, 47: 30.

Traka-Mavrona, E., M. Koutsika-Sotiriou and T. Pritsa, 2000. Response of squash (*Cucurbita* Spp.) as rootstock for melon (*Cucumis melo* L.). *Scientia Horticulturae*, 83: 353-362.

Tsouvaltzis, P.I., A.S. Siomos and K.C. Dogras, 2004. The effect of the two tomatoes grafting on the performance, earliness and fruit quality. *Proc. 21st Pan-Hellenic Congress of the Greek Society for Horticultural Science*. Ioannina, Greece, 8-10 October 2003. Vol. 11: 51-55.

White, R.A.J. 1963. Grafted greenhouse tomatoes give heavier crops. *New Zealand Journal Agriculture*, 106: 247-248.

Journal of Applied Horticulture, 8(1): 8-11, January-June, 2006

Use of a chlorophyll meter and plant visual aspect for nitrogen management in tomato fertigation

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Abstract

This study evaluated the feasibility of using SPAD-502 chlorophyll meter and plant visual aspect for N management in drip fertirrigated tomato plants (*Lycopersicon esculentum* Mill.) under unheated greenhouse. Two separate experiments were carried out at Universidade Federal de Viçosa - MG – Brazil in leached and non-leached soils under greenhouse. Six treatments were evaluated in a randomised complete-block design with four replicates. In treatment 1, N was applied at the time SPAD reading in leaf dropped below a critical value previously established for the specific plant physiological stage (SPAD-1). In treatments 2 and 3, SPAD critical values were increased 20 % (SPAD-2) and decreased 10% (SPAD-3), respectively. In treatment 4, the visual aspect of tomato plant (PVA) was utilized as a criterion of N management. In treatments 5 and 6 (check), N rates were 280 and 0 kg N ha^{-1}, respectively. Total applied N rates ranged from 0 to 594 kg N ha^{-1}. In both the experiments, total and marketable fruit yields were highest in SPAD-1 treatment which only differed from the check plot. All five criteria allowed high total tomato fruit yields but, as experiments average, N use efficiency was highest with the PVA treatment. The highest net income was obtained with SPAD-1 treatment and was associated with the highest yield. The results indicate that a SPAD meter can provide a quantitative measure of the N requirement of the tomato plants as long as appropriate SPAD critical values are established. Visual ratings of plant canopy needs to be more evaluated and improved.

Key words: *Lycopersicon esculentum* Mill, unheated greenhouse, drip irrigation, SPAD, plant nutrition

Introduction

Usually, nitrogen (N) fertilizer recommendation to tomato crop are derived from analysis of yield response to different N rates from a group of experiments (Fontes and Guimarães, 1999). In intensive vegetable cropping systems, as greenhouse tomato production (Fayad *et al.*, 2000), growers tend to add excessive N fertilizer. However, economic, environmental and safety considerations demand that N fertilizer should be applied only in quantities which are strictly justified. Matching agreement between crop demand and supply is one of the prerequisites for efficient N use.

Approaches based on N contents in leaves have been used to increase N fertilizer use efficiency. N management program in tomato production can be attained by suitable evaluation of plant N status (Coltman, 1988; Smith and Loneragan, 1997) which is usually accomplished by a quantitative analysis of the N concentration in the plant dry matter. Alternatively, quick procedures had been proposed as the tomato leaf greenness determination by a hand-held device– Minolta SPAD-502 meter (Sandoval-Villa *et al.*, 1999; Guimarães *et al.*, 1999)

The chlorophyll meter SPAD-502 is for simple, rapid, and non destructive estimation of chlorophyll contents in tomato leaves (Guimarães *et al.*, 1999). As several authors have shown a relationship between chlorophyll and N contents in plant leaves (Scheepers *et al.*, 1992; Sexton and Carol, 2002; Wang *et. al*, 2004), chlorophyll contents can be used as an alternative measure of plant N status (Fontes, 2001). Timely and nondestructive leaf N status detection could allow real time decision and improvement in N management.

Chlorophyll meter utilization to evaluate plant N status at real time is suitable for precision agriculture and canopy greenness might serve as a useful diagnostic tool to assess plant N demand (Wiesler *et al.,* 2002). This is also valid for plant visual aspect as long as evaluation criterion could be established. Very few papers deal with the theme (Ronchi *et al.*, 2001).

The objective of this study was to evaluate the feasibility of using SPAD-502 chlorophyll meter and plant visual aspect for N management in drip fertirrigated tomato plant under unheated greenhouse conditions.

Materials and methods

Two experiments were carried out in unheated greenhouse at the Federal University of Viçosa – MG – Brazil. One experiment was set in a previously leached area (experiment 1) and the other one was set in a non-leached area (experiment 2), in the same greenhouse conditions. Leaching was accomplished by applying excessive water in the soil during 15 days immediately before tomato plant transplantation. Six treatments were evaluated in a randomised complete-block design with four replicates.

In three treatments, Minolta SPAD-502 meter was utilized for measurements on five leaflets of the leaf closest to each specific cluster, at the same day time, from 7:00 to 9:00 a.m., immediately after drip irrigation. A mean SPAD value was calculated for each plot at 28, 42, 56, 70 and 98 days after transplantation (DAT) coinciding to the flowering time of the first, second, third, fourth, fifth, and sixth cluster, respectively. Each SPAD value was the mean of the measurement in 10 leaflets. In treatment 1, (SPAD-1), N was applied at the time SPAD reading dropped below a

Table 1. Previously established SPAD critical values (CV) and SPAD readings at selected tomato plant physiological stages[1] (days after transplantation-DAT) in experiments (Experiment 1 & 2)

DAT[1]	Treatments								
	SPAD-1			SPAD-2			SPAD-3		
	CV	Exp. 1	Exp. 2	CV	Exp. 1	Exp. 2	CV	Exp. 1	Exp. 2
28	45.9	49.0	49.6	55.2	48.0	51.3	41.5	47.1	46.7
42	43.6	49.3	52.1	52.4	51.8	54.2	39.4	48.8	50.5
56	41.2	43.3	48.5	49.6	56.2	51.5	37.3	44.3	45.9
70	38.8	32.8	37.1	46.8	57.6	56.8	35.2	32.6	38.1
84	36.4	55.3	57.5	44.0	61.2	60.2	33.1	53.0	51.9
98	34.0	57.5	50.7	41.2	57.8	57.4	31.0	56.0	54.2

[1] From the first to the sixth cluster.

critical value previously established for the specific physiological stage of the plant. In treatments 2 and 3, SPAD critical values were increased 20% (SPAD-2) and decreased 10% (SPAD-3), respectively (Table 1). SPAD critical values (Y) utilized in the experiment were previously established from the equation $\hat{Y}$ = 50.7179 - 0.170527 x, derived from Guimarães (1998), where x values were 28, 42, 56, 70, 84, and 98 DAT (Table 1). Plants in all three SPAD treatments received 50 kg N ha^{-1} at transplanting and the remaining N was applied as necessary set by SPAD critical values (Table 1) at the rates calculated by equations given in Table 2.

Table 2. Equations utilized to calculate nitrogen fertilizer rate in SPAD treatments[1]

Treatment	Equation
SPAD-1	F={[50.7-(d x 0.17)]-C}x 70
SPAD-2	F={[60.8-(d x 0.20)]-C}x 70
SPAD-3	F={[45.7-(d x 0.15)]-C}x 70

[1] F = N rate (kg N ha^{-1}); d = plant age (days after transplantation) at the moment of SPAD reading; C = SPAD critical values at selected physiological stage; 0.17, 0.20, and 0.15 = daily decreases in the SPAD critical value with tomato plant aging; 70 = N rate (kg N ha^{-1}) to increase 1 SPAD unit.

In treatment 4, tomato plant visual aspect (PVA) was utilized as a criterion for N management. The severity of leaf chlorosis was characterized using a visual rating index (Table 3). Every 14 days, depending on the plant visual rating index it was decided on N sidedress application. Nitrogen rate of 30, 22.5, 15 or 7.5 kg N ha^{-1} was added whenever PVA where bad, regular, good or very good, respectively. A pre-planting 50 kg N ha^{-1}, at the transplanting time, was applied.

In treatment 5 (REFE), N was added @ 280 kg N ha^{-1} following recommendation supported by local experimental results (Fontes and Guimarães, 1999). In the treatment 6 (Check), plants were not fertilized with N.

At the transplanting time, N fertilizer (ammonium sulphate) was placed in open furrows, under the tomato plant. In sidedress, N fertilizer was applied by drip irrigation. N rates applied during the experiment are given in Table 4.

The experiments were conducted using recommended cultural practices (Fontes and Silva, 2002) which includes 25 days old seedlings (hybrid Carmen), plant stems vertically trained with plastic twine, stand of 1.66 plants m^{-2}, drip irrigation, stem tip pruned at 9 cluster, 10 harvests (during 65 days) and 143 days after transplantation cycle, from 10 September to 30 January.

Harvested fruits were separated as marketable and non-marketable; the marketable ones were graded according to Brazilian grade standards for big, medium, and small fruit. Based on different market prices for these three tomato fruit classes, yield was also expressed as "weighted yield" taking into account the big, medium and small fruits being 1, 0.658, and 0.396, respectively. Data were statistically evaluated by analysis of variance and treatment averages were compared with Tukey test (P=0.05).

Results and discussion

In both experiments, treatments led to different N sidedress rates and application dates (Table 4). Total N rates ranged from 0 to 594 kg N ha^{-1}. N requirement for high-yielding tomato fruit (> 80 t ha^{-1}), at field conditions, ranged from 125 to 351 kg N ha^{-1} (Scholberg *et al.,* 2000). In both experiments, increasing (SPAD-2) or decreasing (SPAD-3) SPAD critical values in relation to SPAD-1, led to higher or lower N fertilizer applications rates, respectively (Table 4).

In experiments 1 and 2 (Tables 5 and 6), total and marketable fruit yields were highest at SPAD-1 treatment which only differed significantly from the check plot. Total, marketable, and weighted yield values in this treatment were higher than 97, 75, and 45 previously obtained in the same place (Guimarães *et al.*, 1999). Weighted yield indicates the production cash value as it takes into account the price relationships between each fruit size grade (Fontes, 1997).

All five criteria allowed high total tomato fruit yields but with the PVA treatment, as experiments average, due to lower N addition, the nitrogen use efficiency (NUE) was highest (Table 7). NUE was expressed as: (total fruit yield at each treatment - total fruit yield at check plot)/(N rate in the treatment). Adjusting N rate in association with visual aspect and eliminating evaluator bias may turn the PVA approach useful.

The highest net income was obtained with SPAD-1 treatment (Exp. 2) and was associated with both the highest yield and the highest NUE (Table 7). SPAD-1 treatment led to apply N at 70 days after transplantation (DAT), at almost mid tomato plant cycle, at the beginning of fruit harvest which started at 77 DAT. This was probability due to high N demand by the tomato fruit enlargement. At this time, N demand increases (Tapia and Gutierrez, 1997; Fayad *et al.*, 2000) and soil N contents plus 50 kg N ha^{-1} added at transplantation time were not sufficient to maintain SPAD reading above the critical value. N rate applied in function of SPAD treatment was calculated based upon the criterion to apply 70 kg N ha^{-1} to increase 1 SPAD unit. To increase 1 SPAD

Table 3. Tomato plant visual aspect (PVA) utilized as a criterion for N management in the treatment number 4 and associated characteristics determined during plant cycle

PVA	Characteristic	Days after transplantating					
		14	28	42	56	70	84
Bad	Canopy greenness	YE	YE	YE	YE	YE	YE
	Leaf number	5	11	14	22	25	23
	Plant height (cm)	10	20	25	35	45	50
Regular	Canopy greenness	YG	YG	YG	YG	YG	YG
	Leaf number	6	15	24	26	28	25
	Plant height (cm)	15	30	50	95	105	110
Good	Canopy greenness	LG	LG	LG	LG	LG	LG
	Leaf number	7	18	30	35	34	33
	Plant height (cm)	15	45	90	155	165	170
Very good	Canopy greenness	DG	DG	DG	DG	DG	DG
	Leaf number	8	20	32	38	36	34
	Plant height (cm)	20	50	100	165	170	185

[1] YE = yellow; YG = yellow green; LG = light green; DG = dark green.

Table 4. Sidedress N rates (kg N ha^{-1}) applied during the tomato plant growth cycle in experiments 1 and 2

Treatment	Experiment	Days after transplanting							Total
		14	28	42	56	70	84	98	
SPAD-1	1	0	0	0	0	420	0	0	420
	2	0	0	0	0	116	0	0	116
SPAD-2	1	0	502	42	0	0	0	0	544
	2	0	270	0	0	0	0	0	270
SPAD-3	1	0	0	0	0	180	0	0	180
	2	0	0	0	0	0	0	0	0
PVA	1	22	15	15	15	15	15	0	97
	2	15	15	15	15	15	15	0	90
REFE	1	28	42	42	42	42	42	42	280
	2	28	42	42	42	42	42	42	280
CHECK	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0

Table 5. Total, marketable and weighted yields of tomato as a function of treatments in experiment 1

Treatment	Yield (t ha^{-1})		
	Total	Marketable	Weighted
SPAD-1	99.1 a	97.0 a	52.2
SPAD-2	83.4 ab	77.3 ab	45.4
SPAD-3	82.8 ab	78.7 ab	43.4
PVA	84.5 ab	81.0 ab	44.5
REFE	93.7 ab	91.5 ab	53.7
CHECK	68.3 b	64.5 b	37.5

Table 6. Total, marketable and weighted yields of tomato as a function of treatments in experiment 2

Treatment	Yield (t ha^{-1})		
	Total	Marketable	Weighted
SPAD-1	101.9a	99.7a	61.7a
SPAD-2	86.4ab	82.3ab	49.2ab
SPAD-3	77.7ab	74.9ab	40.9ab
PVA	93.1ab	88.5ab	50.3ab
REFE	94.3ab	89.8ab	55.5ab
CHECK	71.7b	68.2b	40.3b

In each column, means followed by the same letter were not different by Tukey test (P=0.05)

unit in cotton and potato plants it was necessary 25 or 61 kg N ha^{-1}, respectively (Feibo *et al.*, 1998; Gil *et al.*, 2002). Varvel *et al.* (1997) utilized 30 kg N ha^{-1} when SPAD reading was below the critical level to obtain the highest corn yield.

In SPAD-1 treatment, commercial average yield was 688 kg ha^{-1} day^{-1}. Usually, tomato plant cycle in the field is 120 -160 days. But, it can be grown for in the field for longer time and in such cases the fruit productivity will be higher. So, expressing fruit productivity per day plant stay in the field, allow appropriate comparison among research results (Fontes, 1997). Values ranging from 700 (Vooren *et al.*, 1986) to 1.200 kg ha^{-1} day^{-1} (Fontes *et al*., 1997, Papodopoulos and Hao, 1997) have been reported.

Finally, the result suggests a SPAD meter can provide a quantitative measure of the requirement of tomato plants as long as appropriate SPAD critical value are established. To establish precise and universal critical SPAD index is complex process due to the narrow values separating N deficiency from surplus and great number of variables affecting the index, as changes in leaf irradiance and water status (Martinez and Guiamet, 2004), environmental conditions and statistical procedures (Fontes and Ronchi, 2002). Caution is needed regarding the universality of SPAD and N calibrations across geographical

Table 7. Total nitrogen fertilizer rate and cost, net income, nitrogen use efficiency (NUE), agronomic nitrogen efficiency (ANE) for each treatment in experiments 1 and 2[1]

Treatment	Experiment	Total N (kg N ha^{-1})	N cost (US$ ha^{-1})	NPI[2] (US$ ha^{-1})	NUE[3] (kg kg^{-1})	ANE[4] (kg kg^{-1})
SPAD-1	1	470	588	15,072	66	211
	2	166	208	18,302	182	614
SPAD-2	1	594	745	12,875	25	140
	2	320	400	14,360	46	270
SPAD-3	1	230	288	12,732	63	360
	2	50	63	12,207	122	1556
PVA	1	153	191	13,159	106	552
	2	146	183	14,907	147	638
REFE	1	280	350	15,760	91	335
	2	280	350	16,300	81	337
CHECK	1	0	0	11,250	-	-
	2	0	0	12,090	-	-

[1]N price: US$ 1.25 kg^{-1}; selling price of high graded fruit (weighted yield): US$ 0.30 kg^{-1}

[2]Net partial income: (weighted yield x 0.30) – (N fertilizer cost). [3]NUE: (total fruit yield at each treatment - total fruit yield at check plot)/(N rate at treatment).[4]NE: (total fruit yield at each treatment)/(N rate at each treatment).

locations and seasons. To counter these potential problems, users should establish the SPAD critical values for specific environmental condition. Visual ratings of plant canopy needs to be more evaluated. This may facilitate more precise N fertilizer recommendations and thereby help to minimize nitrate contents in the soil.

References

Coltman, R.R. 1988. Yield of greenhouse tomatoes managed to maintain specific petiole sap nitrate. *HortScience*, 23(1): 148-151.

Fayad, J.A., P.C.R Fontes, A.A. Cardoso, F.L. Finger and F.A. Ferreira, 2000. Absorção de nutrientes pelo tomateiro cultivado em condições de campo e de estufa. *Horticultura Brasileira*, 20(1): 90-94.

Feibo, W., W. Lianghuan and X. Fuhua, 1998. Chlorophyll meter to predict nitrogen sidedress requirements for short-season cotton (*Gossypium hirsutum* L.). *Field Crops Res.,* 56: 309-314.

Fontes, P.C.R. 1997. Produtividade do tomateiro: kg/ha ou kg/ha/dia?. *Horticultura Brasileira*, 15(2): 83-84.

Fontes, P.C.R. 2001. *Diagnóstico do estado nutricional das plantas*. Viçosa: UFV, 122p.

Fontes, P.C.R., E.N. Dias, S.R. Zanin and F.L. Finger, 1997. Produção de cultivares de tomate em estufa coberta com plástico. *Revista Ceres*, 44(252): 152-160.

Fontes, P.C.R. and T.G. Guimarães, 1999. Manejo dos fertilizantes nas culturas de hortaliças cultivadas em solo, em ambiente protegido. *Informe Agropecuário*, 20(200&201): 36-44.

Fontes, P.C.R. and C.P. Ronchi, 2002. Critical values of nitrogen indices in tomato plants grown in soil and nutrient solution determined by different statistical procedures. *Pesquisa Agropecuária Brasileira*, 37(10): 1421-1429.

Fontes, P.C.R. and D.J.H. Silva, 2002. *Produção de tomate de mesa*. Viçosa, MG: Aprenda Fácil, 195p.

Gil, P.T., P.C.R. Fontes, P.R. Cecon and F.A. Ferreira, 2002. Índice SPAD para o diagnóstico do estado de nitrogênio e para o prognóstico da produtividade de batata. *Horticultura Brasileira*, 20(4): 611-615.

Guimarães, T.G. 1998. *Nitrogênio no solo e na planta, teor de clorofila e produção do tomateiro, no campo e na estufa, influenciados por doses de nitrogênio*. Viçosa-MG: UFV, 184p. (DS Thesis).

Guimarães, T.G., P.C.R. Fontes, P.R.G. Pereira, V.H.V. Alvarez and P.H. Monnerat, 1999. Teores de clorofila determinados por medidor portátil e sua relação com formas de nitrogênio em folhas de tomateiro cultivados em dois tipos de solo. *Bragantia*, 58(1): 209-216.

Martinez, D.E. and J.J. Guiamet, 2004. Distortion of the SPAD 502 chlorophyll meter readings by changes in irradiance and leaf water status. *Agronomie*, 24(1): 41-46.

Papadopoulos, A.P. and X. Hao, 1997. Effects of three greenhouse cover materials on tomato growth, productivity, and energy use. *Scientia Hortic.*, 69: 1-29.

Ronchi, C.P., P.C.R. Fontes, P.R.G. Pereira, J.C.S. Nunes and H.E.P. Martinez, 2001. Índices de nitrogênio e de crescimento do tomateiro em solo e em solução nutritiva. *Revista Ceres*, 48: 469-484.

Sandoval-Villa, M., C.W. Wood and E.A. Guertal, 1999. Ammonium concentration in solution affects chlorophyll meter readings in tomato leaves. *J. Plant Nut.,* 22(11): 1717-1729.

Scheepers, J.S., D.D. Francis, M. Vigil and F.E. Below, 1992. Comparison of corn leaf-nitrogen concentration and chlorophyll meter readings. *Commun. Soil Sci. Plant Anal.,* 23(17&20): 2173-2187.

Scholberg, J., B.L. McNeal, K.J. Boote, J.W. Jones, S.J. Locascio and S.M. Olson, 2000. Nitrogen stress effects on growth and nitrogen accumulation by field-grown tomato. *Agronomy Journal,* 92: 159-167.

Sexton, P. and J. Carrol, 2002. Comparison of SPAD chlorophyll meter readings vs. petiole nitrate concentration in sugarbeet. *J. Plant Nut*., 25(9): 1975-1986.

Smith, F.W. and J.E. Loneragan, 1997. Interpretation of plant analysis: concepts and principles. In: *Plant analysis - an interpretation manual.* Reuter, D.J., Robinson, J.B. (Eds). Collingwood: CSIRO Publishing, p.2-33.

Tapia, M.L. and V. Gutierrez, 1997. Distribution pattern of dry weight, nitrogen, phosphorus, and potassium thought tomato ontogenesis. *J. Plant Nut*., 20(6): 783-791.

Varvel, G.E., J.S. Schepers and D.D. Francis, 1997. Ability for in-season correction of nitrogen deficiency in corn using chlorophyll meters. *Soil Sci. Soc. Am. J*., 61: 1233-1239.

Vooren, J., G.W.H. Welles and G. Hayman, 1986. Glasshouse crop production. In: *The tomato crop a scientific basis for improvement*. Atherton, J.G., Rudich, J. (Eds.). London: Chapman and Hall, p. 581-623.

Wang, Q., J. Chen and Y. Li, 2004. Nondestructive and rapid estimation of leaf chlorophyll and nitrogen status of peace lily using a chlorophyll meter. *J. Plant. Nut.,* 27: 557-569.

Wiesler, F., M. Bauer, M. Kamh, T. Engels and S. Reusch, 2002. The crop as indicator for sidedress nitrogen demand in sugar beet production – limitations and perspectives. *J. Plant Nutr. Soil Sci.,* 165: 93-99.

Journal of Applied Horticulture, 8(1): 12-14, January-June, 2006

Biodegradable paper/polymerized vegetable oil mulches for tomato and pepper production

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Abstract

This project was undertaken to compare the efficacy of a biodegradable paper/cured vegetable oil mulch with newspaper/straw and bare soil for reducing weed growth and promoting vegetable yields. There were no significant differences in total tomato (*Lycopersicon esculentum*) or pepper (*Capsicum annum*) yields between the different mulch types. The coated paper and newspaper/straw mulches were effective in preventing weed growth around the plants while hand weeding was required for the bare soil plots. After 3 months, there was slight degradation (a few cracks, holes) of the coated paper mulches but not enough to allow noticeable weed penetration or detachment of the buried edge. Paper/cured oil mulch rolls appear to be a convenient and effective alternative to laborious hand weeding or spreading of newspaper and straw for vegetable gardening.

Key words: Degradable mulch, soybean oil, sustainable agriculture, vegetable production

Introduction

There has been growing interest recently in the use of biodegradable mulch films for suppression of weeds, increasing soil temperatures and yields of vegetables and fruits (Greer and Dole, 2003; Halley *et al.*, 2001; Weber, 2003). A biodegradable mulch allows growers to till the mulch into the field at the end of the growing season rather than having to remove and dispose of non-degradable polyethylene mulches, often at considerable cost (Anderson *et al.*, 1995).

Various types of biodegradable mulches have been considered including starch-based films (Halley *et al.*, 2001), polyester films (Dever *et al.*, 1998), fiber slurries (Olsen and Gounder, 2001) and coated paper. Paper alone begins to tear and blow away within 2-3 weeks after field application due to rapid biodegradation and loss of strength when wet (Anderson *et al.*, 1995; Shogren, 2000). Therefore, a number of coatings or laminates on paper have been examined to increase wet strength and slow biodegradation. These include tar (Rivise, 1929), wax, polyethylene (Vandenberg and Tiessen, 1972), latexes (Brault *et al.*, 2002), polyesters (Rangarajan, 2000) and vegetable oils (Anderson *et al.*, 1995).

Vegetable oil coatings are attractive because they are inexpensive, renewable, produced in large quantity in the U.S. and can be polymerized (cured) into water resistant, biodegradable films (Shogren, 1999). Previous studies have shown that kraft paper coated with soybean or linseed oils then allowed to cure via sun and air in the field were effective as polyethylene mulches for growing watermelon (Shogren and Hochmuth, 2004) and cottonwood trees (Shogren and Rousseau, 2005). One disadvantage of these oil saturated paper mulches was the messiness associated with handling oily paper in the field (Shogren and Hochmuth, 2004). In order to avoid this problem, kraft paper was coated with a resin made from epoxidized soybean oil (ESO) and citric acid and then thermally cured (Shogren, 1999; Shogren, 2000). Previous work has shown these compositions to be fully biodegradable in soil but over a longer time span than uncoated paper (several months) (Shogren *et al.*, 2004).

The objective of this study was to assess whether mulches made from paper coated with ESO/citric acid resins would serve as effective weed barriers, promote the growth of tomato and pepper plants and sustain good yields when compared to other weed control methods commonly used in a garden or small farm setting.

Materials and methods

Materials: Brown kraft paper, 30 and 40 lb weights (3000 ft^{-2}), made from 100% recycled fiber was obtained from Carter Paper and Packaging, Peoria, IL. Epoxidized soybean oil (ESO, Paraplex G-62) was from C. P. Hall, Bedford Park, IL. Citric acid (99%) was from Aldrich, Milwaukee, WI. Straw was a mixture of wheat (*Triticum aestivum*) and rye (*Secale cereale*) stems from a local farm. Tomato (*Lycopersicon esculentum*) and sweet green pepper (*Capsicum annum*) plants were donated by Greenview Nursery, Peoria, IL.

Preparation of coated paper: ESO (2950 g) was heated to 100 °C in a 11.5 l stainless steel beaker and 110 g of carbon black (colorant) was added with a motorized propeller stirrer. Carbon black was added to reduce light transmission through the paper mulches. A hot (102 °C) solution of 850 g citric acid in 280 g deionized water was next added to the ESO with stirring. After the mixture reached 105 °C, the beaker was removed from heat and placed in an ice bath to cool to 40 °C.

[*]Product names are necessary to report factually on available data; however the USDA neither guarantees nor warrants the standard of the product, and the use of the name. USDA implies no approval of the product to the exclusion of others that may also be suitable.

Paper coating and heat curing was accomplished using a simple in-house design. This consisted of a stainless steel table with supply and motorized take-up rolls on the lower shelf, a flat aluminum sheet on the top shelf for coating, and an attached flow-through sheet oven for heat-curing. The oven consisted of 2 x 4.5 ft. aluminum sheets separated by 1 in. heated by electrical resistance mats and insulated with 2 in. of fiberglass top and bottom. ESO/citric coatings were dripped onto one side of the paper and were spread into a thin layer by a neoprene rubber blade clamped between 2 aluminum bars. Temperatures and residence times in the oven were 150-180 °C and ~1 min, respectively at coating speeds of 2-3 ft./min. Coating weights were 37 and 42% of paper weight for the 30 and 40 lb papers, respectively.

Field studies: There were four different mulch treatments (30 lb coated paper, 40 lb coated paper, newspaper/straw, bare soil). The field site was located in Peoria, IL and run by the University of Illinois Master Gardeners of Peoria County as part of the Plant-a-Row-for-the-Hungry program. Tomato and bell pepper plants were planted in adjacent 75 ft. long rows with spacings of approximately 1.5 ft. between plants and 4 ft. between row centers. Planting took place on 26 May and mulches were laid on 3 June 2003. Coated paper mulch samples were cut into 6 x 2 ft. lengths then cut from the side with scissors to allow the mulch to be placed around the plants (coated side up). Edges of the mulches were then weighted down with soil (pepper) or held in place by steel cages (tomato). For the newspaper/straw treatment, 3 layers of newspaper were placed around the plants followed by approximately 2 inches of straw on top on the newspaper to help hold the newspaper in place. There were 3 replications for each treatment and were arranged in randomised order. The bare soil plots were hand weeded once per week over the summer. Vegetables were harvested weekly beginning 21 July until 15 September. Total number as well as weight of vegetables deemed marketable were measured weekly.

Soil temperatures were measured in duplicate on 18 June, 24 June, 7 July and 26 August at 8 AM and 2 PM using a digital temperature probe model 4045 (Control Co., Friendswood, TX). Measurements were taken at a depth of approximately 4 in. (10.2 cm) below the surface. Soil samples for moisture determination were collected on 18 June, 24 June, 7 July and 26 August. Mulches were carefully lifted from the edge and the top 3-4 in. of soil (~100 g) was scooped into plastic zip lock bags. Edges of the mulches were reburied after sample collection. Soil samples were then transported to the lab where water content was measured gravimetrically after heating 10 g soil to 105 °C for 20 min. using an Ohaus moisture analyzer, model MB200 (Ohaus Co., Florham Park, NJ). Counts of weeds growing through the mulches were made from detailed photographs of the plots taken on 24 June, 24 July, 4 August and 18 September.

Statistical analyses: A Levene's homogeneity of variance test was carried out to determine if transformation of the vegetable number and weight data were necessary. Four, single-factor Analyses of Variance (ANOVA) were performed comparing the four mulch treatments for number and weight of harvested tomatoes and green peppers at the end of each week and for the season. In case significant F-test was obtained in ANOVA, Duncan's multiple range test was used for multiple comparison procedure, at the P=0.05 level, for determining pairwise differences between the mulch treatments.

Results and discussion

As shown in Table 1, there were no significant differences in the total number or weight of tomatoes or peppers between plants grown on the different mulch types. Soil temperatures for the different mulch treatments are shown in Table 2. Soil temperatures underneath the newspaper/straw were less than the coated paper or bare soil, especially during the hottest part of the day (afternoon). This is probably due to the thick, insulative properties of the straw as well as its light colour which tends to reflect solar radiation. There was a noticeable lightening in colour (bleaching) of the coated papers over the summer and this would likely tend to lessen the soil warming later on. This lightening effect has been noted previously for coated paper mulches (Brault *et al*., 2002). Soil moisture, as shown in Table 2, was generally higher under the newspaper/straw mulch than under the bare soil or coated paper. This is likely due to the lower soil temperature under the newspaper/straw and hence lower evaporation rates. Water permeability of the coated paper mulches has not been measured but a simple test of a drop of water placed on the mulch shows it will pass through within an

Table 1. Total mean yields of tomato and pepper

Mulch	Total mean tomato yield		Total mean pepper yield	
	Number (number/plot)	Weight (kg/plot)	Number (number/plot)	Weight (kg/plot)
30 lb paper/oil	59 a[z]	12.5 a	48 a	3.7 a
40 lb paper/oil	66 a	12.6 a	44 a	3.3 a
Newspaper/straw	58 a	17.0 a	38 a	3.0 a
Bare soil	57 a	12.0 a	41 a	3.2 a

[z]Means with the same letter within a column are not significantly different at P=0.05.

Table 2. Mean soil temperatures and moistures on 7 July

Mulch	8 AM		2 PM	
	Temperature (°C)	Moisture (%)	Temperature (°C)	Moisture (%)
30 lb paper/oil	25.4 a[z]	-	32.2 a	12.1 a
40 lb paper/oil	24.9 a	-	33.0 a	12.9 a
Newspaper/straw	24.0 b	-	27.7 b	17.9 b
Bare soil	25.2 a	-	33.3 a	11.8 a

[z]Means with the same letter within a column are not significantly different at P=0.05.
Ambient air temperatures were 30 and 34.8 °C at 8 AM and 2 PM, respectively

Table 3. Mean[y] number of weeds from six 1.8 x 0.6 m plots

Mulch	Days post-planting			
	29	59	70	114
30 lb paper/oil	0 a[z]	0 a	1 a	1 a
40 lb paper/oil	0 a	0 a	0 a	1 a
Newspaper/straw	0 a	0 a	1 a	1 a
Bare soil	4 b	6 b	14 b	11 b

[y]Mean of six replications (data from tomato and pepper replicates combined)
[z]Means with the same letter within a column are not significantly different at P=0.05.

hour or so, indicating some permeability.

Mean number of weeds penetrating different mulch treatments are given in Table 3. Even after almost 4 months, there was only an average of 1 weed per 6 ft plot penetrating the coated paper mulches. The exposed and buried tuck areas of the coated paper mulches were largely intact, with a few holes. In contrast, there were >10 weeds per plot in the bare soil treatments, even with weekly hand pulling of weeds. There was no significant difference between the 30 and 40 lb coated paper so the thinner paper could be used for better economics. Manual weed removal required about 1 hour per week for the six 6-ft bare ground plots or 16 hours total for the 16 week growing season. The time required for the initial coated paper mulch laying was about 1 hour for six 6-ft plots.

Thus, paper/ESO/citric acid mulches were effective in preventing weed growth and plants grown on these gave yields of tomatoes and green peppers similar to the newspaper/straw mulch or hand-weeded control plots. For many home or community gardeners, hand-weeding is a tedious and difficult task so use of a weed-blocking mulch would be desirable. Application of the paper/oil mulch would be a little easier than newspaper/straw since the former is in a roll form. It could be unrolled first then seedlings planted in cut holes or placed around existing plants as in this study. For larger-scale vegetable growers, the paper/oil rolls would work with conventional plastic mulch-laying equipment, as has been shown previously (Shogren and Hochmuth, 2004). The ESO/citric acid coated paper used here might be more readily accepted commercially since it has been heat cured to give a hard surface rather than the oily paper described previously (Shogren and Hochmuth, 2004).

Paper coated with other types of biodegradable polymers, especially polyesters, have been tested recently (Rangarajan, 2000). These biodegradable polyesters, such as polycaprolactone, polylactic acid, poly(butylene succinate-adipate), are currently rather expensive ($1.5-3/lb.) Epoxidized soybean oil and citric acid are less expensive ($0.30-0.60/lb.), making them more economically attractive. Paper, on an area basis, is however more expensive than polyethylene mulch due to the greater thickness of the paper. Thus the coated paper mulches would be more suited to higher value applications such as home gardening or small farmers or where a biodegradable, water permeable mulch is required.

Acknowledgements

We thank Elizabeth Krietemeyer, Lynn Webb, Helen Nixon and Patrise Swanson for help in field work. We also thank Debra Palmquist, USDA/ARS Midwest area statistician for statistical analyses.

References

Anderson, D.F., M. Garisto, J. Bourrut, M.W. Schonbeck, R. Jaye, A. Wurzberger and R. DeGregorio, 1995. Evaluation of a paper mulch made from recycled materials as an alternative to plastic film mulch for vegetables. *J. Sustainable Agriculture*, 7: 39-61.

Brault, D., K.A. Stewart and S. Jenni, 2002. Optical properties of paper and polyethylene mulches used for weed control in lettuce. *HortScience*, 37: 87-91.

Dever, M., P. Lambdin and B. Ownley, 1998. Biodegradable plastic mulch: An option in vegetable production? *Tennessee Agricultural Science*, 186: 16.

Greer, L. and J.M. Dole, 2003. Aluminum foil, aluminum-painted, plastic, and degradable mulches increase yields and decrease insect-vectored diseases of vegetables. *HortTechnology*, 13: 276-284.

Halley, P., R. Rutgers, S. Coombs, J. Kettels, J. Gralton, G. Christie, M. Jenkins, H. Beh, K. Griffin, R. Jayasekara and G. Lonergan, 2001. Developing biodegradable mulch films from starch-based polymers. *Starch/Stärke*, 53: 362-367.

Olsen, J.K. and R.K. Gounder, 2001. Alternatives to polyethylene mulch film - a field assessment of transported materials in capsicum (*Capsicum annuum* L.). *Australian J. Experimental Agriculture*, 41: 93-103.

Rangarajan, A. 2000. Paper mulch: Can it replace plastic? http://www.hort.cornell.edu-commercialvegetables.

Rivise, C.W. 1929. Mulch paper. *Paper Trade J*., 89: 55-57.

Shogren, R.L. 1999. Preparation and characterization of a biodegradable mulch: paper coated with polymerized vegetable oils. *J. Appl. Polym. Sci.*, 73: 2159-2167.

Shogren, R.L. 2000. Biodegradable mulches from renewable resources. *J. Sustainable Agriculture.*, 16: 33-47.

Shogren, R.L. and R.C. Hochmuth, 2004. Field evaluation of watermelon grown on paper-polymerized vegetable oil mulches. *HortScience*, 39: 1588-1591.

Shogren, R.L. and R.J. Rousseau, 2005. Field testing of paper/polymerized vegetable oil mulches for enhancing growth of eastern cottonwood trees for pulp. *Forest Ecology and Management*, 208: 115-122.

Shogren, R.L., Z. Petrovic, Z. Liu and S.Z. Erhan, 2004. Biodegradation behaviour of some vegetable oil-based polymers. *J. Polymers and the Environment*, 12: 173-178.

Vandenberg, J. and H. Tiessen, 1972. Influence of wax-coated and polyethylene-coated paper mulch on growth and flowering of tomato. *HortScience*, 7: 464-465.

Weber, C.A. 2003. Biodegradable mulch films for weed suppression in the establishment year of matted-row strawberries. *HortTechnology*, 13: 665-668.

Journal of Applied Horticulture, 8(1): 15-20, January-June, 2006

Compact 3U as a novel lighting source for the propagation of some horticultural plants

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Abstract

A novel lighting system (Compact 3U) was successfully applied to the micropropagation of some horticultural plants. *Cymbidium* 'Tim Hot', *Lilium longiflorum* and *Fragaria vesca* cv. 'My Da' shoots were used for this study. To compare *in vitro* growth of plantlets placed under Neon and Compact 3U lighting systems, *Fragaria vesca* cv. 'My Da' shoots were cultured on ½ MS medium supplemented with 1 gl^{-1} activated charcoal, 30 gl^{-1} sucrose and 8 gl^{-1} agar under two lighting sources at 45 $\mu molm^{-2}s^{-1}$. After three weeks of culture, the shoot and root length, leaf area and fresh weight of strawberry plantlets under Compact 3U system were significantly higher than those grown under Neon system. To clarify the effect of irradiance of Compact 3U system on the development of plantlets, *Cymbidium* 'Tim Hot' shoots were cultured on MS medium supplemented with 0.5 mgl^{-1} NAA, 1 gl^{-1} activated charcoal, 100% coconut water, 25 gl^{-1} sucrose and 8 gl^{-1} agar, *Lilium longiflorum* and *Fragaria vesca* cv. 'My Da' shoots were cultured on ½ MS medium supplemented with 1 gl^{-1} activated charcoal, 30 gl^{-1} sucrose and 8 gl^{-1} agar at different irradiances: (1) Neon at 45 $\mu molm^{-2}s^{-1}$ (control), and Compact 3U at: (2) 45 $\mu molm^{-2}s^{-1}$, (3) 60 $\mu molm^{-2}s^{-1}$, and (4) 75 $\mu molm^{-2}s^{-1}$. The results showed that plantlets of the three genera adapted differently to irradiances and lighting sources, but in all, the growth of plantlets were better under the Compact 3U system. Futhermore, *ex vitro* plantlets derived from Compact 3U system also developed better than those from Neon system.

Key words: Compact 3U, Neon, *Cymbidium* 'Tim Hot', *Lilium longiflorum*, *Fragaria vesca* cv. 'My Da'

Introduction

Now-a-days, *in vitro* multiplication is a primary method to rapidly mass-produce horticultural plants. The demand for high quality planting material has been increasing quickly worldwide for reforestation, foods/forage production, urban/indoor horticulture and global environment protection (Kozai *et al.*, 1992). In many cases, since micropropagation gave some superior transplant qualities to seedling production and conventional vegetative production, billions of micropropagated plantlets were produced annually world-wide (Debergh and Zimmerman, 1990). Tissue culture has been carried out in more than 600 companies all over the world. However, the widespread use of micropropagation for major crops in agriculture and horticulture was restricted because of its relatively high production costs caused by high labour cost (Kozai *et al.*, 1992), especially electrical energy consumption.

Control of plantlet growth and morphology is important in micropropagation to obtain high plantlet quality at different growth and developmental stages, and to save labour by automation or robotics (Miyashita, 1995). Many of the growth and morphological characteristics of plants *in* and *ex vitro* are influenced by environmental factors, such as light (quality, intensity, duration and direction), temperature, gaseous composition (CO_2, O_2, H_2O and C_2H_4), and medium composition (Schwabe, 1963; Kozai *et al.*, 1992). Light quality had a significant influence on the growth and morphology of plants *in* and *ex vitro* (Warrington and Michell, 1976; Morgan and Smith, 1981; Smith, 1982; Tibbitts *et al.*, 1983; Mortensen and Stromme, 1987; Economou and Read, 1987; Agrawal, 1992). The total quantity of light that a plant received during illumination directly affected photosynthesis as well as plant growth and yield (Kim and Kozai, 2000). Hence, many lighting systems that effectively used electrical energy in the multiplication of horticultural plants have been studied intensively such as fluorescent, incandescent, luminescent (Sodium high pressure) lighting systems, and recently, light-emitting diode (LED) lighting source. However, tissue cultured plants are almost invariably grown under fluorescent illumination (Collin *et al.*, 1988), especially under cool white fluorescent lamps with a high proportion of its output in the blue and red regions (Hart, 1988).

Previous studies had been done on the effect of light quality and intensity of different lighting sources on the growth and morphology of *in* and *ex vitro* plantlets (Seabrook, 1987; Hayashi *et al.*, 1992; Iwanami *et al.*, 1992; Kozai *et al.*, 1992; Kirdmanee *et al.*, 1993; Gabarkiewicz *et al.*, 1997; Wulster and Janes, 1997; Maas and Bakx, 1997; Kunneman and Ruesink, 1997; Moe, 1997; Faust and Heins, 1997; Murakami *et al.*, 1997; Gabryszewska and Rudnicki, 1997; Walz and Horn, 1997; Miyashita *et al.*, 1997; Nhut, 2002). In our study, Compact 3U lamps were used as a promising lighting source for propagating some horticultural plants such as *Cymbidium*, *Lilium* and strawberry. These plants are highly valuable economic crops in Vietnam as well as all over the world. In this report, we focused on the effects of two different lighting sources (Neon and Compact 3U) as well as some different intensities of Compact 3U lamps (45, 60, and 75 $\mu molm^{-2}s^{-1}$, respectively) on the growth and morphology of these *in vitro* plantlets, and Neon lamp (with cool white emission) as a control system.

Compact 3U lamp (Fig. 1), which saves 80% electrical energy

as compared to incandescent lamps, has a compact size, long life (>6.000 h), and reaches one-fifth the brightness of conventional incandescent lamps. Hence, plant production cost could be decreased.

Materials and methods

Plant materials and culture media: *Cymbidium* 'Tim Hot' shoots (4 cm length), derived from protocorm-like bodies (PLBs) cultured on MS (Murashige and Skoog, 1962) medium containing 0.5 mgl^{-1} α-naphthaleneacetic acid (NAA), 2 mgl^{-1} 6-benzyladenine (BA), 1 gl^{-1} activated charcoal (AC), 20% coconut water (CW), 30 gl^{-1} sucrose and 8 gl^{-1} agar (Haiphong Co., Vietnam), were cultured on MS medium containing 0.5 mgl^{-1} NAA, 1 gl^{-1} AC, 10% CW, 25 gl^{-1} sucrose and 8 gl^{-1} agar.

Lilium longiflorum bulb scales, derived from *in vitro* bulblets cultured on MS medium containing 0.2 - 0.5 mgl^{-1} BA, 30 gl^{-1} sucrose and 8 gl^{-1} agar, were cultured on ½ MS medium supplemented with 1 gl^{-1} AC, 30 gl^{-1} sucrose and 8 gl^{-1} agar.

Fragaria vesca cv. 'My Da' shoots (1.5 cm), derived from meristems cultured on MS medium containing vitamin B_5, 0.2 mgl^{-1} BA, 30 gl^{-1} sucrose and 8 gl^{-1} agar, were cultured on ½ MS medium supplemented with 1 gl^{-1} AC, 30 gl^{-1} sucrose and 8 gl^{-1} agar.

For all experiments, explants were cultured in vessels (500 ml) containing 60 ml medium. pH of media was adjusted to 5.7 before autoclaving at 121°C, 1 atm for 40 min.

Lighting systems: Cool white fluorescent lamps (Neon tubes) (40 W each; Rang Dong Light source and Vacuum Flask Co., Vietnam, FL-40W/T10) and warm white fluorescent lamps (Compact 3U lamps) (18 W each; Rang Dong Light source and Vacuum Flask Co., Vietnam, CFH-3U18W) were used as lighting sources in each experiment.

Irradiances were 45 $\mu molm^{-2}s^{-1}$ under Neon light or 45, 60, 75 $\mu molm^{-2}s^{-1}$ for Compact 3U system according to each experiment. Photosynthetic photon flux density (PPFD) was measured with an illumination meter (Tokyo photoelectric Co., LTD., Japan, ANA-F11) on the empty culture shelf.

Experimental designs

Effect of Compact 3U lighting source on the *in vitro* development of *Fragaria vesca* cv. 'My Da' plantlets: Five strawberry shoots were cultured in each culture vessel, and ten vessels were placed on the shelf in one row under the Compact 3U lighting system with three lamps per shelf, arranged in one row. Ten other vessels were placed in one row on another shelf under the Neon lighting system at 45 $\mu mol\ m^{-2}\ s^{-1}$ (the control lighting system). After three weeks of culture, some morphological parameters (plant height and fresh weight, root length, leaf area) were recorded and the *in vitro* plantlets were transplanted to the greenhouse.

Effect of different irradiances of Compact 3U on the *in vitro* development of *Cymbidium, Lilium* and strawberry plantlets: Each vessel contained five shoots of each plant (*Cymbidium*, *Lilium* and strawberry). There were four shelves (ten vessels per shelf) with different irradiances: three shelves with the Compact 3U lighting system at either 45, 60 or 75 $\mu molm^{-2}s^{-1}$, and the remaining shelf with the Neon lighting system at 45 $\mu molm^{-2}s^{-1}$. Some morphological parameters of strawberry plantlets (plant height, leaf area, root length, and plant fresh weight) were recorded after three weeks of culture, of *Cymbidium* (plant height, root length, leaf area, number of newly formed roots, number of bulbs, bulb diameter, bulb cluster fresh weight and bulb fresh weight) and of *Lilium* (plant height, root length, leaf width, number of roots and plant fresh weight) were recorded after six weeks of culture.

The process to set up the Compact 3U and Neon lighting sources for studying *in vitro* development of some horticultural plants is depicted in Fig. 2.

The *in vitro* plantlets were thereafter transplanted to greenhouse. This subsequent stage of development of *Lilium* and *Cymbidium* plantlets were placed under 6h/day supplemental Compact 3U lighting source. After one and a half months of culture, plant

Fig. 1. Compact 3U lamp.

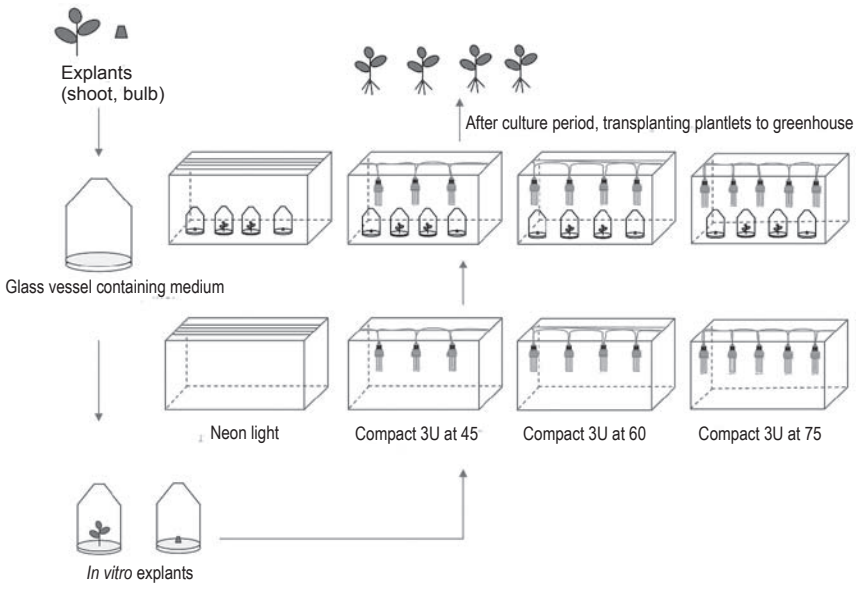

Fig. 2. Setting up the Compact 3U and Neon lighting sources for studying *in vitro* growth and development of some horticultural plants.

height, leaf width, number of leaves, number of roots, root length and plant fresh weight of *Cymbidium* and plant height, bulblet diameter, leaf width, number of leaves, number of roots, root length, and plant fresh weight of *Lilium* were collected.

Culture conditions (*in* and *ex vitro*): *In vitro* cultures were incubated at 25 ± 2°C with a ten-hour photoperiod and 75-80% relative humidity under different lighting systems as treatment.

After six weeks of culture, *Cymbidium* and *Lilium* plantlets were transplanted to the greenhouse and cultured on tree fern fiber substrate in spongy trays at 25 ± 2°C, 80-85% relative humidity and under 6h/day supplemental Compact 3U lighting source. Plantlets were sprayed with an antifungal solution containing 5 gl^{-1} Dithane M-45 (Dow AgroSciences Co., USA) twice a week. In addition, these plantlets were sprayed with a pesticide solution containing 150 gl^{-1} Sumi alpha (Omo Chemical Ltd., Co., Japan) and fertilizer solution containing 100 gl^{-1} NPK, 50 gl^{-1} Komix BFC 201 (Thien Sinh Biochemical Agriculture and Trade Co., Vietnam) and 15 gl^{-1} Miracle Fort (Phu Hung Foundation, Vietnam) once a week. Moreover, plantlets were also watered twice daily.

Statistical analysis: Each treatment was repeated three times and data was recorded at the 3rd or 6th week of culture. The explants in experiments were arranged in a randomized complete block design with five shoots per treatment and three blocks. The data were analyzed for significance using analysis of variance with the mean separation by Duncan's multiple range test (Duncan, 1995).

Results and discussion

Effect of Compact 3U lighting system on the *in vitro* development of *Fragaria vesca* cv. 'My Da' plantlets: The effect of the Compact 3U and Neon lighting source on the *in vitro* growth and development of strawberry plantlets are described in Table 1 and Fig. 3a. There was a significant difference in plant height, root length, leaf area and plant fresh weight between plantlets placed under two lighting systems at 45 $\mu molm^{-2}s^{-1}$. The morphological parameters of strawberry cultured under Compact 3U were higher than those under Neon lighting system.

Table 1. Effect of the Compact 3U lighting source on the *in vitro* development of *Fragaria vesca* cv. 'My Da' plantlets after 3 weeks of culture

Lighting system	Plant height (cm)	Leaf area (mm^2)	Root length (cm)	Plant fresh weight (mg)
Neon (control)	3.7b	45a	2.5b	200b
Compact 3U	4.2a	35b	3.5a	300a

Different letters within a column indicate significant differences ($P = 0.05$) by Duncan's multiple range test.

Effect of different irradiances of Compact 3U lighting source on the *in vitro* development of *Cymbidium, Lilium* and strawberry plantlets

***Fragaria vesca* cv. `My Da'**: The effect of different Compact 3U irradiances on the *in vitro* development of strawberry is shown in Table 2. In general, *in vitro* strawberry shoots placed under the Neon lighting system had a slower growth than those placed under the Compact 3U lighting system. Two lighting sources had different effects on the morphology and biomass of strawberry, whereas different Compact 3U irradiances virtually did not affect the morphological parameters of strawberry (Fig. 3a). Strawberry plantlets were best at 75 $\mu mol\ m^{-2}s^{-1}$. because of the maximum growth and development. However, for commercial purposes, we recommended the use of Compact 3U light at 45 $\mu mol\ m^{-2}s^{-1}$ for the micropropagation of strawberry because of saving electrical energy and still remaining the relatively good growth and development.

Table 2. Effect of different irradiances of Compact 3U on the *in vitro* development of *Fragaria vesca* cv. "My Da" plantlets after 3 weeks of culture

Irradiance (Compact 3U) ($\mu molm^{-2}s^{-1}$)	Plant height (cm)	Leaf area (mm^2)	Root length (cm)	Plant fresh weight (mg)
Neon (45)	3.7c[x]	40.5d	2.5d	200d
45	4.2b	45.4b	3.5b	250c
60	4.2b	41.4c	3.0c	270a
75	4.4a	50.0a	3.9a	260b

[x] Different letters within a column indicate significant differences ($P = 0.05$) by Duncan's multiple range test.

***Cymbidium* cv. 'Tim Hot'**: The effect of different irradiances of Compact 3U on the *in vitro* growth and development of *Cymbidium* plantlets is shown in Table 3. Data showed that there was no significant difference in root length of plantlets placed under the different lighting systems. However, the plantlets were significantly taller at 60 $\mu molm^{-2}s^{-1}$, with considerably more roots at 45 $\mu molm^{-2}s^{-1}$ as compared to Neon at 45 $\mu molm^{-2}s^{-1}$ (Fig. 3b). Besides, the data indicated that the plantlets placed under the Compact 3U lighting system had a better growth than those under the Neon lighting system. The irradiance in this case did not affect the root growth but plantlet fresh weight, which was highest at 60 $\mu molm^{-2}s^{-1}$. The most suitable irradiance of Compact 3U (60 $\mu molm^{-2}s^{-1}$) for growth and morphology of *Cymbidium*, characterized by long shoots and roots, wide leaves and high fresh weight (Fig. 3b), was also the most appropriate intensity

Table 4. Effect of different irradiances of Compact 3U on the *in vitro* development of *Lilium longiflorum* plantlets after 6 weeks of culture

Irradiance (Compact 3U) ($\mu molm^{-2}s^{-1}$)	Plant height (cm)	Root length (cm)	Leaf area (mm^2)	Number of new formed roots	Number of bulbs	Bulb diameter (mm)	Bulb cluster fresh weight (mg)	Bulb fresh weight (mg)
Neon (45)	9.1c*	2.5c	26.1d	11.1b	1.9a	6.4a	750c	510c
45	11.4a	3.8a	40.3a	10.6c	1.6c	5.5d	810b	480a
60	10.3b	2.1d	30.7b	9.7d	1.8b	5.8c	830b	470b
75	9.1c	3.0b	30.0c	13.1a	2.0a	6.1b	930a	460b

* Different letters within a column indicate significant differences ($P = 0.05$) by Duncan's multiple range test

for inducing vigorous growth of the plantlets transplanted to greenhouse.

Table 3. Effect of different irradiances of Compact 3U on the *in vitro* development of *Cymbidium* cv. "Tim Hot" plantlets after 6 weeks of culture

Irradiance (Compact 3U) ($\mu molm^{-2}s^{-1}$)	Plant height (cm)	Root length (cm)	Leaf width (mm)	Number of roots	Plant fresh weight (mg)
Neon (45)	10.1b[w]	3.0a	4.7b	2.0c	620c
45	10.2b	3.0a	4.8a	2.5a	650b
60	10.9a	3.0a	4.8a	2.3b	740a
75	10.2b	2.6b	4.4c	2.6a	610c

[w] Different letters within a column indicate significant differences ($P = 0.05$) by Duncan's multiple range test.

Lilium longiflorum:The effect of different irradiances of Compact 3U on the *in vitro* growth and development of *L. longiflorum* plantlets is indicated in Table 4 and Fig. 3c. The results suggest that the lighting source as well as its PPFD significantly affected the fresh weight of new bulblet clusters derived from initial bulb scales. These results show that *L. longiflorum* bulb scales could be cultured at 75 $\mu molm^{-2}s^{-1}$ under Compact 3U for multiplying new high quality bulblets and under Neon at 45 $\mu molm^{-2}s^{-1}$ for increasing biomass and producing vigorous bulblets before transplanting to greenhouse. In addition, there was a considerable effect of Compact 3U at 45 $\mu molm^{-2}s^{-1}$ on the *in vitro L. longiflorum* morphology. This might be a result of the increase in red light spectrum associated with a low PPFD of Compact 3U lighting source which played a certain role in increasing plant height and leaf area. On the other hand, the *L. longiflorum* root morphology was not affected by different lighting sources.

Subsequent growth of Cymbidium and Lilium plantlets

Cymbidium* cv. "Tim Hot":* The subsequent growth of Compact 3U-derived *Cymbidium* plantlets in the greenhouse after one and a half months culture under 6h/day supplemental Compact 3U lighting source at night is given in Table 5. The results showed that *Cymbidium* plantlets grown in the greenhouse under supplemental Compact 3U lighting source had better development than those derived from Neon lighting source (except for leaf width) (Fig. $4a_1$, $4a_2$).

Lilium longiflorum: Results in Table 6 show that the development of *Lilium* plantlets when transplanted to greenhouse was affected by different lighting sources and intensities. Neon lighting source-derived plantlets had lower growth (plant height and leaf width) but greater number of leaves and better root length than those derived from the Compact 3U lighting source, which yielded variable results under different irradiances (Fig. $4b_1$, $4b_2$).

In summary, the irradiance of Compact 3U had a positively stimulated impact that affected significantly on the development of these three plants. The Compact 3U lighting source had a positive effect on the plant height, and the plant fresh weight of *Lilium*, *Cymbidium*, the number of strawberry shoots cultured *in vitro* as well as on the root length of strawberry and the number of roots of *Cymbidium* cultured *in vitro*. The results obtained in this study showed that the Compact 3U lighting source affected the morphology of these plants, increased their biomass, and enhanced plantlet growth before transplanting to the greenhouse. Furthermore, this data also showed that different plants adapted differently to different lighting sources, but different irradiances from two lighting sources did not affect root development including root fresh weight, root elongation (except for strawberry) and the number of roots (except for *Cymbidium*) of these plants.

In most cases, different Compact 3U irradiances had no obvious impact on plantlet development as compared to those of Neon light. Different irradiances affected *Lilium* and *Cymbidium* plant height and *Lilium* plant fresh weight. In these plants, a lower intensity gave a higher plant quality. Except for the plant fresh weight of *Lilium* that was enhanced when cultured under Compact 3U at 75 $\mu molm^{-2}s^{-1}$, the remaining cases showed that plantlets developed well under lower intensities (45 or 60 $\mu mol\ m^{-2}s^{-1}$). Consequently, Compact 3U confirmed the positive effect

Table 5. Subsequent growth of Compact 3U-derived *Cymbidium* plantlets in the greenhouse after one and a half months of culture under 6h/day supplemental Compact 3U lighting source

Lighting systems ($\mu molm^{-2}s^{-1}$)		Plant height (cm)	Leaf width (mm)	Number of leaves	Number of roots	Root length (cm)	Plant fresh weight (g)
Neon	45	10.4b[*]	5.8a	4.3c	3.7b	2.9b	1.7b
	45	10.5b	5.5b	4.4c	3.6b	3.3a	1.9a
Compact 3U	60	11.1a	5.4b	4.9a	3.7b	3.2a	1.8a
	75	11.2a	5.2c	4.6b	3.9a	2.9b	1.6b

[*] Different letters within a column indicate significant differences ($P = 0.05$) by Duncan's multiple range test.

Table 6. Subsequent growth of Compact 3U-derived *Lilium* plantlets in the greenhouse after 2 months of culture under 6h/day supplemental Compact 3U lighting source

Lighting systems ($\mu mol\ m^{-2}s^{-1}$)		Plant height (cm)	Bulblet diameter (cm)	Leaf width (mm)	Number of leaves	Number of roots	Root length (cm)	Plant fresh weight (mg)
Neon	45	5.5d[*]	0.72c	4.5d	4.7a	4.0c	0.7c	410c
	45	7.6a	0.77b	6.3a	3.5c	4.0c	5.6a	580a
Compact 3U	60	6.8b	0.72c	6.0b	3.7b	4.5b	1.0b	560a
	75	6.1c	0.83a	5.0c	3.7b	4.7a	0.9b	490b

[*] Different letters within a column indicate significant differences ($P = 0.05$) with the mean separation by Duncan's multiple range test.

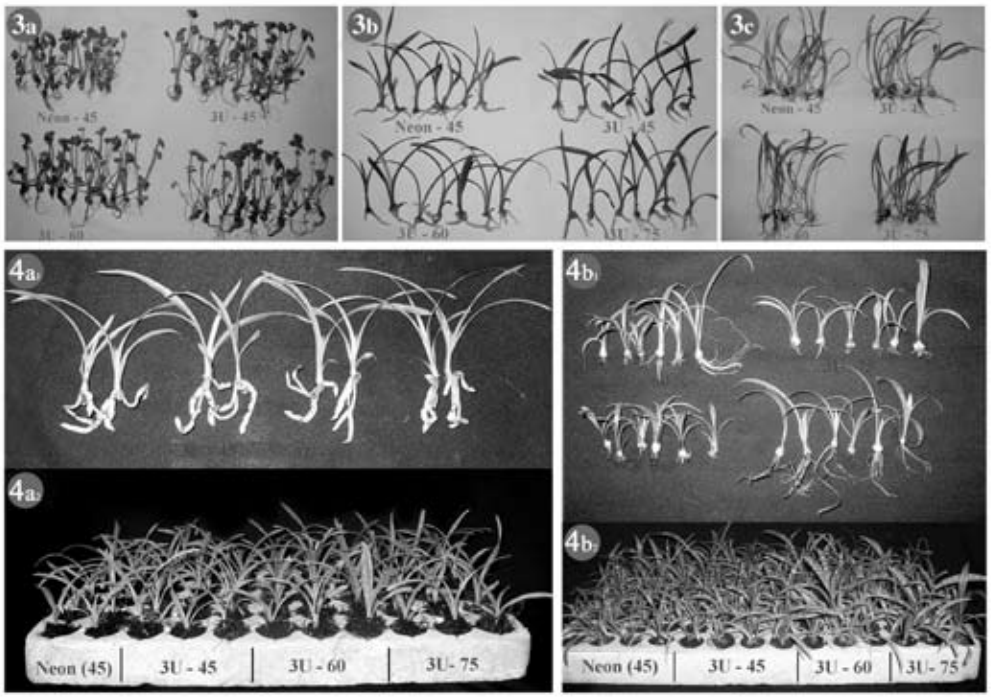

Fig. 3. Strawberry, *Cymbidium* and *Lilium* plantlets cultured under Compact 3U at different irradiances. (a): Strawberry cultured under Neon light at 45 $\mu molm^{-2}s^{-1}$ (top left), Compact 3U at 45 $\mu molm^{-2}s^{-1}$ (top right), 60 $\mu molm^{-2}s^{-1}$ (bottom left), or 75 $\mu molm^{-2}s^{-1}$ (bottom right) after three weeks of culture. (b): *Cymbidium* plantlets cultured under Neon light at 45 $\mu molm^{-2}s^{-1}$ (top left), Compact 3U at 45 $\mu molm^{-2}s^{-1}$ (top right), 60 $\mu molm^{-2}s^{-1}$ (bottom left), or 75 $\mu molm^{-2}s^{-1}$ (bottom right) after six weeks of culture. (c): *Lilium* plantlets cultured under Neon light at 45 $\mu molm^{-2}s^{-1}$ (top left), Compact 3U at 45 $\mu molm^{-2}s^{-1}$ (top right), 60 $\mu molm^{-2}s^{-1}$ (bottom left), or 75 $\mu molm^{-2}s^{-1}$ (bottom right) after six weeks of culture.
Fig. 4. *Cymbidium*, *Lilium* plants after transplanted in greenhouse. (a_1): *Cymbidium* plants transplanted in greenhouse after one and a half months. Left to right: *Cymbidium* plants derived from Neon light at 45 $\mu molm^{-2}s^{-1}$, Compact 3U at 45, 60 or 75 $\mu molm^{-2}s^{-1}$. (a_2): *Cymbidium* plants in spongy tray after one and a half months transplanted in greenhouse. Left to right: *Cymbidium* plants derived from Neon light at 45 $\mu molm^{-2}s^{-1}$, Compact 3U at 45, 60 or 75 $\mu molm^{-2}s^{-1}$. (b_1): *Lilium* plants derived from Neon light at 45 $\mu molm^{-2}s^{-1}$ (bottom left), Compact 3U at 45 $\mu molm^{-2}s^{-1}$ (bottom right), 60 $\mu molm^{-2}s^{-1}$ (top left), or 75 $\mu molm^{-2}s^{-1}$ (top right) after two months in greenhouse. (b_2): *Lilium* plants in spongy tray after two months transplanted in greenhouse. Left to right: *Lilium* plants derived from Neon light at 45 $\mu molm^{-2}s^{-1}$, Compact 3U at 45, 60 or 75 $\mu molm^{-2}s^{-1}$.

on the *in vitro* development of plantlets before transplanting to greenhouse.

These above results were similar to those of Warrington and Michell (1976), Morgan and Smith (1981), Smith (1982), Tibbitts *et al.* (1983), Mortensen and Stromme (1987), Economou and Read (1987), and Agrawal (1992), who all confirmed the significant effects of light quality (related to different lighting sources) on the growth and morphology of *in* and *ex vitro* plants. The effect of irradiance of different lighting sources on the development of plants were also the concern of some studies of Gilslerod and Mortensen (1997), Miyashita *et al.* (1997), and Nhut (2002). In these studies, higher irradiances gave the best plant growth. But in this report, we suggest for use of lower intensities (45 or 60 $\mu molm^{-2}s^{-1}$) of Compact 3U for plant propagation owing to the reduction of electrical energy consumption as well as the increase in the development of some horticultural plants.

The Compact 3U lighting source had a highly significant effect on the development of *Cymbidium* 'Tim Hot', *Lilium longiflorum* and *Fragaria vesca* cv. 'My Da' as in this study. The results indicate that these plants adapted differently with different light sources and intensities. Strawberry shoots had better growth and morphology when cultured under Compact 3U lighting system than those under Neon light system. The preeminence of Compact 3U lighting sytem was also expressed when shoots of three plants were cultured under different irradiances of two lighting system. Though in most cases, different irradiances of Compact 3U had no obvious effect on the development of plantlets as compared to the Neon light, lower irradiance gave higher *Cymbidium* and *Lilium* plantlets quality. Moreover, plantlets derived from Compact 3U lighting system developed better than those from Neon lighting system.

Hence, the Compact 3U lamp, having a suitable light spectrum, resulting in good, high quality plants, saving 75-80% of electrical energy consumption as compared to incandescent lamps, being cheap, and subsequently retrieving initial investments quickly, was expected to be a novel lighting system used in the successful micropropagation and subsequent *ex vitro* growth of some horticultural plants. In fact, because of obvious advantages, the Compact 3U lamp has been used as the lighting source for propagation of many horticulture plants in Dalat, Lam Dong, Vietnam.

References

Agrawal, S.B. 1992. Effects of supplemental UV-B radiation on photosynthetic pigment, protein and glutathione contents in green algae. *Env. Exp. Bot.*, 32(2): 137-143.

Collin, R.N., E.N. Margaret, H. Ted and P. David, 1988. Light quality and light pipes in the micropropagation of woody ornamental plants. *Acta Hortic.*, 226: 413-416.

Debergh, P.C. and R. Zimmerman, 1990. Micropropagation: technology and application. *Kluwer Academic Publishers, Dordrecht,* pp. 484.

Duncan, D.B. 1995. Multiple range and multiple F test. *Biomet.,* 11: 1-42.

Economou, A.S. and P.E. Read, 1987. Light treatments to improve efficiency of *in vitro* propagation systems. *HortSci.*, 22(5): 751-754.

Faust, J.E. and R.D. Heins, 1997. Quantifying the influence of hight-pressure dodium lighting on shoot-tip temperature. *Acta Hortic.*, 418: 85-92.

Gabarkiewicz, B., E. Gabryszewska, R. Rudnicki and D. Goszcynska, 1997. Effects of light quality on *in vitro* growing of Dieffenbachia cv. compacta. *Acta Hortic.*, 418: 159-162.

Gabryszewska, E. and R.M. Rudnicki, 1997. The effects of light quality on the growth and development of shoots and roots of *Ficus benjamina in vitro*. *Acta Hortic.*, 418: 163-168.

Gilslerod, H.R. and L.M. Mortensen, 1997. Effect of light intensity on growth and quality of cut roses. *Acta Hortic.*, 418: 25-32.

Hart, J.W. 1988. Light and Plant Growth. In: *Topics in Plant Physiololgy*, 1: 34-50.

Hayashi, M., N. Fujita, Y. Kitaya and T. Kozai, 1992. Effect of sideward lighting on the growth of potato plantlets *in vitro*. *Acta Hortic.*, 319: 163-166.

Iwanami, Y., T. Kozai, Y. Kitaya and S. Kino, 1992. Effects of supplemental red and far-red lighting using light emitting diodes on stem elongation and growth of potato plantlets *in vitro* (Abstr.). *International Symposium Transplant production Systems, Yokohama, Japan,* pp. 183.

Kim, H.H. and T. Kozai, 2000. Production of value-added transplants in closed systems with artificial lighting. In: Transplant Production in the 21st Century. C. Kubota and C. Chun (eds.), Kluwer Academic Publishers, 137-144.

Kirdmanee, C., Y. Kitaya and T. Kozai, 1993. Effect of supplemental far-red lighting and photosynthetic photon flux density on stem elongation and dry weight increase of *Eucalyptus camaldulensis in vitro* plantlets (Abstr.). XVth International Botanical Congress Yokohama, Japan, pp. 537.

Kozai, T., K. Fujiwara, M. Hayashi and J. Aitken-Christie, 1992. The *in vitro* environment and its control in micropropagation. *Transplant production systems.* Kluwer Academic Publishers. Dordrech, pp. 247-282.

Kunneman, B.P.A.M. and J.B. Ruesink, 1997. Interactions between light, temperature and CO_2 in rooting of conifer cuttings. *Acta Hortic.*, 418: 97-102.

Maas, F.M. and E.J. Bakx, 1997. Growth and flower development in roses as affected by light. *Acta Hortic.*, 418: 127-134.

Miyashita, Y. 1995. Growth and morphology of potato plantlets *in vitro* as affected by light quality, and size and nodal position of the explant. *Graduate School of Science and Technology.* Chiba University, pp. 1-5.

Miyashita, Y., T. Kimura, Y. Kitaya, C. Kubota and T. Kozai, 1997. Effects of red light on the growth and morphology of potato plantlets *in vitro*: Using light emitting diodes (LEDs) as a lighting source for micropropagation. *Acta Hortic.*, 418: 169-176.

Moe, R. 1997. Physiological aspects of supplementary lighting in horticulture. *Acta Hortic.*, 418: 17-24.

Morgan, D.C. and H. Smith, 1981. Non-photosynthetic responses to light quality. In: *Encyclopedia of Plant Physiology.* Springer-Verlag, Berlin, pp. 109-134.

Mortensen, L. M. and E. Stromme, 1987. Effects of light quality on some greenhouse crops. *Sci. Hort.*, 33: 27-36.

Murakami, K., I. Aiga, K. Horaguchi and M. Morita, 1997. Red/far-red photon flux ratio used as an index number for morphological control of plant growth under artificial lighting conditions. *Acta Hortic.*, 418: 135-140.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Plant Physiol.*, 15: 473-497.

Nhut, D.T. 2002. *In vitro* growth and physiological aspects of some horticultural plantlets cultured under red and blue light emitting diodes (LEDs). PhD Thesis, Kagawa University, pp. 163-172.

Schwabe, W.W. 1963. Morphogenetic responses to climate. In: *Environmental Control of Plant Growth.* Academic Press, New York, pp. 311-336.

Seabrook, J.E.A. 1987. Changing the growth and morphology of potato plantlets in vitro by varying the illumination source. *Acta Hortic.*, 212: 401-410.

Smith, H. 1982. Light quality, photoperception and plant strategy. *Annual Rev. Plant Physiol.*, 33: 481-518.

Tibbitts, T.W., D.C. Morgan and I.J. Warrington, 1983. Growth of lettuce, spinach, mustard, and wheat plants under four combinations of high-pressure sodium, metal halide, and tungsten halogen lamps at equal PPFD. *J. Amer. Soc. Hort. Sci.*, 108(4): 622-630.

Walz, F. and W. Horn, 1997. The influence of light quality on gas exchange of dendranthema. *Acta Hortic.*, 418: 53-58.

Warrington, I.J. and K.J. Michell, 1976. The influence of blue- and red-biased light spectra on the growth and development of plants. *Agr. Met.*, 16: 247-262.

Wulster, G. and H.W. Janes, 1997. Effects of supplemental light quality, quantity, and differential temperature on growth and development of Easter Lilium (*Lilium longiflorum*). *Acta Hortic.*, 418: 153-158.

Journal of Applied Horticulture, 8(1): 21-24, January-June, 2006

Effect of slow release fertiliser on the growth of containerised flannel flower (*Actinotus helianthi* Labill.)

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Abstract

Two controlled-release fertiliser (CRF) formulations, Nutricote Total ® 13N : 5.7P : 10.8K (N13) and Nutricote Total ® 18N : 2.6P : 6.6K (N18), were applied at 0, 1.25, 2, 2.5, 5 and 10 kg m^{-3}, to flannel flower (*Actinotus helianthi* Labill.) seedlings grown in soil-less potting mix in containers. After five months, during peak spring flowering, a number of characters relating to the quality of the cut flower product of this species were assessed. As the rate of fertiliser application increased, the plant height, total number of stems, number of flowering stems and number of flowers and buds increased. There were significantly more stems and flowers overall, and more flowering (saleable) stems, in the N18 treatments at all application rates. Plant height was not affected by fertiliser formulation. Basal foliar necrosis, which scored highly in the control treatment (0 fertiliser), was reduced by fertiliser application.

Key words: Nutrition, controlled-release fertiliser, nitrogen, *Actinotus helianthi*, flannel flower, cut flower

Introduction

The flannel flower (*Actinotus helianthi* Labill.) is an erect annual or perennial herb that is covered with a woolly indumentum (Powell, 1992) giving the plant and particularly the inflorescence its characteristic 'flannel' appearance. It occurs naturally in eastern Australia in New South Wales and southern Queensland. This species is generally found on sandy and rocky soils along the coast and also in small sandy patches in the western extent of its distribution.

Belonging to the Apiaceae family, with umbels subtended by large involucral bracts giving an inflorescence reminiscent of daisies, the long-stemmed selections are considered a useful cut flower feature-filler product. Ten years ago, the species was primarily bush harvested for the cut flower market, but in recent years it has been cultivated and export sales are steadily increasing (Worrall *et al.*, 2004).

Originally there were several significant limitations to production of flannel flower, including low seed germination and vegetative propagation rates. These problems have largely been overcome (Offord and Tyler, 1996; von Richter and Offord, 1997, 2000) and attention has recently turned to improving knowledge about cultivation of this species including nutrition, substrate requirements and disease interactions. Little is known about the nutritional requirements of flannel flowers or many other Australian species that occur on low nutrient soils (Brennan *et al.*, 1998).

This paper examines the significance of the effect of two controlled-release fertiliser (CRF) formulations, at increasing application rates, on several reported and unreported growth characteristics of containerised flannel flowers (von Richter and Offord, 1997). Nutricote Total® was used for this experiment as it was readily available in our nursery at that time; however, several other CRF products commercially available would have been equally suitable for this work. CRFs are a commonly used source of nitrogen and other major as well as minor nutrients because they release the nutrients more evenly than conventional soluble fertilisers reducing problems associated with burning or leaching, and without the more labour intensive liquid fertiliser applications that also deliver good plant growth (Cresswell and Weir, 1997; Oliet *et al.*, 2004).

Materials and methods

Plant material: *Actinotus helianthi* seeds were collected in spring (November) from Tea Gardens on the NSW Central Coast (Latitude 32°39′43″S, Longitude 152° 08′58″E). All work on seeds, seedlings and plants was carried out at Mount Annan Botanic Garden (34º04′04″S, 150º46′04″E).

Potting mix, fertiliser formulation and rate: Seeds were sown soon after harvest onto seed raising mix (sand /perlite 1:1 v/v) and the seedlings pricked out at the two leaf stage and planted into 50 mm tubes containing sand/coir mix (4:1 v/v) and 0.5 g L^{-1} $FeSO_4$ and 0.5 g L^{-1} lime. When the seedlings were 80 mm high, 220 seedlings were planted into the sand/coir mix in 140 mm slimline black plastic pots, but with varying application rates of either Nutricote Total® 13N : 5.7P : 10.8K (N13) or Nutricote Total® 18N : 2.6P : 6.6K (N18). The release time for each fertiliser is 270 days at 25ºC. The following rates were applied: 0, 1.25, 2, 2.5, 5 and 10 kg m^{-3}. The rate recommended by the manufacturer is 2 kg m^{-3}.

Experimental design: There were 20 replicates of each treatment. The plants were arranged in a completely random design on raised benches in full sun. Watering was by hand as and when required.

Assessment: At peak flowering time (mid-November), when the plants had been in the different fertiliser treatments for five months, the following measurements were made: plant height,

total stem number, flowering stem number and total number of flowers and buds. Foliar leaf necrosis was scored (1 = basal leaves green; 2 = one basal leaf yellow; 3 = two basal leaves yellow or brown; 4 = all basal leaves brown).

Statistical analysis: Main effects and interactions were analysed by ANOVA and differences between the means compared using LSD (P=0.05). Reponses to the two fertiliser formulations to application rates were analysed by linear regression. All analyses were performed using SYSTAT 11 (SPSS Inc. 2004).

Results

Plant height: Plant height was largely unaffected by the fertiliser formulation, but the rate effect was significant (Table 1), probably mainly due to the much lower zero fertiliser control treatment (Fig. 1A). The regression slopes in Fig. 1A were highly significant for N13 (P = 0.001) and N18 (P = 0.001), indicating that the slopes were not equal to zero and that fertiliser application rate has some effect on plant height. However, the proportions of the total variance explained by the application rates were very low (R^2 = 0.12 and 0.13) (Fig. 1A).

Stem number: Overall, the number of stems produced was greater in the N18 treatment, when compared to the N13 treatments (Fig. 1B). The regression slopes (Fig. 1B) were highly significant for N13(P <0.001) and N18 (P < 0.001), which indicates that the slopes were not equal to zero and that there is a relationship between fertiliser application rate and the stem number produced. Maximum stem numbers were found at N18 at 5 and 10 kg m^{-3} (averages of 6.5 and 8.5 stems); the next highest stem number was at N13 at 10 kg m^{-3} (average of 6 stems). Fertiliser types and rates of application were significant main effects for this variable, and there was also a significant interaction effect detected (Table 1). At the time of measurement, fewer than half of the stems had produced flowers, with the least flowering stems produced at zero fertiliser application (Fig. 1C). The highest number of flowering stems was produced at 10 kg m^{-3} N18 which represented 35% of the total number of stems (Fig. 1B and C).

There was little difference between the formulations in the number of flowering stems produced at 2.5 and 5 kg m^{-3}, but there were small significant differences between the formulations at 1.25 and 2 kg m^{-3} (Fig. 1C). This accounts for the significant and highly significant main effect responses detected for fertiliser formulation and rate respectively (Table 1). Again, the regression slopes for this variable were highly significant for N13 (P <0.001) and N18 (P < 0.001), Fig. 1C), which indicates that the slopes were not equal to zero and that there is a relationship between fertiliser application rate and the flowering stem numbers, but the direction of the regression slopes were less steep (R^2 = 0.2%) (Fig. 1C), than when compared to the total number of stems (R^2 = 0.4 and 0.6%) (Fig. 1B).

Number of flowers and buds: The number of flowers and buds produced was greatly affected by both fertiliser type and rate, although there was no interaction observed between the two (Table 1). The regression slopes were highly significant for N13 (P = 0.001) and N18 (P = 0.001), which indicates that the slopes were not equal to zero and that there is a relationship between fertiliser application rate and flower/bud numbers. Maximum flower numbers were observed at the 10 kg m^{-3} level of both fertilisers (average of 28 and 22 flowers), with the slope of the response being similar (R^2 = 0.49 and 0.5) for both formulations over the range of applications tested (Fig. 1D).

Basal foliar necrosis: Basal foliar necrosis was significantly affected by fertiliser formulation and rate (Table 1), with significantly less necrosis observed for N18, when compared to N13 at the 2 and 2.5 kg m^{-3} application rates (Fig. 1 E), which may account for the significant interaction term for fertiliser x rate. The regression slopes for foliar necrosis were highly significant for N13 (P = 0.001) and N18 (P = 0.001), indicating a relationship between fertiliser application rate and the degree of necrosis observed, despite the weak R^2 values. Overall, fertiliser application, especially N18 greater than 2 kg m^{-3} and N13 at 5 and 10 kg m^{-3}, significantly improved (decreased) the necrosis rating when compared with the control (zero fertiliser).

Discussion

In terms of the quality of the flowering stems produced, flannel flowers appeared to respond positively to all the imposed fertiliser treatments in this study when compared with the control treatment (zero fertiliser application), with no adverse affects observed at any applied level. The recommended rate of 2 kg m^{-3} was exceeded up to five times (to 10 kg m^{-3}) and the plants produced more stems and more flowers as the application rates increased. This high tolerance of applied nutrients is in contrast to some other Australian and South African native species which require little fertiliser to achieve optimal results (Brennan *et al.*, 1998; Clark and Burge, 1999),while others responded in a similar fashion to *A. helianthi* (Lamont *et al.*,1990; Bennell and Williams, 1992; Brennan *et al.*, 2000). It has commonly been observed that flannel flowers are found in abundance after bushfire, at which time there may be increased nutrient levels are often available depending on the nutrient status of the plant material that has been burnt (Ashton and Martin, 1996; Enright *et al.*, 1997). Further work is required to determine the ecological significance of this response.

Relative differences between the two fertiliser treatments are of greatest horticultural interest. The plants responded to the higher nitrogen formulation by producing more saleable product *i.e.* more stems and flowers. Nutricote N13 is recommended for potted flowering plants because of its balance of lower nitrogen when compared to phosphorus and potassium, while the N18 formulation is recommended by the manufacturer (Yates Pty Ltd)

Table 1. Significance (P value) of main effects (fertiliser type and rate) and interactions for characteristics of flannel flower plants five months after fertiliser application

	Height of plant	Number of stems	Stems with flowers	Flower number	Basal foliar necrosis
Fertiliser type	0.960	0.001	0.015	0.001	0.041
Rate	0.001	0.001	0.000	0.001	0.001
Fertiliser x Rate	0.730	0.003	0.482	0.362	0.021

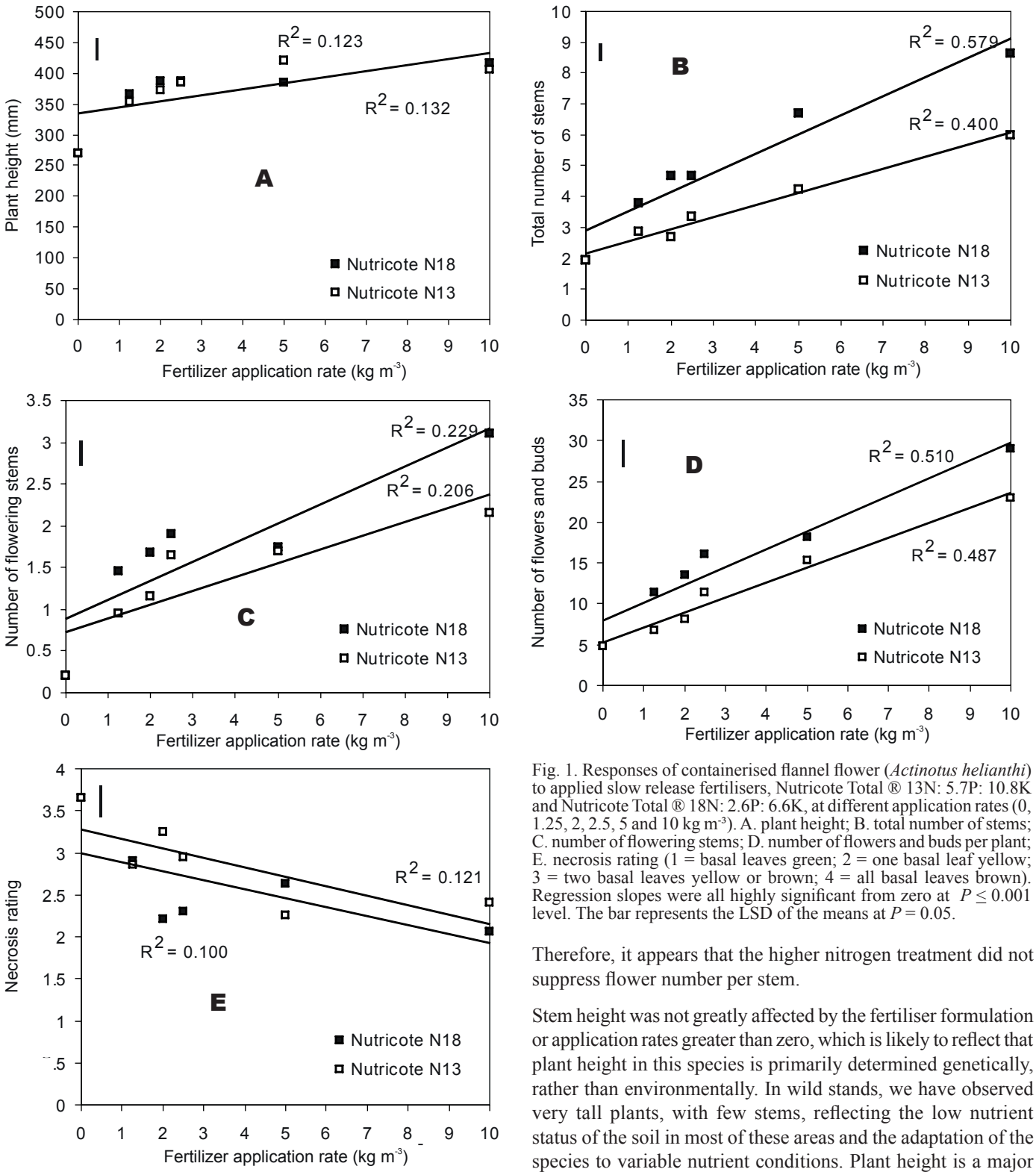

Fig. 1. Responses of containerised flannel flower (*Actinotus helianthi*) to applied slow release fertilisers, Nutricote Total ® 13N: 5.7P: 10.8K and Nutricote Total ® 18N: 2.6P: 6.6K, at different application rates (0, 1.25, 2, 2.5, 5 and 10 kg m^{-3}). A. plant height; B. total number of stems; C. number of flowering stems; D. number of flowers and buds per plant; E. necrosis rating (1 = basal leaves green; 2 = one basal leaf yellow; 3 = two basal leaves yellow or brown; 4 = all basal leaves brown). Regression slopes were all highly significant from zero at $P \leq 0.001$ level. The bar represents the LSD of the means at $P = 0.05$.

for foliage plants because of its higher nitrogen ratio. Certainly, greater flowering stem production is a desired aim in cut flower production in this species, and it would appear that the high nitrogen formulation achieved the best result in this respect. Although the overall number of flowering stems was greatest at high nitrogen, the number of flowers produced per flowering stem (derived from Figs 1 C and D) was very similar for the two fertiliser treatments at each rate. For example, at 2 kg m^{-3} the N18 treatment had an average of 8.0 flowers per flowering stem and N13 had 7.1; at 10 kg m^{-3} N18 had 9.4 and N13 had 10.7. Therefore, it appears that the higher nitrogen treatment did not suppress flower number per stem.

Stem height was not greatly affected by the fertiliser formulation or application rates greater than zero, which is likely to reflect that plant height in this species is primarily determined genetically, rather than environmentally. In wild stands, we have observed very tall plants, with few stems, reflecting the low nutrient status of the soil in most of these areas and the adaptation of the species to variable nutrient conditions. Plant height is a major selection criterion for Flannel flower development, and we have documented the differences between populations, which are maintained by the plants in cultivation (Offord and Tyler, 1996). For example, short headland-growing varieties are being developed for the pot plant market, while inland forest forms are useful for cut flower production because of their naturally long stems.

There are many forms of *Actinotus helianthi* and some of the tall growing cut flower types show basal leaf necrosis much sooner than short pot plant types. Based on evidence from tissue cultured

plants (von Richter, unpublished) there are differences in nutrient requirements for these different forms. Considering the axenic nature of tissue culture, the necrosis is likely to be caused by the movement of nutrients, particularly nitrogen away from the basal leaves to be reutilised in the apical region rather than the effects of a pathogenic infection. The faster growing varieties are likely to have a higher nutritional requirement than smaller, slower growing ones.

The optimal fertiliser regime could not be determined by this study, but it would be expected to not exceed the highest application rate and may be less, especially in terms of the cost of application (Obreza *et al.*, 1999) or leaching into the soil/water environment, mainly in the form of nitrate (Cox, 1993; Huett, 1997). The results of this study are based on seedlings from one population only. The recommendations for fertiliser use found here is only an indicator of the nutrient usage of one flannel flower type and at one point in time. The temporal effects of fertiliser formulations and rates to enable growers to optimise their usage of CRFs alone, or in combination with water soluble fertilisers depends on the varieties being produced and the timing of production required in nursery situations (Jacobs *et al.*, 2005) or in the field (Brennan *et al.*, 1998).

Acknowledgements

We gratefully acknowledge the financial support of the Rural Industries Research and Development Corporation (RIRDC).

References

Ashton, D.H. and D.G. Martin, 1996. Regeneration in a pole-stage forest of *Eucalyptus regnans* subjected to different fire intensities in 1982. *Australian Journal of Botany*, 44: 393-410.

Bennell, M.R. and R.R. Williams, 1992. Cultivation of the pink mulla mulla *Ptilotus exaltatus* Nees. 2. Nutrition and growth regulation. *Scientia Horticulturae*, 51: 107-110.

Brennan, R.F., A.M. Crowhurst and M.G. Webb, 1998. The growth of Yellow Bells (*Geleznowia verrucosa*) seedlings in response to additions of nitrogen, potassium and phosphorus fertiliser. *Australian Journal of Experimental Agriculture*, 38: 385-390.

Brennan, R.F., M.G. Webb and A.M. Crowhurst, 2000. Yield responses of mulla mulla (*Ptilotus exaltatus* Nees.) seedlings to additions of nitrogen, potassium and phosphorus fertiliser. *Australian Journal of Experimental Agriculture*, 40: 847-871.

Clark, G.E. and G.K. Burge, 1999. Effects of nitrogen nutrition on *Sandersonia* cut flower and tuber production in a soil-less medium. *New Zealand Journal of Crop and Horticultural Science*, 27: 145-152.

Cox, D.A. 1993. Reducing nitrogen leaching-losses from containerized plants: the effectiveness of controlled-release fertilizers. *Journal of Plant Nutrition*, 16: 533-545.

Cresswell, G.C. and R.G. Weir, 1997. *Plant nutrient disorders 5, Ornamental Plants and Shrubs.* Inkata Press, Melbourne.

Enright, N.J., D. Goldblum, P. Ata and D.H. Ashton, 1997. The independent effects of heat, smoke and ash on emergence of seedlings from the soil seed bank of a healthy *Eucalyptus* woodland in Grampians (Gariwerd) National Park, western Victoria. *Australian Journal of Ecology*, 22: 81-88.

Huett, D.O. 1997. Fertiliser use efficiency by containerised nursery plants. 2. Nutrient leaching. *Australian Journal of Agricultural Research*, 48: 259-265.

Jacobs, D.F., D.L. Hasse and R. Rose, 2005. Growth and foliar nutrition of Douglas-fir seedlings provided with supplemental polymer-coated fertilizer. *Western Journal of Applied Forestry,* 20(1): 58-63.

Lamont, G.P., G.C. Cresswell and G.J. Griffith, 1990. Nutritional studies of Christmas Bell. *HortScience*, 25: 1401-1402.

Obreza, T.A., R.E. Rouse and J.B. Sherrod, 1999. Economics of controlled-release fertilizer use on young citrus trees. *Journal of Production Agriculture,* 12: 69-73.

Offord, C.A. and J.L. Tyler, 1996. Flannel Flowers. In: *Horticulture of Australian Plants.* Burchett, M. and Johnston K. (Eds), UNSW Press, Sydney, pp. 212-217.

Oliet, J., R. Planelles, M.L. Segura, F. Artero and D.F. Jacobs, 2004. Mineral nutrition and growth of containerized *Pinus halepensis* seedlings under controlled-release fertilizer. *Scientia Horticulturae,* 103: 113-129.

Powell, J.M. 1992. Apiaceae. In: *Flora of NSW*. G.J. Harden (Ed). Vol. 3. New South Wales University Press, Sydney, pp. 87-116.

von Richter, L. and C. Offord, 1997. Flannel flower (*Actinotus helianthi*) as a cut flower crop. RBG 1A. Rural Industries Research and Development Corporation, Canberra, 66 pp.

von Richter, L. and C.A. Offord, 2000. Development of a Flannel flower production system. Rural Industries Research and Development Corporation, Canberra, Publication NO. 00/106, 54pp.

Worrall, R., C. Offord and L. von Richter, 2004. Flannel flower. In: *The New Crop Industries Handbook.* Salvin, S., Bourke, M. and Byrne, T. (Eds), Rural Industries Research and Development Corporation, Canberra, pp. 434-441.

Journal of Applied Horticulture, 8(1): 25-28, January-June, 2006

Growth and flowering response of snapdragons after release from apical dominance

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Abstract

Plants of an early flowering *Antirrhinum* cultivar 'Chimes White' were pinched at 4, 5, 6, and 7 leaf-pair stage to observe the effects on flowering time and plant quality. Though control plants flowered earlier (81 days) than the pinched ones, they produced less number of flower buds. Flower time and rate of progress to flowering in pinched plants increased linearly and significantly. The quality of pinched plants regarding branch numbers, leaf area, plant height, plant fresh weight *etc*. was significantly improved in all treatments. Many plant growth parameters were successfully fitted by the second degree polynomial model whereas linear model indicated a good fit in reproductive development.

Key words: *Antirrhinum majus*, snapdragon, apical dominance, growth, flowering.

Introduction

Snapdragons, apart from been grown as bedding plants and as a cut flower crop, can also be grown as pot plants, as they have a high flower bud to vegetative growth ratio, mature quickly and a range of brightly coloured cultivars are available. Disadvantages when grown as pot plants are that they may grow too tall and so are not self-supporting and a large central flowering inflorescence initially dominates the plant (Wainwright and Irwin, 1987). One possible method of overcoming these problems and producing a well-proportioned flowering pot plant could be to practice pinching technique.

Pinching is the removal of the apical bud to release the lower axillary buds from apical dominance in order to increase branching and stimulate axillary bud development. Apical dominance has been linked to auxins produced in the apical bud, which may indirectly inhibit lateral bud growth. Gocal *et al*. (1991) measured the concentration of IAA in axillary buds after pinching and found that IAA concentrations, firstly, increased and then remained constant. This was accompanied by an increase in the fresh weight of the buds. However, auxins are not the only hormones that control axillary bud growth. Cytokinin content has also been shown to increase after pinching (Mader *et al.,* 2003; Bangreth, 1994; Turnbull *et al.,* 1997). Exogenously applied cytokinin was found to have the same effect as pinching (Bangreth, 1994; Chen *et al.,* 1997). Therefore, there may be an antagonistic relationship between auxin and cytokinin in the control of apical dominance.

Release of apical dominance by pinching has been shown to have significant effects on yield in many crops. In rooted cuttings of chrysanthemum, pinching is used to produce a marketable spray shape. The 'time pinch' must be carried out at a particular time in order to allow a flower bud to initiate in the centre of the spray. This will develop into an arrangement of inflorescences on peduncles of a desired length (Kofranek, 1981). Post (1949) suggested that axillary meristems of chrysanthemum can show a period of insensitivity to photoperiod when released from apical dominance, but a contradictory result was reported by Adams *et al.* (1998), who found that chrysanthemum plants were capable of responding to short days immediately following pinching. This method of removing apical dominance is also found to delay flowering but did not affect plant height or plant width (Starman and Faust, 1999).

In winter cultivars of *Antirrhinum* (Coronette Yellow and Coronette Scarlet), pinching produces plants with significantly reduced plant height, shorter but increased number of flower spikes and lengthened flowering time. The pinching stage does not seem to influence plant height, but pinching carried out at later stage of plant development reduced the spike length and prolonged flowering time. Pinching at the third pair of leaves from the base of plant produced maximum flower spikes in the shortest time. These effects of pinching are a result of a more even distribution of assimilates between several growing points rather than just one. The delay in flowering is a result pushing the plant back to the juvenile phase. In this way, pinching appears to maintain the juvenile phase for longer and the precise cause of the flowering delay, which may be attributed to the axillary shoots being in a less advanced physiological phase than apical shoot (Wainwright and Irwin, 1987). The stage at which the plants are pinched, though, was found to have significant effects on the time of flowering and the length of spike, the following experiment was however undertaken to evaluate the effect of pinching at different stages of plant development (4, 5 and 6 leaf-pair stage) on the flowering and plant growth of *Antirrhinum*.

Materials and methods

Seeds of *Antirrhinum majus* L. cultivar Chimes White were obtained from Colegrave Seeds Ltd., Banbury, U.K., and were sown on 2nd February 2000 into module trays (P135, volume of each cell, 20ml; Plantpak Ltd., Maldon, U.K.) containing a peat-based modular compost (SHL, William Sinclair

Horticulture Ltd., Lincoln, U.K.). Seed trays were watered and held for germination at 20 ± 1°C in a growth room providing a photosynthetic photon flux density of 72 µmol m^{-2} s^{-1} at approx. one meter above tray height from a mixture of white fluorescent and tungsten bulbs (6.3% tungsten by nominal wattage), with a 16 h d^{-1} photoperiod.

After 70% seed germination, plants were transplanted into 9 cm pots (volume 370ml) containing a mixture of peat-based compost (SHL) and perlite (3:1 v/v) and kept in a glasshouse (7.3m x 11.3m). Ten plants in each treatment were pinched when they produced 4, 5, 6, and 7 pair of leaves. Same number of plants were left without pinched as control. In the glasshouse, all plants were equally spaced to avoid the light competition among them and were subjected to 20°C constant temperature (19.8°C actual temperature) until flowering. The set point temperature was maintained with ventilation and a water pipe heating system above 3°C. Temperature was recorded inside the glasshouse compartments using a sensor situated in an aspirated screen attached to a data-logger. In the temperature-controlled compartment, PT100 4 wire platinum resistance sensor was connected to a data-logger (Datataker 500, Data Electronics, Letchworth Garden City, U.K.) recording temperature at every 15s. Tube solarimeters were used to measure the average light transmission into the glasshouse and approximately 6.88MJ. m^{-2}.d^{-1} light integral from emergence to flowering were received by the plants during this experiment.

Plants were irrigated by hand to avoid *Pythium* attack and nutrient solution (Sangral 111, William Sinclair Horticulture Ltd, Lincoln, U.K.) was applied twice a week with the irrigation at conductivity of 1500 µs cm^2 (182 ppm N; 78 ppm P; 150 ppm K), and 5.8 pH. Plants in each treatment were daily observed until first flower opening (corolla fully opened). Flowering and vegetative parameters were recorded at harvest. Data were analysed by using regression and analysis of variance technique of GENSTAT-5, Release 4.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, U.K.).

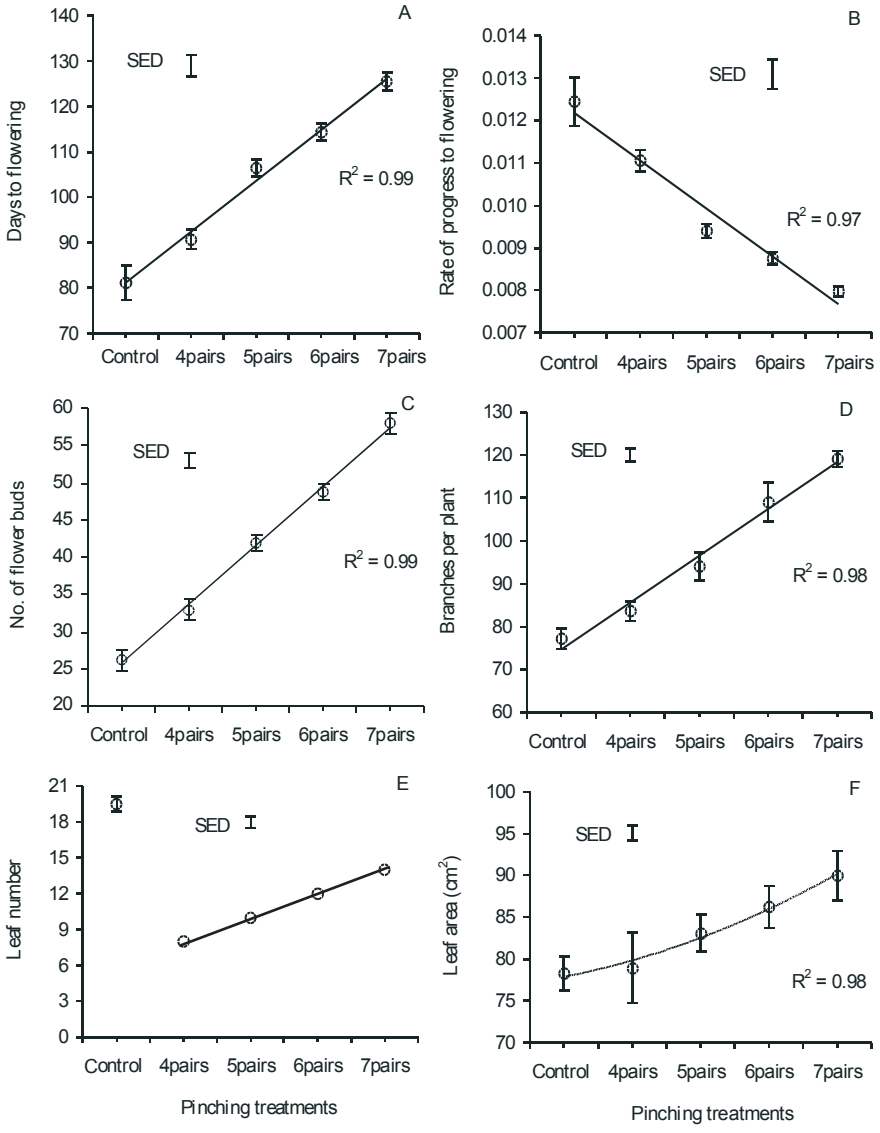

Fig. 1. Effect of different pinching treatments on (A) days to flowering, (B) rate of progress to flowering (1/f), (C) Number of flower buds, (D) No. of branches per plant, (E) leaf numbers, and (F) leaf area (cm2). Vertical bars (where larger than the points on lines) represent the standard error (SE) of variability within replicates, whereas the separate ones represent the standard error of difference (SED) between means.

Results

Directly Measured Parameters

Flowering parameters: Fig. 1A show that time to flowering increased linearly when pinched leaf-pair numbers were increased (P<0.05). An early flower anthesis (81 days) was recorded in control plants followed by 91 days in 4 leaf-pair. However, plants pinched at 7 leaf-pair stage took maximum time to flower (126 days). Similarly, the rate of progress to flowering (1/*f*) was the inverse function to the pinching treatments *i.e.* when plants were pinched late, rate of progress to flowering decreased (Fig. 1B). Similar to time of flowering, a linear relationship between pinching treatments and number of flower buds was observed (P<0.05). Fig. 1C revealed that minimum flower buds (26) were produced by the control plants. Among pinching treatments, 33 flower buds were noted in 4 leaf-pair treatment whilst 58 flower buds were produced in 7 leaf-pair pinching treatment.

Plant quality parameters: A linear increase in number of branches was observed between control and 7 leaf-pair pinching treatments. Minimum branches per plant (77) were produced by control plants followed by 84 in 4 leaf-pair treatment. Branch numbers increased significantly (P<0.05) in all subsequent treatments and up to 119 branches were produced in 7 leaf-pair treatment (Fig. 1D). Leaf numbers below inflorescence did not change following pinching after pinching the plants maintained the same leaf number present at the time of pinching. However, maximum leaf number (20) was produced in control (Fig. 1E). Control plants and those pinched at 4 leaf-pair stage produced minimum leaf area (79 cm^2), which was significantly (P<0.05) different than the other treatments (Fig. 1F). However, it was observed maximum in plants

pinched at 7 leaf-pair stage (90 cm^2). As control plants were not pinched therefore they continued their apical growth and attained maximum height (21 cm) as compared to the pinched plants (12-17 cm), which were forced to have a limited size (Fig. 2A). A curvilinear response was observed in plant fresh (Fig. 2B) and dry weight (Fig. 2C). Plants pinched at 7 leaf-pair stage produced maximum plant fresh (34.12 g) and dry (5.25 g) weights. This trend significantly ($P<0.05$) declined in all preceding treatments including control plants, which produced minimum fresh and dry weights (15.12 and 3.37 g, respectively).

Derived Parameters

A gradual but significant ($P<0.05$) increase in leaf area ratio (LAR) was seen between control and 7 leaf-pair pinched plants (Fig. 2D). Un-pinched plants (control) have minimum LAR (1184.01 $cm^2\ g^{-1}$), which gradually increased in all subsequent treatments. However, maximum LAR (3076.76 $cm^2\ g^{-1}$) was estimated in plant pinched at 7 leaf-pair stage. A fairly similar trend (Fig. 2E) was observed in relative growth rate (RGR) where minimum RGR was observed in control plants (15.12 d^{-1}) whereas it was maximum (34.12 d^{-1}) in plants received 7 leaf-pair pinching treatment. Net assimilation rate (NAR) showed an opposite curvilinear response to those in LAR and RGR parameters (Fig. 2F). As plants in control treatment took minimum time to flower, they had a maximum NAR (0.07 g $cm^{-1}\ d^{-1}$) whereas it was estimated minimum (0.03 g $cm^{-1}\ d^{-1}$) in plants pinched at 7 leaf-pair stage and they took more time to flower.

Discussion

Flowering was generally delayed by pinching, as all of the pinched treatments produced their first flower later than the control plants (Fig. 1A). This delay in flowering seems to be a result of pushing the plants back to the juvenile phase after pinching. The later the pinching occurred, the later the plants flowered. The precise cause of flowering delay may be attributed to the axillary shoots being in a less advanced physiological phase than the apical shoot, as they begin to develop only after pinching. Present results of summer cultivar Chimes White are coincided with winter cvs. Coronette Yellow and Coronette Scarlet (Wainwright and Irwin, 1987). Another possible cause could be the interception of developmental signals from leaves to the apical meristem, which normally carries genes to change growing meristem from vegetative to reproductive phase. If plants are pinched before the perception of development signal in the leaves, induction will never occur and plants never become competent to release the stimulus towards apex (Munir, 2003; McDaniel, 1996). Similarly, in roses, pinching of the apical meristem enhanced the suitability of axillary meristem to determine florally after obtaining a specific nodes/leaf number (Cockshull and Horridge, 1977). The difference in flowering time can be directly linked to the branch numbers, leaf area, plant fresh and dry weights. It can be assumed from the present study that when plants produced more branches, larger leaf area, maximum fresh and dry weights, they took more time to flower. This indicate that after pinching, plants remained juvenile for longer time than the un-pinched ones and as a consequence the release of developmental stimulus was delayed.

The co-ordination of promotive root-sourced cytokinin (CK) and inhibitory shoot apex-sourced auxin (IAA) is central to all current models on lateral bud dormancy release. For example, in chickpea it has been observed that three potential lateral bud growth inhibitors, IAA, ABA, and *cis*-zeatin 9-riboside (ZR), declined sharply in the released buds and xylem following pinching. This is in contrast to potential dormancy breaking CKs like *trans*-ZR and *trans*-zeatin 9-riboside 5' phosphate (ZRMP), which represented strongest correlative changes by increasing 3.5 fold in xylem sap and 22-fold in buds. However, polyamines had

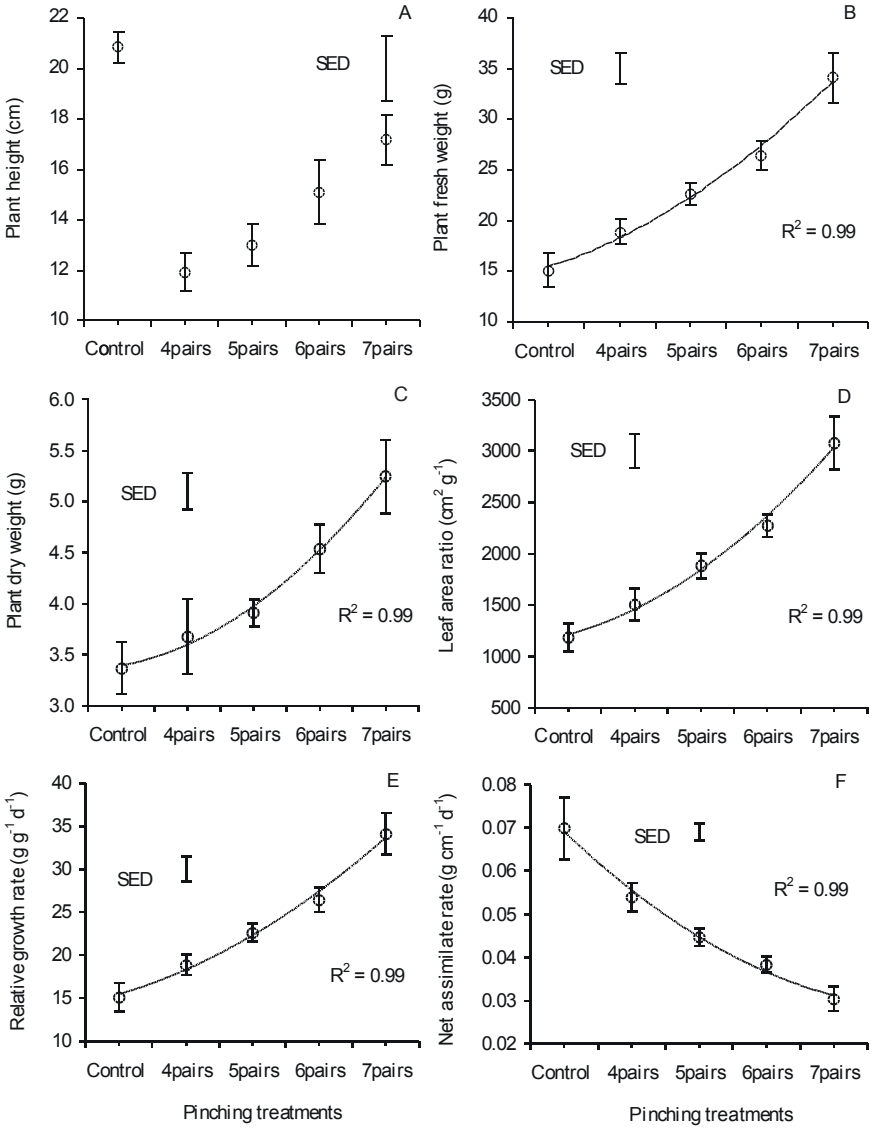

Fig. 2. Effect of different pinching treatments on (A) plant height (cm), (B) plant fresh weight (g), (C) plant dry weight (g), (D) leaf area ratio $cm^2\ g^{-1}$, (E) relative growth rate (d^{-1}), and (F) net assimilate rate (g $cm^{-1}\ d^{-1}$). Vertical bars (where larger than the points on lines) represent the standard error (SE) of variability within replicates, whereas the separate ones represent the standard error of difference (SED) between means.

not changed significantly in buds or other tissues, so they were not directly involved in the breaking of bud dormancy (Mader *et al.,* 2003). This evidence could be related to the present study on *Antirrhinum* that what would happen at the apex and in the xylem when a plant is released from apical dominance.

The major advantage of pinching is that the pinched plants produced maximum flower buds at harvest (Fig. 1C). This could be a result of more even distribution of assimilates between several growing points (in axillary branches) rather than just the apical one (as in control plants). The later the pinching the greater was the number of flower buds, although they took longer to flower. Similar findings were obtained in *Antirrhinum*, chrysanthemum and cowpea (Wainwright and Irwin, 1987; Starman and Faust, 1999; Argall and Stewart, 1984).

Pinching is a method that can be used to produce well-proportioned plants, suitable for selling as high quality pot-plants and also as a method of controlling the time of flowering, when plants are required for selling them flowered at a certain time and occasion. Similarly, plant quality was much improved in plants pinched at 5-7 leaf-pair stage at the expense of flowering time. Therefore, proper manipulation of the above-mentioned protocol can be used commercially to get maximum outcome.

References

Adams, S.R., S. Pearson and P. Hadley, 1998. An appraisal of the use of reciprocal transfer experiments: assessing the stages of photoperiod sensitivity in chrysanthemum cv. Snowdon (*Chrysanthemum morifolium* Ramat.). *J. Exp. Bot*., 49: 1405-1411.

Argall, J.F. and K.A. Stewart, 1984. Effects of decapitation and benzyl adenine on growth and yield of cowpea. *Ann. Bot.*, 54: 439-444.

Bangreth, F. 1994. Response of cytokinin concentration in the xylem exudate of bean (*Phaseolus vulgaris* L.) plants to decapitation and auxin treatment and relationship to apical dominance. *Planta*, 194: 439-442.

Cockshull, K.E. and J.S. Horridge, 1977. Apical dominance and flower initiation in the rose. *J. Hort. Sci.*, 52: 421-427.

Chen, J.G., Y.H. Zhao, X. Zhou, L.S. Mao and X.X. Chen, 1997. Fluctuation in levels of endogenous hormones after decapitation and 6-benzyl amino purine treatment in Azalea and their relationship to apical dominance. *Scientia Hortic.*, 71: 49-58.

Gocal, F.W.G., P.R. Pharis, C.E. Yeung and D. Pearce, 1991. Changes after decapitation in concentrations of indole-3-acetic acid and abscisic acid in the larger axillary bud of *Phaseolus vulgaris* L. cv. Tender Green. *Plant Physiol.*, 95: 344-350.

Kofranek, M.A. 1981. Affecting the spray shape of pompon chrysanthemum during natural season flowering. *Acta Hortic.*, 125: 167-172.

Mader, J.C., R.J.N. Emery and C.G.N. Turnbull, 2003. Spatial and temporal changes in multiple hormone groups during lateral bud release shortly following apex decapitation of chickpea (*Cicer arietinum*) seedlings. *Physiologia Plantarum*, 119: 295-308.

McDaniel, C.N. 1996. Developmental physiology of floral initiation in *Nicotiana tabacum* L. *J. Exp. Bot.*, 47: 465-475.

Munir, M. 2003. A study on the effects of environmental factors affecting the phases of flower development in *Antirrhinum majus* L. Ph.D. Thesis. Department of Horticulture and Landscape, School of Plant Sciences, The University of Reading, U.K.

Ono, M., K.S. Ono, K. Yamada, M. Yasui and M. Okazaki, 1993. Axillary bud flowering after apical decapitation in *Pharbitis* in relation to photoinduction. *Physiologia Plantarum*, 87: 1-6.

Post, K. 1949. Pompon growth after pinching. *New York State Flower Growers Bulletin*, 41: 3.

Starman, W.T. and E.J. Faust, 1999. Field-grown garden chrysanthemum responses to pinching and plant date. *HortScience*, 34: 539-540.

Turnbull, G.N.C., A.A.M. Raymond, C.I. Dodd and E.S. Morris, 1997. Rapid increases in cytokinin concentration in lateral buds of chickpea (*Cicer arietinum* L.) during release of apical dominance. *Planta*, 202: 271-276.

Wainwright, H. and H.L. Irwin, 1987. The effects of paclobutrazol and pinching on *Antirrhinum* flowering pot plants. *J. Hortic. Sci.*, 62: 401-404.

Journal of Applied Horticulture, 8(1): 29-32, January-June, 2006

Postharvest control of soft-rot fungi on grape berries by fungicidal treatment and *Trichoderma*

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Abstract

The present research deals with the effect of postharvest treatment of grape berries with four commonly used fungicides and two forms of *Trichoderma harzianum* on the infection with soft-rot fungi- *Rhizopus stolonifer* and *Mucor piriformis*. This effect was evaluated by comparison of the external diameter of rot-lesion in treated and untreated berries, in addition to comparison of percent reduction in external rot-lesion diameter relative to control. Results indicated that the infection with *R. stolonifer* and *M. piriformis* was significantly reduced ($P<0.05$) in all treated berries in comparison with untreated control berries. The highest reduction in mean external rot-lesion diameter was obtained for both *R. stolonifer* and *M. piriformis* when inoculated berries were treated with Score® (difenoconazole) applied at 0.35%(v/v) or Switch® (cyprodinil + flodioxonil) applied at 0.20%(w/v) or formulated *T. harzianum* conidia in invert emulsion applied at $9.6x10^8$ conidia/ml of formulation (13.5, 13.2, and 19.3 mm, respectively for *R. stolonifer*; 7.2, 7.5, and 19.2mm, respectively for *M. piriformis*). The greatest decrease in percent reduction in external rot-lesion diameter relative to control was also obtained for both the fungal species when inoculated berries were treated with the same type of fungicides (Score® and Switch®) and *Trichoderma* (formulated *T. harzianum* in invert emulsion) (60.9, 61.7, and 44.1%, respectively for *R. stolonifer*; 74.5, 73.4, and 31.9%, respectively for *M. piriformis*). Overall results indicate that the most effective treatment obtained on grape berries could be integrated with other control measures being usually used in grape berry-rot management plans by alternating fungicidal treatment (*e.g.* Score® or Switch®) with application of formulated *T. harzianum* conidia in invert emulsion.

Key words: Grape, *Rhizopus stolonifer*, *Mucor piriformis*, *Trichoderma harzianum*, difenoconazole, captan, cyprodinil + flodioxonil, metalaxyl + mancozeb, postharvest.

Introduction

The rapid and extensive deterioration of the table grapes is mainly caused by the fungal decay. The major organisms that are involved in this decay are: *Botrytis cinerea*, *Aspergillus niger*, *Rhizopus stolonifer* and *Mucor piriformis* (Nelson, 1979). Of these organisms, *R. stolonifer* and *M. piriformis* are the two known fungal species which cause severe losses to table grapes during marketing and export in many countries (Lisker *et al.*, 1996). The reason for these losses is the incidence of soft-rot symptoms on the infected berries during storage at temperatures >8°C. Mechanical wounding dramatically increases the susceptibility of berries to soft-rot fungi and facilitates their penetration into the attacked berries (Lichter *et al.*, 2002).

The conventional method used to avoid the fungal decay in table grapes is by fumigating SO_2 or releasing it from generator pads containing a metabisulfite salt, and packaging of the fruit in polyethylene liners (Lichter *et al.*, 2002). Immersion of the detached berries in 70% ethanol also eliminates most of the fungal and bacterial population on the berry surface. This immersion results in inhibition of berry decay that is equivalent to or better than that achieved by SO_2 released from generator pads (Lichter *et al.*, 2002). High levels of SO_2 can also result in fruit damage, unpleasant aftertaste and allergies (Lisker *et al.*, 1996). Other methods such as acetic acid fumigation (Sholberg *et al.*, 1996), acetylaldehyde vapors (Avissar and Pesis, 1991), and modified atmosphere packaging and generating chlorine gas (Zoffoli *et al.*, 1999) can be used instead of SO_2 fumigation. Benomyl was one of the most widely used fungicides to control postharvest fungal decay especially Mucor rot on grape berries and other fruits in packinghouse (Spotts and Cervantes, 1986). Two other chemicals, orthophenyl phanate as fog and calcium hypochlorite as chlorine vapor that were applied to the surrounding atmosphere, significantly decreased postharvest decay in artificially inoculated berries (Lisker *et al.*, 1996). Vinclozolin (Ronilan 50 WP) can be used effectively against *R. stolonifer* and *B. cinerea* on table grapes, strawberries and kiwifruit (Lima *et al.*, 1997). Finally, Ozone treatment can be considered a possible substitute for SO_2 fumigation for the control of *R. stolonifer* on table grapes (Sarig *et al.*, 1996). The subatmospheric pressures (0.25, 0.50, and 0.75 atmos) for different times (1 to 24h) were used to reduce Rhizopus rot lesion on table grapes and other fruit types in comparison with the control treatment (1 atmos) (Romanazzi *et al.*, 2001).

Biological control as a substitute to chemical control or as practice that could be integrated with other control practices may result in acceptable levels of fruit decay with reduced levels of pesticide use such as using the following antagonistic yeast species: *Pichia membranefaciens* to control postharvest Rhizopus rot on nectarine fruit (Fan and Tian, 2000), *Kloeckera apiculata* and *Candida guilliermondii* to control postharvest diseases of many fruits including table grape (McLaughline *et al.*, 1992), *Cryptococcus laurentii*, *C. flavus*, *C. albicus* to control Mucor rot on pear fruit (Roberts, 1990), *Aureobasidium pullulans* and *Candida oleophila* to control *Botrytis cinerea* and *Rhizopus*

stolonifer on strawberry, table grape and kiwifruit (Lima *et al.*, 1997). Also, using the following antagonistic bacterial species: *Pseudomonas cepacia* to control Mucor rot on apple (Janisiewicz and Roitman, 1987), *Pantoea agglomerans* to control *Rhizopus stolonifer* and *Monilinia laxa* on peach, apricot and nectarine (Bonaterra *et al.*, 2003), and *Enterobacter cloacae* to control Rhizopus rot on peach (Wilson *et al.*, 1987).

Although antagonistic fungus, *T. harzianum* is in widespread use against many fungal plant pathogens such as root rot fungi (*e.g. Pythium*, *Sclerotinia*, *Rhizoctonia* and *Fusarium*) (Fravel, 1998), and postharvest fungal pathogens on different types of fruit (*e.g. Botrytis cinerea*, *Alternaria alternata*, *Penicillium expansum*) (Batta, 1999, 2000, 2001, 2004a, and 2004b), no previous study was carried out to control soft-rot fungi of table grapes using formulated or unformulated *T. harzianum*. Therefore, the objectives of the present research were: i) to test the efficacy of postharvest treatment of grape berries with four types of commonly used fungicides and two forms of Trichoderma (unformulated and formulated *T. harzianum*) on the infection with soft-rot fungi: *R. stolonifer* and *M. piriformis*, ii) to compare the efficacy of this treatment with fungicides and *T. harzianum* forms.

Materials and methods

Grape berries used in the experiments: Healthy mature grape berries (table grape cultivar: Zaini) were used for bioassay of the treatment effect of fungicides and *T. harzianum* against Rhizopus and Mucor soft-rot.

Fungal strains used in the inoculation and treatment: The following fungal strains were used in the experiments: strain RS1 of *Rhizopus stolonifer*, strain MP3 of *Mucor piriformis*, and strain Th_2 of *T. harzianum*. The first two strains were isolated from infected grape berries (cultivar: Beiruti) then subcultured on plates with potato dextrose agar (PDA) medium for production of sporangia and sporangiospores to be used in the inoculation tests. The third strain which belongs to the antagonistic fungus *T. harzianum* was obtained from Faculty of Agriculture in Gembloux (Belgium) and subcultured on plates with oat meal agar (OMA). Young cultures of 14-day old of the above-mentioned strains were used to carry out the various tests of bioassay. The concentration of the conidia or sporangiospores in the suspensions prepared from these cultures was $11.7x10^6$ sporangiospores of strain RS1 per ml, $5.5x10^5$ sporangiospores of strain MP3 per ml, and $9.6x10^8$ conidia of strain Th_2 per ml.

Treatments with fungicides and *T. harzianum* used in the experiments: Four types of treatments with fungicides were applied against Rhizopus and Mucor soft-rot inoculations on grape berries. They were: 0.30% (W/V) metalaxyl + mancozeb (sold as Ridomil® MZ 63.5 WP, concentration of a.i=7.5% metalaxyl + 56% mancozeb); 0.35% (V/V) difenoconazole (sold as Score® 250 EC); 0.35% (W/V) captan (sold as Merpan® 50 WP) and 0.20% (W/V) cyprodinil + flodioxonil (sold as Switch® 62.5 WG, a.i.=375g/kg cyprodinil + 250 g/kg flodioxonil).

Two types of treatments with *T. harzianum* (strain Th_2) were applied against Rhizopus and Mucor soft-rot inoculations on grape berries *viz*., conidial suspension in sterile distilled water; and formulated conidia in invert emulsion. Concentration of the conidia in both the treatments was $9.6x10^8$ conidia/ml. Ingredients of the invert emulsion (water-in-oil type) used in the experiments were identical to those used by Batta (2004a). The conidia of *T. harzianum* (strain Th_2) harvested from 14-day old culture, were introduced into the invert emulsion according to the technique developed by Batta (2004a). Two additional non-treated control treatments were included in the tests: one with blank formulation of invert emulsion and the other with sterile distilled water only (water control treatment).

Rhizopus and Mucor inoculation and treatment effect assessment: Inoculation of *R. stolonifer* and *M. piriformis* was accomplished by depositing 25-µl droplet of spore suspension containing 292,500 sporangiospores of *R. stolonifer* and 13,750 sporangiospores of *M. piriformis* (original suspensions contained $11.7x10^6$ and $5.5x10^5$ sporangiospores/ml, respectively) on the fruit surface after being superficially wounded using sterile scalpel. All fruits used in the tests were disinfected with 0.025% sodium hypochlorite, and then rinsed with sterile distilled water 3 times before inoculation.

For the assessment of treatment effect with fungicides and *T. harzianum* on the rot-lesion development caused by the above-mentioned fungi, the preventive effect (treatment application at the same time of the inoculation) was studied. The same droplet size (25µl) of fungicides' solution (mentioned earlier) or *T. harzianum* conidia formulated in invert emulsion or suspended in sterile distilled water was used in the treatments. The droplet was deposited at the same site of inoculation with sporangiospores of *R. stolonifer* or *M. piriformis* on the fruit surface immediately after the inoculation. Inoculated fruits were incubated in closed plastic containers (9.5cm diameter 6.5cm deep) with 6 fruits per container for 3 days at 20±1°C under humid conditions.

Evaluation of the treatment effect with fungicides and *T. harzianum* on the soft-rot development: Such effect was evaluated by measuring the ability of each type of fungicides or *T. harzianum* form to reduce the development of rot-lesion caused by *R. stolonifer* or *M. piriformis* on grape berries. The external rot-lesion diameter was measured in all replicates 3 days after the treatment at 20±1°C. Mean of external rot-lesion diameter in each treatment type was calculated to be used in the comparison of treatment effect. Mean percent reduction in the external rot-lesion diameter relative to non-treated water control was also calculated in each treatment for comparison of their effect.

Statistical analyses: Data were analyzed using completely randomized design (CRD) with 6 replicates representing 6 fruits per treatment. Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were used to test the significant differences between treatments representing the means of external rot-lesion diameter or the means of percent reduction in external rot-lesion diameter relative to non-treated water control.

Results and discussion

Effect of fungicides and *T. harzianum* on Rhizopus soft-rot development on grape berries: Significant differences (at $P<0.05$) were obtained between treatments with four types of fungicides or two forms of *T. harzianum* when used against Rhizopus soft-rot on grape berries in comparison with the non-treated control (Table 1). Treatments with Switch® (cyprodinil + flodioxonil) and Score® (difenoconazole) resulted in the

least values of external rot-lesion diameter (13.2 and 13.5mm, respectively), followed by the treatment with formulated conidia of *T. harzianum* in invert emulsion (19.3mm). The other treatments used in the experiment resulted in significant higher values of means of external rot-lesion diameter ranging from 24.2 to 34.5mm. The treatment efficacy, in a descending order, was as follows: Switch® or Score®, formulated conidia of *T. harzianum* in invert emulsion, conidial suspension of *T. harzianum* in sterile distilled water, Merpan® or Ridomil®, blank formulation of invert emulsion or sterile distilled water as non-treated control treatments. Similarly, significant differences (at $P<0.05$) were obtained between means of percent reduction in the external rot-lesion diameter relative to control with sterile distilled water (Table 1). Therefore, the highest means of the percent reduction (60.9 and 61.7) were recorded with Score® and Switch®, respectively, followed by formulated *T. harzianum* conidia in invert emulsion (44.1). The rest of the treatments significantly caused lower means of the percent reduction ranging between 29.8 and 3.5. The treatment efficacy, in a descending order, was similar to the above-mentioned trend.

Effect of fungicides and *T. harzianum* on Mucor soft-rot development on grape berries: Significant differences ($P<0.05$) were obtained between treatments with four types of fungicides or two forms of *T. harzianum* when used against Mucor soft-rot on grape berries in comparison with the non-treated control (Table 2). Treatment with Score® (difenoconazole) and Switch® (cyprodinil + flodioxonil) gave the least values of external rot-lesion diameter (7.2 and 7.5mm, respectively), followed by the treatment with Merpan® (captan) (15.7mm), formulated conidia of *T. harzianum* in invert emulsion (19.2mm). The rest of the treatments used in the experiment gave significantly higher values of means of rot-lesion diameter ranging from 24.7 to 28.2mm. The treatment efficacy in a descending order, was as follows: Score® or Switch®, Merpan®, formulated conidia of *T. harzianum* in invert emulsion, conidial suspension of *T. harzianum* in sterile distilled water or Ridomil®, blank formulation of invert emulsion, and sterile distilled water as non-treated control treatment. Significant differences ($P<0.05$) were also obtained between means of percent reduction in the external rot-lesion diameter relative to control with sterile distilled water (Table 2). The highest means of the percent reduction (74.5 and 73.4) were thus caused by the treatment with Score® and Switch®, respectively, followed by Merpan® (44.3) and formulated conidia of *T. harzianum* in invert emulsion (31.9). The rest of the treatments significantly caused lower means of the percent reduction ranging between 12.4 and 1.8 and similar to above mentioned trend of the treatment efficacy.

Overall results obtained in the present investigation indicate that the postharvest treatment of grape berries with Switch® (cyprodinil + flodioxonil) or Score® (difenoconazole) gave the highest percent reduction in the external rot-lesion diameter caused by *R. stolonifer* and *M. piriformis*. This effectiveness of treatment is comparable with that obtained by SO_2 fumigation, conventionally practiced to prevent fungal decay in table grape berries (Lisker *et al.*, 1996; Lichter *et al.*, 2002). SO_2 treatment remains effective as long as SO_2 level is sufficiently high, but the high levels can result in fruit damage as fruit bleeching, unpleasant aftertaste and allergies (Lisker *et al.*, 1996; Lichter *et al.*, 2002). To avoid the undesirable side-effects of chemical treatment against fungal decay of harvested fruit especially in table grape berries, treatment with biocontrol agents is recommended by many investigators (Elad, 1994; Harmann *et al.*, 1996). In the present study, the postharvest treatment of grape berries with *T. harzianum* formulated in invert emulsion also gave a high percent reduction in external rot-lesion diameter caused by *R. stolonifer* and *M. piriformis*. However, this treatment is less

Table 1. Effect of treatment with four types of fungicides and two forms of *T. harzianum* (strain Th_2) on the soft-rot development caused by *Rhizopus stolonifer* (strain RS1) on grape berries 3 days after inoculation and treatment at 20±1°C under humid conditions

Fungicides and *T. harzianum* treatments	External rot-lesion diameter (mm) developed on berries (Mean±SE)	Reduction(%) in external rot-lesion diameter relative to water (control)
Ridomil® (metalaxyl + mancozeb)	30.2±2.8 d [1)]	12.5 b [1)]
Score® (difenoconazole)	13.5±1.9 a	60.9 e
Merpan® (captan)	28.5±3.1 d	17.4 b
Switch® (cyprodinil + flodioxonil)	13.2±1.8 a	61.7 e
Formulated conidia of *T. harzianum* in invert emulsion	19.3±2.4 b	44.1 d
Blank formulation of invert emulsion as control treatment [2)]	33.3±3.3 e	3.5 a
Conidial suspension of *T. harzianum* in sterile distilled water	24.2±2.9 c	29.8 c
Sterile distilled water as control treatment	34.5±3.1 e	

Table 2. Effect of treatment with four types of fungicides and two forms of *T. harzianum* (strain Th_2) on the soft-rot development caused by *Mucor piriformis* (strain MP3) on grape berries 3 days after inoculation and treatment at 20±1°C under humid conditions

Fungicides and *T. harzianum* treatments	External rot-lesion diameter (mm) developed on berries (Mean±SE)	Reduction(%) in external rot-lesion diameter relative to water (control)
Ridomil® (metalaxyl + mancozeb)	24.8±2.5 d [1)]	12.1 b [1)]
Score® (difenoconazole)	7.2±1.3 a	74.5 e
Merpan® (captan)	15.7±2.1 b	44.3 d
Switch® (cyprodinil + flodioxonil)	7.5±1.2 a	73.4 e
Formulated conidia of *T. harzianum* in invert emulsion	19.2±1.9 c	31.9 c
Blank formulation of invert emulsion as control treatment [2)]	27.7±3.1 de	1.8 a
Conidial suspension of *T. harzianum* in sterile distilled water	24.7±2.8 d	12.4 b
Sterile distilled water as control treatment	28.2±3.7 e	

[1)] Means of external rot-lesion diameter within each column followed by different letters are significantly different at $P<0.05$ according to ANOVA and Duncan's multiple range test (DMRT).
[2)] Invert emulsion is composed of the following ingredients (W/W): sterile distilled water (45.25%), glycerine (4.00%), water-soluble wax or Dehymuls K® (0.75%), Tween 20 (2.50%), and a mixture of 19.0% coconut oil + 28.50% soybean oil.

effective than the fungicidal treatment with Switch® or Score®, it has no undesirable side-effects *e.g.* leaving toxic residues or bad flavors or smells in the treated berries. To overcome the problem of harmful side-effects that appear in association with the chemical treatment of grape berries, many investigators have recommended alternation of chemical and biological treatment applications keeping meanwhile the same level of treatment efficacy. The applications of biological treatment could be also integrated with other control measures practiced within the decay-management program of grape berries.

For the other types of grape berry decay (*e.g.* Botrytis bunch rot caused by *Botrytis cinerea*), the decay-management program was performed successfully using both chemical and biological means such as 0.5-1.0 g L^{-1} of *T. harzianum* dry conidia + 0.5 g L^{-1} of vinclozolin or iprodione or 0.25 g L^{-1} of diethofencarb plus carbendazim resulted in up to 78% disease reduction in grape berries (Elad, 1994). Also, treatment with *T. harzianum* dry conidia could replace some applications of iprodione or vinclozolin with little reduction in efficacy against *B. cinerea* on grape berries (Harmann *et al.*, 1996).

The effectiveness of postharvest treatment with *T. harzianum* formulated in invert emulsion was proved by us in controlling other types of fruit decay on various types of fruits at postharvest stage (*e.g.* gray mold on strawberry and apple; blue mold on apple; black fruit spot of persimmon) (Batta, 1999, 2001, 2004a and 2004b). We have also proved in our previous research that this type of postharvest biocontrol treatment has no harmful side-effects since the ingredients used for the invert emulsion are especially oils and emulsifiers which are safe and have no phytotoxic effect on treated fruits. These ingredients are also likely to be non-toxic to humans as they are also used as food additives and in the manufacture of cosmetics.

References

Avissar, I. and E. Pesis, 1991. The control of postharvest decay in table grapes using acetylaldehyde vapor. *Ann. Appl. Biol.,* 118: 229-237.

Batta, Y. 1999. Biological effect of two strains of microorganisms antagonistic to *Botrytis cinerea*: causal organism of gray mold on strawberry. *An-Najah Univ. J. Res. Natural Sci*., 13:67-83.

Batta, Y. 2000. Alternaria leaf spot disease on fig trees: varietal susceptibility and effect of some fungicides and Trichoderma. *The Islamic Univ. J. Gaza,* 8: 83-97.

Batta, Y. 2001. Effect of fungicides and antagonistic microorganisms on the black fruit spot disease on persimmon. Dirasat: *Agric. Sci.,* 28: 165-171.

Batta, Y., 2004a. Postharvest biological control of apple gray mold by *Trichoderma harzianum* Rifai formulated in invert emulsion. *Crop Prot*., 23: 19-26.

Batta, Y. 2004b. Effect of treatment with *Trichoderma harzianum* Rifai formulated in invert emulsion on postharvest decay of apple blue mold. *Int. J. Food Microbiol.,* 96: 281-288.

Bonaterra, A., M. Mari, L. Casalini and E. Montesinos, 2003. Biological control of *Monilinia laxa* and *Rhizopus stolonifer* in postharvest of stone fruits by *Pantoea agglomerans* EPS 125 and putative mechanisms of antagonism. *Int. J. Food Microbiol*., 84: 93-104.

Elad, Y. 1994. Biological control of grape gray mold by *Trichoderma harzianum*. *Crop Prot.*, 13: 35-38.

Fan, Q. and S.P. Tian, 2000. Postharvest biological control of Rhizopus rot of nectarine fruits by *Pichia membranefaciens*. *Plant Dis.*, 84: 1212-1216.

Fravel, D. 1998. Commercial biocontrol products for use against soil borne diseases (dfravel@asrr.arsusda.gov).

Harmann, G.E., B. Latorre, E. Agosin, R. San Martin, D.G. Riegel, P.A. Nielson, A. Tronsomo andR.C. Pearson, 1996. Biological and Integrated control of *Botrytis* bunch rot of grape using *Trichoderma* spp. *Biological Control*, 7: 259-266.

Janisiewicz, W. J. and J. Roitman, 1987. Postharvest mucor rot control on apple with *Pseudomonas cepacia*. *Phytopath.,* 77: 1776.

Lichter, A., Y. Zutkhy, L. Sonego, O. Dvir, T. Kaplunov, P. Sarig and R. Ben-Arrie, 2002. Ethanol controls postharvest decay of table grapes. *Postharvest Biol. Technol*., 24: 301-308.

Lima, G., A. Ippolito, F. Nigro and M. Salerno, 1997. Effectiveness of *Aureobasidium pullulans* and *Candida oleophila* against postharvest strawberry rots. *Postharvest Biol. Technol.*, 10: 169-178.

Lisker, N., Z. Keren-Shachman, P. Sarig, Y. Zutkhy and R. Ben-Arie, 1996. The biology and pathology of the fungus *Rhizopus stolonifer*, cause of black mold disease of table grapes in Israel. *Plant Pathol.*, 45: 1099-1109.

McLaughline, R.J., C.L. Wilson, S. Droby, R. Ben-Arie and E. Chalutz, 1992. Biological control of postharvest diseases of grape, peach and apple with yeasts *Kloeckera apiculata* and *Candida guilliermondii*. *Plant Dis*., 76: 470-473.

Nelson, K.E. 1979. Harvesting and handling California table grape for market. *Univ. California Bull. No.* 1913.

Roberts, R.G. 1990. Biological control of Mucor rot of pear by *Cryptococcus laurentii*, *C. flavus* and *C. albicus*. *Phytopathology,* 80: 1051.

Romanazzi, G., F. Nigro, A. Ippolito and M. Salerno, 2001. Effect of short hypobaric treatment on postharvest rots of sweet cherries, strawberries and table grapes. *Postharvest Biol. Technol*., 22: 1-6.

Sarig, P., Y. Zutkhy, T. Zahavi, S. Yannai, N. Lisker and R. Ben-Arie, 1996. Ozone for control of postharvest decay of table grapes caused by *Rhizopus stolonifer*. *Physoil. Mol. Plant Pathol*., 48: 403-415.

Sholberg, P.L., A.G. Reynolds and A.P. Gaunce, 1996. Fumigation of table grapes with acetic acid to prevent postharvest decay. *Plant Dis*., 80: 1425-1428.

Spotts, R.A. and L.A. Cervantes, 1986. Population pathogenicity and benomyl resistance of *Botrytis* spp., *Penicillium* spp. and*Mucor piriformis* in packinghouse. *Plant Dis*., 70: 106-108.

Wilson, C.L., J.D. Franklin and P.L. Pusey, 1987. Biological Control of *Rhizopus* rot of peach with *Enterobacter cloacae*. *Phytopath.,* 77: 303-305.

Zoffoli, J.P., B.A. Latorre, E.J. Rodrigues and P. Aldance, 1999. Modified atmosphere packaging using chlorine gas generators to prevent *Botrytis cinerea* on table grapes. *Postharvest Biol. Technol*., 15: 135-142.

Journal of Applied Horticulture, 8(1): 33-36, January-June, 2006

Nitrogen metabolism of *Aloe vera* under long-term diluted seawater irrigation

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Abstract

Diluted seawater such as 10% (10 volumes of seawater and 90 volumes of freshwater), 25%, 50%, 75% and 100% were used to irrigate *Aloe vera* L. during four successive years in Ledong region, Hainan Province of China. The effect of seawater irrigation on nitrogen metabolism of aloe plant was studied. Total nitrogen content of aloe leaves ranged from 1.48 to 1.56 % of dry matter, and no significant differences were observed between control (freshwater irrigation) and seawater treatments. The total nitrogen content of *aloe* roots, in the range of 0.74 to 0.85 % of dry matter, was much lower than that in the leaves. There was no significant difference in total nitrogen content of roots between control and seawater treatments. It is suggested that seawater treatments do not affect nitrogen uptake and transport in aloe plant. The nitrate content in aloe leaves irrigated with seawater was much lower than that with fresh water irrigation, and a continuous decline in nitrate content was noted with increasing seawater concentration. The nitrate/total nitrogen ratio also tends to decrease in leaves suggesting that nitrate has been assimilated into osmoregulated substances under seawater stress. The amino acid content of aloe plant was not affected, while the ratios of amino acid/total nitrogen significantly increased under seawater stress as compared with control. The protein content and protein/total nitrogen ratios were not affected by seawater treatment except for 100%, suggesting that there was a favourable transformation from amino acids to proteins under salt stress. It is concluded that a long term irrigation by diluted seawater on leachable sandy soil with excessive annual rain precipitation could effectively maintain yield and improve the quality of aloe.

Key words: Amino acid, aloe qualities, nitrate, protein, total nitrogen

Introduction

The shortage of freshwater resources for irrigation is a well known problem of agriculture (Hamdy *et al*., 2005). The challenge is to maintain crop production without impairing the balance of good quality water: an obvious solution is consequently to explore the sustainable use of non-conventional water resources (Pereira, 2002). When freshwater supply is limited, there is an increasing demand for the use of non-conventional waters. Seawater, as an ample non-conventional water source, was proposed to be used for crop production along the coastal desert 40 years ago (Glenn, 1998).

aloe is commonly used to treat a number of skin irritations, such as dry skin and irritant contact dermatitis (West and Zhu, 2003), healing of burns (Visuthikosol *et al.*, 1995) and cure of certain cancers. *aloe* is widely cultivated in coastal regions of southern China. Since several qualities of aloe plant were realised recently, aloe became a priority subject of many studies (Zheng, 2004; Liu, 2003; Sun *et al.*, 2003; Wu *et al.*, 2003). However, studies on nitrogen metabolism of aloe plant under salt stress are rare. The effect of seawater irrigation on nitrogen metabolism and qualities of aloe plant has not been studied.

The objectives of the present study were (1) to study the nitrogen metabolism of aloe plant under long-term irrigation with diluted seawater, and (2) to assess the qualities of aloe plant under seawater irrigation *vis a vis* increase/decrease of metabolic products.

Materials and methods

Study area and experiment layout: The experiments were conducted at the "863" Research Station, Ledong County, southeast of Hainan Province, China (18º9´N, 108º56´E). The climate is a tropical monsoon with mean annual temperature of 23-25ºC, mean annual precipitation 1000-1200 mm, most of which occurs from late May to October, and mean annual evaporation 2000-2200 mm.

A. vera used in this experiment has salt stress tolerance since the tissue cultured plants used for cultivation were from plants growing under seawater stress conditions. It was grown in 20 m^2 plots (5×4 m). The soil contained 69, 30 and 1% sand, silt and clay, respectively. The total soluble salts content and bulk density were 0.002% and 1.62 g cm^{-3}, respectively. There were six treatments *i.e.* CK (freshwater irrigation) and 10% (EC=4.2 dS m^{-1}), 25% (EC=10.9 dS m^{-1}), 50% (EC=19.1 dS m^{-1}), 75% (EC=28.4 dS m^{-1}) and 100% (EC= 39.2 dS m^{-1}) seawater

Table 1 Some chemical properties of the irrigation water[a)]

Warer used	pH	EC (dS m^{-1})	TN (mg L^{-1})	IN (mg L^{-1})	TP (mg L^{-1})	RP (mg L^{-1})
Seawater	7.8	39.2	1.28	0.43	0.76	0.30
Tap water	7.0	0.11	0.15	0.02	0.03	0.02

[a)] EC: Electrical conductivity; TN: Total nitrogen; IN: Inorganic nitrogen; TP: Total phosphorus; RP: Reactive phosphorus.

treatments, with a randomised complete block design, and each treatment had three replicates.

Seawater used for irrigation had a salinity of about 31.0 g L^{-1}. Prior to irrigation, the seawater and tap water were mixed in a tank, to which a compound fertilizer ($N:P_2O_5:K_2O$=15:15:15) was added to give the final concentration of 0.2%. The prepared mixtures were delivered to the experimental plots by plastic tubes. Basic chemical properties of irrigated water are shown in Table 1.

On August, 2001, plots were separated from each other with a deeply buried plastic film (40 cm depth) so as to prevent lateral permeation of seawater between plots. Before start, the experimental plots were pre-irrigated with sufficient freshwater to keep the soil field capacity moisture. Base fertilizers were applied at the rates of 600 and 75000 kg ha^{-1} for superphosphate and pig manure, respectively. Aloe was grown in the plots with 60 cm between rows and 50 cm between plants in the row. In one of the plots within the replicates, tensionmeter was installed at the depth of 0~40 cm. On December 10, the first irrigation was given using freshwater, and after that irrigation with the seawater treatments was done whenever soil water suction at a depth of 0~40 cm exceeded the value of 3.8 MPa. In all, 218 irrigations were given during the growth stages of aloe in 4 years. The total amount of water supplied in the irrigation reached about 1 m^3 per plot.

Sampling and analysis: Before irrigation, seawater and freshwater samples were analysed for electrical conductivity, pH, bicarbonate and levels of the ions: potassium, sodium, calcium, magnesium, chlorine and sulphate. Electrical conductivity and pH were measured in the field with EC 214 Conductivity Meter (HANNA instruments) and pH Meter (Cyerscan 510), respectively. Certain water parameters and initial soil samples which couldn't be measured in the field were analysed in the Resources and Environmental Laboratory, Nanjing Agricultural University (NJAU).

Aloe was harvested during late April 2004, and three individual plants in each plot were collected for analyzing plant biomass, total nitrogen, nitrite, amino acid and protein content. Analysis of amino acid and protein content was as per Li *et al.* (1999). All other analyses were according to Lu (1999).

Statistical methods: The analysis of variance was performed by the standard procedures using MS-EXCEL 2000 software. The means of different treatments were compared by applying Least Significant Difference Test (P=0.05) using SPSS 13.0 software.

Results and discussion

Effect of seawater irrigation on total nitrogen content in leaves and roots: Total nitrogen content in leaves and roots of aloe under seawater irrigation during four years are shown in Fig.1. The nitrogen content of aloe leaves ranged from 1.48 to 1.56 % of dry matter, and no significant differences were noted between CK- and seawater-treatments. The nitrogen content of aloe in roots, in the range of 0.74 to 0.85 % of dry matter, was much lower than that in leaves. There was no significant difference in nitrogen content of roots between the CK- and the seawater- treatments. These results suggest that seawater irrigation didn't significantly influence the nitrogen uptake and transport. This may be attributed to high irrigation frequency and good water drainage in the sandy soil. Total nitrogen uptake by aloe plant decreased at 75 and 100% seawater treatments due to the decreased biomass under severe seawater stress (Fig. 2). These results are in agreement with earlier studies with different plants species, like Alfalfa (Khan *et al.*, 1994), pumpkin (Aroiee *et al.*, 2005) and bean (Rabie and Almadini, 2005) under salt stress conditions.

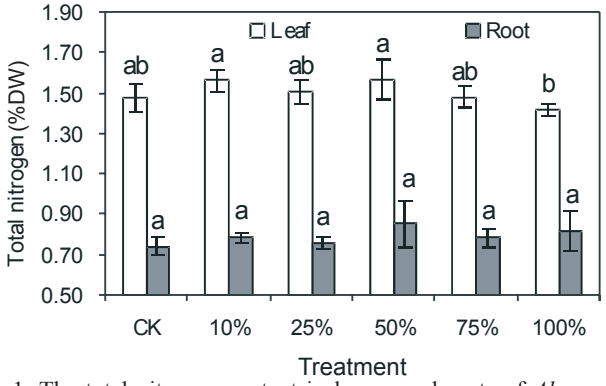

Fig. 1. The total nitrogen content in leaves and roots of *Aloe vera* under diluted seawater stress. CK represents freshwater (tap water) treatment, and seawater treatment, for example, 10% a mixture of 10% seawater and 90% fresh water on volume basis. The same letters on the bars are not significantly different (P=0.05) by Least Significant Difference Test.

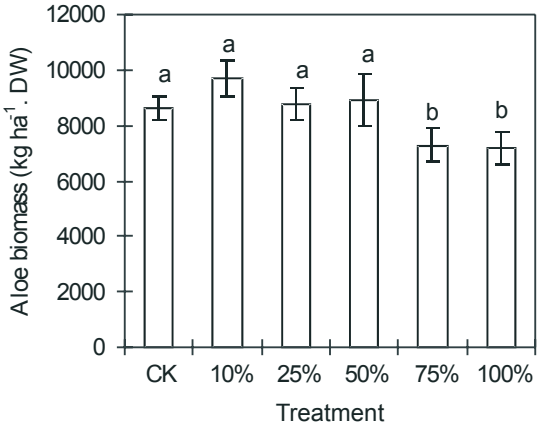

Fig. 2. The aloe biomass at harvest in 2004 under diluted seawater stress. CK represents freshwater (tap water) treatment, and seawater treatment, for example, 10% a mixture of 10% seawater and 90% fresh water on volume basis. The same letters on the bars are not significantly different (p=0.05) by Least Significant Difference Test.

Effect of seawater irrigation on nitrate content and ratios of nitrate to total nitrogen in leaves of aloe: Seawater irrigation significantly decreased nitrate content in leaves of aloe when compared with the CK (Fig. 3A). Among the treatments, the highest value of nitrate content in leaves was obtained in 25%, followed by 10, 50, 75 and 100%. The ratio of nitrate/nitrogen in leaves are presented in Fig. 3B. Seawater irrigation markedly lowered the ratio of nitrate/total nitrogen in the leaves compared to the CK. With 10, 25, 50, 75 and 100% seawater irrigation, nitrate/nitrogen in leaves decreased by 20.8, 11.8, 22.9, 26.0 and 32.3%, respectively as compared to CK. Decrease in the nitrate level of leaves with increasing salinity could be attributed to a build-up of organic osmoregulatory substances. A decreased nitrate level in leaves may also be due to incorporation of

nitrate in sugars and amino acid (proline) (Boggess *et al.*, 1976; Stewart, 1981; Rhodes *et al.*, 1986; Rhodes and Handa, 1989), and this transformation may be an adapting mechanism of plant for maintaining their normal growth under salt stress. It is also likely that an increase in Cl^- concentration in soil after seawater irrigation had reduced the nitrate uptake as reported for other plants (Weigel *et al.*, 1973; Kafkafi *et al.*, 1982; Elia *et al.*, 2004).

Effect of seawater irrigation on organic nitrogen and ratio of organic nitrogen to total nitrogen: Seawater irrigation resulted in a significant increase in amino acid concentration in aloe leaves. The highest content of amino acid was obtained in the 25% treatment. It was 1.16, 1.15, 1.07 and 1.04-folds respectively, when compared with the treatments 10, 50, 75 and 100% (Fig. 4A). Seawater irrigation had increased the ratio of amino acid to total nitrogen as compared with the CK (Fig. 4B).

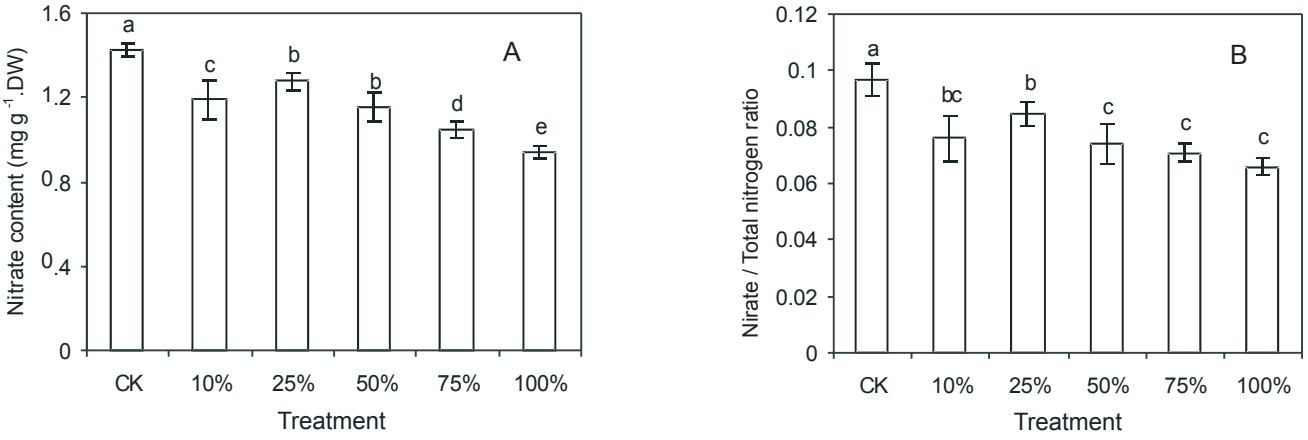

Fig. 3. The nitrate content (A) and the ratio of nitrate/total nitrogen (B) in aloe leaves under diluted seawater irrigation. CK represents freshwater treatment. Seawater treatment, for example, 10% is a mixture of 10% seawater and 90% fresh water on volume basis. The same letters on the bars are not significantly different (P=0.05) by Least Significant Difference Test.

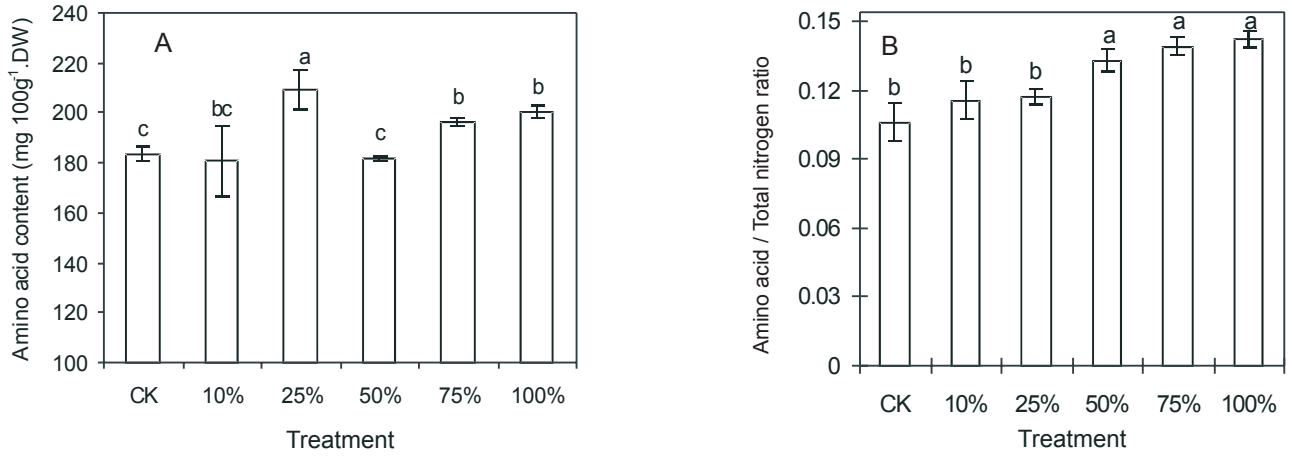

Fig. 4. The amino acid content (A) and the ratio of amino/total nitrogen (B) in aloe leaves under diluted seawater stress. CK represents freshwater treatment, and seawater treatment, for example, 10% is a mixture of 10% seawater and 90% fresh water on volume basis. The same letters on the bars are not significantly different (P=0.05) by Least Significant Difference Test.

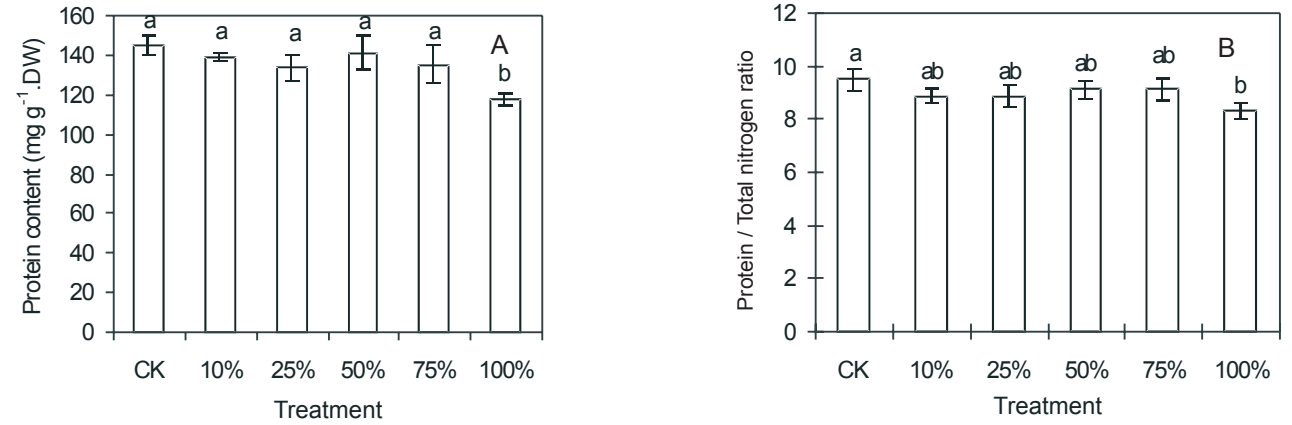

Fig. 5. The protein content (A) and the ratios of protein/total nitrogen (B) in aloe leaves under diluted seawater stress. CK represents freshwater treatment, and seawater treatment, for example, 10% is a mixture of 10% seawater and 90% fresh water on volume basis. The same letters on the bars are not significantly different (P=0.05) by Least Significant Difference Test.

Protein content: A significant increase in protein content of aloe leaves was observed under seawater treatments (except for 100%) indicating that the protein content had not been influenced under moderate seawater stress (Fig. 5A). Seawater irrigation enhanced the ratio of protein/total nitrogen as compared with CK (except for 100%) (Fig. 5B). It may be due to the fact that the presence of salt ions may activate the biosynthesis of glycinebetaine which in turn may improve the protein synthesis, a mechanism adopted by the plants against salinity stress as was suggested by Niazi *et al.* (2004).

Total nitrogen content of aloe did not decline under diluted seawater irrigation as compared to freshwater irrigation. However, nitrate content as well as the ratio of nitrate to nitrogen in aloe leaves was significantly reduced due to seawater stress, suggesting assimilation of nitrate into osmoregulatory substances like soluble sugars and organic acids. Subsequent results showed that seawater irrigation significantly increased the amino acid content of leaves suggesting that nitrate in leaves was largely assimilated into amino acid to resist severe salt stress. However, protein content of aloe in leaves was not affected by seawater except the pure seawater treatment, demonstrating that there is a transformation of amino acids into proteins under salt stress. This study demonstrates that a long term irrigation by diluted seawater on leachable sandy soil with excessive annual rain precipitation could effectively maintain yield and improve the quality of aloe.

Acknowledgement

The research was funded by the National High-Tech 863 Project (Nos. 2002AA2Z4061 and 2003AA627040) of China. We acknowledge the assistance of LIU Lian, CHEN Jian-Miao, YUN Yan and FU Yong-Sheng in trial management, as well as Sun X for careful review of this manuscript.

References

Aroiee, H., M. Azizi and R. Omibaigi, 2005. Effect of salinity and nitrogen nutrition on free proline and oil content of medicinal pumpkin (*Cucurbita pepo* subsp. Pepo convar. Pepo var. styriaca). *Acta Hortic.*, 676: 45-51

Boggess, S.F., D. Aspinall and L.G. Paleg, 1976. Stress metabolism. IX. The significance of end-product inhibition of proline biosynthesis and of compartmentation in relation to stress-induced proline accumulation. *Aust. J. Plant Physiol.*, 3: 513-525.

Elia, A., P. Santamaria and F. Serio, 2004. Artichoke response to nitrogen form ratio under saline conditions. *Acta Hortic.*, 660: 455-460.

Glenn, E P., J.J. Brown and J.W. Leary, 1998. Irrigating crops with seawater. *Scientific American*, 279(2): 56-61

Hamdy, A., V. Sardob and K.A. Farrag Ghanem, 2005. Saline water in supplemental irrigation of wheat and barley under rainfed agriculture. *Agricultural Water Management*, 78: 122-127.

Kafkafi, U., N. Valoras and J. Letey, 1982. Chloride interaction with nitrate and phosphate nutrition in tomato. *J. Plant Nutr.*, 5: 1369

Khan, M.G., M. Silberbush and S.H. Lips, 1994. Physiological studies on salinity and nitrogen interaction in alfalfa. 1. Biomass production and root development. *J. Plant Nut*., 17: 657-668.

Li, H.S. 2000. *Principles and Techniques of Plant Physiological Biochemical Experiment.* Higher Education Press, Beijing. 123p.

Liu, L., Liu, L., Liu, Z.P., Fu, Y.S., Yun, Y., Deng, L.Q. and Chen, M.D., 2003. Study on *Aloe vera* irrigated by seawater in the sea-beach of South China. *Journal of Natural Resources*, 18(5): 589-594.

Lu, R.K. 1999. *Soil and agrochemistry analyses.* Beijing: Agricultural Science and Technology of China Press.

Niazi, B.H., M. Athar and J. Rozema, 2004. Salt tolerance in fodderbeet and seabeet: analysis of biochemical relations. *Bulg. J. Plant Physiol.*, 30(1-2): 78-88

Pereira, L.S., T. Oweis and A. Zairi, 2002. Irrigation management under water scarcity. *Agricultural water management*, 57: 175-216

Rabie, G.H. and A.M. Almadini, 2005. Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. *African Journal of Biotechnology*, 4(3): 210-222

Rhodes, D. and S. Handa, 1989. Amino acid metabolism in relation to osmotic adjustment in plant cells. In: "*Environmental Stress in Plants: Biochemical and Physiological Mechanisms*", NATO ASI Series, Vol. G19 (JH Cherry ed), Springer, Berlin, pp. 41-62.

Rhodes, D., S. Handa and R.A. Bressan, 1986. Metabolic changes associated with adaptation of plant cells to water stress. *Plant Physiol.*, 82: 890-903.

Stewart, C.R. 1981. Proline accumulation: Biochemical aspects. In: "*Physiology and Biochemistry of Drought Resistance in Plants*," (Paleg, L.G. and Aspinall, D. eds), Academic Press, Sydney, pp 243-259.

Sun, S.B., Q.R. Shen, J.M. Wan and Z.P. Liu, 2003. Induced expression of the gene for NADP-malic enzyme in leaves of *Aloe vera* L.under salt stress. *Acta Biomica et Biophysica Sinica*., 35(5): 423-429

Visuthikosol, V., B. Chowchuen, Y. Sukwanarat, S. Sriurairatana and V. Boonpucknavig, 1995. Effect of *Aloe vera* gel to healing of burn wound: a clinical and histologic study. *J Med Assoc Thai.*, 78: 403-409.

Weigel, R.C., J.A. Schillinger, B.A. Mc Caw, H.G. Gauch and E. Hsiao, 1973. Nutrient-nitrate levels and accumulation of chloride in leaves of snapbeans and root of soybeans. *Crop Sci.*, 13: 411-420.

West, D.P. and Y.F. Zhu, 2003. Evaluation of *Aloe vera* gel gloves in the treatment of dry skin associated with occupational exposure. *Am. J. Infect. Control*, 31: 40-42.

Wu, J.M., Z.P. Liu and G.M. Zhao, 2003. Effects of treatment with different concentrations of seawater on chlorophyll content and ultrastructure of Aloe. *Journal of Nanjing Agricultural University*. 26(3): 113-116.

Zheng, Q.S., Z.P. Liu,, Y. L. Liu and L. Liu, 2004. Effects of iso-osmotic salt and water stresses on growth and ionic distribution in *Aloe* seedlings. *Acta Phytoecologica Sinica*, 28(6): 823-827.

Journal of Applied Horticulture, 8(1): 37-41, January-June, 2006

Relationship between soil and leaf mineral nutrient concentration and yield of selected citrus species

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Abstract

Low yields of citrus in Trinidad prompted an investigation to determine whether infield yield variation in citrus was due to differences in plant nutrition induced by field variability. Selected trees of three cultivars (Valencia orange (*Citrus sinensis*), Portugal mandarin (*C. reticulata*) and Ortanique tangor (*C. sinensis* x *reticulata*) were monitored for one to two years and indicators of yield such as percent fruit set, fruit count and fruit quality measured. Leaf nutrient content of the trees and nutritive factors of the soil in the root zone were also determined. Nutrient deficiencies were found in the fields of all the three cultivars. The most common deficiencies were of calcium, zinc and magnesium. There was limited evidence of yield correlation with soil pH ($P = 0.012$), and leaf phosphorus content ($P = 0.02$), Zn ($P = 0.005$) and N ($P = 0.001$). DRIS analysis supported the notion that infield yield variability was associated with nutrients that were limiting. Percent fruit set was associated with Ca/Mg ratio (r = 0.542, P = 0.045; r = 0.607, P = 0.016) and foliar concentration of micro elements Cu (r = 0.738, $P = 0.003$; r = 0.667, $P = 0.007$) and Fe (r = 0.507, $P = 0.064$; r = 0.573, $P = 0.026$) in 1997 for one field each of Valencia orange and Portugal mandarin, respectively. The most commonly derived relationship for fruit quality was a negative relationship of leaf nitrogen concentration with fruit weight. A positive relationship between leaf concentration of manganese and peel thickness occurred in Portugal mandarin for the two years of the study.

Key words: Citrus, Valencia, Ortanique, mandarin, mineral nutrition, yield, fruit set, fruit quality

Introduction

Mean yield for oranges in Trinidad is low (Lucie-Smith, 1953; Cooper, 1956; Ali *et al*., 1973; Andrews *et al*., 2001), less than 1.5 crates per tree (41 kg per crate). It has been observed as well that there is great variation in yielding level between trees in the same field (Andrews, 1994). Increased yield can be accomplished by reduction of tree-to-tree variation and also by increasing the yield of individual trees. It is hypothesized in this study that variation in yield, percent fruit set and fruit quality are mirrored by corresponding variation in nutrient content in leaf or soil.

As in other crops, the routine method of assessing nutritional status and needs have been leaf and soil analysis in citrus (Weir, 1965; Rodriguez *et al.*, 1997). The use of sufficiency ranges and critical values have been most common for foliar analyses (Bar-Akiva *et al.*, 1968; Jorgensen, 1978; Embleton and Labanauskas, 1982; Dey and Singha, 1998). An alternative method of interpreting data from the soil and foliar analyses is the use of mineral nutrient ratios (Weir, 1969). The more recent development of this approach is the use of the Diagnosis and Recommendation Integrated System (DRIS) that allows for determination of relative deficiency or excess (Beverly *et al.*, 1984; Walworth and Sumner, 1987; Rodriguez *et al.*, 1997; Varalakshmi and Bhargava, 1998). DRIS indicates the relative limiting order among the nutrients analyzed and has been applied successfully to many annual and perennial crops (Walworth and Sumner, 1987).

A study was started in 1996 on citrus at the Todds Road estate of Caroni (1975) Limited with a view to determine the major factors influencing yield levels. This report deals with the nutrient levels in soil and leaf and their relationship with yield, fruit set and fruit quality. This information will be useful in developing a strategy for reduction of yield variation and the increase of crop yield.

Materials and methods

Tree selection: The cultivars used were Valencia orange (*Citrus sinensis*), Portugal mandarin (*C. reticulata*) and Ortanique tangor (*C. sinensis* x *reticulata*), the former two selected in two fields each on different soil types (Table 1). Five fields representing three cultivars were used and fifteen trees of similar size were selected from each field for sampling. At the time of selection, the trees represented groups of high, medium and low yielders of the 1996/1997 crop. These trees were located at the Todds Road estate in central Trinidad on Sevilla/L'Ebranche (Dystric gleysol) and Talparo clay (Eutric vertisol) soils, respectively.

Sampling: Soil sampling was done in 1997 in all plots at two depths, 0 to 23 cm and 23 to 46 cm, within the drip circle of the tree. Leaf samples were taken randomly from non-fruiting terminals and were 4 - 7 months old. Leaves from plots were collected in January - February 1997 and additionally for Portugal mandarin in one plot in December 1997 (representative of 1998).

Soil analyses: Analysis was done for pH, K and P only. Samples were extracted with ammonium acetate and read for K with a flame photometer. Phosphorus was extracted with Troug's solution and read at 660 nm using a spectrophotometer.

Foliar analysis: Foliar analysis was conducted on oven dried and ground material by digestion in sulphuric acid for N, P and K. A sample was treated with hydroquinone and ammonium

Table 1. Field characteristics of study plots at Todds Road Estate

Cultivar/Field ID	Soil type	Area (ha)	Number of trees	Year of planting	Topography
Portugal 12036	L'Ebranche clay	2.9	1082	1989	Flat
Portugal 12050	Talparo clay	2.3	954	1989	Slope
Valencia 12069	Sevilla/L'Ebranche	6.1	2325	1988	Flat
Valencia 12071	Talparo clay	4.2	1727	1988	Slope
Ortanique 12083	Sevilla/L'Ebranche	5.5	1862	1988	Flat

molybdate and P read on a spectrophotometer at 660 nm. The sample for N was treated with Nessler's reagent and read with a spectrophotometer at 408 nm. The K sample was diluted with water and read with a Flame Photometer.

Analysis of trace elements was done after dry ashing using the method of Richard (1993). Levels of Ca, Mg, Fe, Zn, and Cu were read using an Atomic Absorption Spectrophotometer. Microelement analyses were done in 1997 and 1998 on leaves from the Portugal mandarin plot at field 12050 and in 1997 only for Valencia orange, field 12069.

Yield and fruit set: Yield level was initially determined subjectively by visual inspection. All levels were associated with yield ranges and were determined by technicians skilled in visual counts and having acceptable levels of consistency (Bekele and Andrews, 1997). In 1998 and 1999, on-tree fruit, counts were used in determining yield levels in Valencia and Ortanique but not Portugal. Percent fruit set was determined by comparing flower counts of labeled branches with fruit number after three months.

Fruit quality: Fruit quality tests were conducted in order to determine mean volume, mean fruit weight, percent juice, brix, percent titratable acidity, number of seeds and peel thickness. Fruit colour was graded on a scale of 1 to 4 based on colour photographs of fruit at different maturity and colour development stages. Sugar:acid ratio was calculated from the preceding data. Fruit volume was determined by water displacement and brix determined using an Atago hand held refractometer. The percentage titratable acidity was done using method B (Wardowski *et al.*, 1991) with acidity expressed as percentage anhydrous citric acid. Juice percentage was calculated as volume over weight of fruit.

Data analysis: The relationship between nutrients and fruit quality was explored for the crop recently harvested or on-tree at the time of leaf and soil sampling. However, the yield and fruit set data were taken for the crop following sampling, as fertilizer application that influenced foliar nutrient levels was applied too late in the year to have affected either fruit set or fruit count of the crop used for fruit quality analyses.

Statistical analysis was done using Minitab Release 13.1. and DRIS analysis was accomplished with the use of the Potash and Phosphate Institute software (Version Beta 1.1) available on the Brazil (POTAFOS) site (http://www.potafos.org/). DRIS makes use of nutrient concentration ratios to interpret tissue analysis in relation to a database of analytical values of high yielding trees.

Results and discussion

Effect of soil and leaf nutrient levels on yield

Valencia: A regression equation was derived for fruit count of Valencia in field 12069 :

Fruit count (1998) = -153 + 7042 % Mg – 1451 % P + 39.9 pH '2' + 6.08 Mn ppm + 168 %Ca – 85.1 Zn ppm – 26.4 P ppm '2'. '2' refers to the 23 – 46 cm soil depth. $P < 0.01$, Adjusted $R^2 = 99.5$

The regression indicates that mineral nutrition was the main cause of yield variation in Valencia field 12069, however, yield variation due to tristeza infection has also been observed in that field (Andrews *et al.*, 2005). Positive yield response to correction of deficient leaf levels of Mg, Ca and Mn have been reported (Chapman, 1968; Embleton *et al.*, 1973) and support the regression formula presented for Valencia. A previous nutrition survey in citrus producing areas in Trinidad revealed widespread symptoms of magnesium, zinc and manganese deficiency (Weir, 1965).

According to sufficiency standards (Embleton *et al.*, 1973; Jorgensen, 1978) zinc, magnesium, calcium and manganese were deficient or considerably low in all three of the yield levels. When yield was considered as high, medium and low (3 levels), analysis of variance showed significant differences for leaf concentrations of zinc (Table 2). An appraisal of leaf nutrient levels of Zn at the three yield levels suggests that reducing levels of this nutrient are associated with increased yield. One possible explanation relates to the practice of applying fertilizer more liberally to high yielding trees. The applied N and K may have aggravated the existing Zn deficiency (Reuther and Smith, 1950). The leaf content of Fe showed a similar trend as Zn (Table 2) and this may have been induced by high K application which can be associated with Fe availability (Chapman, 1968).

Analysis of variance for soil and leaf nutrient concentrations showed no significant differences between yield levels in field 12071; however, soil phosphorus levels were in the range of 4 – 6 ppm and are considered low.

DRIS analysis of foliar nutritional levels for high yielding and low yielding trees in Valencia field 12069 show that in both the high and low yielding trees, zinc had the highest negative index. The second highest negative index was for manganese in high yielding trees, whereas it was magnesium in low yielding trees. The order of limiting nutrients by DRIS analysis was zinc, manganese, magnesium, iron and calcium in the high yield trees. No DRIS analyses were conducted for those fields where leaf data were limited to nitrogen, phosphorus and potassium only.

Table 2. Foliar levels of nitrogen, iron and zinc in February 1997 in relation to subsequent yield levels in Valencia orange field 12069

Yield level-1998 (fruit count)	N (%)	Zn (ppm)	Fe (ppm)
Low (<301)	2.5	10.24	133.4
Medium (301– 450)	2.7	9.47	105.2
High (>450)	2.1	3.80	59.9
Probability	0.09 NS	0.005	0.081 NS

NS – not significant ($P = 0.05$)

Portugal: Soil and leaf nutrient concentrations taken in January 1997 showed no significant difference when compared at the three yielding levels of the 1997/1998 crop of Portugal in field 12036. Field 12050 showed a significant difference (P=0.02) for percent leaf phosphorus only, (Table 3). Soil levels of phosphorus in field 12050 showed no significant difference at the three yield levels but the concentration is considered low. The optimum range of phosphorus in citrus leaves is 0.14 – 0.16% (Jorgensen, 1978) and 0.12 – 0.16% (Embleton *et al.*, 1973). The samples from Portugal field 12050 in 1997 showed means of 0.12, 0.14 and 0.17% (P=0.02) associated with trees giving low, medium and high yields, respectively in the 1997/1998 crop. This suggests that low yielding trees only were low or deficient in phosphorus. Analysis of variance for 1998 leaf samples for field 12050 showed no significant differences for any nutrient at the three yield levels. No regression analysis was done as data were not taken on fruit counts.

DRIS analysis of leaf nutrient data of field 12050 in 1997 indicated that the following nutrients limiting yield, in order of importance, were calcium, zinc, potassium, manganese and iron. When sufficiency ranges were considered (Embleton *et al.*, 1973; Jorgensen, 1978) calcium and zinc were deficient or low at all yielding levels. DRIS analysis of low yielding trees indicated zinc and calcium as the limiting nutrients.

In 1998, the DRIS limiting nutrients, in order of importance, were calcium, zinc, copper and manganese. Application of sufficiency range standards resulted in the same list of limiting nutrients except for manganese for which the concentration was close to critical. The order and magnitude of the DRIS negative indices was not the same for Portugal trees that were high or low yielding, suggesting that yield was lower when certain mineral nutrient limitations were more acute.

Table 3. Soil and leaf concentrations of phosphorus in Portugal trees of field 12050 (January 1997)

Yield level 1998 (fruit count)	P ppm (0 - 23 cm)	P ppm (23 - 46 cm)	P in leaves (%)
Low (<301)	8.8	8.8	0.120
Medium(301– 800)	8.3	9.5	0.140
High (>800)	8.0	10.4	0.177
Probability	NS	NS	0.020

NS – not significant (P=0.05)

Ortanique: Only soil potassium content at the 23 – 46 cm depth was significantly different for the three yield levels (Table 4) but the relationship was not linear. No adequate regression equation was derived. When data for fields 12069 and 12083 were combined because of similar soil type (Table 1) soil pH and leaf nitrogen levels became significant indicators of yield (Table 5). Yield level is inversely related to pH even though the upper limit of mean pH is 6.8 (Table 5). The high pH may have contributed to unavailability of zinc seen in Valencia field 12069 (Table 2). The high nitrogen levels (Table 5) may be reflecting phosphorus deficiency (Bar-Akiva *et al.*, 1968; Rabe and Lovatt, 1986) or calcium deficiency (Weir, 1969) which would explain the associated lower yield level.

Table 4. Mean content of soil nutrients in field 12083 in relation to the following year's yield levels (1997/1998 crop) (February 1997)

Yield level 1998 (fruit count)	K ppm (0 – 23 cm)	K ppm (23 -46 cm)	P ppm (23-46 cm)
Low (<251)	96.4	89.7	18.6
Medium (251 – 300)	146.4	117.6	18.4
High (>300)	160.0	84.0	7.5
Probability	0.072	0.038	0.525

Table 5. Effect of leaf and soil nutrient characteristics on yield level of Valencia and Ortanique grown on Sevilla/L'Ebranche clays

Yield level	pH (0 - 23 cm)	pH (23 - 46 cm)	Foliar nitrogen (%)
Low	6.6	6.8	3.1
Medium	6.5	6.7	3.2
High	6.2	6.3	2.2
Probability	0.062	0.012	0.001

Effect of soil and leaf nutrient levels on percent fruit set: The correlations in Table 6 are more revealing than ANOVA or regression analyses as it is clear that copper and iron and the calcium/magnesium ratio (indicative of calcium nutrition) were positively related to percent fruit set in Valencia and Portugal.

Within the Caroni (1975) Limited environment, leaf nutritional data of January in the years 1997 and 1998 reflect the nutritional status that can have direct effect on flowering and fruit set for the next crop, since no intervening fertilizer application occurred before August (Caroni Research Station, 1999). Only one application of fertilizer was given in those years as supplies were received late. By that time flowering would have occurred and fruit drop stabilized within eight weeks thereafter (Andrews, 1996).

The correlation analyses (Table 6) showed a positive relationship between concentration of copper, iron and calcium/magnesium ratio in the leaves and fruit set in two cultivars. Increased availability of minor element concentration has been associated with increased yield under conditions of deficiency (Chapman, 1968; Alva and Obreza, 1998) but the effect on percent fruit set has not been reported. There is generally a decrease in percent fruit set with increase in flowering (Deidda and Agabbio, 1977; Garcia-Luis *et al.*, 1988). However, Becerra and Guardiola (1984) have determined that, at the same flowering intensity, high yielding trees have higher fruit set percent than low yielding trees.

Ortanique leaf moisture content measured in February 1997 (Table 6) could not have had a direct effect on fruit set some five months later but may have been related to the tree's capacity for carbohydrate production during that period and affect fruit set indirectly.

Effect of soil and leaf nutrient levels on fruit quality

Nitrogen: The effect of nitrogen was common for Portugal and Valencia and the relationship with fruit weight was generally negative (Table 7), as seen in the derived regression equations. Significant nitrogen effect was not observed in 1997 for Portugal, field 12050, but it was present in 1998 for leaf concentration in relation to fruit weight (Table 7). The negative relationship between leaf nitrogen content and fruit size is consistent with the other reported experiences (Embleton *et al.*, 1973).

Phosphorus and potassium: Soil or leaf phosphorus appeared to be important in all three cultivars for various aspects of fruit quality, especially for peel thickness where the relationship was inconsistently positive and negative. The effect of potassium on fruit quality was weak and inconsistent.

Magnesium: Magnesium effect was limited to fruit weight and seed number in Valencia 12069 and to fruit volume and percent acidity in Portugal 12050 in 1998 (Table 7). This positive effect of magnesium content on juice acidity in the Portugal crop (Table 7) confirms to the report by Chapman (1968).

The Mg/Zn relationship with seed number in Valencia 12069 (Table 7) is probably due to the more influence of magnesium than zinc as foliar magnesium deficiency symptoms are more pronounced in seedy cultivars (Chapman, 1968). The removal of foliar magnesium for seed development may be significant even though Valencia is not a seedy cultivar.

Microelements: Effects were observed for the two fields in which foliar analyses for microelements was done (Table 7). Except for the effect of manganese on peel thickness, microelement effects were not consistent by attributes for the different fields and years. The effect of manganese on peel thickness of Portugal mandarin was quite pronounced (Table 7), as the contribution to variability was 50.25% in 1997 and 17.95% in 1998. This relationship has not been previously reported although a positive correlation was found between fruit size (diameter and fresh weight) and flower content of manganese in Valencia orange (Pestana *et al.*, 2001). Some local growers believe that fruit with thick peel are favoured by buyers because of less adherence to the endocarp.

All fields showed some form of nutrient deficiency that would affect yield. There is general evidence to support the idea that yield variation is associated with variation in nutritional factors. The relationship between leaf concentration of manganese and peel thickness in Portugal mandarin suggested by preliminary data should be investigated further, including the attractiveness of peel thickness to the local market.

Table 6. Pearson's correlation of citrus percent fruit set with soil and leaf nutrition variables at Todds Road estate

Cultivar/ Field ID	Fruit set date	Nutrition variable	Correlation coefficient	Probability
Valencia 12069	1997	Fe	0.507	0.064
		Cu	0.738	0.003
		Ca/Mg	0.542	0.045
Portugal 12050	1997	Fe	0.573	0.026
		Cu	0.667	0.007
		Ca/Mg	0.607	0.016
Portugal 12050	1998 [1]	% K	0.580	0.030
Valencia 12071	1997 [1]	pH 0–23 cm (soil)	-0.534	0.074
		pH 23–40 cm (soil)	-0.693	0.013
		K(ppm) 23–40 cm	0.613	0.034
Ortanique 12083	1997	% Water[2]	0.844	<0.001
		K (ppm) 23-40 cm	-0.610	0.016
		P (ppm) 23-40 cm	0.529	0.043

[1]Log transformation of % set data
[2] % Water refers to leaf moisture content

References

Ali, A.G., V. Thomasos, K. Morton-Gittens, W. Augustin-Coryat, K. Beckles and G. Bally, 1973. Report No. 3, Agriculture in County St. George *Land Capability Studies Phase II Trinidad and Tobago*, Ministry of Planning and Development, Trinidad and Tobago.

Alva, A.K. and T.A. Obreza, 1998. By-product iron-humate increases tree growth and fruit production of orange and grapefruit. *Hortscience,* 33(1): 71-74.

Andrews, L. 1994. Germplasm improvement in citrus. *Caroni Research Station Technical Report,* 31(2): 3-9.

Andrews, L. 1996. Fruit retention in citrus. *Caroni Research Station Technical Report,* 33: 175.

Table 7. Effect of soil and leaf nutrient factors on fruit quality of Valencia orange and Portugal mandarin

Cultivar	Field ID	Year	Nutrient factor	Regression equation	Adjusted R^2 (%)	Probability
Valencia orange	12069	1997	N	Fruit weight = 181 + 61.7 %N – 455 %Mg – 5.73 P ppm '2'[a]	62.3	0.005
Valencia orange	12071	1997	N	Fruit weight = 155 + 27.6 pH – 31.7 %N	50.7	0.006
Portugal mandarin	12036	1998	N	Fruit weight = 183 – 57 %N + 796 %P	37.7	0.023
Portugal mandarin	12050	1998	N	Fruit weight = 136 – 11.9 %N + 31.2 %K – 0.143 Fe/Zn	74.7	0.001
Valencia orange	12069	1998	Mg	Fruit weight = 181 + 61.7 %N – 455 %Mg – 5.73 P ppm '2'[a]	62.3	0.005
Valencia orange	12069	1998	Mg	Number of seeds = 4.58 + 0.00665 Mg/Zn	76.3	<0.001
Portugal mandarin	12050	1998	K/Mg	Fruit volume = 102 – 2.67 K/Mg + 29.7 %K	37.5	0.024
Portugal mandarin	12050	1998	Mg	Acidity (%) = 1.03 + 0.421 %Mg – 0.00222 Mn ppm	54.2	0.004
Portugal mandarin	12050	1998	Mn	Peel thickness = 0.95 +0.00359 P ppm + 0.00192 Mn ppm – 0.0589 %N – 0.00591 K ppm	85.5	<0.001
Portugal mandarin	12050	1998	Mn	Peel thickness = 0.186 + 0.00244 Mn ppm	23.3	0.039

[a] '2' refers to the 23 – 46 cm soil depth

Andrews, L., R. Phelps and R.A.I. Brathwaite, 2005. Relationship of citrus yield and virus infection in Trinidad. *Journal of Applied Horticulture,* 6(2): 112-115.

Andrews, L., G. White and P. Jordan, 2001. The use of field attributes database for analysis of citrus yields at Todds Road. NIHERST, 11th Annual Conference on Agricultural Research and Development: 11-22.

Bar-Akiva, A., V. Hiller and J. Patt, 1968. Effects of phosphorus and chicken manure on yield, fruit quality and leaf composition of grapefruit trees. *Proceedings of the American Society for Horticultural Science,* 93: 145-152.

Becerra, S. and J.L. Guardiola, 1984. Inter-relationship between flowering and fruiting in sweet orange, cultivar Navelina. *Proceedings of the International Society of Citriculture,* 1: 190-193.

Bekele, I. and L. Andrews, 1997. Investigation into the accuracy of visual on-tree fruit counts for estimating citrus production. *National Institute of Higher Education Research Science and Technology. 9th Seminar on Agricultural Research and Development*. Port of Spain, NIHERST. p 3-9.

Beverly, R.B., J.C. Stark, J.C. Ojala and T.W. Embleton, 1984. Nutrient diagnosis of 'Valencia' oranges by DRIS. *Journal of the American Society of Horticultural Science,* 109(5): 649-654.

Caroni Research Station, 1999. Citrus - Research and Production Review and Planning Workshop. Caroni (1975) Limited.

Chapman, H.D. 1968. The mineral nutrition of citrus. *The Citrus Industry.* Volume 2. Eds. W. Reuther, L.D. Batchelor, and H.J. Webber. Division of Agricultural Sciences, University of California, pp. 127-274.

Cooper, St. G. 1956. Citrus production in the Santa Cruz and Diego Martin valleys in Trinidad. *Journal of the Agricultural Society of Trinidad and Tobago,* 56: 612-628.

Deidda, P. and M. Agabbio, 1977. Some factors influencing flowering and fruit-set of 'Clementine' mandarin. *Proceedings of the International Society of Citriculture,* 2: 686-692.

Dey, J.K. and D.D. Singha, 1998. Nutritional status of healthy and declining citrus (*Citrus reticulata*) orchards. *Indian Journal of Agricultural Sciences,* 68(2): 139-143.

Embleton, T.W. and C.K. Labanauskas, 1982. Plant analysis as a basis for the mineral fertilization of citrus orchards. *Proceedings of 21st International Horticultural Congress,* 1: 369-383.

Embleton, T.W., W.W. Jones, C.K. Labanauskas and W. Reuther, 1973. Leaf analysis as a diagnostic tool and guide to fertilization.In: *The Citrus Industry*, Volume 3. (W. Reuther, Ed.). University of California, Division of Agricultural Sciences. pp. 183-205.

Garcia-Luis, A., F. Fornes, A. Sanz and J.L. Guardiola, 1988. The regulation of flowering and fruit set in citrus: relationship with carbohydrate levels. *Israel Journal of Botany,* 37: 189-201.

http://www.potafos.org/ppiweb/brazil.nsf

Jorgensen, K.R. 1978. Leaf and soil analysis a guide to fertilizing citrus. *Queensland Agricultural Journal,* 104: 17-21.

Lucie-Smith, M.N. 1953. *Citrus production in Trinidad.* Port of Spain, Trinidad, Port of Spain Gazette Limited.

Pestana, M., P.J. Corriera, A. de Varennes, J. Abada and E.A. Faria, 2001. The use of floral analysis to diagnose the nutritional status of orange trees. *Journal Plant Nutrition,* 24(12): 1913-1923.

Rabe, E. and C.J. Lovatt, 1986. Relative phosphorus dependency of citrus rootstocks is reflected in leaf nitrogen metabolism. *Journal American Society Horticultural Science,* 111(6): 922-926.

Reuther, W. and P.F. Smith, 1950. A preliminary report on the relationship of nitrogen, potassium and manganese fertilization to yield, leaf composition and the incidence of zinc deficiency in oranges. *Proceedings American Society Horticultural Science,* 56: 27-33.

Richard, J.E. 1993. Chemical characterization of plant tissue. In: *Soil sampling and methods of analysis.* (M. Carter, Ed.). Boca Raton, Lewis Publishers pp. 115-121.

Rodriguez, O., E. Rojas, and M. Sumner, 1997. Valencia orange DRIS norms for Venezuela. *Communications in Soil Science and Plant Analysis* 28 (15/16): 1461-1468.

Varalakshmi, L.R. and B.S. Bhargava, 1998. Plant nutrient status and leaf diagnostic norms for acid lime (*Citrus aurantifolia*). *Indian Journal Agricultural Sciences,* 68(7): 375-376.

Walworth, J.L. and M.E. Sumner, 1987. The Diagnosis and Recommendation Integrated System (DRIS). *Advances in Soil Science,* 6: 150-185.

Wardowski, W., J. Soule, J. Whigham and W. Greirson, 1991. *Florida citrus quality tests.* SP 99. Gainesville, University of Florida.

Weir, C.C. 1965. A survey of the mineral nutrition of valencia orange in Trinidad. *Experimental Agriculture,* 1: 179-184.

Weir, C.C. 1969. Nutrient element balance in citrus nutrition. *Plant and Soil,* 3: 405-414.

Journal of Applied Horticulture, 8(1): 42-44, January-June, 2006

Studies with thidiazuron on the vase life of cut rose flowers

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Abstract

Effects of postharvest-applied thidiazuron (TDZ) on the vases life of 7 rose cultivars (*Rosa hybrida* L.) were investigated. Cut rose flowers were pulse-treated with TDZ for 24 hours at 22 °C. Application of 20, 60 and 100 µM TDZ to 'Memoire' rose did not affect vase life when compared with the control (0 µM TDZ). Similarly, pulse treatments with 10 µM TDZ did not affect the vase life of cvs. 'Champagne', 'Laser', 'Magnum', 'Neon' and 'Tresor 2000' roses compared with their untreated controls, but did increase the vase life of 'First Red' by 2 days (+11.5 %). Lateral shoot development was a common side effect of TDZ treatment.

Key words: Postharvest, thidiazuron, vase life, *Rosa hybrida*, lateral shoot, pulse treatment, cut flower, longevity

Introduction

Rosa hybrida is among the most commercially important flowers, with an annual value of $10 billion (Guterman *et al.*, 2002). Roses are used as cut flowers, flowering pot plants and garden plants. The vase life of cut rose flowers is relatively short (Huang *et al.,* 2002; Marisen and Benninga, 2001), and thus, postharvest technologies for improving the keeping quality of roses are sought and applied.

Increase in the postharvest life of cut flowers following cytokinin treatments has been reported (Hicklenton, 1991; Lukaszewska *et al.*, 1994; Paull and Chantrachit, 2001). Bosse and Van Staden (1989) reported that DHZ (dihydrozeatin), used as a pulse treatment for 6-24 hours at a concentration of 2×10^{-4} M, significantly delayed carnation flower senescence. An increase in the longevity of carnation flowers by 15 days was found at 4×10^{-6} M DHZ. Lukaszewska *et al.* (1994) showed that exogenous application of trans-zeatin, trans-zeatin riboside, 2iP (isopentenyladenine) and 2iPA (isopentenyladenosine) in holding solutions delayed rose senescence by 1.3-1.6-fold. Their data also showed that zeatin and zeatin riboside were most effective on roses at 1×10^{-7} M and prolonged longevity by 1.3-fold.

Thidiazuron (N-phenyl-N-1, 2, 3-thidiazol-5-ylurea) is a urea-type cytokinin that is a relatively novel compound for treating cut flowers. It is more commonly used at high concentration as a cotton defoliant (Malik *et al.*, 2002) and at low concentration for regeneration in tissue culture (Singh and Syamal, 2001). TDZ is around 50-100 times more active in inducing cytokinin-like effects than common cytokinins (Genkov and Iordanka, 1995). Treatments with TDZ delayed leaf yellowing in cut Alstroemeria (Ferrante *et al.,* 2002), and was most effective at 10 µM as a pulse treatment or at 1 µM as a continuous (vase) treatment. TDZ treatments of less than 50 µM also reduced flower shedding and induced additional flower buds during vase life of cut phlox inflorescences (Sankhla *et al.,* 2003). TDZ treatment of cut chrysanthemum and tulip inhibited leaf yellowing but did not enhance flower quality (Ferrante *et al.*, 2003).

The present experimentation was conducted to determine the effects of TDZ on the vase lifes of seven cut rose cultivars.

Materials and methods

Rose flower stems were harvested from greenhouses at the tight bud stage and trimmed to 40 cm length and 3 leaves. Postharvest experiments were carried out under vase life evaluation room conditions of 22 ± 1°C, 60-70 % relative humidity and 12 h photoperiod with 15µmol $m^{-2}s^{-1}$ irradiance from cool white florescent lamps. The 'Memoire' rose cultivar for experiment 1 was harvested in December 2003 from near Tehran, Iran. For experiment 2, 'Champagne', 'Neon', 'Tresor 2000', 'Laser' and 'Magnum' cultivars were harvested in February 2004 from near Brisbane, Australia. 'First Red' roses for experiment 3 (May 2004) were harvested from a different grower near Brisbane.

Vase life was recorded as the time in days after harvest (day 0) that flowers reached the end of their longevity due to bent neck or advanced signs of fading on all petals (Mayak and Halevy, 1974; Liao *et al.,* 2000).

Experiment 1: TDZ (Sigma Chemical Co.) was dissolved in 2 mL aliquots of 1M KOH, made up with distilled water to concentrations of 0, 20, 60, and 100 µM and neutralized with 2 mL 1M HCL (Ferrante *et al.,* 2002). Cut 'Memoire' flowers (white) were then pulse-treated for 24 h at 20°C. Distilled water was the control pulse treatment. The post-pulse treatment vase solution was 200 mg L^{-1} HQS (hydroxyquinoline sulphate) in distilled water (Liao *et al.,* 2000). Flower vase life was assessed daily and recorded for each of 7 single bloom replicates per treatment. A completely randomised design was adopted. Data were analyzed by one way ANOVA with Minitab Release 13.1 for Windows (Minitab Inc.).

Experiment 2: 'Champagne' (white), 'Neon' (deep pink), 'Laser' (pink), 'Tresor 2000' (yellow) and 'Magnum' (red) were pulsed with 10 µM TDZ for 24 h at 22°C. Distilled water was the control pulse treatment. The post-pulse treatment vase solution was distilled water containing 10 µL L^{-1} available chlorine supplied

Fig. 1. Lateral shoot development (arrowed) during vase life in cut 'Memoire' (left photograph) and 'First Red' (right photograph) roses pulse-treated with 10 µL L^{-1} TDZ.

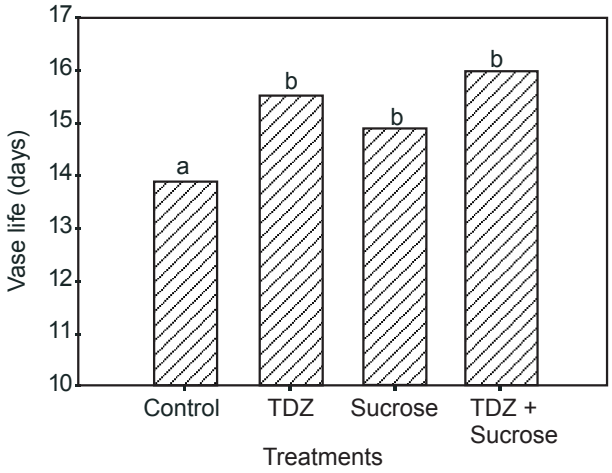

Fig. 2. Effect of TDZ (10 µM), sucrose (4% w/v), and TDZ plus sucrose in combination (all applied as pulse-treatments) on the vase life of 'First Red' rose. Bars topped with the same letter are not significantly different (P<0.05).

as dichloroisocyanuric acid (DICA) (Joyce *et al.,* 2000) Vase life was assessed daily. Each replicate was 5 individual stems of each cultivar for control and TDZ treatments. A completely randomised design was adopted, and t-tests (Minitab Release 13.1) were used for data analysis for each cultivar.

Experiment 3: 'First Red' roses were treated with 10 µM TDZ, 4% w/v sucrose and both in combination as pulses for 24 h at 22°C. Distilled water was the control pulse treatment. Vase life was assessed daily. Ten replicated stems were used for each treatment. A completely randomised design was adopted, and data were analysed by ANOVA using SAS procedures (SAS Institute 1998). The least significant difference (P = 0.05) was calculated to allow comparisons among treatment means.

Results and discussion

Pulse-treatment with TDZ at concentrations of 0, 20, 60 and 100 µM did not differentially affect the vase life of cut 'Memoire' rose. The mean (±SD) vaselife was 14.6±0.25 days. A side-effect of TDZ treatments was enhanced lateral shoot development (Fig. 1). Pulse-treatment with TDZ at 10 µM did not affect the vase lives of 'Champagne' (18 ± 1.4 days), 'Laser' (16 ± 1.3 days), 'Magnum' (19 ± 0.7 days), 'Neon' (18 ± 0.3 days) and 'Tresor 2000' (16 ± 0.3 days). As observed with cv. 'Memoire', lateral shoot development on TDZ-treated stems was observed for these 5 cultivars.

Pulse-treatment of 'First Red' roses with 10 µM TDZ did increase the vase life by 1.5 days (Fig. 1), and also encouraged lateral shoot development as in 'Memoire' (Fig. 1). Pulse-treatment with 4% (w/v) sucrose also increased flower vase life by 1 day. TDZ pulsing in combination with sugar pulsing had a synergistic effect on vase life (Fig. 2).

Paull and Chantrachit (2001) reported that different cultivars of anthurium had different vase life responses to benzyladenine treatment. The responses ranged from a 20% reduction to a 2.5-fold increase in vase life. A lack of response to benzyladenine may have been due to high natural cytokinin levels. Perhaps in cultivars with naturally low levels of cytokinin, TDZ treatment might increase vase life. Further experimentation is required to examine this proposition for cut roses.

In summary, TDZ pulse-treatments had no effect on vase life of several cut roses, but did increase the vase life of cv. 'First Red', one of the more commercially important cut rose cultivars. As a consistent side effect, TDZ treatments promoted shoot development from lateral vegetative stem buds.

Acknowledgments

We thank Alan Lisle for advice and assistance in analysing the data. We also thank Mr Brian Shannon for donating flowers used in one experiment.

References

Bosse, C.A. and J. Van Staden, 1989. Cytokinins in cut carnation flowers. V. Effects of cytokinin type, concentration and model of application on flower longevity. *Journal Plant Physiology*, 135**:** 155-159.

Ferrante, A., D.A. Hunter, P.H. Wesley and M.S. Reid, 2002. Thidiazuron – a potent inhibitor of leaf senescence in Alstroemeria. *Postharvest Biology and Technology*, 25: 333-338.

Ferrante, A., F. Tognoni, A. Mensuali-Sodi and G. Serra, 2003. Treatment with thidiazuron for preventing leaf yellowing in cut tulips and chrysanthemum. *Acta Hoticulturae,* 624: 357-363.

Genkov, T. and I. Iordanka, 1995. Effect of cytokinin-active phenylurea derivatives on shoot multiplication peroxidase and superoxide dismutase activities of *in vitro* cultured carnation. *Bulgarian Journal of Plant Physiology*, 21: 73–83.

Guterman, I., M. Shalit, N. Menda, D. Piestun, M. Dafny-Yelin, G. Shalev, E. Bar, O. Davydov, M. Ovadis, M. Emanuel, J. Wang, Z. Adam, E. Pichersky, E. Lewinsohn, D. Zamir, A. Vainstein and D. Weiss, 2002. Rose scent: genomics approach to discovering novel floral fragrance–related genes. *Plant Cell***,** 14: 2325–2338.

Hicklenton, P.R. 1991. GA_3 and benzylaminopurine delay leaf yellowing in cut Alstroemeria stems. *HortScience*, 26(9): 1198-1199.

Huang, K.L., L.J. Liao, R.S. Shen, W.S. Chen and Y.H. Lin, 2002. The synergistic effect of maleic acid hydrazide (1,2-dihydro-3,6-pyridazinedione) and sucrose on vase life of cut roses. *Australian Journal Experimental Agriculture*, 42: 637-641.

Joyce, D.C., S.A. Meara, S.E. Hetherington and P. Jones, 2000. Effects of cold storage on cut Grevillea 'Sylvia' inflorescences. *Postharvest Biology and Technology*, 18: 49-56.

Liao, L.J., L.J. Liao, K.L. Huang, W.S. Chen and Y.M. Cheng, 2000. Postharvest life of cut rose flowers as affected by silver thiosulfate and sucrose. *Botanical Bulletin Academica Sinica*, 41: 299-303.

Lukaszewska, A.J., J. Binaco, P. Barthe and M.T. Le Page-Degirry, 1994. Endogenous cytokinins in rose petal and the effect of exogenously applied cytokinins on flower senescence. *Plant Growth Regulation*, 14: 119-126.

Mayak, S. and A.H. Halvey, 1974. The action of kinetin in improving the water balance and delaying senescence process of cut rose flowers. *Physiologia Plantarum*, 32: 330-336.

Malik, M.N.A., U.D. Shabab, M.I. Makhdom and F.I. Chaudhry, 2002. Use of thidiazuron as harvest aid in early and late planted cotton. *International Journal Agriculture and Biology*, 4: 71-73.

Marissen, N. and J. Benninga, 2001. A nursery comparision on the vase life of the rose "First Red": Effects of growth substances. *Acta Horticulturae*, 543: 285-291.

Paull, R.E. and T. Chantrachit, 2001. Benzyladenine and the vase life of tropical ornamentals. *Postharvest Biology and Technology*, 21: 303-310.

Sankhla, N., W.A. Mackay and T.D. Davis, 2003. Reduction of flower abscission and leaf senescence in cut phlox inflorescences by thidiazuron. *Acta Hoticulturae*, 628: 837-841.

Singh, S.K. and M.M. Syamal, 2001. A short pre culture soak in thidiazuron or forchlorfenuron improves axillary shoot proliferation in rose micropropagation. *Scientia Hoticulturae*, 91: 169-177.

Journal of Applied Horticulture, 8(1): 45-49, January-June, 2006

Growth behaviour of apple cactus (*Cereus* species) in hyper-arid environment

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Abstract

Introducing new crop with high water use efficiency into the hyper-arid environment will participate in curb rising demand of water. Apple cactus (*Cereus* species) characteristics fit with most of the requirements of a drought tolerant crop with very high water-use efficiency. Several *Cereus* species were introduced into a desert, characterized with rare rainfall and high temperatures. The introduced fruiting species were *Cereus hexagonus*, *C. peruvianus*, *C. peruvianus monstrose* and *C. validus*. *C. pachanoi* was introduced as a rootstock. *C. peruvianus* cuttings survived storage up to eight months. Horizontal position of the cuttings during storage encouraged the development of lateral branches. Plants were propagated by cuttings, acclimatized and then transplanted into the field in the desert. Growth and development of the introduced species were assessed under the new environment. All the introduced species grew successfully except *C. validus* that was eliminated during the first summer. *C. peruvianus monstrose* was characterized with dramatic contraction of the stem in the dry condition. The main stem of *C. peruvianus*, *C. peruvianus monstrose*, *C. hexagonus* and *C. pachanoi* grew 9.2, 10.2, 8.1 and 15 cm/month, respectively. *C. peruvianus* developed the highest number of sprouts. *C. Peruvianus*, *C. peruvianus monstrose* and *C. hexagonus* unite with the *C. pachanoi* to form successful grafts with percentage of success 80, 53 and 86.5, respectively. *C. validus* failed completely to unite with *C. pachanoi*. *C. peruvianus* and *C. peruvianus monstrose* were the most promising in the new hyper-arid environment in terms of adaptability and healthy growth.

Key words: Apple cactus, *Cereus*, drought resistance, hyper-arid environment, water use efficiency.

Introduction

As a natural hazard, drought imposes differential vulnerability on society in the hyper-arid region (Wilhite, 2000). Introducing new crops with high water use efficiency in the hyper-arid environment will participate in curb rising demand of water (ElObeidy, 2004). Apple cactus (*Cereus* sp.) characteristics fit with most of the requirements of a drought tolerant crop with very high water-use efficiency. Apple cactus has physiological and morphological methods of exploiting environments that would soon desiccate other plant species (Nerd *et al.*, 1988; Wallace and Gibson 2002). In addition, apple cactus is characterized with crassulacean acid metabolism pathway that improve water-use efficiency (Malda *et al.*, 1999; Cushman and Borland, 2002).

Apple cactus, a popular group of cacti from South America ,consists of about 60 species. These vigorous-growing cacti are easy to grow in marginal, infertile, dry lands where common crops fail (Felger and Moser, 1976; Wallace and Gibson 2002). Apple cactus produces unique fruits that are thornless and vary in skin colour from violet-red to yellow. The flesh, which is the edible part of the fruit, is white and contains small, edible, and crunchy seeds (Felger and Moser, 1976; Nerd *et al*., 2002). Apple cactus fruits may be eaten fresh, dried, or can be made into a juice (Scheinvar, 1985).

Apple cactus fruits can offer commercial opportunities (Felger and Moser, 1976). However, at present, it is an unexplored, underutilized fruit (Morton, 1987; Inglese *et al.*, 2002).

The aim of this study was to investigate vegetative growth, development, storing ability of cuttings and grafting success of several apple cactus species introduced in a hyper-arid desert.

Materials and methods

Plant material: Cuttings from four *Cereus* species were obtained from private nurseries of California, USA. The introduced species were *C. hexagonus*, *C. pachanoi*, *C. peruvianus* and *C. validus*. The subspecies *C. peruvianus monstrose* was also introduced. Forty cuttings with 30 cm length from each species were used for propagation. Forty more cuttings of *C. peruvianus* and C. *peruvianus monstrose* were used in the storage experiment.

Cutting healing and storage: Thirty scions of each of *C. hexagonus*, *C. pachanoi*, *C. peruvianus*, *C. peruvianus monstrose*, *C. validus* and *C. pachanoi* were grafted on *C. pachanoi*. Cuttings were allowed to heal in a dry area at 30°C for ten days. Other cuttings of *C. peruvianus* and *C. peruvianus monstrose* were stored at 35°C up to eight months.

Propagation by cuttings: Pots (25 cm in diameter) were filled to about one-third full of potting mix consists of 1 part peat moss: 2 parts sand. Each cutting was planted in the center of a pot. Pots were completed to two-third full with the potting mix. Pots were watered and kept in the greenhouse at 27°C.

Propagation by grafting: Plants of *C. hexagonus*, *C. peruvianus*, *C. peruvianus monstrose*, *C. validus* and *C. pachanoi*, grown in 25 cm diameter pots, were used as scions. *C. pachanoi* plants grown in 25 cm diameter pots were used as rootstocks. The diameter of the stems of *C. hexagonus*, *C. pachanoi*, *C. peruvianus*, *C. peruvianus monstrose* and *C. pachanoi* plants was 9-12 cm. However the diameter of the stems of *C. validus* plants was 5 cm. With a sharp sterile knife, the top of the rootstocks was cut off 20 cm above the soil surface. The bottom of the scion plants was cut off 15 cm from the top. The scions and rootstocks

were joined together by lining up the two cylindrical parts and gently rotated to squeeze out any air bubbles. Two rubber bands of appropriate size were affixed over the scion and under the bottom of the pot. Rubbers could exert a steady pressure on the scion, pressing it against the stock to support the graft while the cut edges heal. The two rubber bands were placed over the scion at 90 degrees from each other, in order to prevent the scion from shifting. The grafts were supported with stakes, then kept in the greenhouse. The rubber bands were removed after two months of grafting.

Orchard location: The orchard was located in Al-Ain desert, United Arab Emirates (UAE), characterized with high temperatures and rare rainfall. Maximum temperature reaches 49°C during the summer and average annual rainfall is 77mm. Soil and irrigation water samples from the orchard were taken to determine salinity.

Transplanting: In the fall (15th September), plants propagated by cuttings were taken from the greenhouse to the shade for ten days acclimatization. The plants were then transplanted into the orchard. Hole was dug about 15 cm wider than the container and a couple inches deeper. The plant was slipped carefully out of its container. Heavy gloves and 40 cm forceps were used to avoid injuring the technicians or plants. The plant was placed into the hole and soil was firmed lightly around.

Irrigation and fertilization: The source of irrigation is the underground aquifer. Drip irrigation system was used in the orchard. Two liters of water was added per plant during two hours every two days. Fertilizer 12:4:24 (150g per plant) was applied one month after transplanting into orchard, then every two months during the growing season.

Data collection and analysis: Data were collected in two successive seasons (2001/2002 and 2002/2003) on plant survival, stem length and diameter and sprout or lateral branch development during the growing season (October – May). Other observations on growth behaviour were recorded. In storage experiment, each cutting was weighed at the beginning and every month during the storage period. In grafting experiment the healing and success of grafts was observed.

Completely randomised design was used for statistical analysis and mean comparisons were made using Duncan's Multiple Range test at 5% significant level (Duncan, 1955).

Results and discussion

Four apple cactus species were introduced into hyper-arid desert in the UAE. Orchard soil found to be sandy with 2200 ppm salinity. Salt concentration in the irrigation water ranged from 1800 to 2000 ppm.

Cuttings of the introduced *Cereus* species were allowed to dry in a warm, dry area for ten days to permit the cut surface to heal and develop callus. The callus helps prevent rotting during propagation (Hartmann *et al.*, 2001).

The introduced species grew in the hyper-arid desert successfully except *C. validus* that was eliminated during the first summer. All the plants of *C. peruvianus* and *C. peruvianus monstrose* survived in the new environment.

The main stem of *C. peruvianus* showed 9.2 cm increase in height (Fig. 1) and 0.9 cm increase in diameter per month (Fig. 2). Five sprouts developed per plant (Figs. 3 and 4). *C. peruvianus*, found in southeastern coast of South America, has already attracted attention as a potential fruit-crop (Backeberg, 1984). The fruit is smooth and spineless and varies from yellow to deep red (Weiss *et al.*, 1993). *C. peruvianus* was found to be very promising fruiting cactus (Morton, 1987; Inglese *et al.*, 2002).

C. peruvianus cuttings survived storage up to eight months. Cuttings lost about 50% of the original weight at the end of the storage period (Fig. 5). Horizontal position of the cuttings during storage encouraged the development of lateral branches (Fig. 6). Lateral branching averaged at four branches per cutting.

C. peruvianus monstrose cuttings did not survive more than three months of storage (Fig. 5). In the field, the main stem of *C. peruvianus monstrose* increased 10.2 cm in length and 1.1 cm in diameter per month (Figs. 1 and 2) and four sprouts developed per plant. *C. peruvianus monstrose* exhibited dramatic expansion and contraction of the stem as water availability changed (Fig. 7). Such mechanism enables the stem to conserve water and survive in dry, hot conditions (Wallace and Gibson, 2002).

Over millions of years, through natural selection, only the most adapted species survived in desert environment (John, 2001).

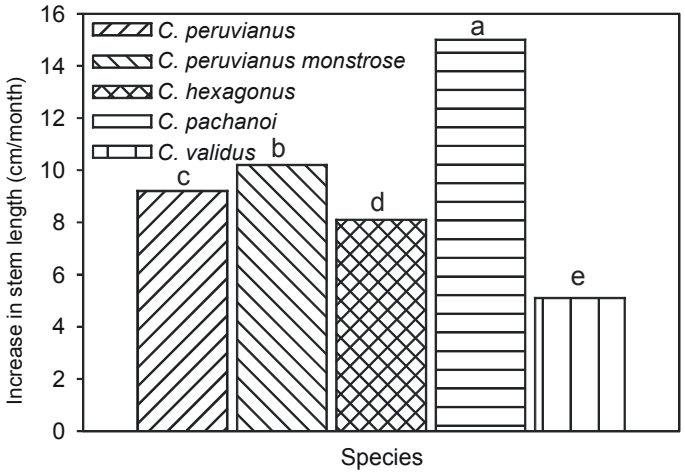

Fig. 1. Rate of the increase in main stem length of the introduced *Cereus* species. Columns labeled with the same letter are not significantly different (P>0.05).

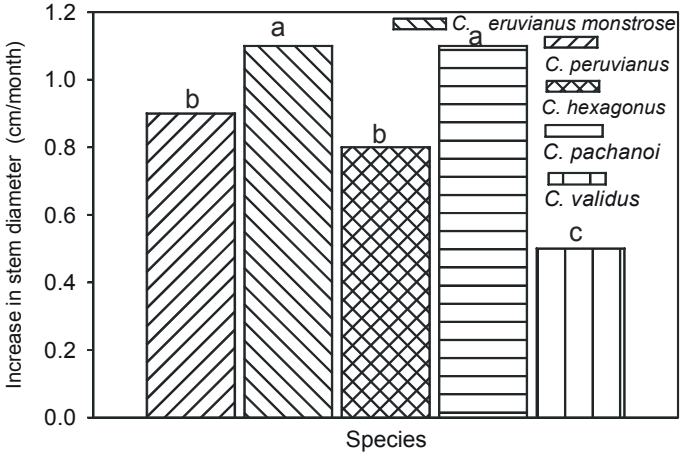

Fig. 2. Rate of the increase in main stem diameter of the introduced *Cereus* species. Columns labeled with the same letter are not significantly different (P>0.05).

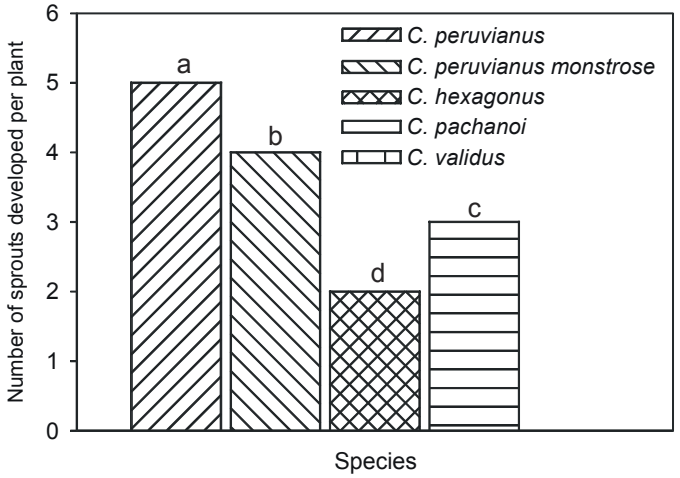

Fig. 3. Average number of the developed sprouts per plant during the first two seasons. Columns labeled with the same letter are not significantly different (P>0.05).

Generally, the family of Cactaceae, contains many plants that are highly adaptable to a new environment and able to tolerate drought, heat and saline soil. Apple cactus, as a member in the family Cactaceae has devolved to be well adapted to extremely xeric conditions (Mauseth, 2000; John, 2001). The pathway of photosynthesis in *Cereus* is the crassulacean acid metabolism in which stomata open at night (when evaporation rates are usually lower) and are usually closed during the day. The CO_2 is converted to an acid and stored during the night. During the day, the acid is broken down and the CO_2 is released to RUBISCO for photosynthesis. When conditions are extremely arid, *Cereus* plants can just leave their stomata closed night and day. Oxygen given off in photosynthesis is used for respiration and CO_2 given off in respiration is used for photosynthesis (Winter and Smith, 1996). Importance of crassulacean acid metabolism species increases in the face of expansion of desertification around the world (Cushman and Borland, 2002).

C. hexagonus is another introduced *Cereus* species with 73% of survival in the new arid environment. The main stem growth rate of *C. hexagonus* was 8.1 and 0.8 cm/month in height and diameter, respectively (Figs. 1 and 2). Each *C. hexagonus* plant developed two sprouts during the first two seasons (Fig. 3). *C. hexagonus* is known to give ovoid red fruits with soft and juicy, white to pinkish pulp and numerous edible small black seeds. *C. hexagonus* was originated in Brazil (Anderson, 2001).

C. validus grew 5.1 cm in height and 0.5 cm in diameter per month during the first season (Figs. 1 and 2). However, it could not survive the high temperature during the summer months. All plants were dried and finally died during the first season.

All *C. pachanoi* plants survived the new environment. *C. pachanoi* grew very fast, averaging up to a fifteen centimeter a month of new growth (Fig. 1). Its stem diameter increased 1.1 cm per month (Fig. 2). Three sprouts developed per plant during the first two seasons (Fig. 3). *C. pachanoi* was introduced as a rootstock for grafting other species. *C. pachanoi* is popular as grafting stock for smaller, slower growing cacti (Ostolaza, 1984).

C. peruvianus, *C. peruvianus monstrose* and *Cereus hexagonus* united with *C. pachanoi* to form grafts with the success of 80, 53 and 86.5%, respectively. *C. Validus,* however, failed completely to unite with *C. Pachanoi* (Fig. 8). This failure to form grafts may

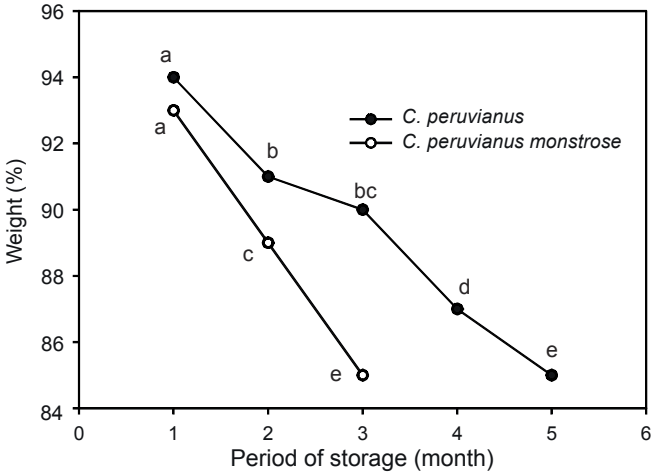

Fig. 5. Effect of the storage period on weight of *C. peruvianus* and *C. peruvianus monstrose* cuttings. Values labeled with the same letter are not significantly different (P>0.05).

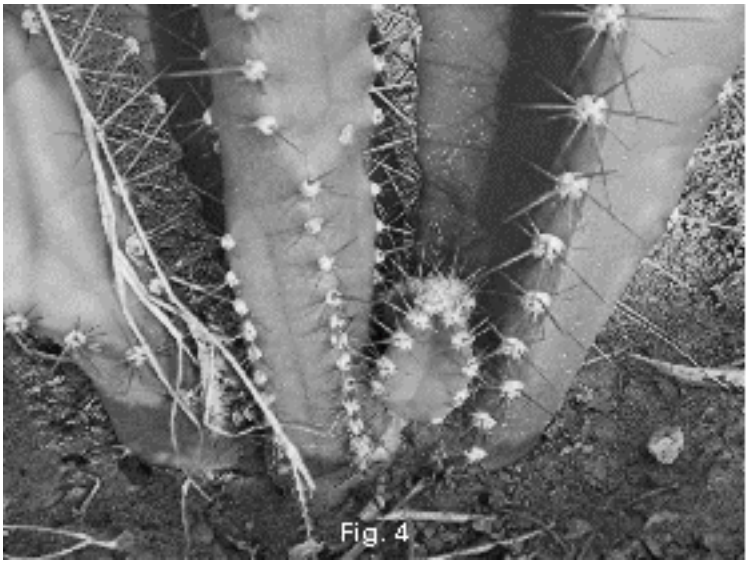

Fig. 4. Sprouts were ramified from the base of the main stem of *C. peruvianus*.

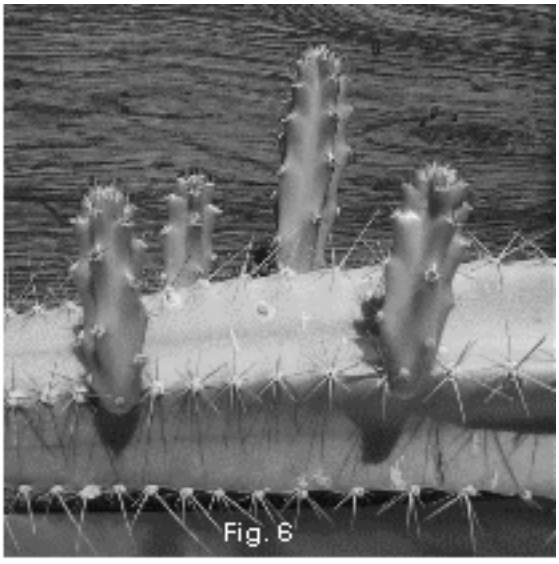

Fig. 6. Horizontal position of *C. peruvianus* cuttings during storage encouraged the development of lateral branches.

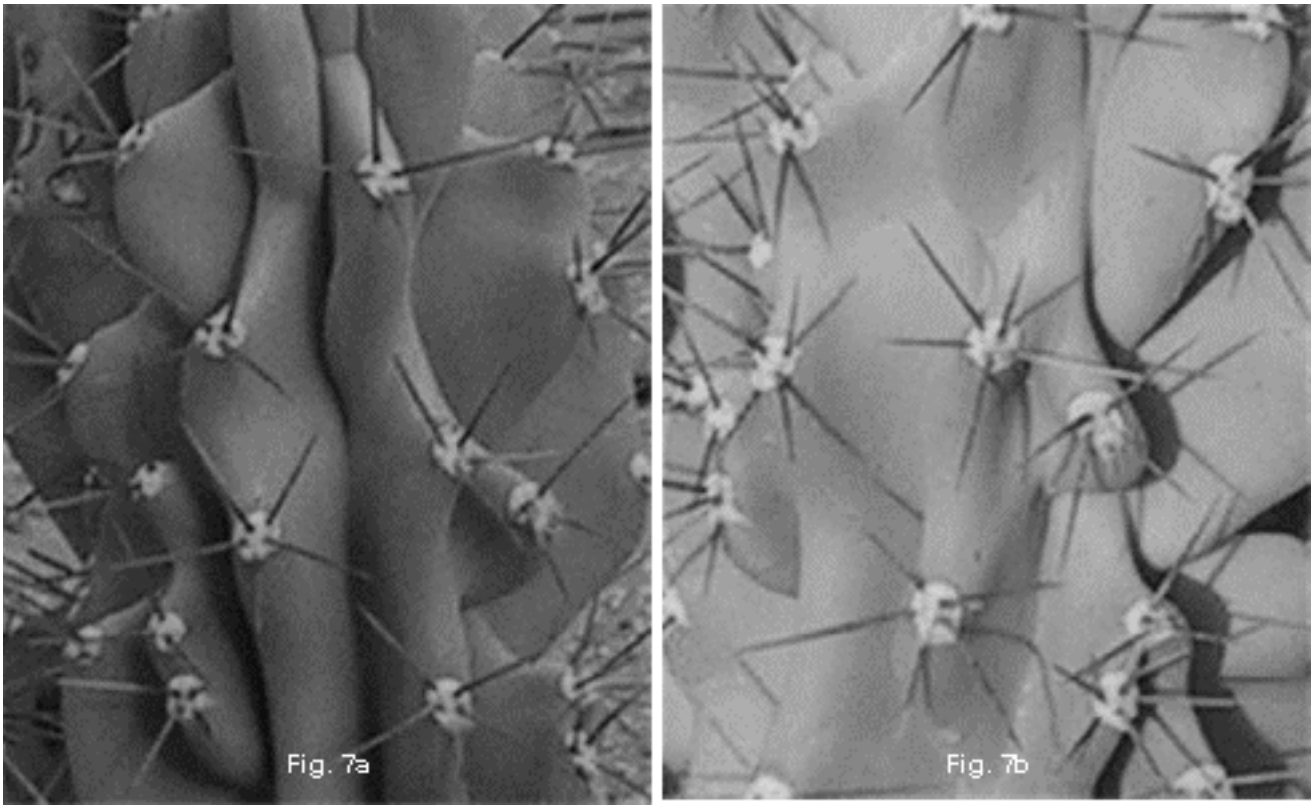

Fig. 7. Dramatic contraction (A) and expansion (B) of *C. peruvianus* monstrose stem as water availability changes.

be due to the incompatibility or the difference in the diameter between the scion and the rootstock. Incompatibility between the scion and the rootstock affects the healing, and could ruin the graft. The difference in diameter between the scion and the rootstock prevent meeting the cambial layers of the scion and stock resulting failure of healing. The risk is smaller if the scion and the rootstock are about the same diameter (Hartmann *et al.*, 2001). However, self grafted *C. Pachanoi* healed with 93% of successful grafts.

Grafting in cactus is really best used as a technique for saving a dying cactus from certain death, speeding up breeding programs or growing less hardy species. Often in cactus genetic improvement, hybridization, and clonal selection programs, the researchers need a more rapid method to prove or disprove the merits of a fruiting clone. To speed the evaluation process for fruit production and to adapt certain clones to local conditions, grafting was done onto mature plants in the ground (Huffman, 2003). A grafted cactus can grow faster, flower sooner and often be used with cactus species having sensitive soil requirements (Hartmann *et al.*, 2001).

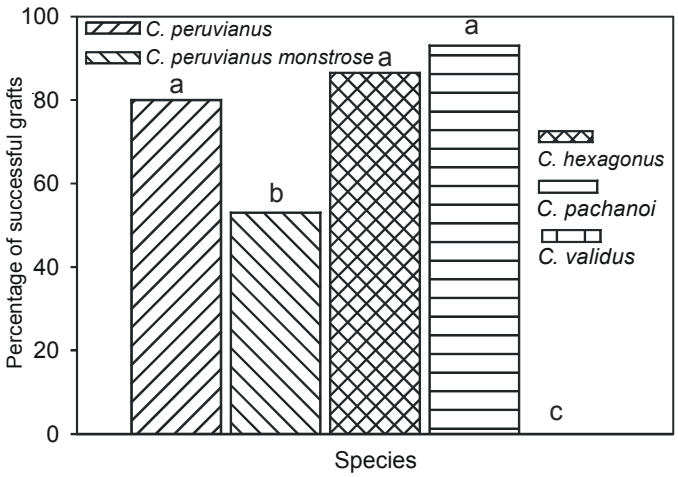

Fig. 8. Percentage of succeeded grafts of different *Cereus* species on *Cereus pachanoi*. Values labeled with the same letter are not significantly different (P>0.05).

The hyper-arid region has limited renewable freshwater supplies (Wilhite, 2000). So, a large portion of the hyper-arid region is desert, the majority of which is uninhabitable because of lacking the biological production (Al Alawi and Abdulrazzak, 1993). It is vital that the kind of agriculture practiced in hyper-arid region should use as little water as possible. In addition, agricultural activities should produce materials that can have economic potential. Apple cactus species *C. peruvianus* and the subspecies *C. peruvianus monstrose* were the most promising in the new hyper-arid environment in terms of adaptability and healthy growth. Apple cactus may give the inhabitants of the hyper-arid and marginal lands a way of making a living.

Acknowledgement

The author would like to thank Mrs. Susan Nelson-Kluk, Program Director, Foundation Plant Materials Service, University of California, for her assistance in collecting the plant material.

References

Al Alawi, J. and M. Abdulrazzak, 1993. Water in the Arabian peninsula: Problems and Perspectives. In: *Water in the Arab World: Perspectives and Prognoses* (Rogers P., Lydon P., eds), Division of Applies Sciences. Harvard University, USA.

Anderson, E.F. 2001. *The Cactus Family*, Timber Press, Portland Oregon. USA.

Backeberg, C. 1984. *Die Cactaceae*. Gustav Fisher, Stuttgart, Germany.

Cushman, J.C. and A.M. Borland, 2002. Induction of crassulacean acid metabolism by water limitation. *Plant, Cell & Environment*, 25: 295-310.

Duncan, D.B. 1955. Multiple range and multiple F test. *Biometrics*, 11: 1-42.

ElObeidy, A.A. 2004. Introducing and growing some fruiting columnar cacti in a new arid environment. *Journal Fruit and Ornamental Plant Research*, 12: 127-136.

Felger, R.S. and M.B. Moser, 1976. Seri Indian food plants: desert subsistence without agriculture. *J. Ecol. Food. Nutr*., 5: 13-27.

Hartmann, H.T., D.E. Kester, R.L. Geneve and F.T. Davies, 2001. *Plant Propagation: Principles and Practices*. Prentice Hall, NJ, USA.

Huffman, M. 2003. Cactus Grafting Methods. *J. PACD*: 106- 113.

Inglese, P., F. Basile and M. Schirra, 2002. Cactus pear fruit production. In: *Cacti: Biology and Uses* (Nobel, P.S., ed), University of California Press, California. USA.

John, S. 2001. *Desert ecology. An introduction to life in the arid Southwest*. University of Utah Press, Salt Lake City, USA.

Malda, G., R.A. Backhaus and C. Martin, 1999. Alterations in growth and carassulacean acid metaolism (CAM) activity of in vitro cultured cactus. *Plant Cell, Tissue and Organ Culture*, 58: 1-9.

Mauseth, J.D. 2000. Theoretical aspects of surface-to-volume ratios and water-storage capacities of succulent shoots. *American Journal of Botany*, 88: 1107-1115.

Morton, J.F., 1987. Cactaceae, strawberry pear. In: *Fruits of warm climates* (Morton J.F., ed), FL, USA.

Nerd, A., J.A. Aronson and Y. Mizrahi, 1988. Introduction and domestication of rare and wild fruit and nut trees. In: *New Crops* (Janick J., Simon J.E., eds.), Timber, Portland, OR, USA.

Nerd, A., N. Tel-Zur and Y. Mizrahi, 2002. Fruits of vine and columnar cacti. In: *Cacti: Biology and Uses* (Nobel, P.S., ed), University of California Press, California. USA.

Ostolaza, C. 1984. Trichocereus pachanoi. *Cactus and Succulent Journal*, 56: 102-104.

Scheinvar, L. 1985. *Flora Ilustrada Catarinese Cactaceae*. Itajai. Santa Catarina, Brazil.

Wallace, R.S. and A.C. Gibson, 2002. Evolution and systematics. In: *Cacti: Biology and Uses* (Nobel, P.S., ed), University of California Press, California. USA.

Weiss, J., A. Nerd and Y. Mizrahi, 1993. Development of *Cereus peruvianus* (Apple Cactus) as a new crop for the Negev desert of Israel. In: *New crops* (Janick J., Simon J.E., eds.), Wiley, New York, USA.

Wilhite, D.A. 2000. Drought as a natural hazards. Concepts and definitions. In: *Drought, A Global Assessment* (Wilhite D., ed.), Volume I, Routledge, London and New York, USA.

Winter, K. and J.A.C. Smith, 1996. *Crassulacean Acid Metabolism - Biochemistry, Ecophysiology and Evolution*, Springer Verlag, New York. USA.

Journal of Applied Horticulture, 8(1): 50-52, January-June, 2006

Assessment of genetic diversity and relationships among some grape varieties using ISSR markers

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Abstract

As a result of large-scale introduction, the origin and authenticity of many grape varieties is unclear and the subject of some controversy. This has led to confusion regarding their correct identification. Molecular markers have proved to be useful to analyze the genetic relationships as well as diversity between different grape varieties. In the present study, 34 grape varieties have been characterized using Inter Simple Sequence Repeat (ISSR) markers. Out of 93 ISSR primers screened initially, 11 showed good polymorphism. Total 174 bands were obtained, out of which 145 were polymorphic. The pair wise similarity indices were calculated from the band data. Cluster analysis of the varieties resulted in the formation of two main clusters, one belonging to *Vitis vinifera* and other to *V. labrusca*. Varieties belonging to *V. vinifera* appeared more diverse and formed distinct sub-clusters based on their colour, flavour and seeds. Out of 34 varieties screened, 10 varieties with green/yellow berries were grouped together in one subcluster and 15 with red/black berries in the other. Three varieties with green/ yellow berries, Italia, Queen of Vineyard and Thompson seedless were grouped with the varieties with red/black berries. The cluster of labrusca varieties showed homogeneity and had five varieties except Dakh, which belongs to vinifera. Concord separates initially from all other varieties. Incidentally, Concord is a pure selection from *V. labrusca*, while other varieties like Bangalore Blue, Black Muscat, Catawba and Muzzafar Nagar in labrusca group, may be the hybrids of *V. abrusca* x *V. vinifera*. The current study thus revealed that genetic relationships among grape cultivars could be assessed using ISSR markers.

Key words: Diversity, genetic relationships, ISSR markers, grape varieties

Introduction

Grape is one of the most important and oldest fruit crop throughout the world. In India, more than 90% of grape produce is utilized for table purpose and a small quantity for raisins, juice and wine making. The long history of viticulture, vegetative propagation of cultivars and the reliance on ampelography pose difficulties in accurate cultivar identification. Most of the commercially cultivated grape cultivars are introductions from exotic sources and the genetic relationships among them are not clear which is important for planning breeding programme and conservation of germplasm.

The use of DNA markers has been proposed as an objective and viable alternative for ampelography (Thomas *et al.*, 1993). There are number of reports involving use of DNA markers for studying genetic relationships, fingerprinting of clones and cultivars as well as parentage studies (Bowers *et al.,* 1993, 1996; Moreno *et al.,* 1995; Cervera *et al.*, 1998; Sefc *et al.,* 1998). Particularly, PCR based DNA markers, provide powerful tools for genetic analysis because of their simplicity and ease of handling. Markers generated by Inter Simple Sequence Repeat amplification (ISSR; Zietkiewicz et al., 1994) have been shown to be useful for detecting polymorphisms and overcome many technical limitations of RFLP and RAPD analyses. ISSR analyses have been applied to grapes earlier mainly for detecting intravarietal differences (Moreno *et al.,* 1998) and distinguishing cultivars (Herrera *et al.,* 2002). In the present work; we have used ISSR markers to characterize seeded grape varieties from India.

Materials and methods

Plant material: A total of 32 seeded grape varieties were analysed in the present study. Two seedless varieties, Thompson Seedless and Flame Seedless were also included in the analysis as standard varieties. All these were obtained from the germplasm maintained at National Research Centre for grapes, Pune. List of varieties is given in Table-1

Table 1. List of varieties analysed in present study

	Green / Yellow berries		Red / Black berries
1	Anab-e-Shahi	18	Black Muscat
2	Angur Kalan	19	Black Prince
3	Banqui Abyad	20	Catawba
4	Cheema Sahebi	21	Coarna Regia
5	Chenin Blanc	22	Concord
6	Gold	23	Convent Large Black
7	Itatia	24	Dakh
8	Jaos Beli	25	Diamond Jubilee
9	Muller Thergau	26	Muscat Hamburg
10	Palomino	27	Muzzafar Nagar
11	Queen of Vineyard	28	Red Globe
12	Sahebi Ali	29	Red Muscat
13	Sundekhani	30	Shiraz
14	Walthom Cross	31	Spin Sahebi
	Red / Black berries		**Standards**
15	Bangalore Blue	32	Thompson Seedless
16	Bharat Prince	33	Flame Seedless
17	Black Champa	34	Gulabi

DNA isolation: DNA was extracted from young, fully expanded leaves by modified CTAB method (Lodhi *et al.,* 1994). The isolated DNA was processed, quantified and used for PCR reactions.

Table 2. List of ISSR primers used and polymorphic bands

S. No.	Primer	Sequence	Number of bands	Polymorphic bands	Polymorphism (%)
1	807	AGA GAG AGA GAG AGA GT	11	7	64.1
2	827	ACA CAC ACA CAC ACA CG	14	13	93
3	855	ACA CAC ACA CAC ACA CYT	13	7	54
4	856	ACA CAC ACA CAC ACA CYA	23	9	39
5	857	ACA CAC ACA CAC ACA CYG	16	16	100
6	859	TGT GTG TGT GTG TGT GRC	6	6	100
7	860	TGT GTG TGT GTG TGT GRA	16	15	94
8	888	BDB CAC ACA CAC ACA CA	19	14	73.7
9	889	DBD ACA CAC ACA CAC AC	19	17	89.5
10	890	VHV GTG TGT GTG TGT GT	23	23	100
11	891	HVH TGT GTG TGT GTG TG	24	18	75
		Total	174	145	83.3

Y=C/T; R=A/G; B=C/G/T; D=A/G/T; H=A/C/T; V=A/C/G

ISSR amplifications and Gel electrophoresis: ISSR amplifications were carried out using Primer set#9; obtained from University of British Columbia, Vancouver, Canada in 25µl reaction volume. Reaction mixture contained 1X PCR buffer containing 1.5mM $MgCl_2$, 12.5ng of genomic DNA, 0.5 U Taq DNA Polymerase, 0.1mM of each dNTP, 0.04 mM spermidine, 2% formamide and 0.3 µM of primer. The thermal cycling was performed in PTC 200 Thermal Cycler (MJ Research Inc, USA) following the protocol of Nagaoka and Ogihara (1997). The PCR reaction was performed at least three times for each primer to ensure reproducibility. The amplification products were separated on 1.5% agarose gels. The gels were stained with ethidium bromide and visualized on a UV transilluminator.

Data Analysis: Bands in the amplification profiles were recorded as present (1) and absent (0). Based on the band data, the similarity matrix was calculated using Dice coefficient and the cluster analysis was carried out using SAHN module in NTSYS pc 2.1 software.

Results and discussion

Total 93 ISSR primers from set #9 were screened initially for polymorphism and 11 were finally selected based on the basis of clear scorable band pattern. The list of the primers used and polymorphic bands recorded is given in Table 2. The amplification profile obtained with primer UBC 857 is shown in Fig. 1.

Total 174 bands were obtained with the 11 selected primers; out of which 145 were polymorphic. The bands ranged from 70 bp to 1.4 kb in size. Primer 891 showed the maximum number of bands. The number of polymorphic bands ranged from 6 (UBC 859) to 23 (UBC 890). All these primers contained dinucleotide repeats. Primers with $(AC)_n$ repeats were maximum in number (5/11) followed by $(TG)_n$ (3/11). In general, percentage polymorphism obtained by primers containing $(AC)_n$ repeats was lower (75%) as compared to those with $(TG)_n$ (89.66%). All the bands obtained with primers 857, 859 and 890 were polymorphic. Few genotype specific bands were observed with primers 856 and 860 in variety Black Prince and primer 889 in varieties Concord and Diamond Jubilee.

Based on the band data, similarity matrix was generated using Dice coefficient and cluster analysis was carried out. The dendrogram generated by UPGMA algorithm using NTSYS pc 2.1 software is shown in Fig. 2. The similarity coefficient ranged from 0.48 to 0.89. Two major clusters were observed; one consisting of *V. labrusca* and its derivatives and the other of varieties from *V. vinifera*. Variety Concord separated initially from all others and showed least similarity. Cluster I consisted of mainly *V. labrusca* or *labrusca* x *vinifera* hybrids except Dakh. The cluster II consisted mainly of *V. vinifera* varieties. In this cluster, Black Prince and Red Muscat separated initially from other vinifera varieties. Several subgroups based on their colour, flavour and seeds were observed in this cluster indicating the diverse nature of these varieties. A distinct sub cluster of 10 varieties with green / yellow berries could be distinguished. Similarly, 15 varieties with (red / black) berries were grouped together. Three varieties, Italia, Queen of Vineyard and Thompson Seedless having yellowish green berries were also grouped with these varieties. Varieties Anab-e-Shahi, Angur Kalan and Cheema Sahebi, all high yielding and hard seeded were grouped together, while three varieties in the other subgroup namely Gold, Muller Thergau and Sundekhani, have soft seeds and muscat flavour. Four flavoured varieties; Italia, Bharat Prince, Muscat Hamburg and Gulabi were grouped together in the same group.

The cluster of labrusca varieties showed homogeneity and had five varieties including Dakh, which however, belongs to vinifera. Varieties Catawba and Dakh showed maximum similarity of 89%. Such a close relationship and the grouping of Dakh in *labrusca* group is surprising since Dakh belongs to *V. vinifera* (Chadha and

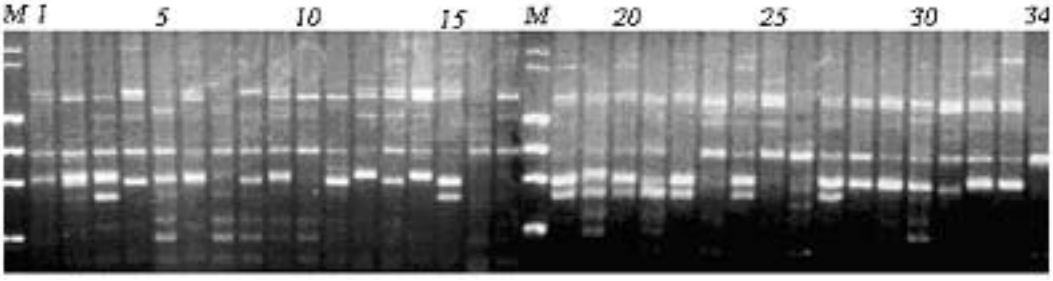

Fig. 1. Amplification profile obtained with UBC primer 857. M: Molecular weight marker, PhiX174DNA/ HaeIII digest. 1-34 DNA samples as listed in Table 1

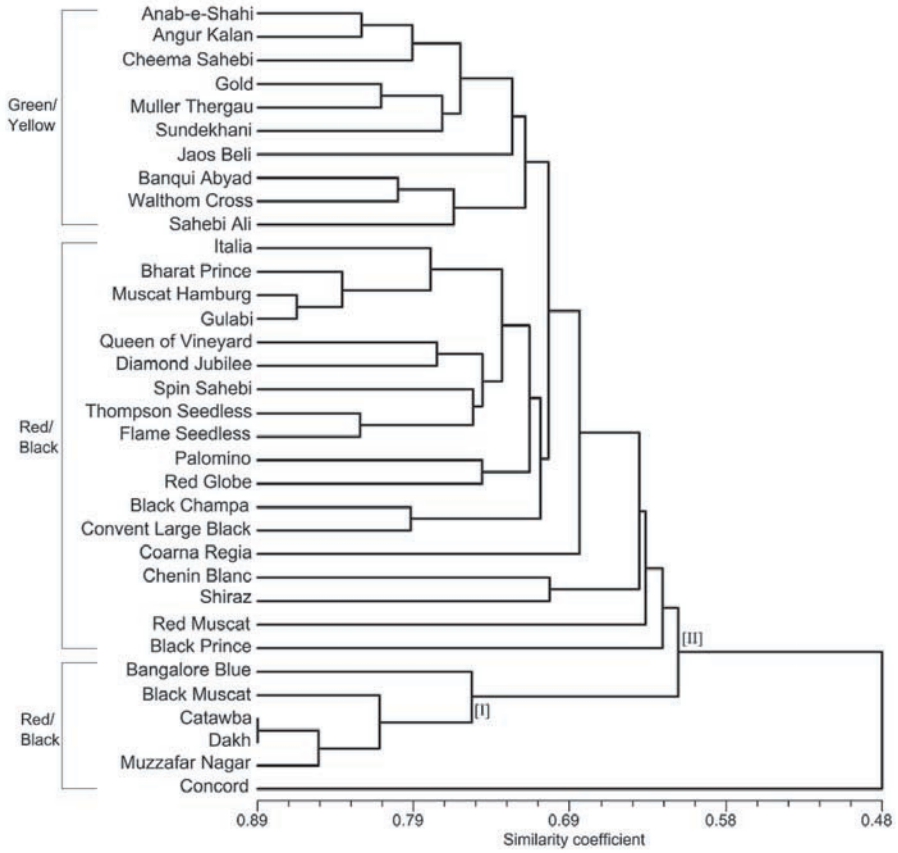

Fig. 2. Dendrogram based on ISSR band data and UPGMA algorithm showing relationships among the grape varieties [I]: *Vitis labrusca* cluster [II]: *Vitis vinifera* cluster

Randhawa, 1974). Therefore, it appears to be a case of mistaken identity. The separation of Concord from *V. labrusca* group is also unexpected since it is one of the oldest *labrusca* cultivar. According to Hedrick (1938), Concord has all the morphological characters of the American species (*V. labrusca*), while, several other workers have reported this variety to be a hybrid between *V. labrusca* and *V. vinifera* (Chadha and Randhawa, 1974). In our earlier analysis using RAPD markers also it had grouped along with other *labrusca* varieties (Tamhankar *et al.*, 2001). The analysis of multiple samples collected from different sources will be useful to confirm these results. Although ISSR markers are efficient and reliable for the assessment of genetic relationships among grape varieties, application of other marker systems like microsatellites (STMS) and AFLP is necessary for resolving the controversial grouping of some varieties.

Acknowledgements

The financial assistance from the Department of Biotechnology (DBT), Ministry of Science and Technology, New Delhi is gratefully acknowledged.

References

Bowers, J.E., E.B. Bandman and C.P. Meredith, 1993. DNA fingerprint characterization of some wine grape cultivars. *Am. J. Enol. Viticult.,* 44: 266-274.

Bowers, J.E., G.S. Dangl, R. Vignani and C.P. Meredith, 1996. Isolation and characterization of new polymorphic simple sequence repeat loci in grapes (*Vitis vinifera* L). *Genome,* 39: 628-633..

Cervera, M.T., J.A. Cabezas, J.C. Sancha, De Martinez, F. Toda and J.M. Martinez Zapater, 1998. Application of AFLPs to the characterization of grapevine *Vitis vinifera* L. genetic resources. A case study with accessions from Roija (Spain). *Theor. Appl. Genet.,* 97: 51-59

Chadha, K.L. and G.S. Randhawa, 1974. *Grape varieties in India-Description and classification,* ICAR Technical Bulletin No.48, ICAR, New Delhi.

Hedrick, U.P. 1938. *Cytology of hardy fruits.* The Macmillan co., New York.

Herrera, R., V. Cares, M.J. Wilkinson and P.D.S. Caligari, 2002. Characterization of genetic variation between *Vitis vinifera* cultivars from central Chile using RAPD and Inter Simple Sequence Repeat Markers. *Euphytica,* 124: 139-145.

Lodhi, M.A., G.N. Ye, N.F. Weeden and B.I. Reisch, 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant Mol. Biol. Reporter,* 12: 6-13.

Moreno, S., Y. Gogorcena and J.M. Ortiz, 1995. The use of RAPD markers for identification of cultivated grape vine (*Vitis vinifera* L). *Scientia Hortic.,* 62: 237-243.

Moreno, S., J.P. Martin and J.M. Ortiz,1998. Inter-simple sequence repeats PCR for characterization of closely related grape vine germplasm. *Euphytica,*101: 117-125.

Nagaoka, T. and Y. Ogihara, 1997. Applicability of inters simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theor. Appl. Genet.*, 94: 597-602.

Sefc, K.M., F. Regner, J. Glossl and H. Steinkellner, 1998. Genotyping of grapevine and rootstock cultivars using microsatellite markers. *Vitis*, 37:15-20.

Tamhankar, S.A., S.G. Patil, V.S. Rao, 2001. Assessment of the genetic diversity of some important grape genotypes in India using RAPD markers. *Vitis,* 40: 157-161.

Thomas, M.R., S. Matsumoto, P. Cain and N.S. Scott, 1993. Repetitive DNA of grapevine: classes present and sequence suitable for cultivars identification. *Theor. Appl. Genet.*, 86: 173-180.

Zietkiewicz, E., A. Rafalski and D. Labuda, 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymorphism chain reaction amplification. *Genomics,* 20: 176-183.

Journal of Applied Horticulture, 8(1): 53-57, January-June, 2006

Impact of polyethylene glycol-induced water stress on growth and development of shoot tip cultures from different banana (*Musa* spp.) cultivars

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Abstract

Shoot tip explants of the Egyptian banana cultivars Maghraby, Valery, Grand Nain and Hindy were tested for their tolerance to water stress. Shoot survival, shoot growth and root growth stimulation in presence of polyethylene glycol (PEG) was strongest in cultivar Hindy followed by Grand Nain, Maghraby and Valery. The accumulation of soluble sugars and proline in shoots was positively correlated with the applied polyethylene glycol concentration, while the reverse was true for N, P and K content. The cultivar Hindy exhibited higher metabolite accumulation response and cultivar Maghraby the least. The effects were most clear on liquid medium whereas solid (agar) medium exerted some additional effects increasing the osmotic stress at low PEG concentrations and alleviating the PEG effect at high PEG concentrations. In conclusion, the cultivar Hindy appeared to be the most tolerant to water stress because of strong accumulation of compatible solutes and greater stimulation of root development.

Keywords: Banana (*Musa* spp. L.), medium (solid/liquid), micropropagation, osmotic stress, polyethylene glycol (PEG), proline, sugars

Introduction

Banana (*Musa* spp.) fruits represent a staple food for about one billion people all over the world (FAO, 1992). It has become one of the strategic crops in tropical countries due to its high income potential for the local farmers. Since they are seedless, the plant has to be propagated vegetatively or *in vitro*. The *in vitro* propagation is preferable in modern breeding because it allows the production of a large number of virus-free plants in a relatively short time and in a small space (Cronauer and Krikorian,1984, 1985; Khalil *et al.,* 2002; Sagi *et al.,*1995).

Banana is cultivated in tropical climates wherever a steady year-round water supply is available. Recently it was introduced into tropical regions which have relatively low water availability. This initiated the investigation of banana plant response to water stress during micropropagation and under greenhouse conditions. Water stress can be induced in the micropropagation media by adding a compatible osmoticum such as polyethylene glycol (PEG). Despite the numerous studies on micropropagation of banana, there is no information about the effect of polyethylene glycol on regenerating or regenerated banana tip explants. Since the drought stress tolerance differs among plant species and the cultivars of a given species (Dodd and Donovan, 1999), the *in vitro* response of four banana cultivars which are important for Egyptian horticulture, to polyethylene glycol-induced water stress was studied.

Materials and methods

Plant material: Four cultivars of *Musa* spp. obtained from the experimental farm El-Kanater El-Khayreia, Kalubiya of the Agricultural Development Systems Project (Giza, Egypt), served as the source material for shoot tips during the study period (2000-2002). The cultivars were Maghraby, Valery, Grand Nain, which belong to the semi-dwarf Cavendish group and Hindy is a strain of dwarf Cavendish. All are of triploid *Acuminata* type.

Aseptic cultures were established from shoot tips which were surface-sterilized in 3 % NaOCl solution (contained 0.1 % Tween 20 as a wetting agent) for 20 min. Thereafter, the tips were rinsed several times in sterilized distilled water to remove all traces of chlorine. After removal of the outside tissues, apical meristems were vertically cultured for 4 weeks on Murashige-Skoog basal medium (Murashige and Skoog, 1962) supplemented with benzyladenine (3 mg/l) and agar (6 g/l). The growing explants were recultured, at 4 week intervals, on fresh media until the onset of proliferation (ca. 2 months). This culture period was called starting phase. In order to obtain sufficient number of explants, the produced shoots were subcultured four times on solid Murashige-Skoog basal media supplemented with benzyladenine (5 mg/l) in a so-called multiplication phase. The obtained plantlets were used for the following experiments (Fig.1).

Incubation with polyethylene glycol: To determine the lethal concentration of polyethylene glycol for each cultivar, PEG-6000 was added to solid basal media at levels of 0, 5, 15, 25, 35 and 45 g/l. The produced shoots were cultured for 4 weeks on these media and the percentage of survival was determined.

Polyethylene glycol (0, 10 and 20 g/l) was added to basal media which were either supplemented with benzyladeneine (5 mg/l) to produce shoots or α-naphthalene acetic acid (1 mg/l) to produce roots. After 4 weeks, shoot or root growth and development were determined. This procedure was applied in liquid and in solid media (0.6 % agar). Shoot strength (or vigour) was determined according to Pottino (1981) on a rating scale: 1 = no growth, 2 = below average, 3 = average, 4 = above average, 5 = excellent.

Analytical methods: The metabolites were determined from oven-dried shoots. Mixed-acid digestion method was used in preparing the sample solution used for determination of N, P and K (Ebrahim and Aly, 2002). Total-nitrogen content (N) was estimated using the micro-Kjeldahl method (Jacobs, 1958). Phosphorus (P) content was spectrophotometrically determined by molybdenum-blue method (Page, 1982). Potassium (K) was determined according to Allen *et al.* (1974). Sugars were extracted in borate buffer pH 8 (0.1 g DW/5 ml buffer), then total soluble sugars were determined by the method adopted by Shaffer and Hartmann (1921). Proline, was quantified in ethanol extract according to Bates *et al.* (1973).

All media were filled into 200 ml Pyrex-glass jars (25 ml/jar), autoclaved for 20 min at 121°C and 1.2 kg/cm^2 pressure, then cooled and kept for 4-15 days before use. In all media, pH was adjusted to 5.7 (before adding agar). Growth was in a growth chamber at 25±3 °C and 40 µmol m^{-2}s^{-1} continuous photosynthetic photon flux provided by cool white fluorescent lamps.

Statistical analysis: All experiments were repeated twice and conducted by using a completely randomised design in factorial arrangement with at least 4 replicates. All data were averaged and statistically analysed by using two-and three-way analysis of variance (ANOVA). In case of percentages, the original data were arcsine-transformed prior to statistical analysis. The least significant difference (LSD) at the 0.05 level was used to compare between means directly (Steel and Torrie, 1980) or indirectly by the multiple range test of Duncan (Duncan, 1955).

Results

Polyethylene glycol is a hydrophilic alcohol polymer with high water solubility and low toxicity (Fontana *et al.*, 2001). We used it in our study as an inert non-penetrating osmoticum (Almansouri *et al.*, 2001). Explant survival in the multiplication phase was significantly decreased by high PEG levels (≥ 15 g/l) (Fig. 2), but the inhibition was more evident in case of the cultivars Maghraby and Valery than Grand Nain and Hindy. The latter survived partly up to 45 g PEG /l.

PEG treatments reduced shoot multiplication and biomass per shoot, especially in the cultivars Maghraby and Valery and less in Grand Nain and Hindy (Table 1). Increasing PEG concentration in the rooting medium increased both root number and, slightly, root length. This increase was highest in Hindy, followed by Grand Nain, Valery and Maghraby (Table 1). This may be interpreted as an adaptive response of roots to alleviate the reduced water availability. Thus cultivar Hindy appeared to be the most adapted to water shortage.

The comparison of shoot and root growth at 20 g/l PEG on solid (agar) medium and in liquid medium revealed that growth was better in liquid medium (Table 2). This finding agrees with previous results by Ebrahim (2004) with Calla and Ebrahim and Ibrahim (2000) on Maranta, who attributed this response to a better availability for growth substances and nutrients and better aeration in liquid media. Also agar may add an osmotic effect in addition to PEG. The cultivar tolerance to PEG with respect to growth was again greatest in the order Hindy>Grand Nain>Valery>Maghraby (Table 2).

From the growth response of the 4 banana cultivars it was obvious that cultivar Hindy could adapt best to water stress, followed by Grand Nain and then Maghraby and Valery (with the latter two virtually identical). The analysis of cell contents should show whether the stress tolerance was correlated with accumulation of nutrient salts or production of compatible osmotica, especially soluble sugars and proline. N, P and K content of shoots decreased with increasing levels of PEG, somewhat less in cultivar Hindy than in Grand Nain or Maghraby (Fig. 3). On solid medium the N, P and K contents were marginally but consistently lower than in liquid medium.

A different, complex picture emerged for sugars and proline. Explants of all cultivars in liquid medium exhibited increasing levels of sugars and proline with increasing PEG concentrations. On solid medium there was the same trend, but the difference between no PEG treatment and 10 g/l PEG was small or absent. In addition the level of sugars and proline were higher on solid medium than in liquid medium when PEG was absent, but lower

Table 1. Shoot growth, shoot development, root growth and root development of *in vitro* cultured banana as affected by the cultivar and PEG concentrat□
supple□
acid (1 mg/l, rooting medium) (Mean ±SD)

Cultivar	PEG (g/l)	Shoots/explant	Shoot fresh weight (g)	Roots/shoot	Root length (cm)
	0	3.02 ± 0.13	0.88 ± 0.04	3.4 ± 0.12	5.2 ± 0.18
Maghraby	10	1.96 ± 0.04	0.73 ± 0.04	4.2 ± 0.17	5.3 ± 0.14
	20	2.12 ± 0.05	0.41 ± 0.01	4.9 ± 0.20	5.6 ± 0.12
	0	3.00 ± 0.11	0.86 ± 0.04	3.6 ± 0.16	5.4 ± 0.18
Valery	10	2.01 ± 0.09	0.70 ± 0.05	4.3 ± 0.18	5.5 ± 0.14
	20	1.04 ± 0.05	0.44 ± 0.02	5.3 ± 0.24	5.8 ± 0.19
	0	3.04 ± 0.04	0.83 ± 0.03	3.8 ± 0.10	5.6 ± 0.26
Grand Nain	10	2.51 ± 0.10	0.74 ± 0.05	5.0 ± 0.18	5.6 ± 0.18
	20	1.57 ± 0.12	0.56 ± 0.02	6.1 ± 0.26	5.8 ± 0.14
	0	3.02 ± 0.12	0.85 ± 0.05	3.9 ± 0.14	5.6 ±0.18
Hindy	10	2.62 ± 0.13	0.80 ± 0.03	4.9 ± 0.18	5.7 ± 0.18
	20	1.96 ± 0.06	0.66 ± 0.03	6.2 ± 0.25	5.9 ± 0.19

Table 2. Shoot growth, shoot development, root growth and root development of *in vitro* cultured banana in presence of 20 g/l PEG on solid and liquid medium. Explants were cultured with 20 g/l PEG for 4 weeks on Murashige-Skoog basal medium containing benzyladenine (5 mg/l, multiplication medium) or on Murashige-Skoog basal medium containing naphthyl acetic acid (1 mg/l, rooting medium) (Mean ±SD)

Cultivar	Medium	Shoots/ explant	Shoot frresh weight (g)	Shoot vigour	Leaves/ shoot	Roots/ shoot	Root length (cm)
Maghraby	Solid	1.12 ±0.04	0.41±0.02	1.4 ± 0.05	2.8 ± 0.14	4.3 ± 0.19	5.6 ± 0.24
	Liquid	1.50 ±0.05	0.57 ±0.02	1.8 ± 0.06	3.2 ± 0.17	4.9 ± 0.21	6.9 ± 0.28
Valery	Solid	1.04 ±0.04	0.44 ±0.02	1.2 ± 0.04	2.9 ± 0.11	4.3 ± 0.18	5.8 ± 0.26
	Liquid	1.57 ±0.08	0.57 ±0.03	1.7 ± 0.07	3.2 ± 0.12	5.3 ± 0.24	7.1 ± 0.32
Grand Nain	Solid	1.57 ±0.06	0.56 ±0.03	2.0 ± 0.07	3.2 ± 0.14	4.8 ±0.22	5.8 ±0.24
	Liquid	1.93 ±0.04	0.66 ±0.04	2.3 ±0.08	3.8 ± 0.14	6.1 ± 0.25	8.1 ± 0.33
Hindy	Solid	1.96 ±0.04	0.66 ±0.05	2.7 ± 0.09	3.4 ± 0.11	4.8 ± 0.21	5.9 ± 0.22
	Liquid	2.22 ±0.08	0.71 ±0.04	3.1 ± 0.06	4.2 ± 0.15	6.2 ± 0.25	8.4 ± 0.32

Fig. 1. Regenerating banana plantlets from meristem tip culture. Successive stages of micropropagation of *Musa* spp. (cultivar. Hindy): (A) shoot tips were cultured on Murashige-Skoog basal medium containing benzyladenine (3 mg/l, starting stage), (B) shoot explants were proliferated on basal medium supplemented with benzyladenine (5 mg/l, multiplication stage), and (C) shoot explants were rooted on basal medium containing naphthyl acetic acid (1 mg /l, rooting stage).

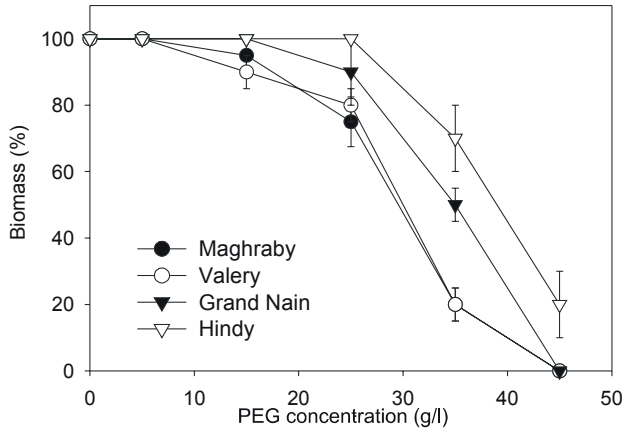

Fig. 2. Percentage of shoot survival of *in vitro* cultured banana cultivars in presence of PEG. Explants were cultured for 4 weeks on Murashige-Skoog basal medium supplemented with benzyladenine (3 mg/l, starting medium). (The results for Maghraby and Valery coincide at high PEG-concentrations) (Mean ±SD).

on solid medium than in liquid medium when PEG was present (Fig. 4). The different banana cultivars showed the same order in compatible solute content as in tolerance towards PEG, namely cultivar Hindy containing the highest levels of nutrients and compatible solutes followed by cultivar Grand Nain and then Maghraby.

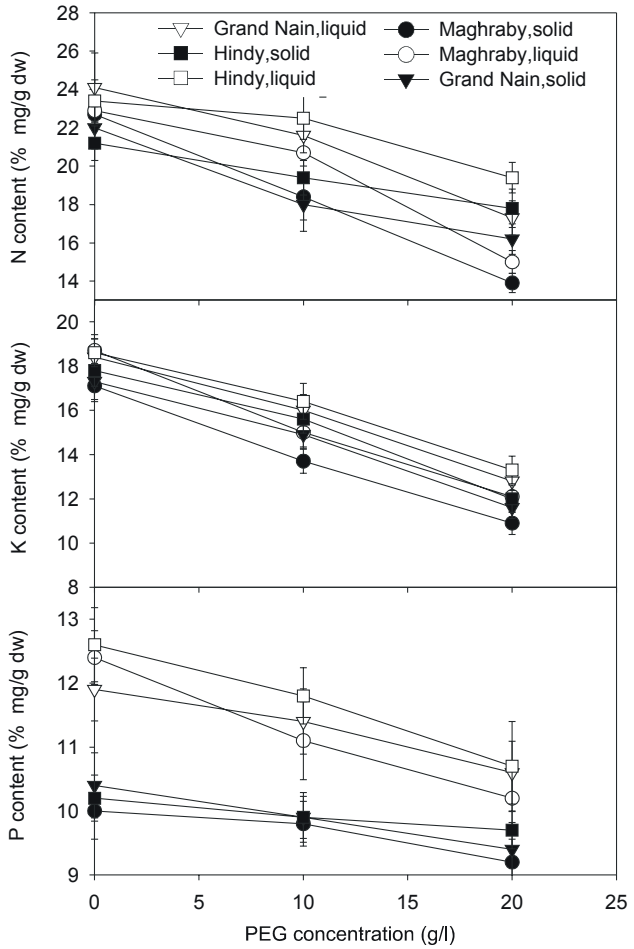

Fig. 3. N, P and K-content of shoots of *in vitro* cultured banana plantlets in presence of PEG on solid and liquid medium. Explants were cultured for 4 weeks on Murashige-Skoog basal medium containing

Discussion

Effects of PEG on osmotic performance had been reported for many plants (Bandurska, 2000; Pushpam and Rangasamy, 2000; Ronde *et al.,* 2000; Liu *et al.,* 2001). Predictably the growth of the small, micropropagated banana plants of cultivars Maghraby, Valery, Grand Nain and Hindy was influenced by application of PEG and, slightly, by the type of medium, but the magnitude of the response was consistently cultivar-dependent. The cultivar

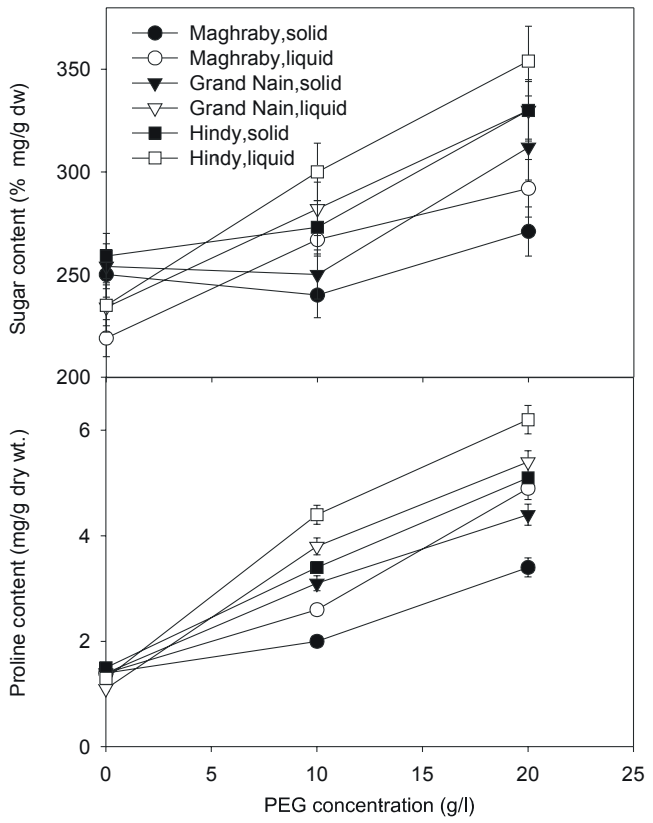

Fig. 4. Content of soluble sugars and proline in shoots of *in vitro* cultured banana plantlets in presence of PEG on solid and liquid medium. Explants were cultured for 4 weeks on Murashige-Skoog basal medium containing benzyladenine (5 mg/l, multiplication medium)

Hindy proved to be the most tolerant to PEG-induced water stress and cultivar Grand Nain was more tolerant than cultivars Valery and Maghraby. PEG in the medium led to less shoot growth and longer roots, thereby shifting the shoot/root ratio to lower values, an effect known for plant stress conditions (Brouwer, 1962). The cultivar Hindy, which turned out to be the most tolerant to PEG-treatment, was the one which had less shoot growth inhibition, but the strongest stimulation of root growth, *i.e.* it reacted strongly to the osmotic stress by favouring root growth and thereby minimized shoot inhibition. The cultivar Hindy was also the cultivar which exhibited the strongest stress response by increasing the content of proline 4-fold and of soluble sugar, mostly sucrose, 1.5-fold. The concentration of proline (4 mg/g d.wt.) however was much less than of sugars (200 mg/g d.wt.). Thus proline seems more indicative for the osmo-compatible solutes than really protective against osmotic stress. Nitrogen, potassium and phosphorus content decreased parallel with osmotic stress with only marginal cultivar differences. The absence of potassium increase was surprising because potassium does replace sugar as osmoticum in some sugar storing monocotyledons such as sugarcane (Glasziou and Gayler, 1972).

The comparison of solid and liquid medium appeared complex at first sight, but it may be explained on the basis of two simultaneous effects. Firstly agar exerts a small osmotic effect by itself, and secondly it slows down solute flux to the explants compared to flux in liquid medium. Therefore the banana plantlets in medium without PEG felt a small osmotic stress on agar and responded with higher values of sugar on solid medium compared to on liquid medium. In contrast, the addition of PEG to the medium had a larger effect on plantlets in liquid medium because they felt the osmotic stress immediately without noticeable diffusion barriers in their surroundings, whereas on agar a lower concentration of osmoticum may exist in the close environment of the roots due to localized nutrient uptake and extracellular metabolic degradation of solutes. In general it appears that plants on liquid medium give a better and clearer response to PEG treatments than plants on solid medium. Cultures in liquid media are also known to perform better in post-thaw regeneration (Khalil *et al.,* 2002).

Since it had been proven that banana plants from micropropagation show no differences in in-field behaviour compared to conventionally propagated plants (Cote *et al.,* 2000), it is suggested that the cultivar-specific osmotic response of the *in vitro* micropropagated banana plants is indicative of the drought tolerance of grown-up plants with the conclusion that cultivar Hindy may be the best for water-tight plantation conditions.

References

Allen, S.G., H.M. Grimshaz, J.A. Parkinson and C. Quarmby, 1974. *Chemical Analysis of Ecological Materials.* Blackwell Scientific Publishing, Oxford.

Almansouri, M., J.M. Kinet and S. Lutts, 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant and Soil*, 21: 243-254.

Bandurska, H. 2000. Does proline accumulated in leaves of water deficit stressed barley plants confine cell membrane injury?. I. Free proline accumulation and membrane injury index in drought and osmotically stressed plants. *Acta Physiol. Plant*, 22: 409-415.

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline under water stress studies. *Plant and Soil*, 9: 205-207.

Brouwer, R. 1962. Nutritive influences on the distribution of dry matter in the plant. *Neth. J. Agricult. Sci.*, 10: 361-375.

Cote, F., O. Goue, R. Domergue, B. Panis and C. Jenny, 2000. In-field behaviour of banana plants (*Musa* AA sp.) obtained after regeneration of cryopreserved embryogenic cell suspensions. *Cryo. Letters*, 21: 19-24.

Cronauer, S.S. and A.D. Krikorian, 1984. Multiplication of *Musa* from excised stem tips. *Ann Bot.*, 53: 321-328.

Cronauer. S.S. and A.D. Krikorian, 1985. Reinitiation of vegetative growth from aseptically cultured terminal floral apex of banana. *Am. J. Bot.*, 72: 1598-1601.

Dodd, G.L. and L.A. Donovan, 1999. Water potential and ionic effects on germination and seedling growth of two cold desert shrubs. *Am. J. Bot.,* 86: 1146-115.

Duncan, D.B. 1955. Multiple range test and multiple E test. *Biometrics,* 11: 1-42.

Ebrahim, M.K.H. 2004. Comparison, determination and optimizing the conditions required for rhizome and shoot formation, and flowering of *in vitro* cultured calla explants. *Scientia Hortic.*, 101: 305-313.

Ebrahim, M.K.H. and M.M. Aly, 2002. Wheat response to foliar application of zinc and inoculation with some bacterial fertilizers. Proceedings, Plants and Industrial Pollution, *Egypt. Bot. Soc., Cairo*. pp. 59-72.

Ebrahim, M.K.H. and I.A. Ibrahim, 2000. Influence of medium solidification and pH value on *in vitro* propagation of *Maranta leuconeura* cultivar. Kerchoviana. *Scientia Hortic*., 86: 211-221.

FAO, 1992. *Food and agriculture organization production*. Year-Book, Vol. 46. Rome, FAO Secretariat (281p).

Fontana, G., M. Licciardi, S. Mansueto, D. Schillaci and G. Giammona, 2001. Amoxicillin-loaded polyethylcyanoacrylate nanoparticles: Influence of PEG coating on the particle size, drug release rate and phagocytic uptake. *Biomaterials,* 22: 2857-2865.

Glasziou, K.T. and K.R. Gayler, 1972. Storage of sugars in stalks of sugar cane. *Bot. Rev.*, 38: 471-490.

Jacobs, M.B. 1958. *The chemical analysis of food and food products*. D. Van Nostr and Co. Inc., New York.

Khalil, S.M., K.T. Cheah, E.A. Perez, D.A. Gaskill and J.S. Hu, 2002. Regeneration of banana (*Musa* spp. AAB cultivar Dwarf Brazilian) via secondary somatic embryogenesis. *Plant Cell Rep*., 20: 1128-1134.

Liu, T., J. Staden and S.J. Van, 2001. Partitioning of carbohydrates in salt-sensitive and salt-tolerant soybean callus cultures under salinity stress and its subsequent relief. *Plant Growth Regulation*, 33: 13-17.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*., 15: 473-497.

Page, A.L. 1982. Chemical and microbiological properties (Part 2). In: *Methods of soil analysis*. AL Page, DE Baker, J Roscoe-Ellis, DR Keeney, RH Miller and J. D. Rhoades (eds), Second ed., Madison, Wisc, USA.

Pottino, B.G. 1981. *Methods in plant tissue culture.* Department of Horticulture, Agricultural College, College Park, MD, p. 8-25.

Pushpam, R. and S.R.S. Rangasamy, 2000. Effect of salinity on protein and proline contents of callus and seedlings of rice. *Crop Research*, 20: 192-196.

Ronde, J.A., M.H. Spreeth, W.A. Cress and J.A. De-Ronde, 2000. Effect of antisense L-Δ1-pyrroline–5-carboxylate reductase transgenic soybean plants subjected to osmotic and drought stresses. *Plant Growth Regulation*, 2: 13-26.

Sagi, L., B. Panis, S. Remy, H. Schoofs, K. De Smet, R. Swennen and B.P. Cammun, 1995. Genetic transformation of banana and plantain (*Musa* spp.) via particle bombardment. *Biotechnology,* 13: 481-485.

Shaffer, P.A. and A.F. Hartmann, 1921. The iodometric determination of copper and its use in sugar analysis. *J. Biol. Chem.*, 45: 65-67.

Steel, R.G.D. and J.A. Torrie, 1980. *Principles and procedure of Statistics.* 2nd edition. Mc-Graw- Hill, New York.

Journal of Applied Horticulture, 8(1): 58-61, January-June, 2006

Effect of gibberellin treatment on parthenocarpic ability and promotion of fruit swelling in papaya

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Abstract

To improve the productivity of vegetable papaya in subtropical regions, 1) fruit setting rate (parthenocarpic ability) and fruit productivity between sex types (females and hermaphrodites) and among cultivars; and 2) effect of gibberellins (GAs) on fruit swelling, was studied. In both sex types, the number of fruits per tree correlated more closely with fruit yield than with individual fruit weight. Females produced higher number of fruits per tree, thus attaining a higher fruit yield than hermaphrodites. A variation in parthenocarpic ability was observed among cultivars, and this ability was higher in female plants than in hermaphrodites. These results suggest that it is possible to grow female cultivars with high parthenocarpic ability. However, parthenocarpic fruits were significantly smaller than those produced by pollination. GA treatment was found to be effective for promoting fruit swelling under greenhouse conditions. Thus, in the greenhouse production of papaya, GA treatment was more efficient than hand pollination. Based on these results, we suggest that in subtropical regions, efficient production of papaya fruit for use as a vegetable may be realized by selection and cultivation of female cultivars with high parthenocarpic ability and promotion of fruit swelling by GA treatment.

Key words: Fruit swelling, gibberellins, papaya (*Carica papaya*), parthenocarpy, sex types.

Introduction

Papaya (*Carica papaya* L.) is a polygamous species with three sex types: male, female, and hermaphrodite (Hofmeyr, 1938; Storey, 1938). The ripe fruits from female and hermaphrodite trees are used commonly as a dessert, and in Southeast Asia the green fruits are also cooked as vegetables (Manshardt, 1992; Nakasone and Paull, 1998). Recently, the demand for green papaya fruits has increased in the Okinawa Islands, Japan (L26˚12'51", N127˚40'28").

The hermaphroditic Hawaiian 'Solo' variety of papaya is preferred in the international market, however, this variety is not highly productive under Okinawa Islands conditions. This low productivity may be related to sexual reversal in the flowers of hermaphrodites (Hofmeyr, 1939; Manshardt, 1992; Nakasone and Paull 1998; Ray, 2002). In contrast, female trees remain stable in their sexual expression throughout the year (Hofmeyr, 1939; Manshardt, 1992; Nakasone and Paull, 1998; Ray, 2002).

Male plants are usually intercropped with females to allow pollination and increase fruit yield (Allan, 1976; Samson, 1986; Aquilizan, 1987; Nakasone and Paull, 1998). However, intercropping is not practiced in the Okinawa Islands, where papaya plants are grown in greenhouses to protect them from typhoon damage and virus infections. Pollination does not occur naturally under greenhouse conditions, even when male or hermaphrodite trees are intercropped with females. Although hand pollination can be done to promote fruit swelling in greenhouses, it is labour-intensive and costly. Therefore, an alternative to hand pollination is needed to improve fruit productivity.

Nakasone and Paull (1998) and Ray (2002) described natural parthenocarpy in papaya. Rodriquez-Pastor *et al.* (1990) also reported variation in parthenocarpic ability among female cultivars. Although the enhancement of parthenocarpic ability will be vital for increasing productivity of the female plants, the parthenocarpic nature of papaya has not been investigated in detail. Moreover, plant growth regulators such as synthetic auxins, gibberellins, and cytokinins, have been successfully used to promote fruit swelling in other horticultural crops (Lurie, 2000), artificial induction of parthenocarpy may provide another route to fruit yield improvement. In order to improve the productivity of vegetable papaya under subtropical conditions, we investigated differences in fruit yield and parthenocarpic ability between sex types among cultivars, and tested the potential of gibberellin treatment for the promotion of fruit swelling.

Materials and methods

Difference in fruit yield between females and hermaphrodites: Female and hermaphrodite trees of the cultivars 'Dantesu', 'Fruit tower' and 'Kansen' (Wakaba Seed Co., Okinawa, Japan) were investigated. Seeds were sown in black plastic pots on 10 October, 2002 and plants were transplanted to 3 m-wide ridges, 2.5 m apart, in a greenhouse on 3 December, 2002. The surface of the ridges was covered with straw mulch and fertilizer (N: 5.4, P: 7.7, P: 4.7 kg per 10 m^2) was applied to the soil before planting. Five to 6 plants per cultivar (2 to 3 plants per sex type) were used for the study.

Female and hermaphrodite flowers were left unbagged. Fruits were harvested from 3 August to 28 November, 2003 (2 months

post-flowering) and at every harvest times, the presence of seeds in the fruits was confirmed. The number of fruits per tree, fresh weight per fruit, and fruit yield per tree, were recorded. Regression analyses were performed between fruit yield and number of fruits per tree, and between fruit yield per tree and individual fruit weight, for both females and hermaphrodites of cultivars tested.

Variation in parthenocarpic ability of sex types and cultivars: Seven cultivars, 'Dantesu', 'Fruit Tower', 'Kansen', 'Perfect', 'Tropicana' (Wakaba Seed Co., Okinawa, Japan), 'Taino-2' and 'Taino-5' (Noyu Seed Co., Taipei, Taiwan) were used. Six plants each of females and hermaphrodites from each cultivar were used in the experiment, with the exception of 'Taino-2', for which only female plants were available. Sowing and transplantation dates, as well as cultivation conditions, were as described above.

Two experimental plots (pollination and non-pollination) were established to evaluate differences in fruit-setting rates (percentage of flowers that set fruit: parthenocarpic ability) between sex types and among cultivars. To prevent natural pollination, all flowers of female and hermaphrodite plants were covered with paper bags 2 days before flowering. In both plots, the anthers of hermaphrodite flowers were emasculated before bagging. In the pollination plot, female and hermaphrodite flowers were hand-pollinated on the day of flowering with fresh pollen collected from male plants cultivated in another greenhouse. In the non-pollination plot, female and emasculated hermaphrodite flowers were left bagged for a week, until the stigmatic lobes turned brown.

Data for fruit weight were collected 60 days after bagging, from 16 September to 31 October, 2003. Number of fruits per sex type per cultivar varied from 8 to 29 in the hand-pollination and from 6 to 23 in the non-pollination plots. Differences in fruit weight between the plots were analysed using the Mann-Whitney U-test. Fruit-setting rates were determined for both sex types of 'Dantesu', 'Fruit tower', 'Kansen', and 'Taino-5' and for females of 'Taino-2'. Ten to 30 flowers per sex type were used, and parthenocarpic ability was compared between females and hermaphrodites using the chi-square test on summed data of each sex type.

Promotion of fruit swelling by GA treatment: The cultivar, 'Fruit Tower', was used for the experiment. Seedlings were raised from 12 October to 1 December, 2003. From the seedling population, only female plants were selected using a sex-specific DNA molecular marker (Urasaki *et al.*, 2002). Cultivation conditions were as described above.

Three experimental plots (pollination, non-pollination, and GA treatment) were established and treatments were conducted from 30 April to 2 September, 2004. In the pollination plot, fresh pollen collected from male plants raised in another greenhouse was used; in the non-pollination plot, flowers were not pollinated. In the GA treatment, a commercial GA lanolin paste with 2.7% GAs (containing 85% active GA_3; Kyowa hakko, Co. Tokyo, Japan) was applied to flower peduncles with a soft brush. Approximately 100 mg paste per peduncle was applied to flowers 1 day before flowering (-1), flowering day (0), 1 or 2 days after flowering. Fruits were harvested 37 days after the treatment, and their weight was recorded and analysed by the Scheffe's test. All statistic analyses were conducted using a computer program, StaView (ver. 4.5, SAS Institute Inc., 1998).

Results

Difference in fruit yield between females and hermaphrodites: In all the three cultivars, female fruits contained few seeds, but the hermaphrodite ones had abundant seeds (data not shown). In the 'Dantesu', fruits from hermaphrodites were heavier than those from females, although there was little difference in the number of fruits per tree between the sexes (Fig. 1). Thus, the hermaphrodites produced a greater fruit yield per tree than female plants. In the 'Fruit Tower' and 'Kansen', fruits from hermaphrodites were heavier than those from females. However, in both cultivars, females produced a higher number of fruits per tree than hermaphrodites; thus, fruit yield per tree was higher in the former as compared to the latter.

In the three cultivars, fruit yield per female tree was positively correlated with number of fruits per tree (R^2= 0.904, P= 0.001), and with individual fruit weight (R^2= 0.615, P= 0.036). In hermaphrodite trees, fruit yield per tree also correlated with the number of fruits per tree (R^2= 0.747, P= 0.0056), but not with individual fruit weight (R^2= 0.006, p= 0.850). These results suggest that an increase in the number of fruits per tree predominantly contributed to the improvement of fruit yield per tree.

Variation in parthenocarpic ability of sex types and cultivars: Female plants exhibited a significantly higher fruit-setting rate than hermaphrodites in both pollination (x^2= 8.959, P= 0.0028) and non-pollination treatments (x^2= 10.338, P= 0.0013) (Table 1). There were differences in the fruit-setting rates between females and hermaphrodites and among the cultivars (Fig. 2). In the pollination plot, the fruit-setting rate of female plants varied from 80 % ('Kansen') to 100 % ('Dantesu', 'Fruit tower', and 'Taino-2'), whereas hermaphrodites varied from 37.5 % ('Fruit Tower') to 94.7 % ('Taino-5'). In the non-pollination plot, the rate in females ranged from 60 % ('Kansen') to 100 % ('Dantesu' and 'Fruit tower'); the hermaphrodites ranged from 20 % ('Fruit tower') to 81.3 % ('Kansen').

Table 1. Fruit setting rates between female and hermaphrodite papaya in pollinated and non-pollinated flowers

Sex type[1]	Pollinated				Non-pollinated			
	Number of flowers used	Number of fruits developed	Number of flowers abscised	Fruit setting rate (%)**	Number of flowers used	Number of fruits developed	Number of flowers abscised	Fruit setting rate (%)**
Female	95	79	16	83.2	90	65	25	72.2
Hermaphrodite	145	95	50	65.5	92	45	47	48.9

[1] Data from female or hermaphrodite cultivars were summed up. ** Significant at P<0.0028

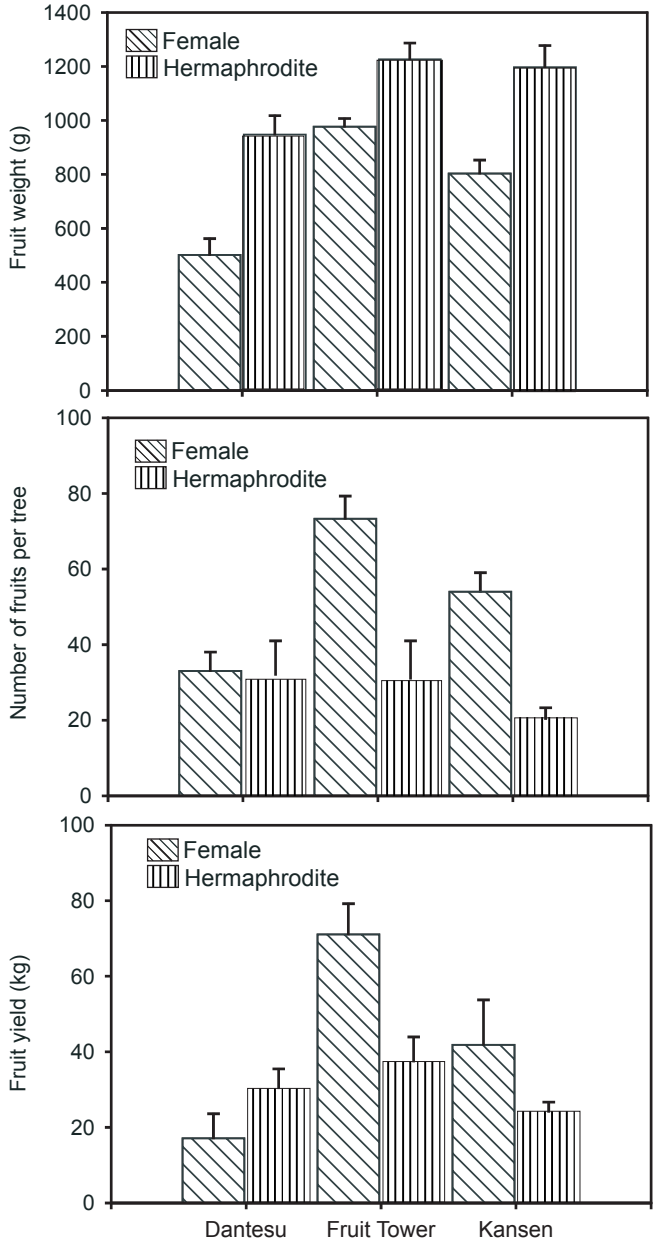

Fig. 1. Number of fruits per tree, fruit weight, and fruit yield per tree in female and hermaphrodite plants

The pollinated fruits were significantly heavier than non-pollinated ones in both sex types of all the cultivars, *i.e.*, pollinated fruits were twice as heavy as non-pollinated ones in both sex types (Fig. 3). Among cultivars, the females of 'Fruit tower' and 'Tropicana' yielded the heaviest fruits from pollinated flowers. In addition, pollinated fruits had thicker flesh than non-pollinated in both sex types (data not shown).

Promotion of fruit swelling by GA treatment: GA treatment effectively promoted fruit swelling (Fig. 4). The pollinated fruits were twice as heavy as the non-pollinated ones. The GA treatment further increased fruit weight, *i.e.*, the fruits were 3 times as heavy as the non-pollinated fruits and 1.5 times of the pollinated ones.

The optimal flower age for the GA treatment ranged from flowering day to 2 days after flowering (Fig. 4). Flowers treated 2 or 3 days after flowering yielded significantly heavier fruits than those treated 1 day before flowering.

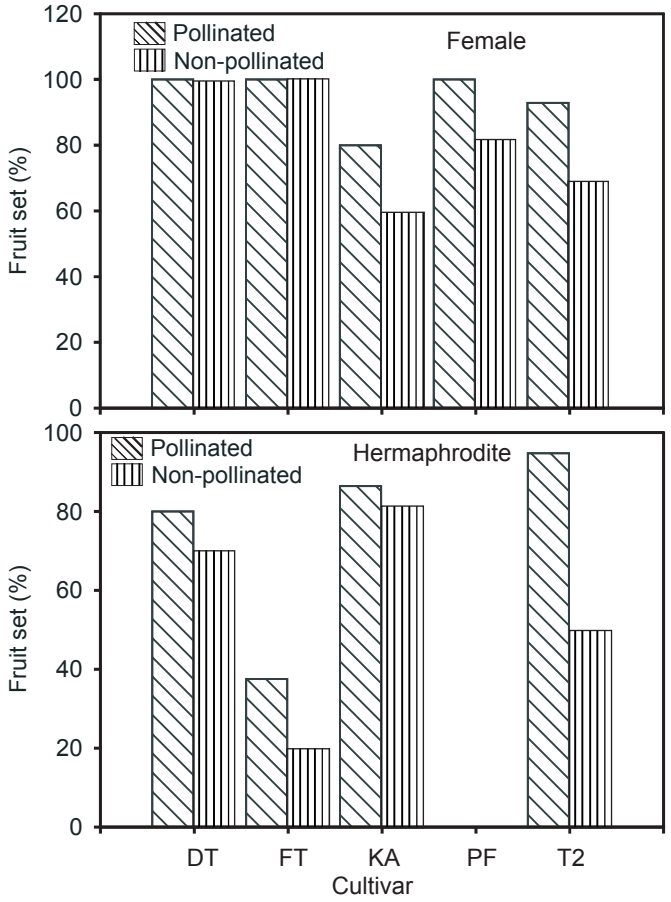

Fig. 2. Fruit-setting rates in pollinated and non-pollinated female and hermaphrodite plants

Discussion

The primary determinant of fruit yield in the papaya plants was the number of fruits per tree; the secondary determinant was individual fruit weight. Thus, increasing the number of fruits per tree appears to be a critical factor in improving fruit yield. In addition, female plants were superior to hermaphrodites at increasing the number of fruits per tree during the hot summer season. This is likely due to the stability of female flowers with respect to sex changes. Therefore, in subtropical regions such as Australia (Aquilizan, 1987) and Okinawa, female papaya plants are considered to be better suited to fruit production than hermaphrodites.

In subtropical regions, the breeding of female cultivars with high fruit-setting rates (parthenocarpic ability) will be important for the improvement of fruit productivity. In this study, we confirmed that both female and hermaphrodite plants exhibit parthenocarpic ability and that female plants possess significantly higher parthenocarpic ability than hermaphrodites. This ability varied among cultivars. The intraspecific variation of this trait suggests a potential possibility of improving papaya genotypes with high parthenocarpic ability. Recently, Rimberia *et al.* (2005) reported an anther culture technique for papaya, by which only female plants were produced from microspores. Such *in vitro* techniques may prove useful in raising female genotypes with high parthenocarpic ability.

As observed in this study, parthenocarpic fruits were smaller than pollinated fruits. However, GA treatment resulted in a

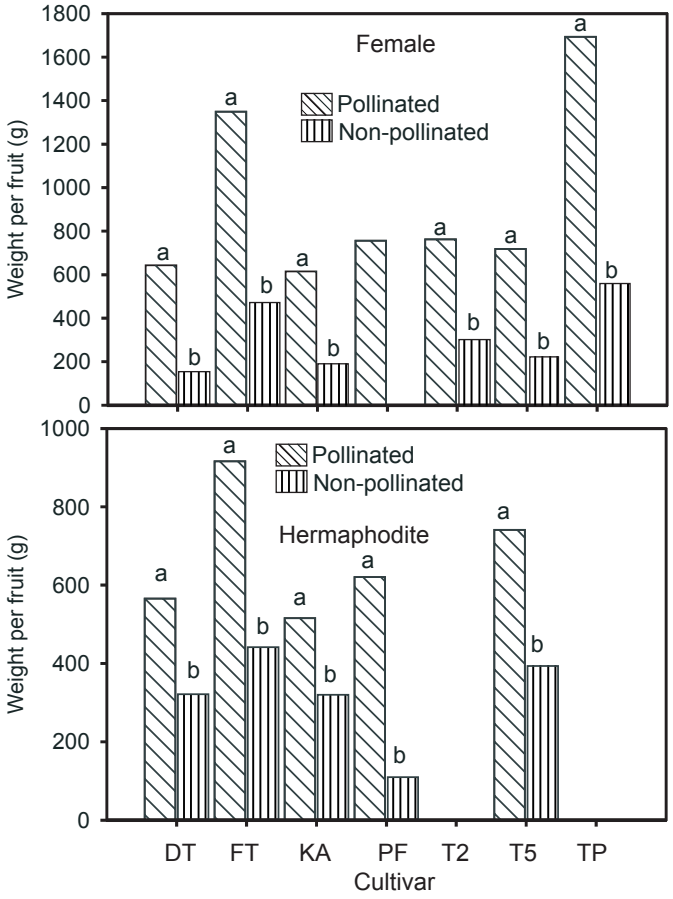

Fig. 3. Differences in fruit weight between hand-pollinated and non-pollinated fruits in female and hermaphrodite cultivars. DT: 'Dantesu', FT: 'Fruit tower', KA: 'Kansen', PF: 'Perfect', T-2: 'Taino-2', T-5: 'Taino-5', and TP: 'Tropicana'. Bars having different letters indicate significant differences between pollination and non-pollination by Mann-Whitney's U-test (P<0.03).

significant increase in parthenocarpic fruit size, when compared to either non-pollination or pollination treatments. Moreover, the GA paste could be applied over a range of flower ages, from day of flowering through to 3 days post-flowering, whereas optimal results for fruit development from hand pollination are restricted to the day of flowering itself (Ray, 2002). These results suggest that GA treatment provide greater flexibility than a conventional hand pollination schedule, and may represent a feasible alternative for fruit production from female papaya plants, under greenhouse conditions.

Based on the results obtained, we propose that an efficient production system of vegetable papaya can be realized by: 1) selection of females with high parthenocarpic ability; 2) cultivation of only female plants separated by sex-diagnostic PCR techniques; and 3) promotion of fruit swelling by GA treatments.

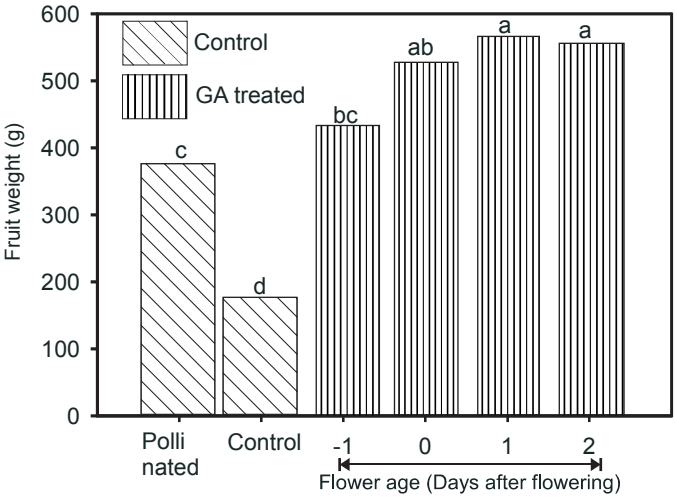

Fig. 4. Effect of GA on fruit swelling. Bars having different letters indicate significant differences by Scheffe's test (P<0.015).

References

Allan, P. 1976. Out of season production of pawpaws (*Carica papaya* L.) in cool subtropical areas. *Acta Hortic.*, 57: 97-103.

Aquilizan, F.A. 1987. Breeding systems for fixing stable papaya inbred lines with breeding potential for hybrid variety production. *Proceedings of an international symposium on the breeding of horticultural crops held at Chung Hsing University, Taiwan, December 1986*. FFTC Book Series No. 35. Taipei, Taiwan, pp. 101-106.

Hofmeyr, J.D.J. 1938. Genetical studies of *Carica* papaya L. *South African J. Sci.,* 35: 300-304.

Hofmeyr, J.D.J. 1939. Sex reversal in *Carica papaya* L. *South African J. Sci.,* 36: 286-287.

Lurie, S. 2000. *In: Plant Growth Regulators in Agriculture and Horticulture.* A.S. Basra (Ed.). Haworth Press Inc., New York, pp. 175-196.

Manshardt, R.M. 1992. In: *Biotechnology of Perennial Fruit Crops*. F.A. Hammerschlag and R.E. Litz (Eds). C.A.B. International, pp. 489-511.

Nakasone, H.Y. and R.E. Paull, 1998. Tropical fruits. C.A.B. International, pp. 239-269.

Ray, P.K. 2002. *Breeding Tropical and Subtropical Fruits*. Narosa publishing house, New Delhi, pp. 106-128

Rimberia, F. K., H. Sunagawa, N. Urasaki, Y. Ishimine and S. Adaniya, 2005. Embryo induction via anther culture in papaya and sex analysis of the derived plantlets. *Scientia Hortic.,* 103: 199-208.

Rodriquez-Pastor, M.C., V. Galan-Sauco and M. Herero-Romero, 1990. Evaluation of papaya autogamy. *Fruits,* 45 (4): 387-391.

Samson, J. A. 1986. *Tropical fruits*. Longman Scientific and Technical. John Wiley & Sons, Inc., New York, pp. 256-269.

Storey, W. B. 1938. Segregations of sex types in solo papaya and their application to the selection of seed. *Proc. Amer. Soc. Hort. Sci.,* 35: 83-85.

Urasaki, N., K. Tarora, T. Uehara, I. Chinen, R. Terauchi and M. Tokumoto, 2002. Rapid and highly reliable sex diagnostic PCR assay for papaya (*Carica papaya* L.). *Breeding Science*, 52: 333-335.

Journal of Applied Horticulture, 8(1): 62-64, January-June, 2006

Effect of mineral concentration on *in vitro* explant growth of almond (*Prunus amygdalus* var. Binazir)

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Abstract

A study was undertaken to determine the potential of mineral dependent growth of almond *in vitro.* Shoot-tip of almond (*Prunus amygdalus* L. var. Binazir) was subcultured on four different concentrations (4, 6, 8, 10 gl^{-1}) of gelled modified de Fossard medium (de Fossard, 1976) with four relative concentrations (0X, 0.2X, 1X and 2X basal medium) containing BA 0.75 mgl^{-1} and NAA 0.75 mgl^{-1}. As mineral concentration increased, both growth and multiplication rate significantly (P=0.05) increased. But increase was not proportional. There was a negative relationship between mineral concentration and root formation. Agar concentration affected the percentage of root formation and hyperhydration. The greatest amount of growth (fresh weight 29%, and dry weight 0.30%) were obtained in the high (2X) mineral concentration with low agar (6 gl^{-1}) treatment after 8 weeks culture period. The highest multiplication rate (7-8 number $month^{-1}$) was also obtained in the same treatment (2X mineral and 6 gl^{-1} agar concentrations). No hyperhydration was observed in high agar concentration treatments. This means, increasing agar concentration resulted in decreased hyperhydration phenomenon, however, growth and multiplication rate decreased as agar concentration increased. Highest percentage (68%) of root formation was obtained in low mineral and low agar concentration treatment. Multiplication rate was 2-4 $month^{-1}$ at low (0.5X) concentration and increased to 7-8 at high (2X) concentration.

Key words: Hyperhydration, medium composition, multiplication, root formation, tissue culture, *Prunus amygdalus*

Introduction

Almond (*Prunus amygdalus* L.) is one of the main nut fruits. It is traditionally propagated by seedling, budding or grafting on to seedling which is laborious and slow. Non uniform germination, prolonged seedling emergence, and disease susceptibility to mycoplasma are other problems related to tradtional propagation (Hammerschlag, 1986; Hartmann *et al.*, 1990). Many woody species are able to regenerate a whole plant from an *in vitro* cultured shoot-tip (Murashige and Huang, 1987). "Shoot tip culture" techniques have been used for the accelerated growth and multiplication rate and forms an important and advantageous tool for rapid mass propagation of disease-free plants. But there are still certain problems which limit its widespread use for almond (Rugini *et al.,* 1987). One of the major shortcomings of shoot tip culture technique in almond is low quantity and quality of growth and occurrence of hyperhydration (translucency) (Vieitez *et al.,* 1985; Rugini *et al.,* 1987; Pasqualetto *et al.,* 1988). Hyperhydration (succulency and glassiness) refers to a physiological and morphological disorder in tissue culture grown plants. It is a major problem in the tissue culture industry since it can affect shoot multiplication and culture vigour (Hammerschlag, 1986) and can impede the successful transfer of micropropagated plants to *in vivo* conditions. Up to 60% of affected plants fail to acclimatise (Paques and Boxus, 1987).

Hyperhydricity could be controlled by agar concentration (Debergh *et al.,* 1981; Paques and Boxus, 1987; Ghashghaie *et al.,* 1991). There is strong connection between the culture medium hardiness, the proliferation rate and vitrification. Lowering the nutrient gel hardiness increased the proliferation and vitrification rate of artichoke (Debergh *et al.,* 1981). Hyperhydration can be caused by high concentrations of minerals (nitrate ammonium) (Pasqualetto *et al.,* 1988). The gelling agent used in the medium can be another factor inducing hyperhydration *e.g.* Gelrite induces hyperhydration in apple (Pasqualetto *et al.,* 1988). Rugini *et al.* (1987) reduced vitrification drastically in almond replacing sucrose by fructose (45 gl^{-1}); Paques and Boxus (1987) avoided hyperhydration in liquid or solid medium with BAP (1 mgl^{-1}). They used a hydrosoluble agar fraction called "antivitrification complex". These materials are complex polysaccharides which, when dissolved in hot water or ionic solution, form cross-links between the macromolecules to create a solid medium (Williams, 1993). Physical parameters of gelled media such as water potential, medium conductivity and solute diffusion, which are responsible for better growth, are affected by gel brand and its concentration (Williams, 1993). Inherent properties (the chemical and physical) which control the availability of water and hence growth quality (hyperhydration), is related to gel concentration (Smith and Spomer, 1994; Williams, 1993).

Although the gelling agents reduce the incidence of hyperhydricity they have inhibited explant growth, compared to growth on liquid medium (Amiri, 2000). In this work, the effects of medium composition (minerals and gelling agent) were examined on almond.

Materials and methods

To measure the effect of medium constituents (minerals and gelled agent) on growth quantity (fresh and dry weight) and growth quality (plant appearance and hyperhydration) of almond, four relative mineral concentrations (0X, 0.2X, 1X and 2X basal medium) were used with de Fossard medium (de Fossard, 1976),

along with four different concentrations (4, 6, 8, 10 gl^{-1}) of agar (Bacto BiTeck agar). These treatments were supplemented with BA 0.75 mgl^{-1}. and NAA 0.75 mgl^{-1}, sucrose 3%, thiamine, myo-inositol and L-tyrosine. pH was adjusted to 5.6 by HCl 0.5 N and NaOH 0.5 N before autoclaving. Then medium was autoclaved by 101 KPs, 120° C for 15 min. Thirty ml of solution in each 250 ml polycarbonate container was dispensed (with 5 replicates of each treatment and the control).

Four uniform shoot tips (3-5 mm) of almond (*Prunus amygdalus* L. var. Binazir) were cultured on each medium treatment and subcultured aseptically in different containers. All explants were kept in a growth room at a temperature of 25 ± 2°C, with 55% relative humidity and cool white fluorescent tubes with a light intensity ranging from 16-50 µmol m^{-2} s^{-1}.

Fresh and dry weights (as growth rate) and multiplication rate, percentage of rooting and hyperhydration were measured at 6th and 8th week. Containers did not occupy fixed positions on culture shelves but were moved around randomly during visual examination every week.

Data was analysed by ANOVA and means subjected to LSD test (P=0.05) NEVA (Burr, 1980). All calculations were performed by Microsoft Excel. Four shoot tips were inoculated per container and four containers were maintained for each treatment. Each unit in the container was considered a replicate and data were analysed for a factorial experiment involving four levels of agar and four levels of mineral treatments.

Results and discussion

Both growth (fresh and dry weights) and multiplication rate of almond explants were dependent upon medium composition (minerals and agar). As mineral concentration increased, both growth and multiplication rate significantly (P=0.05) increased. But increase was not proportional. Whereas, there was a negative relationship between mineral concentration and root formation (Table 1). Furthermore, gelling agent affected the percentage of root formation and hyperhydration (Table 1). The greatest amount of growth (fresh weight 29%, and dry weight 0.30%) were obtained in the high (2X) mineral concentration with low agar (6 gl^{-1}) treatment after 8 weeks culture period (Fig. 1). The highest multiplication rate (7-8 no mon^{-1}) was also obtained in the same treatment (2X mineral and 6 gl^{-1} agar concentrations) (Fig. 2). The highest percentage (43%) of hyperhydration was observed in high (2X) mineral concentration with very low agar (4 gl^{-1}) treatment. In other words, growth quality (hyperhydration) of almond explants was significantly influenced by both mineral and agar concentrations in the medium. For example, hyperhydration of explants was observed in high concentration (2X) of minerals in low gelled medium. The greater amount of multiplication rate, fresh weight and dry weight as final growth of almond explants in low agar-gelled medium (6 gl^{-1}) treatment compared to high agar-gelled (10 gl^{-1}) medium (Table 1) has been reported previously by many authors (Debergh *et al.*, 1981; Bornman and Vogelmann, 1984; Kordan, 1988; and Ghashghaie *et al.,* 1991). The greatest multiplication rate and highest amount of growth (fresh and dry weight) in high mineral concentration (2X) with low agar concentration (6 gl^{-1}) treatment correspond with higher total uptake of minerals and water availability. Low agar-gelled medium behaves differently from high gelled medium, in many manners like water potential, mineral solubility, mineral mobility and availability to the explant. In other words, plant growth *in vitro* depends on mineral uptake and mineral availability. Mineral availability to the explant depends on mineral solubility and

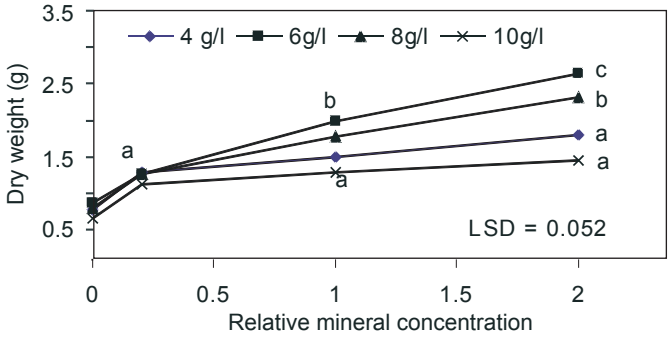

Fig.1. Final growth (dry weight) of four shoot-tips cultured almond (*Prunus amygdalus* L. var. Binazir) explants as affected by four levels (X0, X0.2, X1, and X2) of mineral supply and four levels (4, 6, 8, 10 agar gl^{-1}) of agar concentrations (at week 8).

Table 1. Effects of
and hyperhydration of almond (*Prunus amygdalus* L. var. Binazir) during 8 weeks culture.

Relative mineral concentration	Agar conentration (gl^{-1})	Fresh weight (g)	Dry weight (g)	Multiplication rate (number $month^{-1}$)	Rooting (%)	Hyperhydration (%)
0X	4	7.7	0.08	2.0	58	3.1
	6	8.6	0.09	2.5	56	0.0
	8	7.3	0.08	2.6	51	0.0
	10	6.0	0.07	2.0	46	0.0
0.2X	4	13.4	0.14	2.5	52	8.3
	6	13.1	0.14	3.5	47	6.2
	8	19.9	0.14	3.2	42	5.0
	10	26.9	0.12	2.4	38	0.0
1X	4	15.4	0.16	3.2	45	23.0
	6	21.2	0.22	6.0	40	13.4
	8	17.9	0.20	4.9	38	9.2
	10	12.6	0.14	2.8	30	5.3
2X	4	19.2	0.20	4.0	27	43.2
	6	28.8	0.30	7.8	23	28.8
	8	23.4	0.26	6.0	11	17.3
	10	14.4	0.16	3.0	2	12.8
LSD (P=0.05)		1.25	0.02	1.51	6.38	4.86

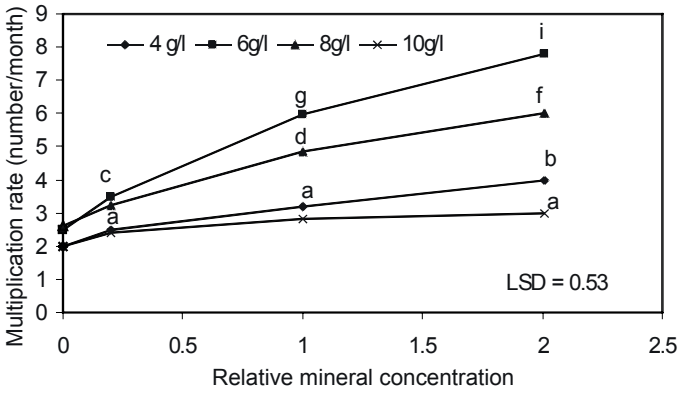

Fig. 2. Effect of four levels (X0, X0.2, X1, and X2) of mineral supply and four levels (4, 6, 8,10 agar gl^{-1}) of agar concentrations on multiplication rate of almond (*Prunus amygdalus* L. var. Binazir).

mineral transport through gel; both these depend on availability of free water (Amiri, 2000).

High rate of multiplication and non physiological disorders (hyperhydration) are the most important economical factors for successful mass propagation of almond. Although, increasing mineral concentration increased rate of multiplication, hyperhydration can be caused by high concentration of minerals (especially nitrate ammonium) (Pasqualetto *et al.*, 1988). Hyperhydration is reported to occur more often on rich (2X concentration) culture media such as Murashige and Skoog's (1962). The evidence of role of ammonium concentration on hyperhydration has been demonstrated by Letouze and Daguin (1983) on *Salix babylonica*. They induced hyperhydration in *Salix* by introducing an ammonium concentration equal to that of an ammonium MS medium into a Knop medium deprived of growth substances. These observations have been confirmed by the findings of Vieitez *et al.* (1985) on *Castanea sativa.* Furthermore, potassium and calcium ions concentration has been described much more higher in vitreous tissues than in normal ones (Pasqualetto *et al.,* 1988).

It can be concluded that improvements in micropropagation of almond is achievable by altering medium composition and water potential (agar and sucrose). It is possible to avoid hyperhydration and to reverse it if the phenomenon is not too advanced. Medium composition, especially, mineral concentrations and gelling agent, must be carefully adjusted.

Acknowledgments

This work has been supported by Grant No. NRCL 4255 of National Research Projects and with the support of National Research Council of Islamic Republic of Iran.

References

Amiri, M.E. 2000. Mineral uptake by banana (*Musa acuminata* L.) *in vitro*. *Acta Horticulturae,* 560: 387-391.

Bornman, C.H. and T.C. Vogelmann, 1984. Effect of rigidity of gel medium on benzyladenine-induced adventitious bud formation and vitrification *in vitro* in *Picea abies*. *Physiol. Plant.,* 61: 505-512.

Burr, E.J. 1980. *Analysis of variance for complete factorial experiments*. Third edition. University of New England, Armidale, NSW, Australia, Pp. 1-25.

de Fossard, R.A. 1976. *Tissue culture for plant propagation.* Univ. of New England, Armidale, NSW, Australia, pp. 123-87.

Debergh P., Y. Harbaoui and R. Lemeur, 1981. Mass propagation of globe artichoke (*Cynara scolymus*): Evaluation of different hypotheses to overcome vitrification with special reference to water potential. *Physiol. Plant.*, 53: 181-187.

Ghashghaie, J., F. Brenckmann and B. Saugier, 1991. Effects of agars concentration on water status and growth of rose plants cultured *in vitro*. *Physiol. Plant.,* 82: 73-78.

Hammerschlag, F.A. 1986. Temperate fruits and nuts. In*: Tissue Culture as a Plant Production System for Horticultural Crops.* Zimmerman R.H., Griesbach R.J., Hammerschlag F.A. & Lawson R.H. (eds.) Martinus Nijhoff, Dorbrecht. pp. 221-236.

Hartmann H.T., D.E. Kester and F.T. Davies, 1990. *Plant Propagation, Principles and Practices.* Prentice-Hall International, New Jersey, USA. pp.145-89.

Kordan, H.A. 1988. Inorganic ions present in commercial agars. *Biochem. Physiol. Pflanzen,* 183: 355-359.

Letouze, R. and F. Daguin, 1983. Manifestation spontanee et aleatoire d'une croissance anormale en culture *in vitro*. Recherche de marqueurs metaboliques. *Rev. Can. Biol. Exp.,* 42: 23-28.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.,* 15: 473-497.

Murashige, T. and L.C. Huang, 1987. Cloning plants by tissue culture: Early years, current status and future prospects. *Acta Hortic.*, 212: 35-42.

Paques, M. and P. Boxus, 1987. Vitrification: review of literature. *Acta Hortic.*, 212: 155-165.

Pasqualetto, P.L., R.H. Zimmerman and I. Fordham, 1988. The influence of cation and gelling agent concentrations on vitrification of apple cultivars *in vitro. Plant Cell Tiss. Org. Cult.,* 14: 31-40.

Rugini, E., P. Tarini and M.E. Rossodivita, 1987. Control of shoot vitrification of almond and olive grown *in vitro*. *Acta Hortic.*, 212: 177-183.

Smith, M.A.L. and L.A. Spomer, 1994. Vessels, gels, liquid media, and support systems. In: *Automation and Environmental Control in Plant Tissue Culture.* Aitken-Christie *et al.* (eds.), Kluwer Academic Publishers. pp. 371-404.

Vieitez, A.M., A. Ballester, M.C. San-jose and E. Vieitez, 1985. Anatomical and chemical studies of vitrified shoots of almond regenerated *in vitro*. *Physiol. Plant.,* 65: 177-184.

Williams, R.R. 1993. Mineral nutrition *in vitro*- A mechanistic aproach. *Aust. J. Bot.*, 41: 237-251.

Journal of Applied Horticulture, 8(1): 65-69, January-June, 2006

Chemical effect of reclaimed water on soil and rose plant grown in soil and tuff media

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Abstract

The effect of three irrigation regimes of low quality water (the effluent of reclaimed wastewater from Ramtha treating plant) on soil, drained water and plant tissue chemical composition of First Red cut flower rose cultivar grown on three rootstocks *Rosa indica, Rosa canina,* and Natal Briar was investigated for two successive years 2003 and 2004 in two planting media soil and Zeotuff. Phosphorus showed intermediate levels in both depths. Potassium in soil accumulated at high levels, especially at 0-20 cm depth. Manganese, copper, and zinc showed no accumulation in soil, iron reached high levels in both depths of soil. Less salinity build up was shown by the three irrigation treatments in soil than water drained from tuff beds regardless of rootstock used for the First Red rose cultivar during the first year, 2003. Both EC and SAR reached a steady status throughout the second year 2004. Based on the nutrient standards mentioned for rose tissue in the literature, the only macro and micro element accumulation was recorded for sodium in the tissue of First Red rose planted in both media during both years and iron in both media during the first year only, regardless of water treatment.

Key words: Rose, *R. indica, R. canina, R. hybrida,* salinity, reclaimed water, media, rootstock, sodium, tuff

Introduction

In middle east region, the challenge for agriculture is represented by the extreme difficulty to sustain high consumption levels of water currently required by growers, particularly due to limited water resources. The rapidly expanding population of the region has generated an ever-increasing volume of reclaimed water, which has raised question as how this type of water should be managed and possibly recycled for the benefit of the society. The main potential risks of reclaimed water reuse in agriculture is heavy metals accumulation in the soil and acidification impact (Water Corporation, 2003; Amin, 2001; and Kretschmer *et al.*, 2002). However, beneficial influences can be gained from this water such as conserving fresh water sources, reducing use of synthetic fertilizer, and improving soil properties (soil fertility) and producing higher yields (Kretschmer *et al.*, 2002).

Reclaimed water is applied mainly to field crops (Middle East Water Shortage, 2000), citrus trees irrigation in Florida (Parsons *et al.,* 1997) and the highway landscapes in Egypt (Heliopolis, 2001). In Jordan, there is a scope to explore potential alternative crops to make benefit and reuse of this low quality reclaimed water. To give new dimensions on reclaimed water reuse in agriculture, one of the proposed alternatives is use for cut flower crops roses (*R. hybrida*) since it is planted on profitable and sustainable bases.

Roses have been classified as high salinity tolerant up to 3-4 dS/m level (Kotuby *et al.*, 2000), or sensitive (Chimonidou, 1997) or highly salt sensitive (Western Australia Department of Agriculture, 2003) with EC level as low as 0.8-1.0 dS/m. Moreover, it is also reported that roses could resist up to 6 dS/m without affecting yield and quality of roses produced (Chimonidou, 1997). This study was conducted for two successive years at the National Center for Agricultural Research and Technology Transfer "NCARTT", Jordan. to assess prospects of reclaimed water reuse and its chemical effects on soil and plants of cut flower rose cultivar grafted onto three rootstocks in soil and zeotuff (soilless culture system) under plastic house conditions.

Materials and methods

This study was carried out during 2003 and 2004 using Mini-plants of First Red cut flower rose cultivar grafted onto three rootstocks: *R. indica,* Major; *R. canina,* Inermis; and *R. hybrida,* Natal Briar. The plants were planted in a plastic house of 360m^2 area, controlled by pad and fan system in Ramtha area 60 km North of Amman. Two planting media in two separate experiments in the plastic house were used, the natural soil (soil chemical characteristics are shown in Table 1), and volcanic rock Zeotuff (soilless culture system). Experimental plots were made as 0.6 x 1 m area and 8 plants were planted in two rows spaced 25 × 40 cm for both cultural media. Soilless plots were made by 700µ black polyethylene mulch, sloped to 1.5% for excess water drain.

The plants were irrigated by three irrigation regimes of the outlet reclaimed water of the Ramtha wastewater treatment plant with EC, 2.5-3.0 dS/m (water chemical characteristics used in irrigation are shown in Table 2) as follows: daily irrigation at levels of 120, 100 and 80% of the pan evaporation readings for the soilless system, and 100 % of the evaporation reading, every other day, every two days, and every three days for the soil experiments. Drip irrigation system was used with three filtering (sand, screen, and disc) process without any addition of fertilizers. Rose plant combinations and water treatments were arranged as Split-Plots in a randomised complete block design (RCBD) with four replications for each experiment. Disease pest control program was done when needed during the experiment duration. To assess chemical effects of reclaimed water reuse on

soil and plants, data on the following parameters were collected: (i) soil chemical analysis at the end of each season, (ii) chemical analysis of drained water from soilless beds at the end of each season, (iii) salinity EC and Sodium Adsorption Ratio (SAR) values of soil and drained water during the experiment time. (iv) Chemical plant tissue analysis at the end of each season

All the results were statistically analysed and mean separation was performed using LSD (P=0.05).

Table 1. Soil chemical characteristics before planting

Parameter	Soil depth (cm)	
	0 - 20	20 - 40
pH	7.80	7.80
EC dS/m	0.65	0.55
Total(+)	6.31	5.43
Ca (Meq/L)	2.20	1.70
Mg (Meq/L)	3.00	2.66
Na (Meq/L)	1.11	1.07
Cl (Meq/L)	20.00	15.00
HCO_3 (Meq/L)	2.49	2.49
P (ppm)	24.50	21.10
K (ppm)	369.00	331.00
Cd (ppm)	0.03	0.03
Mn (ppm)	0.57	0.57
Cu (ppm)	0.07	0.07
Fe (ppm)	1.05	1.05
Zn (ppm)	2.30	4.80
NO_3 (ppm)	12.80	18.60
SAR	0.69	0.73

Results and discussion

There was no significant difference between the three water levels in their effect on the chemical composition of soil and drained water from the tuff beds. Table 3 shows that irrigation with saline reclaimed water caused noticeable increase in all macro and micro- elements concentrations at the end of the first year at both depths of the soil, except for the zinc that showed a decrease in its concentration at the end of the first year.

During the second year of irrigation with saline reclaimed water, no changes in magnesium, manganese, and nitrate concentration were recorded compared to their concentrations in both soil depths at the end of the first year (Table 3). Although concentrations of sodium, chloride, phosphorus, potassium, calcium, iron, and zinc increased at the end of the second year of irrigation in both depths of soil compared to their concentrations in the first year (Table 3).

For the drained water from tuff beds (Table 4) there was a high increase in the concentrations of sodium, magnesium, calcium, chloride, nitrate, copper, and cadmium elements during the first year of irrigation compared to the water source composition. However, most of the elements showed no further increase during the second year compared to the first year (Table 4).

The only increase in concentrations was recorded for sodium and chloride in the drained water from tuff beds compared to concentrations at the end of the first year (Table 4). The three

Table 2. Reclaimed water chemical characteristics used in irrigation

Characterstics	Value
pH	7.50
EC dS/m	3.07
TDS (ppm)	1964.00
Na (Meq/L)	13.40
Mg (Meq/L)	7.40
Ca (Meq/L)	7.30
Cl (Meq/L)	13.00
HCO_3 (Meq/L)	8.25
P (ppm)	2.00
K (ppm)	47.40
NO_3 (ppm)	48.70
Na(%)	47.60
Zn (ppm)	0.044
Fe (ppm)	0.421
Cu (ppm)	0.006
Mn (ppm)	0.019
Cd (ppm)	0.006
SAR	4.490

Table 3. Soil chemical characteristics of rose beds irrigated with reclaimed water for two years

Parameter	First year		Second year	
	0 – 20	20 – 40	0 – 20	20 – 40
pH	7.76	7.63	7.70	7.60
Ca (Meq/L)	11.33	10.22	18.99	15.10
Mg (Meq/L)	9.88	9.22	8.77	9.05
Na (Meq/L)	17.00	14.01	28.93	22.43
Cl (Meq/L)	17.21	16.38	34.16	28.11
HCO_3 (Meq/L)	2.44	2.44	2.48	2.48
P (ppm)	72.01	50.73	86.08	57.33
K (ppm)	754.84	589.43	832.06	663.56
Cd (ppm)	0.09	0.09	0.21	0.20
Mn (ppm)	2.70	2.70	2.98	2.98
Cu (ppm)	1.50	1.50	1.60	1.60
Fe (ppm)	8.01	8.15	30.56	20.58
Zn (ppm)	1.80	1.80	9.61	8.19
NO_3 (ppm)	88.30	88.30	90.50	90.50

levels of saline reclaimed water showed similar trends of progressive salinity build in the first year in the soil (regardless of depth) and drained water from tuff beds until July month (Fig. 1). After that they showed almost steady state to the end of the year 2003. However, salinity was greater in the drained water from tuff beds than in the soil beds. SAR values also increased progressively during the first year in both depths of soil 0-20 cm and 20-40 cm (Fig.1A).

It reached higher values in the upper depth 0-20 cm than in the lower depth 20-40 cm. In the drained water from the tuff beds, SAR reached higher values than in the soil (Fig. 1B). During the second year, no change occurred in salinity and the SAR values in the soil nor salinity in the drained water from tuff beds when irrigated with the three levels of saline reclaimed water (Fig. 2A).

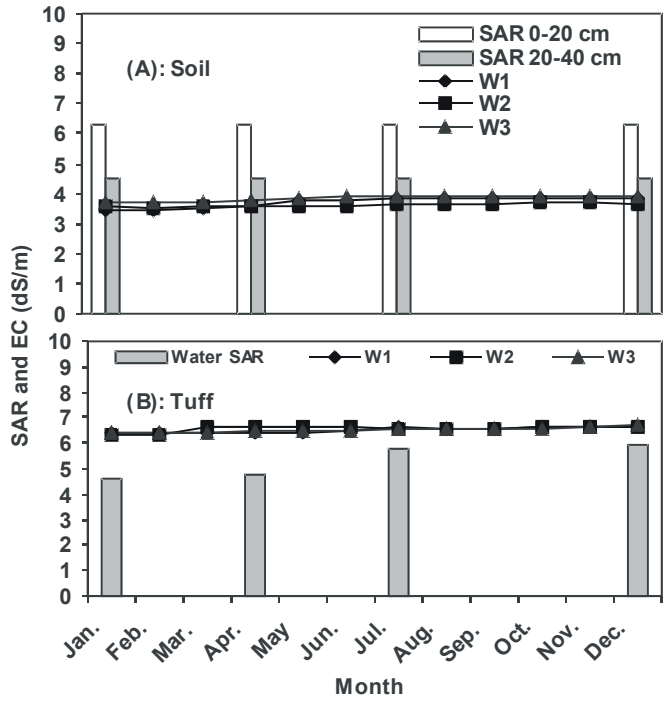

Fig. 1. Salinity and SAR variations in soil (A) and drained water of zeotuff (B) for experimental plots during season 2003 planted with First Red rose cultivar grown on three rootstocks. Water levels: For Soil: W1=(Every other day); W2= (Every two days); W3= (Every three days). And For Tuff: W1=(120%);W2=(100%); W3=80%) of the evaporation pan reading.

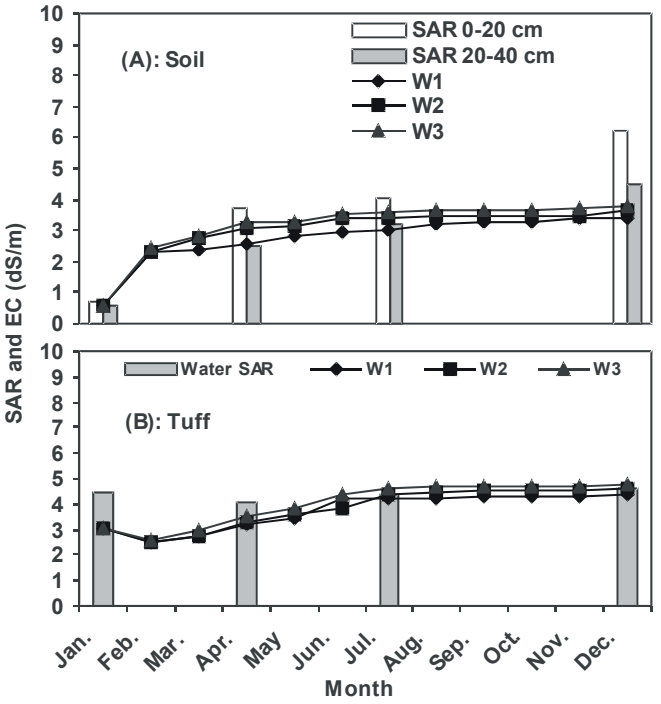

Fig. 2. Salinity and SAR variations in soil (A) and drained water of zeotuff (B) for experimental plots during season 2004 planted with First Red rose cultivar grown on three rootstocks.Water levels: For Soil: W1=(Every other day); W2= (Every two days); W3= (Every three days). And For Tuff: W1=(120%);W2=(100%); W3=80%) of the evaporation pan reading.

While only slight increase occurred to the SAR valus during the second year of experiment (Fig. 2B).

Rose tissue mineral composition was compared with the optimum nutrient levels decleared by the Agriculture Western Australia, 1998. There was no significant difference between the three rose rootstocks with regard to their effect on chemical composition of the tissue of First Red rose cultivar irrigated with the three levels of saline reclaimed water during both years planted in both media. Fig. 3 shows that there were no accumulation of nitrogen, phosphorus, potassium, calcium, and magnesium in the rose plant tissue (regardless of rootstock) planted in both media when irrigated with the three levels of saline reclaimed water during both the years. Accumulation was recorded only for sodium in the tissue of rose planted in tuff medium during the first year and both media during both the years irrigated with the three levels of water (Fig. 3). During the first year of experiment, the only micro-element accumulation in the tissue of rose plants was iron in both the media, when irrigated with the three levels of water (Fig. 3).

No accumulation was recorded for other micro-elements manganese, zinc, and copper in the rose plant tissue during this year. Additinally, no accumulation was noticed in the tissue of rose planted in both media during the second year of experiment irrigated with the three levels of saline reclaimed water (Fig. 3). During the first year in soil only the higher level of water (every other day) caused this accumulation of sodium in the rose tissue compared to the other two water levels, every two days and every three days.

In second year of irrigation with reclaimed wastewater (Table 3), calcium concentrations in soil reached intermediate levels (18.99, 15.1 meq/L) while magnesium reached up to very high levels (8.77, 9.05 meq/L) for both depths compared to the FAO (1980) limits (17.6-40 meq L^{-1} for calcium and >8 meq L^{-1} for magnesium). Sodium concentrations in soil reached (28.93, 22.43 meq/L) however, it still less than the high levels (32 meq/L) of Ilaco (1985). Phosphorus showed intermediate levels (86.08, 57.33 ppm) for both depths compared to Bookers (1984) category (80-200 ppm) for the USA, while high concentration of potassium accumulated in soil, specially at 0-20 cm depth , 754.84 and 832.06 ppm (Table 3) for both years, respectively compared to the limits (156 ppm) of FAO (1980).

Manganese, copper, and zinc showed no accumulation in both depths of the soil at the end of both years (Table 3). They were within lower levels as per standards of FAO (1980), while iron reached high levels in both years of irrigation at the two depths of soil.

All properties and nutrient contents of the reclaimed water used in irrigation (Table 2) were within the Jordanian standards limits of 2002 for reclaimed domestic water allowed for agricultural irrigation. After two years of irrigation with such water (Table 4), drained water from tuff beds had characteristics that are still within the limits of the Jordanian standards (2002). No accumulation for any of the macro and micro nutrients was recorded.

Less salinity build up was shown by the three irrigation treatments in soil than the tuff drained water when planted with First Red rose cultivar regardless of rootstock used during the first year 2003 (Fig. 1). Soil plots showed salinity build up to 3.7 dS/m, while the drained water of the tuff plots reached up to 4.7 dS/m. This was accompanied by gradual increase of the SAR value in

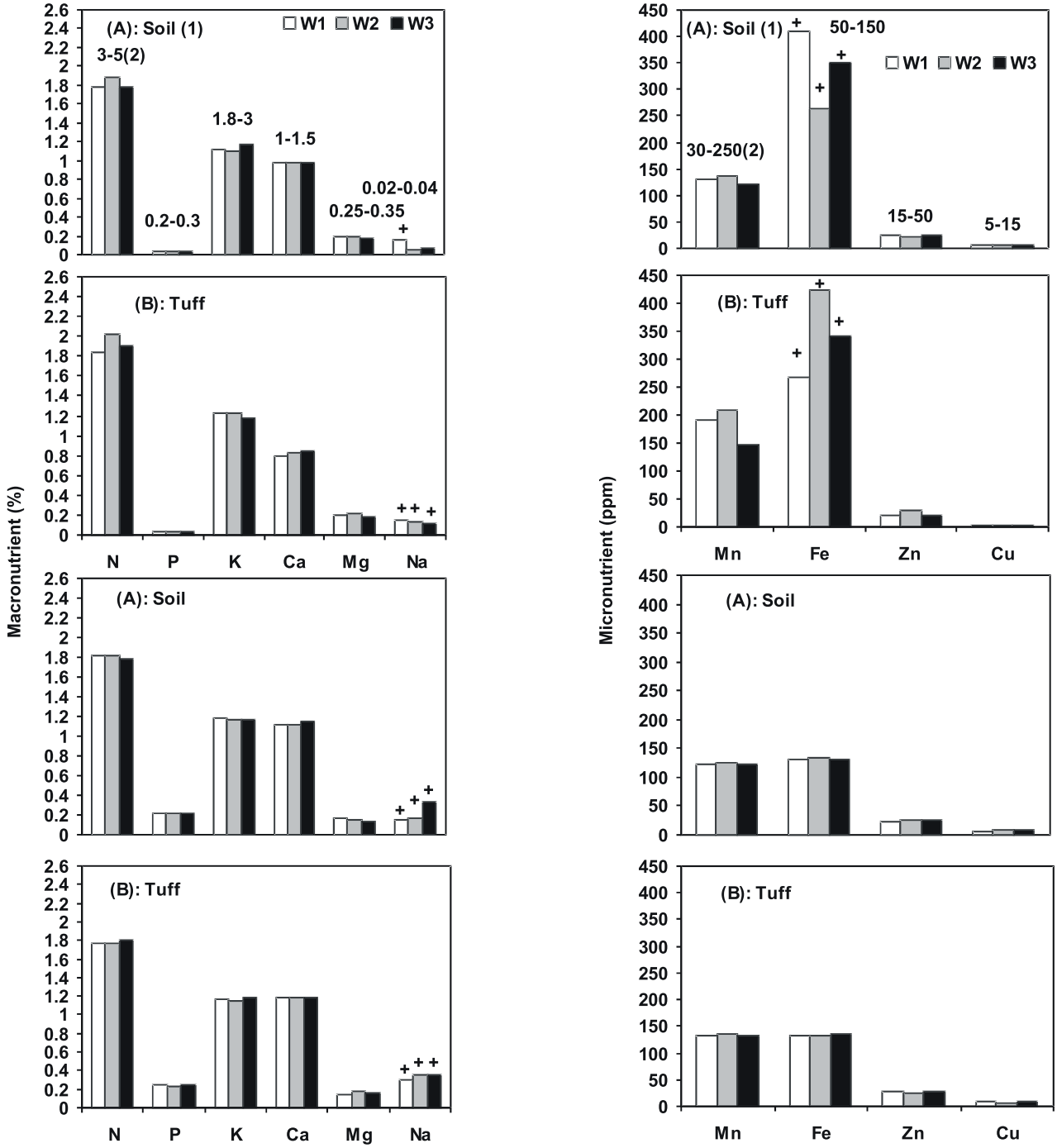

Fig. 3. Macro and micro-element contents in rose plant tissue irrigated with three levels of reclaimed water from Ramtha during 2003 and 2004. (1): Water levels: For Soil: W1=(every other day); W2= (every two days); W3= (every three days) and for Tuff: W1=(120%);W2=(100%); W3=80%) of the pan evaporation reading.

both the media. However, SAR value of the soil was still less than the FAO (1980) standards at 0-20 cm depth and much less at the 20-40 cm depth (Fig. 2).

Both EC and SAR reached a steady status throughout the second year 2004 (Fig. 2). By the end of year 2004, soil salinity, 3.92 dS/m (Fig. 2) was still within the very slightly saline category of the USDA (1969), 2-4 dS/m. Salinity (6.68 dS/m) of drained water from tuff beds exceeded the 3.2 dS/m allowed by the Jordan standards (2002). While the SAR value of 5.9 was much less than 9 that stated in the standards.

According to the optimum nutrient levels in rose tissue given by the Agriculture Western Australia (1998), the only macro and micro element accumulation was recorded for sodium in the tissue of First Red rose planted in both media during both years and iron in both media during the first year only, regardless of water treatment (Fig. 3)

After two years of irrigation with reclaimed wastewater phosphorus showed intermediate levels in both depths, potassium considerably accumulated in soil, especially in the depth 0-20 cm. Manganese, copper, and zinc showed no accumulation in soil, iron reached high levels in both years of irrigation in both depths of soil.

Less salinity build up was shown by the three irrigation treatments in soil than the tuff drained water when planted with First Red

rose cultivar regardless of rootstock used during the first year (2003). Both EC and SAR reached a steady status in the second year (2004).

Table 4. Chemical characteristics of drained water from tuff beds irrigated with RW for the two years

Parameter	Year	
	First	Second
pH	7.90	8.00
Na (Meq/L)	19.21	25.32
Mg (Meq/L)	14.80	15.40
Ca (Meq/L)	19.80	20.82
Cl (Meq/L)	37.80	38.90
HCO_3 (Meq/L)	6.32	6.30
P (ppm)	2.01	2.20
K (ppm)	30.32	32.84
NO_3 (ppm)	318.40	331.50
Zn (ppm)	0.04	0.04
Fe (ppm)	0.11	0.10
Cu (ppm)	0.01	0.01
Mn (ppm)	0.01	0.01
Cd (ppm)	0.01	0.01

As per published optimum nutrient levels for rose, the only macro and micro element accumulation was recorded for sodium in the tissue of First Red rose planted in both media during both years and iron in both media during the first year only.

Acknowledgement

All thanks and gratitude are extended to the Arab Fund for Economic and Social Development for their financial support and funding the implementation of this project.

References

Agriculture Western Australia. 1998. Fertilizers for Greenhouse Roses. Bulletin 4303. The Highway to Horticulture CD. http://www.agric.wa.gov.au/agency/pubns/h2h/5c744d6.htm

Amin, J.S. 2001. Waste as resource. http://lists.isb.sdnpk.org/pipermail/ngo-list/2001-december/001526.html

Bookers. 1984. *Tropical Soil Manual*, Booker Agriculture International Ltd, Longmans.

Chimonidou, P.D. 1997. Response of Roses to Salinity and Irrigation. Proceeding of the 1st Trans-National Meeting on "Salinity as Limiting Factor for Agricultural Productivity in The Mediterranean Basin" Napoli, Italy, 24-25 March 1997,189-199.

FAO. 1980. Soil and Plant Testing as a Base of fertilizer Recommendations. FAO, Calcareous Soils, Rome.

Hasek, R.F. 1980. Carnations. In: *Introduction to Floriculture*. Larson, R.A. Academic Press. Harcourt Brace Jovanovich.

Heliopolis, 2001. Egypt Goes Green. http://www.heliopolisegypt.com/012001/earth.htm

Ilaco. 1985. Agricultural Compendium for Rural Development in Tropics and Sub-Tropics, Elsevier.

Jordan Standards. 2002. Water-Reclaimed Domestic Wastewater. JS 893/2002.

Kotuby, Amacher J., R. Koeing and B. Kitchen, 2000. Salinity and Plant Tolerance. Utah State University Extension. http://www.extension.usu.edu/publica/agpubs/agso03.pdf.

Kretschmer, N., L. Ribbe and H. Gaese, 2002. Wastewater Reuse for Agriculture. http://www.tt.fh-koeln.de/publications/ittpub301202_4.pdf

Middle East Water Shortage, 2000. http://weather.nmsu.edu/hydrology/wastewater/wastewater.htm

Parsons, L., A. Wheaton and J. Jackson, 1997. Reclaiming Waste Water for Irrigation in Florida. http://www.agweb.okstate.edu/agbase/agbex99-19.htm

USDA. 1969. Diagnosis and Improvement of Saline and Alkali Soils Agriculture Handbook No. 60, USDA.

Water Corporation, 2003. Treated Wastewater Re-Use. Bulletin No.2. www.watercorporation.com.au

Western Australia Department of Agriculture. 2003. Salinity Tolerance Chart. http://staneyo.com/news_files/water/salinity_chart.html.

Journal of Applied Horticulture, 8(1): 70-74, January-June, 2006

Partial ringing and liquid nitrogen effects on shoot growth and fruit quality of peach

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Abstract

Effect of partial ringing and liquid nitrogen application on the growth and fruit quality of peach was studied. Twelve five–year–old peach trees (*Prunus persica* [L.] Batsch.), cv. 'Hikawa Hakuho' grafted on wild peach rootstock were randomly selected for this experiment in April 2004. A 4 cm wide partial ring of bark was removed from eight of them at a height of 25 cm above the graft union leaving a 5 mm connecting strip. Four of the ringed trees were treated with liquid nitrogen at the ringed portions while the rest were intact trees as controls. Both partial ringing and partial ringing plus liquid nitrogen treatment led to reduced shoot length, fruit acidity, total shoot length and weight of pruned branches but increased soluble solids content. Liquid nitrogen had little additive effect on partial ringing in terms of these parameters. Both treatments had a similar effect on tree and fruit characteristics as evidenced by similar bark width recovery and fruit diameter. The use of partial ringing plus liquid nitrogen application in commercial peach orchards promises to be slightly more efficient in causing shoot length reduction while improving fruit quality.

Key words: Brix, dwarfing techniques, liquid nitrogen, partial ringing, total shoot length

Introduction

Fruit producers are always looking for ways to reduce farm operation costs while maintaining high fruit quality. The changing trends from agrochemical based production to green farming has made farmers to seek for practices that are less harmful to the environment and leave less or no chemical residue in fruit. Dwarf trees are easy to manage and early maturing. Dwarfing has been accomplished in other fruit trees like apples by using dwarfing rootstocks. The main factor limiting the use of size controlling rootstocks in stone fruit production is the lack of suitable rootstocks with a wide range of compatibility among cultivars (De Jong *et al.*, 2001). This calls for the need to explore alternative dwarfing techniques. Partial ringing or an enhanced application of the technique is being proposed as a method that can be used to meet some of the current challenges in the fruit tree industry by reducing tree height.

Ringing severes phloem vascular vessels thereby preventing translocation of photosynthates from the source to sinks located below the girdle until the wound heals. Thus, ringing has an indirect effect of reducing sink size and increasing the amount of photosynthates available to fruits and other active meristems above the girdled region (Krezdorn and Brown, 1970; Poll *et al.*, 1991). Ringing not only affects basipetal movement of assimilates (Schaper and Chacko, 1993) but also that of phytohormones (Cohen, 1981; Monselise, 1986).

A number of workers have reported useful data on the application of various forms of girdling in fruit production. Ebell (1971) used overlapping, half – circumference – band girdles in which 25 mm wide strips of bark and phloem were removed from opposing sides of the stem. Wheeler *et al.* (1985) working on Douglas fir compared partial – overlapping – band girdles to similar girdles applied with a pruning saw. They found both methods increased cone yield. Onguso *et al.* (2004) reported that partial ringing and partial ringing plus trunk heating had led to a reduction in shoot length while improving fruit quality.

Ringing imposes several effects on the plant, including direct injury and as such it elicits several responses (Cohen, 1981). Partial ringing and partial ringing plus liquid nitrogen application experiments were carried out to study their possible contribution to dwarfing in peach trees. Iwahori *et al.* (1976) in their work on improvement of fruit quality in ponkan reported earlier fruit colour and soluble solid enhancement from xylem ringing compared to phloem ringing. It is postulated that increase in total soluble solids from girdling or related effects occurs in treatments, which also reduce water movement considerably such as xylem ringing (Iwahori *et al.*, 1990) and trunk strangulation (Yamanishi, *et al.*, 1993; Yamanishi, *et al.*, 1995). Therefore, we treated ringed trees with liquid nitrogen to investigate its effect on xylem transport. Application of liquid nitrogen was to effectively kill the regenerative cambium that manufactures new phloem tissues to try and bridge the wound (Nix, 2005). The burning effect of liquid nitrogen would possibly extend to the xylem below and affect water and mineral salts transport hence exerting an effect on the sinks above the ring.

Although stem girdling has received substantial attention, there is limited literature on the combination of partial girdling and liquid nitrogen application on shoot growth and fruit quality. In the present study, the interaction of bark width and shoot growth in peach has been investigated.

Materials and methods

The experiment was conducted at the Ehime University Experimental Farm located in southern Japan, 33°57' N, 132° 47' E at an elevation of about 20 m above sea level. Twelve

five–year–old peach trees (*Prunus persica* [L.] Batsch.), cv. 'Hikawa Hakuho' grafted on wild form rootstocks were used for this study starting from April 2004. A 4 cm wide partial ring of bark was removed from eight of them at a height of 25 cm above the graft union to leave a 5 mm connecting strip. Four of the ringed trees were treated with liquid nitrogen at the ringed portions using a pair of forceps and cotton wool while the rest were controls. The tree form was central leader and trees were spaced at 3 x 2.5 m in a complete randomised design. Routine cultural practices were carried out as required. The growth of the trees was monitored weekly by measuring shoot length of ten terminal shoots, and diameter of ten fruits from each tree. The growth of the 5 mm bark that was left after ringing was also monitored weekly using vernier calipers.

At harvesting, final fruit diameter, fruit number and weight were recorded. Juice was extracted from ten fruits per tree and titratable acidity was determined by acid-base titration using 0.1 N NaOH. The soluble solids content in the juice (Brix) was also measured by means of a refractometer (Atago PR-1). These were repeated at harvesting time of the following year (2005). In February 2005 the percentage of flower buds, total shoot length and weight of pruned branches were also taken.

Results and discussion

The shoots of trees subjected to partial ringing plus liquid nitrogen recorded the shortest lengths (Fig. 1). They were followed by those subjected to partial ringing alone while the control had the highest shoot length throughout the study period.

The total shoot length was greatly reduced in the trees subjected to partial ringing plus liquid nitrogen (Fig. 2). Those subjected to partial ringing alone had a reduced shoot length too but to a lesser degree. This shows the cumulative effect of partial ringing and partial ringing plus liquid nitrogen on tree growth.

The weight of pruned branches from the trees subjected to partial ringing plus liquid nitrogen was greatly reduced compared to those where partial ringing alone was done and the controls (Fig. 3). This shows that application of partial ringing plus liquid nitrogen can decrease labor costs incurred for pruning and thinning.

Bark recovery steadily increased in the trees subjected to both partial ringing and partial ringing plus liquid nitrogen though it was slightly higher in partial ringing plus liquid nitrogen (Fig. 4). There was a positive correlation between shoot length and bark growth for both partial ringing and partial ringing plus liquid nitrogen (Fig. 5). This implies that an increase in bark healing

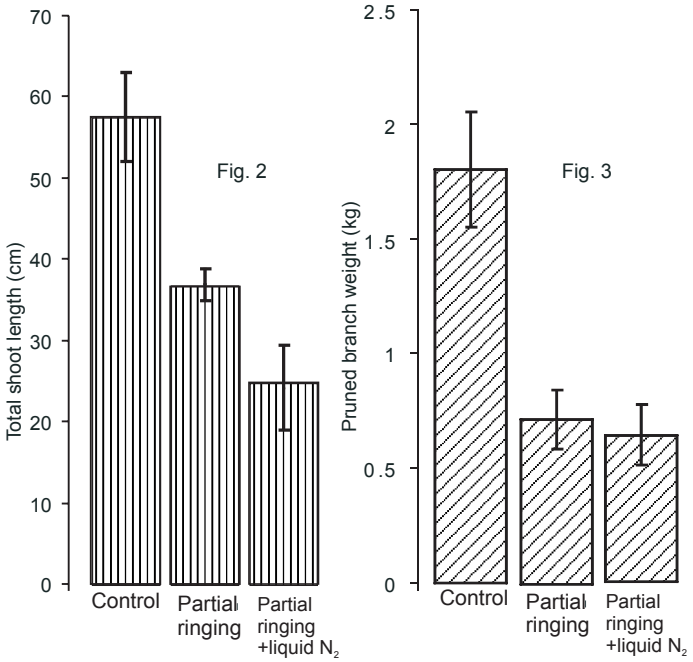

Fig. 2. The total shoot length of peach trees subjected to partial ringing and partial ringing plus liquid nitrogen application. Vertical bars represent SE.
Fig. 3. The weight of branches pruned from peach trees subjected to partial ringing and partial ringing plus liquid nitrogen application. Vertical bars represent SE.

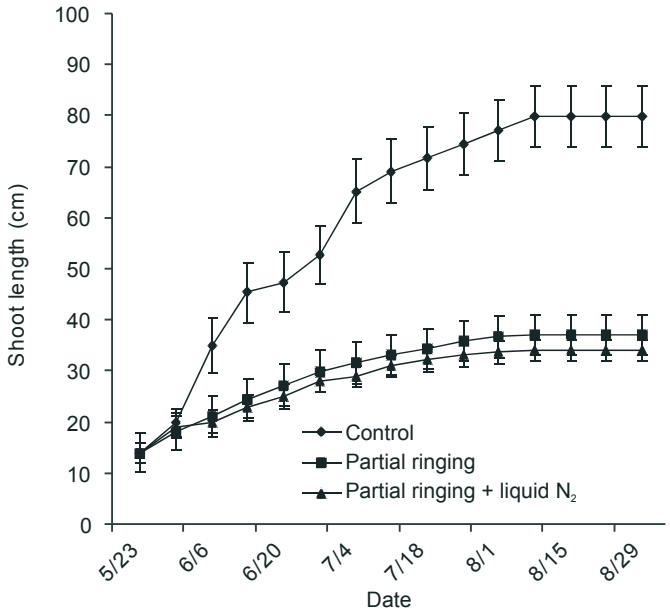

Fig. 1. The shoot length of peach trees as influenced by partial ringing and partial ringing plus liquid nitrogen application. Vertical bars represent SE.

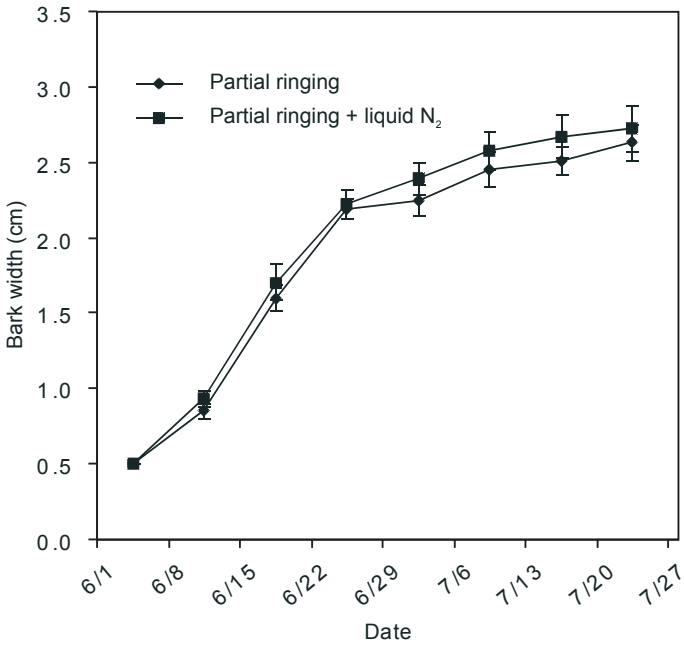

Fig. 4. The bark width of peach trees as influenced by partial ringing and partial ringing plus liquid nitrogen application. Vertical bars represent SE.

Table 1. Fruit quality at harvest in peach trees as affected by partial ringing and partial ringing plus liquid nitrogen application (2004)

Treatment	Fruit weight (g)	Fruit number/tree	Brix (%)	Acid content (%)
Control	181.6±15.5	9.1±1.6	9.46±0.13	0.35±0.08
Partial ringing	203.6± 9.2	9.3±2.1	12.25±0.27	0.26±0.03
Partial ringing +liquid nitrogen	186.6±13.9	9.3±1.4	12.63±0.25	0.29±0.02

Table 2. Fruit quality at harvest in peach trees in the following year as affected by partial ringing and partial ringing plus liquid nitrogen (2005)

Treatment	Fruit weight (g)	Fruit number/tree	Brix (%)	Acid content (%)
Control	197.0± 6.1	12.0±0.8	14.11±0.45	0.37±0.06
Partial ringing	210.8±13.2	14.0±2.4	14.23±0.58	0.26±0.01
Partial ringing +liquid nitrogen	213.2±11.1	14.3±4.3	14.29±0.18	0.24±0.01

Values are means ± standard error (n=4).

contributed to increased shoot length. Increased bark width leads to higher translocation of photosynthates as well as water and mineral salts thus leading to increased shoot length.

Fruit diameter showed a similar growth pattern for all the experimental trees (Fig. 6). This shows that both treatments maintained the normal fruit growth characteristics thus helping to assure acceptable consumer quality.

The fruit weight from ringed trees was slightly higher than that

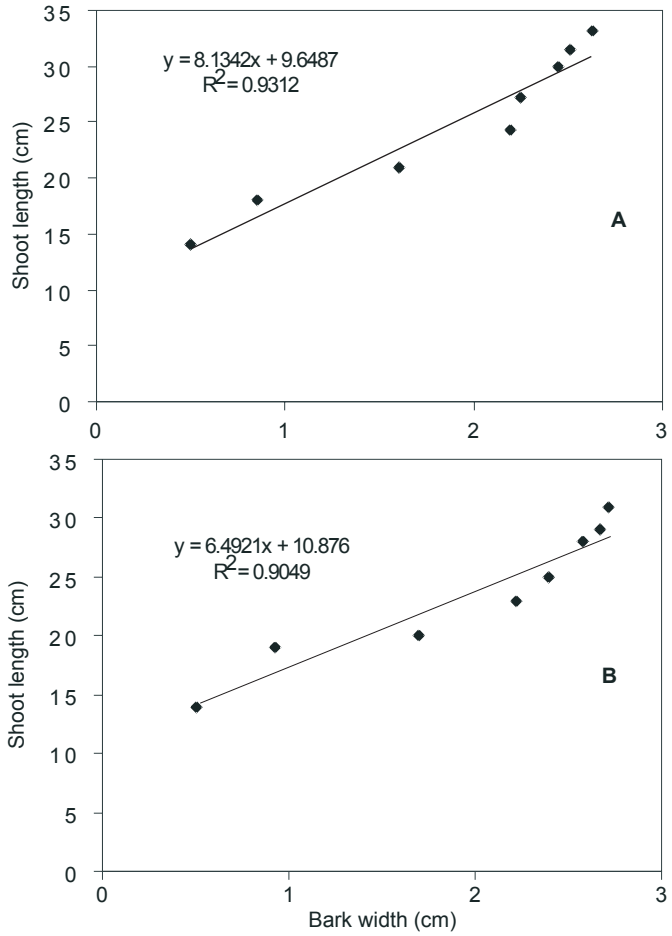

Fig. 5. The correlation between bark width regrowth and shoot length in peach trees subjected to partial ringing (A) and partial ringing plus liquid nitrogen application (B).

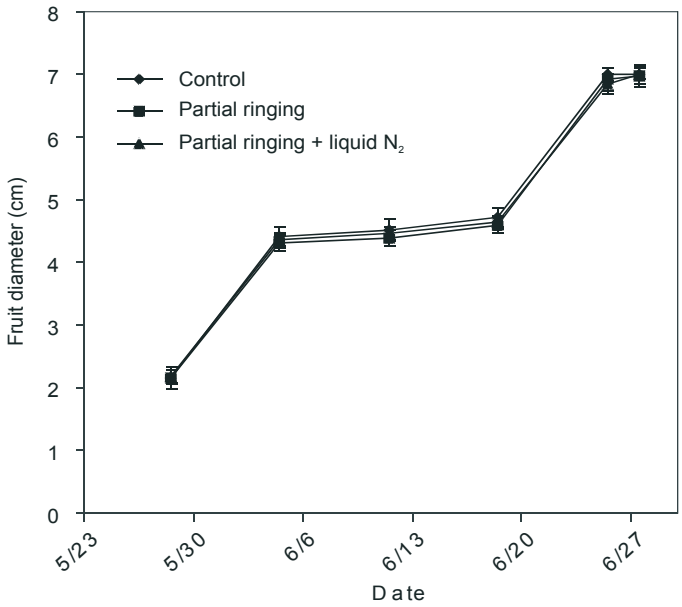

Fig. 6. The fruit diameter of peach trees as influenced by partial ringing and partial ringing plus liquid nitrogen application. Vertical bars represent SE.

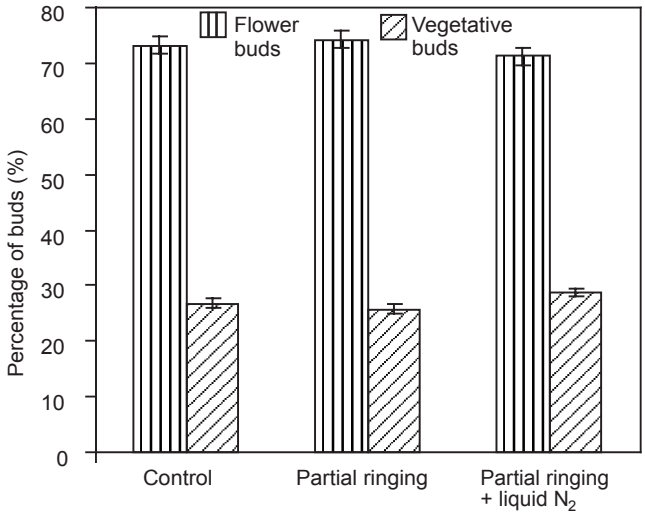

Fig. 7. The percentage of flower and vegetative buds in peach trees the following year as influenced by partial ringing and partial ringing plus liquid nitrogen. Vertical bars represent standard error.

from partial ringing plus liquid nitrogen and from control ones which had similar values in the first year (Table 1). This implies that ringing alone might be effective in improving fruit weight. In the following year, the fruit weight was generally lower in all the trees than was the case in the previous year (Table 2). This might be attributed to the prolonged drought that hit the area during the year. The partially ringed trees were more seriously affected than the partially ringed plus liquid nitrogen application and the control ones. Fruit number per tree was slightly enhanced by the treatment as all the treated trees recorded higher values than the controls in the first year (Table 1). In the following year, the same trend prevailed though the fruit number per tree was much higher with partial ringing plus liquid nitrogen application showing the highest improvement (Table 2).

The yield per tree was slightly improved by partial ringing plus liquid nitrogen compared to partial ringing alone in the first year (data not shown). In the following year, the same trend was maintained though the overall yield drastically reduced due to the drought (data not shown). Partial ringing plus liquid nitrogen application still led to highest yield. Stem girdling is known to improve fruit yield and quality (Iwahori *et al.*, 1990; Cohen, 1981; Monselise, 1986; Krajewski and Rabe, 1995; Yamanishi *et al.*, 1993; Yamanishi *et al.*,1995). Girdling severes phloem vascular vessels thereby preventing translocation of photosynthates from the source to sinks located below the girdle until the wound heals. Thus, girdling has an indirect effect of reducing sink size and increasing the amount of photosynthates available to fruits and other active meristems above the girdled region (Krezdorn and Brown, 1970; Poll *et al.*, 1991). The 5 mm connecting strip that was left in our treatments however, allowed some photosynthates to move to the sink below the ring hence resulting in only a slight increase in yield.

Partial ringing plus liquid nitrogen caused an increase in soluble solids content (SSC) as compared to partial ringing alone in the first year (Table 1) implying that partial ringing plus liquid nitrogen had some additive effect. The control showed a lower SSC than both partial ringing and partial ringing plus liquid nitrogen. In the following year, overall SSC was higher in all the trees with the treated trees recording better values than the control (Table 2). Fujishima *et al.* (2005) reported that girdling of 'Pione' grapevine led to a significant increase in SSC, coloring and anthocyanin content. Iwahori *et al.* (1976) in their work on improvement of fruit quality in ponkan reported early fruit colour development and soluble solid enhancement from xylem ringing compared to phloem ringing. It is postulated that increase in total soluble solids from ringing or related effects occurs in treatments which also reduce water movement in the xylem.

Both partial ringing and partial ringing plus liquid nitrogen led to reduced acidity (Table 1) though partial ringing alone caused a higher reduction in the first year. This might be due to bark width recovery as well as the diminishing effect of liquid nitrogen applied with time. The values were almost similar for the treated trees but the acid content in the fruit from the control trees slightly increased in the second year (Table 2).

There was a slight improvement in the percentage of flower buds in partially ringed trees due to the treatment (Fig. 7). The trees subjected to partial ringing plus liquid nitrogen had a slight increment in vegetative buds. This was in agreement with Arakawa *et al.* (1997) who stated that flowering in apple trees was significantly increased by girdling. Mataa *et al.* (1998) also reported a slight increment in flower number in ponkan mandarin due to ringing.

Girdling blocks the translocation of sucrose from leaves to the root zone through phloem bundles. The block causes a decrease in starch content in the root system (Schneider, 1954) and an accumulation of sucrose in the leaves (Plaut and Reinhold, 1967). Partial girdling however allowed partial flow of sucrose and starch along the 5 mm strip that was left. Onguso *et al.* (2004) reported that partial ringing plus trunk heating had little additive effects on shoot length and fruit quality of peach compared to partial ringing alone.

Our results demonstrate that it is possible to control total shoot length by combining partial ringing and liquid nitrogen application. Further, there is the added improvement of fruit quality in terms of increased Brix and reduced acidity.

Acknowledgement

The authors are grateful to the Ministry of Education, Culture, Sports, Science and Technology of Japan for supporting this work. They also thank Dr. K.L. Rutto for proof reading the manuscript and Mr. J. Nakamura, Ms. Y. Fujita and Ms. M. Shiota for their useful discussions and technical assistance.

References

Arakawa, O., K. Kanno, A. Kanetsuka and Y. Shiozaki, 1997. Effects of girdling and bark inversion on tree growth and fruit quality of apple. *Acta Hortic.*, 451: 579-586.

Cohen, A. 1981. Recent developments in girdling citrus trees. *Proc. Int. Soc. Citric*., 1: 196-199.

De Jong, T.M., A. Weibel, W. Tsuji, J.F. Doyle, R.S. Johnson and D. Ramming, 2001. Evaluation of size controlling rootstocks for California peach production. *Acta Hortic.*, 557: 103-110.

Ebell, L. F. 1971. Girdling: its effect on carbohydrate status and reproductive bud on cone development of Douglas fir. *Can. J. Bot.,* 49: 453-466.

Fujishima, H., M. Shiraishi, S. Shimomura and Y. Horie, 2005. Effects of girdling on berry quality of 'Pione' Grapevine. *Hort. Res.*, 4: 313-318.

Iwahori, S., R. Matsumoto and J.T. Oohata, 1976. The influence of ringing and defoliation on maturation of ponkan (*Citrus reticulata* Blanco). *Bull. Fac. Agric*., Kagoshima Univ. 27: 1-6.

Iwahori, S., A. Garcia-Luis, P. Santamarina, C. Monerri and J.L. Guardiola, 1990. The influence of ringing on bud development and flowering in satsuma mandarin. *J. Exp. Bot.*, 41: 1341-1346.

Krajewski, A.J. and E. Rabe, 1995. Citrus flowering: a critical evaluation. *J. Hort. Sci.*, 70: 357-374.

Krezdorn, A.H. and H.D. Brown, 1970. Increasing yields of the 'Minneola', 'Robinson' and 'Osceola' varieties with gibberellic acid sprays and girdling. *Proc. Fla. State Hort. Soc*., 83: 29-34.

Mataa, M., S. Tominaga and I. Kozaki, 1998. The effect of time of girdling on carbohydrate contents and fruiting in ponkan mandarin (*Citrus reticulata* Blanco). *Scientia Hortic*., 73: 203-211.

Monselise, S.P. 1986. Citrus: fruit ontogeny. In: *Handbook of Fruit Set and Development.* S.P. Monselise (Ed.), CRC Press, Boca Raton, FL, pp. 87-108.

Nix, S. 2005. Killing a tree without chemicals: organic weed-tree control. http://forestry.about.com/od/treephysiology/a/kill_tree_organ.htm.

Onguso, J.M., F. Mizutani and A.B.M. Sharif Hossain, 2004. Effects of partial ringing and heating of trunk on shoot growth and fruit quality of peach trees. *Bot. Bull. Acad. Sin*., 45: 301-6.

Plaut, Z. and L. Reinhold, 1967. The effect of water stress on the movement of ^{14}C-sucrose of tritiated water within the supply of leave of young bean plants. *Aust. J. Biol. Sci*., 20: 297-307.

Poll, van der D., J.E. Miller and P. Allan, 1991. The effect of girdling on the physiological factors controlling bud take, bud burst and scion growth in citrus. *J. S. Afr. Soc. Hort. Sci*., 1: 55-58.

Schaper, H. and E.K. Chacko, 1993. Effect of irradiance, leaf age, chlorophyll and branch-girdling on gas exchange of cashew (*Anacardium occidentale* L.) leaves. *J. Hort. Sci*., 68: 541-550.

Schneider, H. 1954. Effect of trunk girdling on phloem of trunk of sweet orange trees on sour orange root-stocks. *Hilgardia*, 22: 593-601.

Wheeler, N.C., C.T. Masters, S.C. Cade, S.D. Ross, J.W. Keeley and L.Y. Hsin, 1985. Girdling: an effective and practical treatment for enhancing seed yields in Douglas fir seed orchards. *Can. J. For. Res.*, 15: 505-510.

Yamanishi, O.K., Y. Nakajima and K. Hasegawa, 1993. Influence of trunk strangulation on growth and water absorption by young pummelo trees exposed to high temperatures. *J. Jpn. Soc. Hort. Sci.*, 62: 345-352.

Yamanishi, O.K., Y. Nakajima and K. Hasegawa, 1995. Effect of trunk strangulation degrees in late season on return bloom, fruit quality and yield of pummelo trees grown in a plastic house. *J. Jpn. Soc. Hort. Sci*., 64: 31-40.

Journal of Applied Horticulture, 8(1): 75-77, January-June, 2006

Performance of three sweet orange varieties grafted on four rootstocks under Jordan Valley conditions

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Abstract:

Fruit quality of three orange varieties: 'Salustiana', 'Pineapple' and 'Hamlin' grafted on four rootstocks *viz.,* Sour orange (*Citrus aurantium*), 'Cleopatra' mandarin (*C. reticulata*), *C. volkameriana* and *C. macrophylla* were evaluated in Jordan Valley. Results indicated that sweet orange grafted on *C. macrophylla* and *C. volkameriana* gave the largest fruit weight, diameter and length, while those grafted on 'Cleopatra' mandarin gave the smallest fruit. In addition, 'Salustiana' on *C. macrophylla*, 'Pineapple' on 'Cleopatra' mandarin and 'Hamlin' on both *C. volkameriana* and 'Cleopatra' mandarin gave the highest juice percentage, however, 'Salustiana' on sour orange, 'Pineapple' on *C. macrophylla* and 'Hamlin' on sour orange and *C. macrophylla* had the least. Orange trees on sour orange and 'Cleopatra' mandarin gave the highest TSS percentage, while on *C. volkameriana* and *C. macrophylla* it was low. Moreover, 'Salustiana' grafted on *C. macrophylla* gave low juice pH while on 'Cleopatra' mandarin it gave high juice pH, the opposite was observed for 'Pineapple' and 'Hamlin'.

Key words: Fruit quality, rootstocks, sweet orange, *Citrus sinensis,* juice content

Introduction

Citrus is a major crop throughout the world as well as in Jordan covering about 6200 hectare (Ministry of Agriculture, 2002). In Jordan, most of citrus trees are grafted on sour orange, which is known for its resistance to gummosis, and high tolerance to wet calcareous soils (Wutscher, 1979), making it well adapted to surface irrigation system used by many farmers in the Jordan Valley. The fact that sour orange is susceptible to viral diseases such as 'Tristeza', and to avoid risk of incidence in future in citrus orchards in Jordan Valley, several rootstocks had been introduced and tested for its compatibility, tolerance and adaptability.

Citrus rootstocks have been used for a long time and their effects on the performance and characteristics of citrus scion cultivars have been reported and it differ in their effects on tree size, vigour, productivity and fruit quality, disease and pest resistance, and tolerance to different soil conditions such as salinity and acidity (Wutscher, 1979). Differences in production and tree size has been noticed from 'Washington navel', 'Valencia' orange, and 'Minneola' tangelo grafted on several rootstocks (Roose *et al.*, 1989). In addition, 'Shamouti', 'Jaffa', 'Valencia' and 'Navel' sweet orange grafted on different rootstocks differed in respect to tree size and growth and fruit quality (Ghnaim, 1993; Mehrotra *et al.*, 2000 and Zekri and Al-Jaleel, 2004).

'Valencia' orange grafted on *C. volkameriana* rootstock gave the highest production and rind thickness compared with those on sour orange and 'Cleopatra' mandarin, while trees grafted on both rootstocks gave the highest juice content (Reyes *et al.*, 1984). 'Hamlin' and 'Valencia' sweet orange produced smaller trees, better yield and fruit quality and economic returns on a moderate vigour rootstock than those on a vigorous rootstock (Wheaton *et al.*, 1991 and Wheaton *et al.*, 1995). 'Hamlin' orange grafted on *C. volkameriana* gave the largest fruit weight and diameter and rind thickness than those on sour orange which gave moderate values except for juice %, TSS, and acidity which were the highest (Wutscher and Bistline, 1988). 'Marsh' and 'Red blush' grapefruit grafted on Palestine sweet lime and *C. volkameriana* gave the highest production compared to those on sour orange, also, rootstocks affected grapefruit volume, weight, rind thickness, juice content, and TSS % (Economides *et al.*, 1993; Fallahi *et al.*, 1989 and Ramin and Alirezanezhad, 2005).

This study was carried out to evaluate fruit characteristics of three sweet orange varieties ('Salustiana', 'Pineapple' and 'Hamlin') grafted on four citrus rootstocks grown in the Jordan Valley.

Material and methods

Citrus orchard was established in 1980 to study the performance of sweet orange varieties (*C. sinensis* Osbeck) grafted on four rootstocks: Sour orange (*C. aurantium* L.), 'Cleopatra' mandarin (*C. reticulata* Blanco.), Volkamer lemon (*C. volkameriana* L.*)*, and Macrophylla (*C. macrophylla* Wester.) and spaced at 6 x 6 m. The trees received uniform standard cultural practices as practiced by orchardists in the Jordan Valley. The experiment was designed in Randomised Complete Block Design with three replicates and one tree on each rootstock per replicate.

Presented data is the average of 1992 to 2000 seasons and for three sweet orange varieties: 'Salustiana', 'Pineapple' and 'Hamlin'. Rootstock effect on sweet orange varieties was evaluated in relation to fruit characteristics, so in each season 10 kg of fruit from each replicate for each variety on different rootstocks were collected and analysed for fruit weight, length and diameter, seed number, peel thickness, juice % (w/w), total soluble solids and juice pH.

Data was statistically analysed by ANOVA and mean separation was by Least Significant Differences (LSD) value (P=0.05).

Results and discussion

Physical Fruit Characteristics

Average fruit weight: Results indicated that sweet orange varieties grafted on 'Cleopatra' mandarin had the smallest fruit. 'Salustiana' trees grafted on *C. macrophylla* gave the highest fruit weight (213.5 gm) but with no significant difference with *C. volkameriana* and Sour orange rootstocks (Table 1). While 'Pineapple' trees grafted on *C. volkameriana* gave the highest fruit weight (184.1 gm) but with no significant difference with those on *C. macrophylla*. Moreover, 'Hamlin' grafted on *C. macrophylla* significantly gave the highest fruit weight (199.7 gm) (Table 1).

Table 1. Effect of rootstocks on average fruit weight (g) for three sweet orange varieties

Treatments	Average fruit weight (g)		
	'Salustiana'	'Pineapple'	'Hamlin'
'Cleopatra' mandarin	167.8 b	163.3 c	154.4 b
Sour Orange	193.3 a	167.6 bc	155.2 b
C. macrophylla	213.5 a	178.5 ab	199.7 a
C. volkameriana	204.8 a	184.1 a	170.1 b

* Mean separation within columns by LSD test, values that don't share the same letters are significantly different (P=0.05).

Fruit diameter and length: Results also indicate that sweet orange varieties grafted on 'Cleopatra' mandarin had least fruit diameter. 'Salustiana' trees on *C. volkameriana* rootstock recorded the largest fruit diameter (78.4 mm) but with no significant differences with Sour orange and *C. macrophylla* (Table 2). For 'Pineapple', no significant differences were observed among the rootstocks. However, 'Hamlin' trees grafted on *C. macrophylla* significantly gave the largest fruit diameter (71.5 mm) followed by those on *C. volkameriana* and Sour orange (69.1 and 67.7, respectively) (Table 2).

Table 2. Effect of rootstocks on average fruit diameter for three sweet orange varieties

Treatments	Average fruit diameter (mm)		
	'Salustiana'	'Pineapple'	'Hamlin'
'Cleopatra' mandarin	70.9 b	69.5 a	65.5 c
Sour Orange	74.3 ab	70.9 a	67.7 b
C. macrophylla	73.8 ab	70.1 a	71.5 a
C. volkameriana	78.4 a	70.5 a	69.1 b

* Mean separation within columns by LSD test, values that don't share the same letter are significantly different (P=0.05).

As in fruit diameter, the similar trend was observed for fruit length in which sweet orange varieties grafted on 'Cleopatra' mandarin significantly gave the least fruit length. 'Salustiana' trees grafted on *C. macrophylla* rootstock gave the largest fruit length (76.0 mm) but with no significant differences with *C. volkameriana* (Table 3). 'Hamlin' trees grafted on *C. macrophylla* significantly gave the largest fruit length (72.9 mm) followed by those on *C. volkameriana* and Sour orange (Table 3).

Table 3. Effect of rootstocks on average fruit length for three sweet orange varieties

Treatments	Average fruit length (mm)		
	'Salustiana'	'Pineapple'	'Hamlin'
Cleopatra mandarin	67.7 c	67.6 a	65.1 c
Sour Orange	71.2 bc	68.7 a	66.4 bc
C. macrophylla	76.0 a	70.9 a	72.9 a
C. volkameriana	74.1 ab	70.1 a	67.8 b

* Mean separation within columns by LSD test, values that don't share the same letters are significantly different (P=0.05).

Fruit seed number: Results show that there was no significant difference among rootstocks for both 'Salustiana' and 'Pineapple' oranges, however, trees grafted on *C. macrophylla* gave the highest seed number. The opposite was observed for 'Hamlin', in which trees grafted on *C. macrophylla* gave the lowest seed number (2.2 seeds), while trees grafted on 'Cleopatra' mandarin, Sour orange and *C. volkameriana*, gave high fruit seed number (Table 4).

Table 4. Effect of rootstocks on seed number per fruit for three sweet orange varieties

Treatments	Average seed number		
	'Salustiana'	'Pineapple'	'Hamlin'
Cleopatra' mandarin	2.7 a	16.0 a	4.1 a
Sour Orange	2.4 a	16.8 a	3.9 a
C. macrophylla	3.0 a	17.4 a	2.2 b
C. volkameriana	2.4 a	16.3 a	3.8 a

* Mean separation within columns by LSD test, values that don't share the same letter are significantly different (P=0.05).

Chemical Fruit Characteristics

Juice percentage (w/w): Data in Table 5 indicate that 'Salustiana' trees grafted on *C. macrophylla* rootstock had highest fruit juice percentage (46.9 %), followed by those grafted on *C. volkameriana*, and 'Cleopatra' mandarin, however, trees grafted on Sour orange gave the lowest fruit juice content (40.7 %). For 'Pineapple', no significant difference was observed among rootstocks. In addition, 'Hamlin' trees grafted on *C. volkameriana* and 'Cleopatra' mandarin significantly gave high fruit juice percentage (52.4 and 50.0%, respectively), however, trees on Sour orange and *C. macrophylla* significantly gave low fruit juice percentage (45.5 and 45.6%, respectively) (Table 5).

Table 5. Effect of rootstocks on juice % (w/w) for three sweet orange varieties

Treatments	Juice % (w/w)		
	'Salustiana'	'Pineapple'	'Hamlin'
'Cleopatra' mandarin	42.6 b	44.4 a	50.0 a
Sour Orange	40.7 c	43.0 a	45.5 b
C. macrophylla	46.9 a	42.4 a	45.6 b
C. volkameriana	42.1 bc	41.8 a	52.4 a

* Mean separation within columns by LSD test, values that don't share the same letters are significantly different (P=0.05).

Total soluble solids: Results indicated that sweet orange varieties grafted on Sour orange gave the highest total soluble solids percentage followed by those grafted on 'Cleopatra' mandarin. For 'Salustiana', trees grafted on *C. volkameriana* gave the lowest fruit TSS (10.8 %) (Table 6). For 'Pineapple', trees grafted on *C. macrophylla* significantly gave the lowest fruit TSS percentage (11.7 %). In addition, 'Hamlin' trees on *C. macrophylla* significantly gave the lowest TSS (10.0 %) (Table 6).

Juice pH: No significant difference was observed among rootstocks for 'Salustiana' trees, however, trees grafted on *C.*

Table 6. Effect of rootstocks on total soluble solids of three sweet orange varieties

Treatments	TSS (%)		
	'Salustiana'	'Pineapple'	'Hamlin'
'Cleopatra' mandarin	12.6 a	12.3 ab	12.0 b
Sour Orange	13.1 a	13.1 a	13.0 a
C. macrophylla	11.6 b	11.7 b	10.0 c
C. volkameriana	10.8 c	12.4 ab	11.2 b

* Mean separation within columns by LSD test, values that don't share the same letters are significantly different (P=0.05).

macrophylla gave low fruit juice pH (3.76), while trees grafted on 'Cleopatra' mandarin had high juice pH (4.08) (Table 7). The opposite was observed for 'Pineapple' and 'Hamlin' oranges, in which trees grafted on *C. macrophylla* gave high juice pH (3.84 and 4.01, respectively) while those on Sour orange and 'Cleopatra' mandarin gave low fruit juice pH (Table 7).

Table 7. Effect of rootstocks on juice pH for three sweet orange varieties

Treatments	Juice pH		
	'Salustiana'	'Pineapple'	'Hamlin'
'Cleopatra' mandarin	4.08 a	3.64 b	3.71 b
Sour Orange	3.77 a	3.63 b	3.80 b
C. macrophylla	3.76 a	3.84 a	4.01 a
C. volkameriana	3.94 a	3.75 ab	3.75 b

* Mean separation within columns by LSD test, values that don't share the same letters are significantly different (P=0.05).

Several investigations have been conducted on the rootstock effect on citrus fruit quality and there are conflicting results since it would not be realistic to expect a rootstock to induce a radical change in fruit quality. Results indicated that rootstocks and scions interact in many ways including at least 14 fruit quality factors influenced by the rootstock (Wutscher, 1979). In general, several researchers found that trees on sour orange can produce medium-sized to large fruit (Wutscher, 1979), and this was observed in this study for all the three varieties in respect to fruit weight, diameter and length.

In addition, trees grafted on sour orange produce fruit with high total soluble solids (TSS) and high juice acidity (Wutscher, 1979) and this statement agrees with our findings. Trees grafted on sour orange are also expected to produce fruit with high juice acidity and this statement generally agrees with our findings. However, for fruit juice percentage, the result of this study do not agree with the results of Wutscher and Bistline (1988), who found that 'Hamlin' orange grafted on sour orange gave the highest fruit juice percentage.

On the other hand, many researchers found that trees grafted on lemon rootstocks (*C. volkameriana* and *C. macrophylla*), produce usually larger fruit with poor fruit quality: thick rinds, low total soluble solids and low juice acidity (Reyes *et al.*, 1984; Salibe and Mischan, 1984 and Wutscher and Bistline, 1988) and this view corroborates with our results.

Trees grafted on 'Cleopatra' mandarin produced small fruits with high total soluble solids and juice acidity than on other rootstocks (Wutscher, 1979). These findings are, in general, agreement with the results of this study except for juice acidity in 'Salustiana' variety.

The study revealed that Sour orange, 'Cleopatra' mandarin, Volkamer lemon (*C. volkameriana* L.) and Macrophylla (*C. macrophylla* Wester.) rootstocks affected external and internal fruit quality of 'Salustiana', 'Pineapple' and 'Hamlin' sweet orange varieties including fruit weight, length, diameter, seed number, juice content, total soluble solids and juice pH. Trees on sour orange produced medium-sized fruits with high TSS and juice acidity. While, those on *C. volkameriana* and *C. macrophylla* produced larger fruit with low TSS and juice acidity. Whereas, trees on 'Cleopatra' mandarin produced small fruits with high TSS and juice acidity.

References

Economides, C.V and C. Gregrious, 1993. Growth, yield and fruit quality of nucellar Frost 'Marsh' grapefruit on fifteen rootstocks in Cyprus. *J. Amer. Soc. Hort. Sci.,* 118(3): 326-329.

Fallahi, E., J.W. Moon and D.R. Rodney, 1989. Yield and quality of 'Red blush' grapefruit on twelve rootstocks. *J. Amer. Soc. Hort. Sci.*, 114(2): 187-190.

Ghnaim, H., 1993. Performance of 'Shamouti' orange grafted on some citrus rootstocks in Jordan Valley. MSc. Thesis, University of Jordan, p.65.

Mehrotra, N.K., H. Kumar, V.K. Vij and P.S. Aulakh, 2000. Performance of Jaffa cultivar of sweet orange (*Citrus sinensis* (L.) Osbeck) on different rootstocks. *J. Research Punjab Agricultural University*, 37(1&2): 56-60.

Ramin, A. and A. Alirezanezhad, 2005. Effects of citrus rootstocks on fruit yield and quality of Ruby Red and Marsh grapefruit. *Fruits*, 60(5): 311-317.

Reyes, F.J., E. Monteverde and M. Espinoza, 1984. Preliminary results of 'Valencia' orange on several rootstocks in Venezula. *Proc. Int. Soc. Citriculture,* 1: 34-35.

Roose, M.L., D.A. Cole and R.S. Atkupper, 1989. Yield and tree size of four citrus cultivars on 21 rootstocks in California. *J. Amer. Soc. HortSci.,* 114 (4): 678-684.

Salibe, A.A. and M.M. Mischan, 1984. Effect of ten rootstocks on tree size, early bearing and fruit quality of Satsuma mandarin trees. *Proc. Int. Soc. Citriculture*, 1: 55-57.

Ministry of Agriculture, The Hashemite Kingdom of Jordan, 2002. Annual Statistical Report.

Wheaton, T.A., W.S. Castle, J.D. Whitney and D.P.H. Tucker, 1991. Performance of citrus scion cultivars and rootstocks in a high-density planting. *HortScience*, 26(7): 837-840.

Wheaton, T.A., J.D. Whitney, W.S. Castle, R.P. Muraro, H.W. Browning, and D.P.H. Tucker, 1995. Citrus scion and rootstock, topping height, and tree spacing affect tree size, yield, fruit quality, and economic return. *J. Amer. Soc Hort. Sci.*, 120(5): 861-870.

Wutscher, H.K. 1979. Citrus rootstocks. *Horticultural Reviews*, 1: 237-269.

Wutscher, H.K. and F.W. Bistline, 1988. Performance of 'Hamlin' orange on 30 citrus rootstocks in southern Florida. *J. Amer. Soc Hort. Sci.,* 113: 493-497.

Zekri, M. and A. Al-Jaleel, 2004. Evaluation of rootstocks for Valencia and Navel orange trees in Saudi Arabia. *Fruits*, 59: 91-100.

Journal of Applied Horticulture, 8(1): 78-81, January-June, 2006

Comparison of bananas ripened by two methods for textural sale-grades

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Abstract

This study relects on varied maturity levels of available raw and ripe bananas at market level, scope of improvements in quality of bananas by ripening technique and to generate newer avenues for value addition. Raw bananas from the market were ripened in June month both by crude market and standard BIS methods. The final ripeness textural range differed due to ripening methods used {28.56 N (crude market method) and 46.57 N (BIS method) a 63.06 % increase} as compared to available ripe grades in market (same month –June) (13.01 ± 0.99 N) entering after ripening by crude method. Initial texture of available raw grades used as above (June month) was 99.36 ± 10.84 N. The over-ripe bananas (~15 % of bananas available for sale in mandi) if used for beverage yielded an alcoholic drink (with ~8 % alcohol). The processed over-ripe bananas were compared to sale of over-ripe bananas to show potential value addition.

Key words: Banana handling, artificial ripening, texture, Ethephon, banana beverage, texture, and banana grades.

Introduction

India contributes 23.69 % of the world's share of bananas (*Musa* sp.) (UNCTAD, 2000). Transit losses amount to 8.0 – 10 % and 5- 8 % (producer to wholesaler and wholesaler to consumer respectively) in a single wholesale market (mandi) of Bhopal (NHB, 2000). The major problems of festooning banana quality are physical damage, decay and uncrown with unpredictable ripening. Proper maturity at harvest, freedom from defects, care and speed in handling with avoidance of chill injury may reduce the mixed ripe problem in the banana industry (Marchal, 1997; Peacock and Blake, 1970). Ripening characteristics [colour, firmness, and weight of banana (Gutierrez, 1999), vary with country of origin, days in transit, season of the year, maturity when harvested. Pre-mature ripening is probably the biggest cause of loss to the banana trade. There has been difficulty in determining climacteric size of bananas [to reach the market in green condition (Peacock and Blake, 1970)] due to seasonal changes in maturity parameters during growth itself, harvest maturity (as related to bunch age and green life) that varied from one harvest date to another (Montoya *et al.*, 1984). Under these difficulties standard ripening methods that defined banana quality (BIS, 1988) are available but merchants use crude methods. There was thus a need to provide demonstrable results that may be helpful for extension workers to explain the usefulness of standard ripening methods. Our study thus focused on comparing crude method with available standard method for raw bananas ripening.

Materials and methods

Overview and survey of artificial ripening in mandi revealed some traders used calcium- carbide (a banned chemical still used in mandis) (~ 250 g / 100 stacked bunches without ice). We used ethephon at recommended levels @1000ppm (Bureau Indian Standards, 1988) in our experimental set up on artificial ripening. Raw bananas that arrived in wholesale market (Nav Bahar Market, Bhopal) were sampled for 7 different months. Individual banana weights differed if they were in upper or lower portion of the bunch as also noted earlier (Peacock, 1975). Thus due care was taken to get even grade of bunches, collecting them from nearby positions on same main stalk (with bunches having similar curve angle of bananas). Banana samples from bunches were drawn randomly and observed for initial texture and other parameters. Ripe bananas, sampled randomly (as for raw bananas) from 3-4 retailers of the wholesale market (for good representation), were used (sampled in triplicate) for physical texture, colour and physiological parameters {mass, and moisture contents of pulp and peel (% wb)}. Pulp growth increases with the declining growth of peel, especially towards commercial maturity and provides a quantitative estimate of fruit development in relation to postharvest performance (Simmonds, 1966). Thus Pulp: Peel ratio of bananas was observed which gave an estimate of the stage of fruit maturity.

The texture (force of compression of bananas- N at three different positions of the bananas) was measured on a Texture analyzer TA X- 2Ti with a hemispherical plastic probe. The colour (average of three replicates) was observed with a Hunter colour Lab Spectrophotometer with Universal V 3.71 software. The 'CIE-Lab' scale readings were taken using (10/D65 scale) wherein illuminant was D 65 at 10^{o} observer angle. The values were 'L' 0-100 (low to high scale); 'a' more red (+) or more green (-); 'b' more yellow (+) more blue (-). Also a scale of 1 to 8 (von Loesecke, 1949): 1=hard green; 2 = sprung and green; 3 = more green than yellow; 4 = more yellow than green; 5 = green tipped; 6 = fully yellow; 7 = flecking; 8 = browning and over ripe was used to differentiate visibly. Standard deviations on triplicate observations of each month in artificial ripening were represented.

A comparative study of bananas for changes in texture, pulp colour and other physiological parameters, during green to ripe condition, was done (in June month) in banana ripened by two methods: "Crude market" and 'BIS' method (BIS, 1988), simultaneously. Firstly bananas sampled (three replicates) from bunches were observed for physical and physiological changes. Then the BIS method was compared to crude market method of artificial ripening, to demonstrate scope of improving quality (textures, reduced weight loss in ripened condition) of bananas.

For BIS method of ripening, one set (triplicate) of sampled banana bunches were surface dipped in Ethephon (39 % Ethrel solution) @ 1000 ppm of ethylene released from it (*i.e.* ~46 ml/5 l water for 10 min). They were placed in cool chamber (16 –18 °C as per specified by BIS). The other set of raw banana bunches (triplicate) were surface dipped in Ethephon solution (3 lid full =21 ml/10 l water for 10 min) as done in crude market method. They were covered with a gunny bag and over them was placed a slab of ice (~250 g) (in such a way that ice load was not on the bananas directly and only helped to cool as it trickled on melting) and placed in a corner of the room. Fresh ice was placed every 24 h. The approximate temperature under the gunny bag ranged between 19-36 °C. The ripened bananas were observed till 4 d [by 24 h ripening difference observations].

Sampled fresh fruit mass of ripening bananas, moisture contents of peel and pulp were observed during artificial ripening period (Ranganna, 1991).

A survey of the market on the availability of over-ripe bananas was also undertaken. The total over ripe bananas actually present in throwaway condition (sold by 'thela' vendors) was estimated. The monetary losses incurred due to over ripe bananas were estimated to establish the scope of value addition by processing over ripe bananas into banana alcoholic beverage. Over-ripe bananas were processed to an alcoholic beverage (Caputi *et al*, 1965; Pethe, *et al.*, 2003). Alcoholic beverage yield and cost of processing was compared to the losses incurred by sale of over-ripe bananas to show scope of value addition over sale of low quality (over-ripe) bananas if any. Data was analyzed applying one way and two way ANOVA in CRD using MSTATC software

Results and discussion

The banana grades, available in different months in wholesale market of Bhopal (India), differed in maturity (texture, colour and physiological) (Fig. 1, 3a). The compressible texture of raw grades from any month when compared to ripe grades showed higher compressible force texture (Fig. 1) with variations in all months. The raw and ripe grades ranged from 121.18 to 153.19 N (mean 137.81 ± 12.66 N) and 11.46 to 27.44 N (mean 16.18 ± 5.99 N) texture force of compression, respectively. The unevenness in maturity of bananas (Fig. 1, 3a, b, c) as available in market - sale grades was thus a result of uneven harvest maturity (Pp:Pl ratio of raw grades), artificial ripening (of uneven grades used) and also the natural variations due to spiral maturity that already existed in a single stalk of bananas [top to bottom- (Peacock, 1975)]. The raw and ripe grades of bananas varied visibly (von Loesecke, 1949) on 1 to 2 and 7 to 8 scales, respectively. The colour in raw and ripe grades showed variations on L, a, b scales {raw: L 60.54 ±3.64, a -0.35 ±11.42, b 35.98 ±3.86; ripe L 43.87 ±9.47, a 0.75 ±6.24, b 24.82 ±9.83, respectively). Also the moisture contents in pulp in the different months varied between [62.27 ±1.42 to 79.27 ±0.21 nean 73.05 ± 6.32] in raw and [69.22 ±2.51 to 93.51 ± 0.66 mean 78.93 ±7.90] in ripe grades, respectively.

No chill injury was present in the bananas. The cause of variation in pulp colour among different stalks was described primarily to site origin (Hughes and Wainwright, 1994). Results were consistent with earlier reports (Simmonds, 1966) that pulp growth in bananas increased but the growth ratio of peel declined, especially towards commercial maturity (Fig 3 a) and provided a quantitative estimate of fruit development. There were negligible texture and colour (whiteness of pulp) variations (average of triplicate readings) in different sections of a single banana fruit (Fig. 2) of a bunch.

Banana fruit can ripe when pulp: peel ratio reaches 0.5 (Simmonds, 1966). The Pp:Pl ratio range in raw and ripe bananas in market was between 0.54 to 1.97 (mean 1.17 ± 0.46) and 0.82 to 3.08 (mean 2.2 ±0.95), respectively in different months, high ratios for raw banana (Fig. 3a). Also the moisture contents in pulp that contributed to varied texture in raw (62.27 ±1.42 to 79.27 ±0.21 mean 73.05 ±6.32) and ripe (69.22 ±2.51 to 93.51 ±0.66 mean 78.93±7.90) grades, respectively (Fig. 3b). Obviously farmers harvested bananas at different growth stages (in different months) with variations in maturity and hence in ripened grades. The pulp mass content in the raw and ripe bananas available in market varied in different months (Fig. 3b, c) (mean mass: 114.27 ±16.71, 102.02 ±24.82 in raw and ripe grades).

Since for fruit harvested earlier than normal, green life increased by 3-5 d but bunch weight reduced by almost 10 % (Peacock, 1975). Also a relation was established (Montoya *et al.*, 1984) between bunch age and green life, which varied from one harvest date to another. Thus there was need to look into the physiological maturity of bananas harvest according to seasonal variations in temperatures that differed from region to region.

Artificial ripening by the two methods as experimented, showed changes in texture (compressible force on bananas), average mass (g), moisture in pulp (%) and fresh Pp: Pl ratio. Final texture-force of compression (N) of ripened bananas by BIS method was as much as 46.57 ± 0.64 N compared to a texture of 28.56 ± 1.22 N by crude method (Fig. 4).

The bananas ripened by BIS method showed better texture quality.

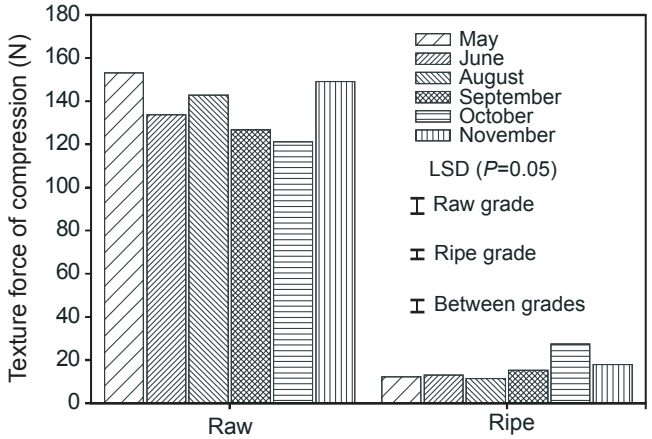

Fig. 1. Variation in texture of bananas (raw and ripe grades) in different months. Vertical bar show LSD (*P*=0.05) for raw and ripe grades.

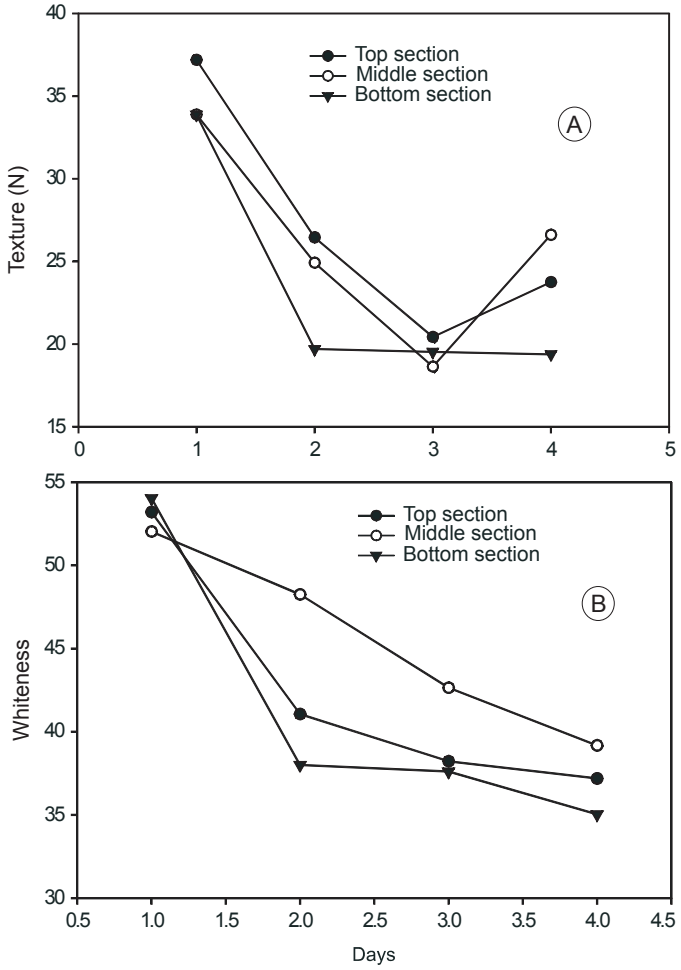

Fig. 2. Texture(A) and colour(B) changes in different sections of ripe banana pulp (on single bunch) during ripening (room conditions).

Colour of pulp, by both methods showed negligible differences. There was enough scope to improve (about 63.06 % higher compressible force) the texture of ripened banana grades by BIS method (rapid procedure). The overall quality of the ripened bananas (BIS method) was better with lesser pulp mass loss in ripening than bananas ripened by crude method (Table 1).

Experimental banana ripening was accomplished at temperature of 16 to 18ºC with high relative humidity 80 % (BIS method to be usually best when ethylene used). A higher Pp: Pl ratio with a good ripeness (17-18 % TSS) was achieved till the last day (4 d) of ripening. Actual usefulness (scope to improve the textural and related physiological quality) of ripened bananas by the BIS standard method has been revealed in this study. This is perhaps an advantage as the consumer prefer a fruit grade that would be better in texture and maturity when retailed.

Thus standard method needs to be perpetuated. There is need to establish studies on suitable stage(s) in raw banana harvesting for green-life and harvest maturity in banana during different seasons. A challenge to orderly marketing was to harvest bananas at proper maturity to get even grades of raw banana fruit (Montoya *et al.*, 1984).

Under the cost considerations BIS method required a cold chamber to be operated at 16 ºC- 18 ºC under maintained 80 % humidity (with say saturated solution of potassium nitrate). If the initial costs (chamber {size according to the need}, chemicals)

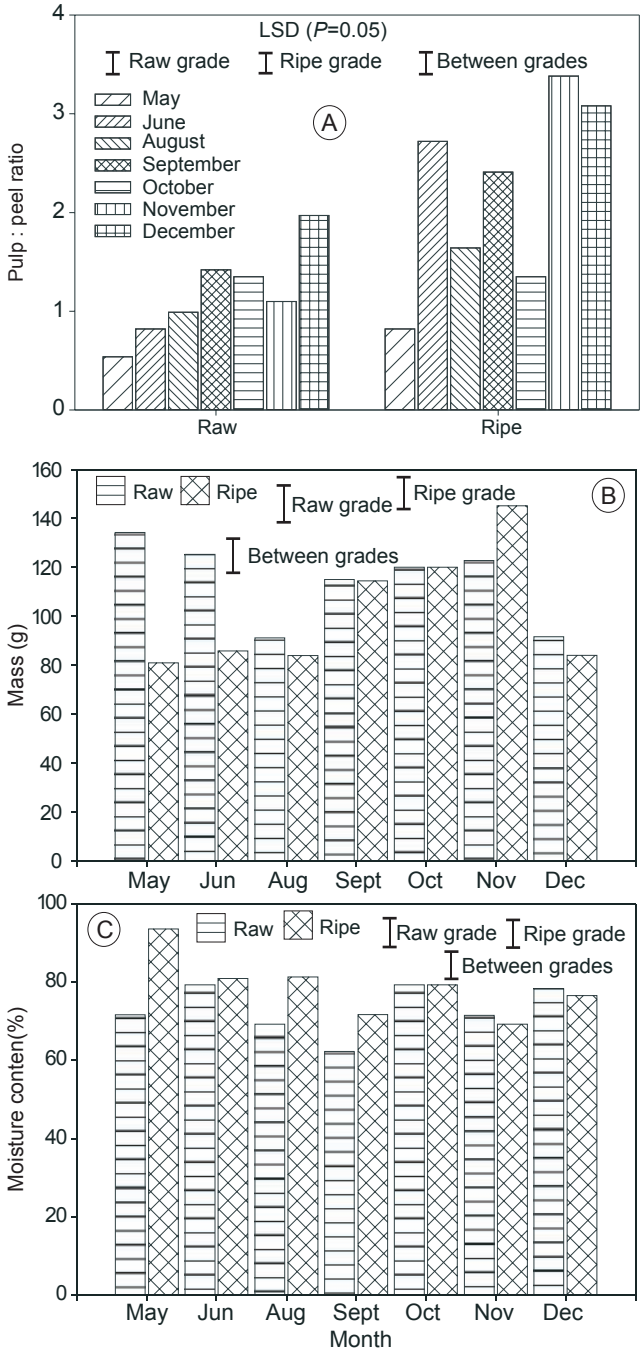

Fig. 3. A) Pulp: peel ratios of bananas grades (raw and ripe) in different months; B) & C) mass and % moisture content (pulp) of bananas (raw and ripe grades) in different months.

was met it could lead to availability of good quality of ripened bananas in market. The unclean conditions caused by ice in crude market method could be avoided.

This may also lead to the reduced use of calcium carbide (banned but still under use), a carcinogen (a major health concern in the market). Centralized ripening systems (CRS) (catalytic generators: web site) available may be checked for their working in the future works under such aspects with features like leak detection, safety, convenience.

Over ripe bananas were abundantly seen in market. Exposing ripe bananas to temperatures (higher than those in the ripening range) after artificial ripening, hastened softening and decay, weakened the neck and peel, and may cause poor colour (Kotecha and Desai,

1995). Thus handling after ripening also needed attention in a tropical country like India. This can be minimized by careful postharvest attention to the fullness of the fruit at harvest, the necessity for speedy loading before the transport.

Table 1**.** Changes in bananas ripening by two different methods in June month

Changes in parameters*	Ripening methods	
	Crude market	BIS
Reduced mass %	13.80 (± 36.08)	5.64 (± 18.63)
Mositure (%) in pulp	3.09 (± 4.67)	3.36 (± 1.31)
Pulp : peel ratio (Fresh)	2.17 (± 0.16	6.76 (± 0.07)

*Changes from initial raw bananas used.

Table 2. Yield of fermented beverage from day's available over-ripe bananas.

Total available over-ripe bananas#	5700
Weight of over-ripe bananas*	460 kg
%Yield of beverage (alcoholic drink)	69.88
Beverage from pulp	321 kg
Over-ripeness in bananas and monetary losses incurred:	
Loss due to over-ripeness	58.3%
Rate of ripe grade	Rs 12/-(max)
Rate of over-ripe grade	Rs 5/- (min)
Loss (difference)	Rs 7 per doz
Monetary loss (due to over ripeness)	7/12 x 100 = 58.3 %

#Estimated value: 15% over ripe bananas, from all retailers in a single day's pick of 19 thelas (selling the different grades of bananas on each thela$ present) in mandi ($ four wheeled manual pulling carts/ carriers); *80.66g (mean weight of one banana).

Table 3. Value addition scope by processing of over ripe bananas

Average earning from sale of 5700 bananas 475 doz. @ Rs 5/-)	Rs 3531/-
Beverage cost @ Rs 11/kg (processed from 5700 bananas)(321 kg)	Rs. 2375/-
Value addition by beverage	$\frac{3513-2375}{2375}100 = 48\%$

The over-ripe grades of bananas were processed to alcoholic banana for likeable good quality beverage (Pethe, *et al.*, 2003). Such a banana beverage (with ~8% alcohol) yielded 69.88 % from pulp used. The value addition so obtained (Table 2, 3) showed a scope of ~48% monetarily.

Since the different levels of moisture and pulp: peel ratios were found in bananas and appears to be responsible for bananas maturing differently to over ripe grades. There is need to disseminate standard ripening procedures in the market to be ultimately followed by traders.

Acknowledgements

The authors wish to thank The Director CIAE and Head Agro Processing Division, CIAE, Bhopal for providing all necessary facilities in completing this work. The authors also wish to thank Dr KN Singh (I/C Statistics and Computer section, IISS, Bhopal) for help in statistical analysis.

References

Bureau of Indian Standards (BIS), 1988. Green bananas- ripening conditions: Indian standards. BIS, Manak Bhavan, ISO: 11977 (1987). New Delhi 2.

Caputi, A.J., M. Ueda and T. Brown, 1965. Spectrophotometric determination of ethanol in wine. *Am. J. Enol. Vitic.*, 19: 160-165.

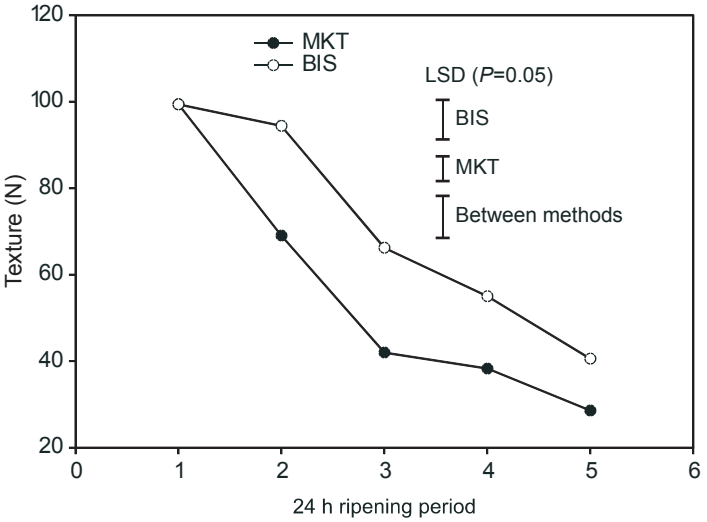

Fig. 4. Lowering of texture during ripening (4 d period) (observed in June) by banana ripening methods (Crude market /BIS).

Catalyticgenerators, 2004. Web site*:* http://www.catalyticgenerators.com/cenripsys.html.

Gutierrez, H.M., 1999. Regression equations for changes in quality characteristics of ripening banana (*Musa* sp.). *USM Research-and-Development Journal* (Philippines), 7 (1): 38-48.

Hughes, P.A. and P.J. Wainwright, 1994. Influence of site and fruit position on the pulp colour and texture of bananas. *Trop. Sci.*, 34: 211-215.

Kader, A., B. Mitchain and B. Hers-Pierce, 1996. Optimum procedures of ripening bananas. Management of fruit ripening. California Univ. Davies, USA.

Kotecha, P.M. and B.B. Desai, 1995. Banana. In: *Handbook of Fruit Science and Technology.* Marcel and Dekker Inc. New York. pp 67-89.

Marchal, J. 1997. An overview of post harvest aspects of banana. In: *Int. Symposium on 'Banana in the sub tropics'*, Puerto dela Cruz. 11: 10-14.

Montoya J., J. Marriott, V.H. Quimi and J.C. Caygill, 1984. Age control of bananas harvesting under Ecuadorean conditions. *Fruits*, 5: 293-296.

NHB, 2000. Fruit and vegetable wholesale market Bhopal-A Profile. Nat. Hort. Board, Min. Agric., Govt of India. Gurgaon 122001.

Peacock, B.C. and J.R. Blake, 1970. Some effects of non damaging temperatures on the life and respiratory behaviour of bananas. *Queensland J. Agricultural and Animal studies*. 27:147-168.

Peacock, B.C., 1975. 'Mixed ripe' a problem for banana industry. *Queensland Agriculture J*., 101: 201-204.

Pethe, Monesh, Sunita Singh, S.D. Kulkarni and S. Patel, 2003. Use of α- amylase enzyme for clarification of banana beverage. *Beverage and Food World*, 30(7): 54-56.

Ranganna, S. 1991. *Handbook of analysis and quality control for fruit and vegetable products.* 2nd Ed. Tata McGraw-hill Pub. Co. Ltd.

Simmonds, N.W. 1966. *Bananas.* 2nd Ed. Longman, London

Singh, Sunita S.D. Kulkarni and K.K. Singh, 2003. Handling Banana Chain - Management Aspects for an Agri-Industrial Approach. In: *The 6th Agricultural Science Congress* (The National Academy of Agricultural Sciences). Feb 13th –15th held at IISS, Bhopal. 263.

UNCTAD, 2000. Web site http://r0.unctad.org/infocomm/anglais/banana/market.htm#prod.

von Loesecke, H.W. 1949. *Banana.* 2nd edition, InterScience Pub. NewYork, USA**.**

Journal of Applied Horticulture, 8(1): 82-84, January-June, 2006

Constraints in production and marketing of pistachio in Iran and concerned policies

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Abstract

Pistachio is one of the most important agricultural crop in Iran. The country earns sizable income from pistachio export. To be globally competitive, the production and trade of pistachio must be economically viable especially in long run. This paper aims to analyze the constraints in production and marketing of Pistachio in Iran. Necessary data were collected through personal interview of randomly selected sample of farmers and exporters/ processors. One hundred farmers and ten processor/ exporters interviewed in Kerman province and Tehran city (Iran), in the crop year 2003-04. The Garret ranking technique was adopted to identify the constraints. Farmers and traders were asked to rank the problems considered. Farmers ranked 14 problems into 9 different categories. Differences between scores adopted for different categories were low and they varied from 74 to 87, indicating that all the problems are important from producer's point of views. On the other hand, traders ranked only 12 given problems among 17. They classified each and every problem into a distinct category. Score variations were comparatively high varying from 19 to 60 indicating that there is significant difference between different categories of problems. The results of tabular analysis of export data showed that pistachio industry of Iran was facing a negative growth rate of production, productivity, export quantity and export value during the period 1991-2002.

Key words: Pistachio, Iran, export, production problems, productivity, economics

Introduction

Pistachio is cultivated in dry areas of Iran, where precipitation is quite low and no other crop could be produced economically. Currently pistachio export earnings stand next to petroleum. Around 10 percent of non-petroleum export value is being realized from pistachio. The average size of pistachio gardens is around 2.5 hectares. Pistachio cultivation alone provides employment to over 7, 60,000 people. Considering the people who were employed in pistachio processing, marketing and export, the employment adds up to over one million. Hence, pistachio is a crop of great importance in Iran's economy (Sedaghat, 2002, 2006). According to the Agricultural Ministry Data (2002), pistachio is cultivated in 3,80,000 hectares in Iran, with 70 percent bearing and 30 percent nonbearing gardens. Pistachio production was 3,20,000 metric tones, with the yield bearing 1.2 tones per hectare in bearing gardens. Foreign exchange earnings from the export of pistachio have been around 350 million US dollars per year during the last five years. Iran stands first both in production and export of pistachio in the world. According to the data from FAOSTAT Information Bank, Iran accounted for 52.89, 58.00, 64.79 and 65.84 percent of world production, cultivation area, export and export value, respectively during the last decade (FAO, 2003).

To be globally competitive, the viability of production and processing should be attained in long run. Otherwise loosing the current position is expected in future. This study aimed at identifying the main obstacles and bottlenecks faced by producers and exporters of pistachio in Iran. Also the feasibility of a contract system was assessed as an alternative system, to reduce the current problems.

Methods and materials

Study area: Pistachio is produced in different provinces, but Kerman is the major province of pistachio gardens in Iran (with 270,000 hectares, accounting for 71 percent of total area under pistachio across the country). Kerman province is located in south east of Iran bounded by Fars, Yazd, Hormozgan, Sistan and Khorasan provinces. The climate in this province tends to be very warm in summers and very cold in winters. The temperature ranges between -10°C to and 48°C. The average rainfall is around 150 mm per year. Rafsanjan city in Kerman province was purposefully selected for this study. More than 50 percent of pistachio gardens are located in this area. Moreover, most of the processing industries and exporters are operating from Rafsanjan.Iran's Pistachio Research Institute and Pistachio Producer's Cooperative are also located in Rafsanjan. Thus, this city is well known all over the world as the most important region of pistachio production.

Database: Rafsanjan consists of five districts, *viz*., Kabootarkhan, Noogh, Anar, Koshkooeya and Hoome. By using a multistage random sampling technique, 20 sample farmers were chosen from each district. The sample spread across the three major varieties of pistachio in the province (Fendoghi, Kaleghoochi and Akbari). Also, a sample of 10 main traders/exporters was interviewed for the purpose of the study. Farmers and traders were questioned about their challenging hindrances and were asked to rank the problems.

Methods of analysis: Tabular analysis was used to identify the growth rates of production, harvested area, yield/hectare, export quantity and export value during the period 1991-2002.

Garret's ranking technique (Garret and Woodworth,1969) was adopted to analyze the factors considered . The respondents were asked to rank the factors. The orders of merit, assigned by the respondents were converted into ranks using the following formula:

Percent position of each rank = 100 (R_{ij} - 0.5) / N_j
R_{ij}= Rank given for i_{th} factor by j_{th} individual
N_j= Number of factors ranked by j_{th} individual

The percentage position of each rank then converted into scores referring to table given by Garret and Woodworth (1969). For each factor, the scores of individual respondents were added together and divided by the total number of respondents for whom scores were added. These mean scores for all the factors were arranged in descending order, ranks were given and the most limiting factors were identified.

Results and discussion

Growth rate of production, harvested area, productivity and export in Iran compared to other major pistachio producing countries: The mean growth rate calculated for the first 2 years and the last two years during 1991-2002, are presented in Table 1. The reason to use 2 years average is the alternate bearing habit in pistachio. The growth rates for all the variables, except harvested area, were negative in the case of Iran. The growth rates of production, productivity and harvested area were negative but that of export and export value were positive in the case of Turkey. The growth rates were positive for all the variables in the case of USA. As already mentioned, Iran's situation for the above mentioned variables is not satisfactory. It is worth mentioning here that if the current situation remain consistent, the future of pistachio industry will become worse.

Table 1. Growth rates of pistachio production, harvested area, yield and export for major producing countries during (1991-2002)

Particulars	Iran	USA	Turkey
Production			
Share in first 2 years (%)	56.5	15	13.00
Share in last 2 years (%)	46.5	24	10.75
Change in share (%)	-17.7	60	-17.30
Harvested area			
Share in first 2 years (%)	55	7.5	10.00
Share in last 2 years (%)	66	9	9.00
Change in share (%)	20	20	-10.00
Yield / hectare			
Share in first 2 years (%)	102.5	199	130.25
Share in last 2 years (%)	71	278	120.50
Change in share (%)	-30.73	39.7	-7.48
Export quantity			
Share in first 2 years (%)	73	8	0.40
Share in last 2 years (%)	57.5	12	2.37
Change in share (%)	-21.23	50	493.75
Export value			
Share in first 2 years (%)	71	7.7	0.67
Share in last 2 years (%)	61	12	3.36
Change in share (%)	-14.08	55.84	401.86

Source: FAO (2003)

Problems faced by producer and traders in Iran: The ranking results of the factors considered by the producers and traders are depicted in Tables 2 and 3, respectively. Farmers ranked all the 14 factors and they classified them in to 9 categories (Table 2). Some of the factors have been given the same rank by the producers. The score variation was very low varying from 74 to 87. Lack of adequate agricultural water received rank I, unsuitable domestic market structure, inter and intra year fluctuations of prices and low price of pistachio in the market all together received rank II. Lack of appropriate chemical fertilizers received rank III. Traders ranked only 12 factors out of 17 identified ones and they sorted them into 12 distinct classes (Table 3). No factor has been given the same score by the traders. The score variation was comparatively high varying from 19 to 60. Aflatoxin contamination, inappropriate and changing governmental policies and rules in export and irregular supply of product to market during the year received ranks I , II and III, respectively.

Table 2. Constraints in production, processing and marketing of pistachio from the producers' point of view in Iran

Constraints	Score	Rank
Lack of appropriate agricultural machinery	79	7
Lack of adequate agricultural water	87	1*
Lack of appropriate pesticides	81	5
Lack of appropriate chemical fertilizers	83	3*
Lack of extension and advisory services	79	7
Inadequate credits for production	82	4
Inadequate processing plants	78	8
Unsuitable domestic market structure	85	2*
Inter and intra year fluctuations of prices	85	2*
Low price of pistachio in the market	85	2*
High discount rate in domestic market undertaken by buyers	80	6
Paying late by buyers	78	8
High cost of production	82	4
Others	74	9

*The most important problems

Table 3. Constraints in production, processing and marketing of Pistachio from the Traders' point of view in Iran

Constraints	Score	Rank
Lack of extension services to the producers and processors and low quality of final product	-	Not Ranked
Lack of modern pistachio processing plants	20	11
Domestic market structure	47	4
World market structure	-	Not Ranked
Inter and intra year fluctuation of prices in world market	-	Not Ranked
High discount rate in domestic market	44	6
High and increasing trend for cost of production	19	12
Low productivity (yield per hectare)	43	7
Lack of credit to marketing agencies	46	5
Low and constant trend of prices in world market	-	Not Ranked
In appropriate and changing Government policies and rules for export	53	2*
In adequate transportation system	28	9
Inadequate of packaging industries	24	10
Irregular supply of product to market during the year	51	3*
Aflatoxin pollution	60	1*
Poor quality of product in international market	37	8
Others	-	Not Ranked

*The most important problems

Nature of the problems and how to reduce them with applying a contract system: Based on the information discussed in the previous sections, it could be obviously revealed that the Iran's pistachio industry suffers from many hindrances which could be divided in two major categories: those pertaining to producers and those concerned to traders. Both type of the problems and constraints exist due to inappropriate policies in production, marketing and export of pistachio in Iran.

As a part of the existing system, inputs and services are mainly provided through government channels which are not the product buyers, Pistachio cooperative and some private agencies buy the product from the farmers. As a result, those who are providing services to the farmers are not much bothered about the timeliness and cost effectiveness in the availability of the inputs and services. Again those who are the buyers of produce don't have any mechanism to provide services to the farmers at proper time, though the safety and quality of product is very important for them. This has created an inconsistency in the production and marketing system of pistachio in Iran, leading to the problems/ constraints already explained.

For betterment of the pistachio industry, a well managed contract system can be applied with an equal right for all the stake holders.

Contract farming refers to a system where a central processing or exporting unit purchases the harvests of independent farmers and the terms of the purchase are arranged in advance in a contract base approach. The terms of the contract vary and usually specify how much product the contractor will buy and what price they will pay. The contractor frequently provides credit, inputs and technical advices. Contracting is fundamentally a way of allocating risk between producer and contractor; the former takes the risk of production and the latter the risk of marketing (Charles and Andrew, 2001).

Feasibility assessment of contract farming system for pistachio industry in Iran: Nature of the product can significantly affect acceptability and applicability of any contract scheme. Pistachio has got many characteristics that encourage undertaking a contract system instead of the existing system, the major characteristics are as follows: Pistachio is produced in dry areas with poor quality of land and water where no other crop can be produced economically (i) large quality variation and the need for quality in final product, (ii) Perishability and the need for quick processing, (iii) The value per unit is high and so it is economic to transport the product, (iv) Acceptable homogeneity between producers either in ethnic or economic aspects, (v) Existence of quasi- monopsony market system for pistachio, (vi) The need to provide and control the credit to the farmers in the region especially at the peak season (vii) Political importance of the crop, (viii) To transfer and adopt the technology required and to offer the services for the farmers (ix) Pistachio being the only product in the region and hence all the inputs and services go to the commodity.

Despite a good position in world pistachio production and trade, Iran's share of global production, productivity and export has declined during the last decade. Productivity of pistachio gardens in Iran is low compared to other competing countries. Cost of production has increased significantly in recent years. The prices received by the farmers do not compensate for the effect of low productivity and increasing production cost. As a result, profitability of production has come down and it may further decline if the current situation continues. There are many constraints and bottlenecks faced by both farmers and exporters, mainly due to inappropriate managerial system for pistachio industry in Iran. These problems exist due to an inconsistency in the market. Experience achieved over the last three decade reveal that, government interference in the production and marketing of pistachio was not efficient. On the other hand, the existing condition is not fully ready for running a free market system. In such a situation, contract system can play a major role to bring efficiency and profitability to the system and benefit to both the producers and traders. Since there is a need for new contract laws, regulations as well as new policy perspective, it is suggested that government take the responsibility of policy making of the contract schemes for effective operation of the system than a direct participation.

References

Charles, E. and W.S. Andrew, 2001. *Contract Farming Partnerships for Growth.* FAO Agricultural Services Bulletin, FAO.

FAO, 2003. *FAOSTAT information bank.* Food and Agriculture Organization of UN.

Garret, H.E. and R.S. Woodworth, 1969. *Statistics in Psychology and Education*, Vakils, Feffer and Simons Pvt. Ltd., Bombay. p. 329.

Sedaghat, R. 2002. The Study of Economical Theory in Poverty and Under Development vicious Circle- A Case study of Pistachio Cultivation Regions in Iran. *Agricultural Economic and Development Journal of APERI,* 10(3): 187-201.

Sedaghat, R. 2006. *An Economic Analysis of Pistachio Production, Processing and Trade in Iran* . Ph.D. Thesis, Department of Agricultural Economics, University of Agricultural Science, G.K.V.K, Bangalore, India.

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Thesis : Marsh, H. 1999. *Phenotypic variation of kiwifruit in a factorial mating design*. M.Appl.Sc. Thesis, Massey University, Palmerston North, New Zealand.

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