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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 × 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.
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) at different growth periods in greenhouse and open-field conditions

<table>
<thead>
<tr>
<th>DAT</th>
<th>B</th>
<th>BP</th>
<th>BB</th>
<th>BH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenhouse</td>
<td>30</td>
<td>42.70b</td>
<td>48.44c</td>
<td>36.80a</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>83.06a</td>
<td>91.88a</td>
<td>82.75a</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>95.88a</td>
<td>106.38a</td>
<td>100.75a</td>
</tr>
<tr>
<td>Open-field</td>
<td>34</td>
<td>53.75bc</td>
<td>46.44a</td>
<td>51.06a</td>
</tr>
<tr>
<td></td>
<td>89</td>
<td>67.75b</td>
<td>62.50a</td>
<td>64.38ab</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>75.31b</td>
<td>69.31a</td>
<td>72.00ab</td>
</tr>
</tbody>
</table>


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

<table>
<thead>
<tr>
<th>Cluster number</th>
<th>DAT</th>
<th>Number of flowers/cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenhouse</td>
<td>1st</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>5th</td>
<td>96</td>
</tr>
<tr>
<td>Total</td>
<td>5th</td>
<td>96</td>
</tr>
<tr>
<td>Open-field</td>
<td>1st</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>5th</td>
<td>97</td>
</tr>
<tr>
<td>Total lowers</td>
<td>5th</td>
<td>97</td>
</tr>
</tbody>
</table>

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

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).
Effect of grafting on growth and yield of tomato

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

<table>
<thead>
<tr>
<th>Characteristics/ part</th>
<th>Greenhouse</th>
<th>Open-field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BH</td>
<td>BP</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>DW</td>
</tr>
<tr>
<td>Stem</td>
<td>204.30a</td>
<td>283.78a</td>
</tr>
<tr>
<td></td>
<td>36.30a</td>
<td>60.69a</td>
</tr>
<tr>
<td>Leaves</td>
<td>884.08a</td>
<td>980.28a</td>
</tr>
<tr>
<td></td>
<td>139.84a</td>
<td>153.54a</td>
</tr>
<tr>
<td>Flowers</td>
<td>13.35a</td>
<td>26.98b</td>
</tr>
<tr>
<td></td>
<td>2.23a</td>
<td>4.70b</td>
</tr>
<tr>
<td>Fruits*</td>
<td>1776.63a</td>
<td>2788.77a</td>
</tr>
<tr>
<td></td>
<td>59.38a</td>
<td>55.80a</td>
</tr>
<tr>
<td>Total DW/FW %</td>
<td>8.86a</td>
<td>9.15a</td>
</tr>
<tr>
<td>Total leaf area (cm²)</td>
<td>10923.10a</td>
<td>8646.20a</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>127.75a</td>
<td>135.00a</td>
</tr>
</tbody>
</table>

*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 between the treatments. 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

<table>
<thead>
<tr>
<th>DAT</th>
<th>Fruit weight (g) plant⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BH</td>
</tr>
<tr>
<td>Greenhouse</td>
<td></td>
</tr>
<tr>
<td>1st 0-84</td>
<td>628.76ab</td>
</tr>
<tr>
<td>2nd 85-155</td>
<td>5066.90a</td>
</tr>
<tr>
<td>3rd 156-192</td>
<td>1872.50a</td>
</tr>
<tr>
<td>Total</td>
<td>7568.16b</td>
</tr>
<tr>
<td>Open-field</td>
<td></td>
</tr>
<tr>
<td>1st 0-84</td>
<td>420.94a</td>
</tr>
<tr>
<td>2nd 85-121</td>
<td>1137.81a</td>
</tr>
<tr>
<td>3rd 122-130</td>
<td>537.50b</td>
</tr>
<tr>
<td>Total</td>
<td>2096.25a</td>
</tr>
</tbody>
</table>

Means followed by the same letter are statistically not significant according 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


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 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 non-destructive 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
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 \( Y = 50.7179 - 0.170527x \), 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPAD-1</td>
<td>( F = \left[50.7 - (d \times 0.17)\right] \times C \times 70 )</td>
</tr>
<tr>
<td>SPAD-2</td>
<td>( F = \left[60.8 - (d \times 0.20)\right] \times C \times 70 )</td>
</tr>
<tr>
<td>SPAD-3</td>
<td>( F = \left[45.7 - (d \times 0.15)\right] \times C \times 70 )</td>
</tr>
</tbody>
</table>

\( F = \) 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 was present, 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 at 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 probably 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
Use of a chlorophyll meter and plant visual aspect for nitrogen management in tomato fertigation

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 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

<table>
<thead>
<tr>
<th>PVA</th>
<th>Characteristic</th>
<th>14</th>
<th>28</th>
<th>42</th>
<th>56</th>
<th>70</th>
<th>84</th>
<th>98</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bad</td>
<td>Canopy greenness</td>
<td>YE</td>
<td>YE</td>
<td>YE</td>
<td>YE</td>
<td>YE</td>
<td>YE</td>
<td>YE</td>
</tr>
<tr>
<td></td>
<td>Leaf number</td>
<td>5</td>
<td>11</td>
<td>14</td>
<td>22</td>
<td>25</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plant height (cm)</td>
<td>10</td>
<td>20</td>
<td>25</td>
<td>35</td>
<td>45</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>Canopy greenness</td>
<td>YG</td>
<td>YG</td>
<td>YG</td>
<td>YG</td>
<td>YG</td>
<td>YG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaf number</td>
<td>6</td>
<td>15</td>
<td>24</td>
<td>26</td>
<td>28</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plant height (cm)</td>
<td>15</td>
<td>30</td>
<td>50</td>
<td>95</td>
<td>105</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>Canopy greenness</td>
<td>LG</td>
<td>LG</td>
<td>LG</td>
<td>LG</td>
<td>LG</td>
<td>LG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaf number</td>
<td>7</td>
<td>18</td>
<td>30</td>
<td>35</td>
<td>34</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plant height (cm)</td>
<td>15</td>
<td>45</td>
<td>90</td>
<td>155</td>
<td>165</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>Very good</td>
<td>Canopy greenness</td>
<td>DG</td>
<td>DG</td>
<td>DG</td>
<td>DG</td>
<td>DG</td>
<td>DG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaf number</td>
<td>8</td>
<td>20</td>
<td>32</td>
<td>38</td>
<td>36</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plant height (cm)</td>
<td>20</td>
<td>50</td>
<td>100</td>
<td>165</td>
<td>170</td>
<td>185</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment</th>
<th>Days after transplanting</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 28 42 56 70 84 98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAD-1</td>
<td>0   0   0   420   0   0</td>
<td>420</td>
<td></td>
</tr>
<tr>
<td>SPAD-2</td>
<td>0   0   0   0   0   0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SPAD-3</td>
<td>0   0   0   0   0   0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PV A</td>
<td>0   0   0   0   0   0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>REF E</td>
<td>0   0   0   0   0   0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CHECK</td>
<td>0   0   0   0   0   0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield (t ha(^{-1}))</th>
<th>Total</th>
<th>Marketable</th>
<th>Weighted</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPAD-1</td>
<td>99.1 a</td>
<td>97.0 a</td>
<td>52.2</td>
<td></td>
</tr>
<tr>
<td>SPAD-2</td>
<td>83.4 ab</td>
<td>77.3 ab</td>
<td>45.4</td>
<td></td>
</tr>
<tr>
<td>SPAD-3</td>
<td>82.8 ab</td>
<td>78.7 ab</td>
<td>43.4</td>
<td></td>
</tr>
<tr>
<td>PV A</td>
<td>84.5 ab</td>
<td>81.0 ab</td>
<td>44.5</td>
<td></td>
</tr>
<tr>
<td>REF E</td>
<td>93.7 ab</td>
<td>91.5 ab</td>
<td>53.7</td>
<td></td>
</tr>
<tr>
<td>CHECK</td>
<td>68.3 b</td>
<td>64.5 b</td>
<td>37.5</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield (t ha(^{-1}))</th>
<th>Total</th>
<th>Marketable</th>
<th>Weighted</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPAD-1</td>
<td>101.9 a</td>
<td>99.7a</td>
<td>61.7a</td>
<td></td>
</tr>
<tr>
<td>SPAD-2</td>
<td>86.4ab</td>
<td>82.3ab</td>
<td>49.2ab</td>
<td></td>
</tr>
<tr>
<td>SPAD-3</td>
<td>77.7ab</td>
<td>74.9ab</td>
<td>40.9ab</td>
<td></td>
</tr>
<tr>
<td>PV A</td>
<td>93.1ab</td>
<td>88.5ab</td>
<td>50.3ab</td>
<td></td>
</tr>
<tr>
<td>REF E</td>
<td>94.3ab</td>
<td>89.8ab</td>
<td>55.5ab</td>
<td></td>
</tr>
<tr>
<td>CHECK</td>
<td>71.7b</td>
<td>68.2b</td>
<td>40.3b</td>
<td></td>
</tr>
</tbody>
</table>

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


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 ft2), 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
days post-planting

<table>
<thead>
<tr>
<th>Mulch</th>
<th>29</th>
<th>59</th>
<th>70</th>
<th>114</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 lb paper/oil</td>
<td>0  a</td>
<td>0  a</td>
<td>1  a</td>
<td>1  a</td>
</tr>
<tr>
<td>40 lb paper/oil</td>
<td>0  a</td>
<td>0  a</td>
<td>0  a</td>
<td>1  a</td>
</tr>
<tr>
<td>Newspaper/straw</td>
<td>0  a</td>
<td>0  a</td>
<td>1  a</td>
<td>1  a</td>
</tr>
<tr>
<td>Bare soil</td>
<td>4  b</td>
<td>6  b</td>
<td>14 b</td>
<td>11 b</td>
</tr>
</tbody>
</table>

*Mean of six replications (data from tomato and pepper replicates combined).

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


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, Fragarica vesca cv. ‘My Da’ shoots were cultured on ½ MS medium supplemented with 0.5 mg l⁻¹ NAA, 1 g l⁻¹ activated charcoal, 30 g l⁻¹ sucrose and 8 g l⁻¹ agar at different irradiances: (1) Neon at 45 µmol m⁻² s⁻¹ (control), and Compact 3U at: (2) 45 µmol m⁻² s⁻¹, (3) 60 µmol m⁻² s⁻¹, and (4) 75 µmol m⁻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 (CO₂, O₂, H₂O and C₂H₅), 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; Ivanami et al., 1992; Kozai et al., 1992; Kirmene et al., 1993; Gabarkiewicz et al., 1997; Walister 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 µmol m⁻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 mg/l 6-naphthaleneacetic acid (NAA), 2 mg/l 6-benzyladenine (BA), 1 g/l activated charcoal (AC), 20% coconut water (CW), 30 g/l sucrose and 8 g/l agar (Haiphong Co., Vietnam), were cultured on MS medium containing 0.5 g/l NAA, 1 g/l AC, 10% CW, 25 g/l sucrose and 8 g/l agar.

Lilium longiflorum bulb scales, derived from in vitro bulblets cultured on MS medium containing 0.2 - 0.5 mg/l BA, 30 g/l sucrose and 8 g/l agar, were cultured on ½ MS medium supplemented with 1 g/l AC, 30 g/l sucrose and 8 g/l agar.

Fragaria vesca cv. ‘My Da’ shoots (1.5 cm), derived from meristems cultured on MS medium containing vitamin B<sub>1</sub>, 0.2 mg/l BA, 30 g/l sucrose and 8 g/l agar, were cultured on ½ MS medium supplemented with 1 g/l AC, 30 g/l sucrose and 8 g/l 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 μmolm⁻²s⁻¹ under Neon light or 45, 60, 75 μmolm⁻²s⁻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 μmol 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 μmolm⁻²s⁻¹, and the remaining shelf with the Neon lighting system at 45 μmolm⁻²s⁻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
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 μmol·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**

<table>
<thead>
<tr>
<th>Lighting system</th>
<th>Plant height (cm)</th>
<th>Leaf area (mm²)</th>
<th>Root length (cm)</th>
<th>Plant fresh weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neon (control)</td>
<td>3.7b</td>
<td>45a</td>
<td>2.5b</td>
<td>200b</td>
</tr>
<tr>
<td>Compact 3U</td>
<td>4.2a</td>
<td>35b</td>
<td>3.5a</td>
<td>300a</td>
</tr>
</tbody>
</table>

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 μmol·s⁻¹ because of the maximum growth and development. However, for commercial purposes, we recommended the use of Compact 3U light at 45 μmol·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**

<table>
<thead>
<tr>
<th>Irradiance (Compact 3U) (μmol·s⁻¹)</th>
<th>Plant height (cm)</th>
<th>Leaf area (mm²)</th>
<th>Root length (cm)</th>
<th>Plant fresh weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neon (45)</td>
<td>3.7c</td>
<td>40.5d</td>
<td>2.5d</td>
<td>200d</td>
</tr>
<tr>
<td>45</td>
<td>4.2b</td>
<td>45.4b</td>
<td>3.5b</td>
<td>250c</td>
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<tr>
<td>60</td>
<td>4.2b</td>
<td>41.4c</td>
<td>3.0c</td>
<td>270a</td>
</tr>
<tr>
<td>75</td>
<td>4.4a</td>
<td>50.0a</td>
<td>3.9a</td>
<td>260b</td>
</tr>
</tbody>
</table>

* 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 μmol·s⁻¹, with considerably more roots at 45 μmol·s⁻¹ as compared to Neon at 45 μmol·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 μmol·s⁻¹. The most suitable irradiance of Compact 3U (60 μmol·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 3. Effect of different irradiances of Compact 3U on the *in vitro* development of *Cymbidium* plantlets after 3 weeks of culture**

<table>
<thead>
<tr>
<th>Irradiance (Compact 3U) (μmol·s⁻¹)</th>
<th>Plant height (cm)</th>
<th>Root length (cm)</th>
<th>Leaf area (mm²)</th>
<th>Number of formed roots</th>
<th>Number of bulbs</th>
<th>Bulb diameter (mm)</th>
<th>Bulb cluster fresh weight (mg)</th>
<th>Bulb fresh weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neon (45)</td>
<td>9.1c</td>
<td>2.5c</td>
<td>26.1d</td>
<td>11.1b</td>
<td>1.9a</td>
<td>6.4a</td>
<td>750c</td>
<td>510c</td>
</tr>
<tr>
<td>45</td>
<td>11.4a</td>
<td>3.8a</td>
<td>40.3a</td>
<td>10.6c</td>
<td>1.6c</td>
<td>5.5d</td>
<td>810b</td>
<td>480a</td>
</tr>
<tr>
<td>60</td>
<td>10.3b</td>
<td>2.1d</td>
<td>30.7b</td>
<td>9.7d</td>
<td>1.8b</td>
<td>5.8c</td>
<td>830b</td>
<td>470b</td>
</tr>
<tr>
<td>75</td>
<td>9.1c</td>
<td>3.0b</td>
<td>30.0c</td>
<td>13.1a</td>
<td>2.0a</td>
<td>6.1b</td>
<td>930a</td>
<td>460b</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Irradiance (Compact 3U) (µmol m⁻² s⁻¹)</th>
<th>Plant height (cm)</th>
<th>Root length (cm)</th>
<th>Leaf width (mm)</th>
<th>Number of roots</th>
<th>Plant fresh weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neon (45)</td>
<td>10.1b</td>
<td>3.0a</td>
<td>4.7b</td>
<td>2.0c</td>
<td>620c</td>
</tr>
<tr>
<td>45</td>
<td>10.2b</td>
<td>3.0a</td>
<td>4.8a</td>
<td>2.5a</td>
<td>650b</td>
</tr>
<tr>
<td>60</td>
<td>10.9a</td>
<td>3.0a</td>
<td>4.8a</td>
<td>2.3b</td>
<td>740a</td>
</tr>
<tr>
<td>75</td>
<td>10.2b</td>
<td>2.6b</td>
<td>4.4c</td>
<td>2.6a</td>
<td>610c</td>
</tr>
</tbody>
</table>

* 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 µmol m⁻² s⁻¹ under Compact 3U for multiplying new high quality bulblets and under Neon at 45 µmol m⁻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 µmol m⁻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 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, 4a.).

**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, 4b.).

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 µmol m⁻² s⁻¹, the remaining cases showed that plantlets developed well under lower intensities (45 or 60 µmol m⁻² s⁻¹). Consequently, Compact 3U confirmed the positive effect of a 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, 4a.).

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

<table>
<thead>
<tr>
<th>Lighting systems (µmol m⁻² s⁻¹)</th>
<th>Plant height (cm)</th>
<th>Leaf width (mm)</th>
<th>Number of leaves</th>
<th>Number of roots</th>
<th>Root length (cm)</th>
<th>Plant fresh weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neon 45</td>
<td>10.4b</td>
<td>5.8a</td>
<td>4.3c</td>
<td>3.7b</td>
<td>2.9b</td>
<td>1.7b</td>
</tr>
<tr>
<td>45</td>
<td>10.5b</td>
<td>5.5b</td>
<td>4.4c</td>
<td>3.6b</td>
<td>3.3a</td>
<td>1.9a</td>
</tr>
<tr>
<td>Compact 3U 60</td>
<td>11.1a</td>
<td>5.4b</td>
<td>4.9a</td>
<td>3.7b</td>
<td>3.2a</td>
<td>1.8a</td>
</tr>
<tr>
<td>75</td>
<td>11.2a</td>
<td>5.2c</td>
<td>4.6b</td>
<td>3.9a</td>
<td>2.9b</td>
<td>1.6b</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Lighting systems (µmol m⁻² s⁻¹)</th>
<th>Plant height (cm)</th>
<th>Bulblet diameter (cm)</th>
<th>Leaf width (mm)</th>
<th>Number of leaves</th>
<th>Number of roots</th>
<th>Root length (cm)</th>
<th>Plant fresh weight (mg)</th>
</tr>
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<tbody>
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* Different letters within a column indicate significant differences (P = 0.05) with the mean separation by Duncan’s multiple range test.
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), Tibbits 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 Gilslrod and Mortensen (1997), Miyashita et al. (1997), and Nhat (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 μmol m⁻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 system 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 plantlet 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


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 (34°04′04″S, 150°46′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 FeSO₄ 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 25°C. 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 (R² = 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 (R² = 0.2%) (Fig. 1C), than when compared to the total number of stems (R² = 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 (R² = 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. 1E), 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 R² 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 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Fertiliser type | Height of plant | Number of stems | Stems with flowers | Flower number | Basal foliar necrosis |
| Rate            | 0.960           | 0.001           | 0.015             | 0.001         | 0.041            |
| Fertiliser x Rate | 0.001           | 0.001           | 0.000             | 0.001         | 0.019            |
| 0.730           | 0.003           | 0.482           | 0.362             | 0.021         |
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

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References


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
Growth and flowering response of snapdragons after release from apical dominance

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 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, they produced 4, 5, 6, and 7 pair of leaves. (19.8°C actual temperature) until flowering. The set point temperature was maintained with ventilation and a water pipe 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⁻¹ (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.).

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 cm²), which was significantly (P<0.05) different than the other treatments (Fig. 1F). However, it was observed maximum in plants.

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.
pinched at 7 leaf-pair stage (90 cm²). 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 \( (1184.01 \text{ cm}^2 \text{ g}^{-1}) \), which gradually increased in all subsequent treatments. However, maximum LAR \( (3076.76 \text{ cm}^2 \text{ g}^{-1}) \) 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 \( (15.12 \text{ d}^{-1}) \) whereas it was maximum \( (34.12 \text{ d}^{-1}) \) 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 \( (0.07 \text{ g cm}^{-1} \text{ d}^{-1}) \) whereas it was estimated minimum \( (0.03 \text{ g cm}^{-1} \text{ d}^{-1}) \) 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 indicates 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

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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 \( \text{cm}^2 \text{ g}^{-1} \), (E) relative growth rate \( \text{g cm}^{-1} \text{ d}^{-1} \), 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.

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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**


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$^4$ conidia/ml of formulation (13.5, 13.2, and 19.3 mm, respectively for *R. stolonifer*; 7.2, 7.5, and 19.2 mm, 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 atm) 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 atm) (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 Monnilinia 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) (Favel, 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 Th2 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, 5.5x10^6 sporangiospores of strain MP3 per ml, and 9.6x10^6 conidia of strain Th2 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 + fludioxonil (sold as Switch® 62.5 WG, a.i.=375g/kg cyprodinil + 250 g/kg fludioxonil).

Two types of treatments with T. harzianum (strain Th2) 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 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 Th2) harvested from 14-day old culture, were introduced into the invert emulsion according to the technique developed by Batta (2004a). Two additional nontreated 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 spor suspension containing 292,500 sporangiospores of R. stolonifer and 13,750 sporangiospores of M. piriformis (original suspensions contained 11.7x10^6 and 5.5x10^6 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 abovementioned 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 + fludioxonil) and Score® (difenoconazole) resulted in the
least values of external rot-lesion diameter (13.2 and 13.5 mm, respectively), followed by the treatment with formulated conidia of *T. harzianum* in invert emulsion (19.3 mm). The other treatments used in the experiment resulted in significantly higher values of means of external rot-lesion diameter ranging from 24.2 to 34.5 mm. 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 observed 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® (difenconazole) and Switch® (cyprodinil + fludioxonil) gave the least values of external rot-lesion diameter (7.2 and 7.5 mm, respectively), followed by the treatment with Merpan® (captan) (15.7 mm), formulated conidia of *T. harzianum* in invert emulsion (19.2 mm). The rest of the treatments used in the experiment gave significantly higher values of means of rot-lesion diameter ranging from 24.7 to 28.2 mm. 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 treatments. 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 + fludioxonil) or Score® (difenconazole) 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 SO₂ fumigation, conventionally practiced to prevent fungal decay in table grape berries (Lisket *et al.*, 1996; Lichter *et al.*, 2002). SO₂ treatment remains effective as long as SO₂ level is sufficiently high, but the high levels can result in fruit damage as fruit bleaching, unpleasant astringence and allergies (Lisket *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 effective than the Switch® or Score®, Merpan®, formulated conidia of *T. harzianum* in invert emulsion, conidial suspension of *T. harzianum* in sterile distilled water developed on berries (Mean±SE) and reduction (%) in external rot-lesion diameter relative to control:

<table>
<thead>
<tr>
<th>Fungicides and <em>T. harzianum</em> treatments</th>
<th>External rot-lesion diameter (mm) developed on berries (Mean±SE)</th>
<th>Reduction (%) in external rot-lesion diameter relative to control (water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ridomil® (metalaxy + mancozeb)</td>
<td>30.2±2.8 d</td>
<td>12.5 b</td>
</tr>
<tr>
<td>Score® (difenconazole)</td>
<td>13.5±1.9 a</td>
<td>60.9 e</td>
</tr>
<tr>
<td>Merpan® (captan)</td>
<td>28.3±3.1 d</td>
<td>17.4 e</td>
</tr>
<tr>
<td>Switch® (cyprodinil + fludioxonil)</td>
<td>13.2±1.8 a</td>
<td>61.7 e</td>
</tr>
<tr>
<td>Formulated conidia of <em>T. harzianum</em> in invert emulsion</td>
<td>19.3±2.4 b</td>
<td>44.1 d</td>
</tr>
<tr>
<td>Blank formulation of invert emulsion as control treatment</td>
<td>33.3±3.3 e</td>
<td>3.5 a</td>
</tr>
<tr>
<td>Conidial suspension of <em>T. harzianum</em> in sterile distilled water</td>
<td>24.2±2.9 c</td>
<td>29.8 e</td>
</tr>
<tr>
<td>Sterile distilled water as control treatment</td>
<td>34.5±3.1 e</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungicides and <em>T. harzianum</em> treatments</th>
<th>External rot-lesion diameter (mm) developed on berries (Mean±SE)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Ridomil® (metalaxy + mancozeb)</td>
<td>24.8±2.5 d</td>
<td>12.1 b</td>
</tr>
<tr>
<td>Score® (difenconazole)</td>
<td>7.2±1.3 a</td>
<td>74.5 e</td>
</tr>
<tr>
<td>Merpan® (captan)</td>
<td>15.7±2.1 b</td>
<td>44.3 d</td>
</tr>
<tr>
<td>Switch® (cyprodinil + fludioxonil)</td>
<td>7.5±1.2 a</td>
<td>73.4 e</td>
</tr>
<tr>
<td>Formulated conidia of <em>T. harzianum</em> in invert emulsion</td>
<td>19.2±1.9 c</td>
<td>31.9 e</td>
</tr>
<tr>
<td>Blank formulation of invert emulsion as control treatment</td>
<td>27.7±3.1 e</td>
<td>1.8 a</td>
</tr>
<tr>
<td>Conidial suspension of <em>T. harzianum</em> in sterile distilled water</td>
<td>24.7±2.8 d</td>
<td>12.4 b</td>
</tr>
<tr>
<td>Sterile distilled water as control treatment</td>
<td>28.2±3.7 e</td>
<td></td>
</tr>
</tbody>
</table>

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 Dehmuls K® (0.75%), Tween 20 (2.50%), and a mixture of 19.0% coconut oil + 28.5% 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


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 vera* was studied. Total nitrogen content of *Aloe vera* 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 vera* 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 vera* plant. The nitrate content in *Aloe vera* 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 vera* 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 vera*.

**Key words:** Amino acid, *Aloe vera* 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 vera* 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 vera* is widely cultivated in coastal regions of southern China. Since several qualities of *Aloe vera* plant were realised recently, *Aloe vera* 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 vera* plant has not been studied.

The objectives of the present study were (1) to study the nitrogen metabolism of *Aloe vera* plant under long-term irrigation with diluted seawater, and (2) to assess the qualities of *Aloe vera* 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 (18°9’N, 108°56’E). The climate is a tropical monsoon with mean annual temperature of 23-25°C, mean annual precipitation 1000-1200 mm, most of which occurs from late May to October, and mean annual evaporation 2000-2200 mm.

*Aloe 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 m² 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>
<thead>
<tr>
<th>Water used</th>
<th>pH</th>
<th>EC (dS m⁻¹)</th>
<th>TN (mg L⁻¹)</th>
<th>IN (mg L⁻¹)</th>
<th>TP (mg L⁻¹)</th>
<th>RP (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seawater</td>
<td>7.8</td>
<td>39.2</td>
<td>1.28</td>
<td>0.43</td>
<td>0.76</td>
<td>0.30</td>
</tr>
<tr>
<td>Tap water</td>
<td>7.0</td>
<td>0.11</td>
<td>0.15</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
</tr>
</tbody>
</table>

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:P₂O₅:K₂O=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 m³ 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 (Cyersan 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 (NIAU).

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.

**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 glycine betaine 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

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References


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 (\textit{Citrus sinensis}), Portugal mandarin (\textit{C. reticulata}) and Ortanique tangor (\textit{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 \textit{et al}., 1973; Andrews \textit{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 \textit{et al}., 1997). The use of sufficiency ranges and critical values have been most common for foliar analyses (Bar-Akiva \textit{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 \textit{et al}., 1984; Walworth and Sumner, 1987; Rodriguez \textit{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 (\textit{Citrus sinensis}), Portugal mandarin (\textit{C. reticulata}) and Ortanique tangor (\textit{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
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Table 1. Field characteristics of study plots at Todds Road Estate

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

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:

\[
\text{Fruit count}(1998) = -153 + 7042 \% \text{Mg} - 1451 \% \text{P} + 39.9 \text{pH} '2' + 6.08 \text{Mn ppm} + 168 \% \text{Ca} - 85.1 \text{Zn ppm} - 26.4 \text{P ppm} '2'.
\]

'2' refers to the 23 – 46 cm soil depth. \( P < 0.01, \text{Adjusted } R^2 = 99.5\)

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

<table>
<thead>
<tr>
<th>Yield level-1998 (fruit count)</th>
<th>N (%)</th>
<th>Zn (ppm)</th>
<th>Fe (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt;301)</td>
<td>2.5</td>
<td>10.24</td>
<td>133.4</td>
</tr>
<tr>
<td>Medium (301–450)</td>
<td>2.7</td>
<td>9.47</td>
<td>105.2</td>
</tr>
<tr>
<td>High (&gt;450)</td>
<td>2.1</td>
<td>3.80</td>
<td>59.9</td>
</tr>
<tr>
<td>Probability</td>
<td>0.09 NS</td>
<td>0.005</td>
<td>0.081 NS</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Yield level 1998 (fruit count)</th>
<th>P ppm (0 - 23 cm)</th>
<th>P ppm (23 - 46 cm)</th>
<th>P in leaves (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt;301)</td>
<td>8.8</td>
<td>8.8</td>
<td>0.120</td>
</tr>
<tr>
<td>Medium (301–800)</td>
<td>8.3</td>
<td>9.5</td>
<td>0.140</td>
</tr>
<tr>
<td>High (&gt;800)</td>
<td>8.0</td>
<td>10.4</td>
<td>0.177</td>
</tr>
<tr>
<td>Probability</td>
<td>NS</td>
<td>NS</td>
<td>NS = not significant ($P=0.05$)</td>
</tr>
</tbody>
</table>

**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)

<table>
<thead>
<tr>
<th>Yield level 1998 (fruit count)</th>
<th>K ppm (0 – 23 cm)</th>
<th>K ppm (23-46 cm)</th>
<th>P ppm (23-46 cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt;251)</td>
<td>96.4</td>
<td>89.7</td>
<td>18.6</td>
</tr>
<tr>
<td>Medium (251 – 300)</td>
<td>146.4</td>
<td>117.6</td>
<td>18.4</td>
</tr>
<tr>
<td>High (&gt;300)</td>
<td>160.0</td>
<td>84.0</td>
<td>7.5</td>
</tr>
<tr>
<td>Probability</td>
<td>0.072</td>
<td>0.038</td>
<td>0.525</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Yield level 1998 (fruit count)</th>
<th>pH (0 - 23 cm)</th>
<th>pH (23-46 cm)</th>
<th>Foliar nitrogen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>6.6</td>
<td>6.8</td>
<td>3.1</td>
</tr>
<tr>
<td>Medium</td>
<td>6.5</td>
<td>6.7</td>
<td>3.2</td>
</tr>
<tr>
<td>High</td>
<td>6.2</td>
<td>6.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Probability</td>
<td>0.062</td>
<td>0.012</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cultivar/Field ID</th>
<th>Nutrient variable</th>
<th>Correlation coefficient</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valencia 12069</td>
<td>Fe</td>
<td>0.507</td>
<td>0.064</td>
</tr>
<tr>
<td>1997</td>
<td>Cu</td>
<td>0.738</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Ca/Mg</td>
<td>0.542</td>
<td>0.045</td>
</tr>
<tr>
<td>Portugal 12050</td>
<td>Fe</td>
<td>0.573</td>
<td>0.026</td>
</tr>
<tr>
<td>1997</td>
<td>Cu</td>
<td>0.667</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Ca/Mg</td>
<td>0.607</td>
<td>0.016</td>
</tr>
<tr>
<td>Portugal 12050</td>
<td>% K</td>
<td>0.580</td>
<td>0.030</td>
</tr>
<tr>
<td>1998</td>
<td>pH 0-23 cm (soil)</td>
<td>-0.534</td>
<td>0.074</td>
</tr>
<tr>
<td>Valencia 12071</td>
<td>pH 23-40 cm (soil)</td>
<td>-0.693</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>K(ppm) 23-40 cm</td>
<td>0.613</td>
<td>0.034</td>
</tr>
<tr>
<td>Ortanique 12083</td>
<td>% Water²</td>
<td>0.844</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>K (ppm) 23-40 cm</td>
<td>-0.610</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>P (ppm) 23-40 cm</td>
<td>0.529</td>
<td>0.043</td>
</tr>
</tbody>
</table>

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

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 mangoes 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.

### References


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

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Field ID</th>
<th>Year</th>
<th>Nutrient factor</th>
<th>Regression equation</th>
<th>Adjusted R² (%)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valencia orange</td>
<td>12069</td>
<td>1997</td>
<td>N</td>
<td>Fruit weight = 181 + 61.7 %N – 455 %Mg – 5.73 P ppm ‘2”</td>
<td>62.3</td>
<td>0.005</td>
</tr>
<tr>
<td>Valencia orange</td>
<td>12071</td>
<td>1997</td>
<td>N</td>
<td>Fruit weight = 155 + 27.6 pH – 31.7 %N</td>
<td>50.7</td>
<td>0.006</td>
</tr>
<tr>
<td>Portugal mandarin</td>
<td>12036</td>
<td>1998</td>
<td>N</td>
<td>Fruit weight = 183 – 57 %N + 796 %P</td>
<td>37.7</td>
<td>0.023</td>
</tr>
<tr>
<td>Portugal mandarin</td>
<td>12050</td>
<td>1998</td>
<td>N</td>
<td>Fruit weight = 136 – 11.9 %N + 31.2 %K – 0.143 Fe/Zn</td>
<td>74.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Valencia orange</td>
<td>12069</td>
<td>1998</td>
<td>Mg</td>
<td>Fruit weight = 181 + 61.7 %N – 455 %Mg – 5.73 P ppm ‘2”</td>
<td>62.3</td>
<td>0.005</td>
</tr>
<tr>
<td>Valencia orange</td>
<td>12069</td>
<td>1998</td>
<td>Mg</td>
<td>Number of seeds = 4.58 + 0.00665 Mg/Zn</td>
<td>76.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Portugal mandarin</td>
<td>12050</td>
<td>1998</td>
<td>K/Mg</td>
<td>Fruit volume = 102 – 2.67 K/Mg + 29.7 %K</td>
<td>37.5</td>
<td>0.024</td>
</tr>
<tr>
<td>Portugal mandarin</td>
<td>12050</td>
<td>1998</td>
<td>Mg</td>
<td>Acidity (%) = 1.03 + 0.421 %Mg – 0.00222 Mn ppm</td>
<td>54.2</td>
<td>0.004</td>
</tr>
<tr>
<td>Portugal mandarin</td>
<td>12050</td>
<td>1998</td>
<td>Mn</td>
<td>Peel thickness = 0.95 + 0.00359 P ppm + 0.00192 Mn ppm – 0.0589 %N – 0.00591 K ppm</td>
<td>85.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Portugal mandarin</td>
<td>12050</td>
<td>1998</td>
<td>Mn</td>
<td>Peel thickness = 0.186 + 0.00244 Mn ppm</td>
<td>23.3</td>
<td>0.039</td>
</tr>
</tbody>
</table>

* “2” refers to the 23 – 46 cm soil depth

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


Studies with thidiazuron on the vase life of cut rose flowers

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Abstract

Effects of postharvest-applied thidiazuron (TDZ) on the vase 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 (Hickleton, 1991; Lukaszewska et al., 1994; Paull and Chanrachit, 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-thiazol-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 pulse-treated 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...
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).

Paufl 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


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 curbing 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 curbing 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 sprout 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).
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 (Mausteth, 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...
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).

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

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References


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


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 Vitis 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. australis 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

<table>
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<th>Green / Yellow berries</th>
<th>Red / Black berries</th>
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<tr>
<td>Anab-e-Shahi</td>
<td>Black Muscat</td>
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<tr>
<td>Angur Kalan</td>
<td>Black Prince</td>
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<tr>
<td>Banqui Abyad</td>
<td>Catawba</td>
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<td>Coarna Regia</td>
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<td>Chemin Blanc</td>
<td>Concord</td>
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<td>Gold</td>
<td>Convent Large Black</td>
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<tr>
<td>Itatia</td>
<td>Dakh</td>
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<tr>
<td>Jaos Beli</td>
<td>Diamond Jubilee</td>
</tr>
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<td>Muller Thergau</td>
<td>Muscat Hamburg</td>
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<td>Palomino</td>
<td>Muzzafar Nagar</td>
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<td>Red Globe</td>
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<tr>
<td>Walthom Cross</td>
<td>Spin Sahebi</td>
</tr>
<tr>
<td>Bangalore Blue</td>
<td>Thompson Seedless</td>
</tr>
<tr>
<td>Bharat Prince</td>
<td>Flame Seedless</td>
</tr>
<tr>
<td>Black Champa</td>
<td>Gulabi</td>
</tr>
</tbody>
</table>

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.
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 1.4 kb in size. Primer 891 showed the maximum number of bands. 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.

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 of *V. vinifera*. Variety Concord separated initially from all others and showed least similarity. Cluster I consisted of mainly *V. labrusca or labrusca × 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](image)
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**


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 A. acuminate type.

Aseptic cultures were established from shoot tips which were surface-sterilized in 3 % NaOCl solution (containing 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 benzyladenine (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/cm² 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.

Table 1. Shoot growth, shoot development, root growth and root development of in vitro cultured banana as affected by the cultivar and PEG concentration

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

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. 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.
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.

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

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...
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 (Glassziou 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 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


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 parthenocarpic in papaya. Rodriguez-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 m²) 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 and cultivars using the chi-square test on summed data for females of ‘Taino-2’. Ten to 30 flowers per sex type 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.

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; Kyowa hakkō, 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 (R²= 0.904, P = 0.001), and with individual fruit weight (R²= 0.615, P = 0.036). In hermaphrodite trees, fruit yield per tree also correlated with the number of fruits per tree (R²= 0.747, P = 0.0056), but not with individual fruit weight (R²= 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 (x²= 8.959, P = 0.0028) and non-pollination treatments (x²= 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 | 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
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.

**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
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


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 mg l⁻¹ and NAA 0.75 mg l⁻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 traditional 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 hardness, the proliferation rate and vitrification. Lowering the nutrient gel hardness 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 hyperhydricity 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 mg l⁻¹). They used a hydroa soluble 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 g l⁻¹) of agar (Bacto BiTeck agar). These treatments were supplemented with BA 0.75 mg l⁻¹, and NAA 0.75 mg l⁻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 g l⁻¹) treatment after 8 weeks culture period (Fig. 1). The highest multiplication rate (7-8 no month⁻¹) was also obtained in the same treatment (2X mineral and 6 g l⁻¹ agar concentrations) (Fig. 2). The highest percentage (43%) of hyperhydration was observed in high (2X) mineral concentration with very low agar (4 g l⁻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 g l⁻¹) treatment compared to high agar-gelled (10 g l⁻¹) medium (Table 1) has been reported previously by many authors (Debergh et al., 1981; Bornman and Vogelmans, 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 g l⁻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 availability to the explant.

### Table 1. Effects of relative mineral concentration and hyperhydration of almond (Prunus amygdalus L. var. Binazir) during 8 weeks culture.

<table>
<thead>
<tr>
<th>Relative mineral concentration</th>
<th>Agar concentration (g l⁻¹)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Multiplication rate (number month⁻¹)</th>
<th>Rooting (%)</th>
<th>Hyperhydration (%)</th>
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<tbody>
<tr>
<td>0X</td>
<td>4</td>
<td>7.7</td>
<td>0.08</td>
<td>2.0</td>
<td>58</td>
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<td></td>
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<td>2.5</td>
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<td>7.3</td>
<td>0.08</td>
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<td></td>
<td>10</td>
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<td>0.07</td>
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<tr>
<td>0.2X</td>
<td>4</td>
<td>13.4</td>
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<td>52</td>
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<tr>
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<tr>
<td></td>
<td>8</td>
<td>19.9</td>
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<td>42</td>
<td>5.0</td>
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<td></td>
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<tr>
<td>1X</td>
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<td>45</td>
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<td>2.8</td>
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<td>3.0</td>
<td>2</td>
<td>12.8</td>
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<tr>
<td>LSD (P=0.05)</td>
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<td>1.51</td>
<td>6.38</td>
<td>4.86</td>
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</table>

![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 g⁻¹) of agar concentrations (at week 8).](image-url)
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 babonica. 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

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References


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 360m² 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 x 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soil depth (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>pH</td>
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</tr>
<tr>
<td>EC dS/m</td>
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<tr>
<td>Total(+)</td>
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<tr>
<td>Mg (Meq/L)</td>
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<tr>
<td>Cl (Meq/L)</td>
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<td>HCO$_3$ (Meq/L)</td>
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<tr>
<td>P (ppm)</td>
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<tr>
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<td>Cd (ppm)</td>
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<tr>
<td>NO$_3$ (ppm)</td>
<td>12.80</td>
</tr>
<tr>
<td>SAR</td>
<td>0.69</td>
</tr>
</tbody>
</table>

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 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 beds (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).
While only slight increase occurred to the SAR value during the second year of experiment (Fig. 2B).

Rose tissue mineral composition was compared with the optimum nutrient levels declared 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. Additionally, 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
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

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.
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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First</th>
<th>Second</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.90</td>
<td>8.00</td>
</tr>
<tr>
<td>Na (Meq/L)</td>
<td>19.21</td>
<td>25.32</td>
</tr>
<tr>
<td>Mg (Meq/L)</td>
<td>14.80</td>
<td>15.40</td>
</tr>
<tr>
<td>Ca (Meq/L)</td>
<td>19.80</td>
<td>20.82</td>
</tr>
<tr>
<td>Cl (Meq/L)</td>
<td>37.80</td>
<td>38.90</td>
</tr>
<tr>
<td>HCO₃ (Meq/L)</td>
<td>6.32</td>
<td>6.30</td>
</tr>
<tr>
<td>P (ppm)</td>
<td>2.01</td>
<td>2.20</td>
</tr>
<tr>
<td>K (ppm)</td>
<td>30.32</td>
<td>32.84</td>
</tr>
<tr>
<td>NO₃ (ppm)</td>
<td>318.40</td>
<td>331.50</td>
</tr>
<tr>
<td>Zn (ppm)</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Fe (ppm)</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>Cu (ppm)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Mn (ppm)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Cd (ppm)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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**


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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

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**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. 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. 4.** The bark width of peach trees as influenced by partial ringing and partial ringing plus liquid nitrogen application. Vertical bars represent SE.

**Fig. 5.** The percentage of flower buds, total shoot length and weight of pruned branches from the trees subjected to partial ringing plus liquid nitrogen application.
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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit weight (g)</th>
<th>Fruit number/tree</th>
<th>Brix (%)</th>
<th>Acid content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>181.6±15.5</td>
<td>9.1±1.6</td>
<td>9.46±0.13</td>
<td>0.35±0.08</td>
</tr>
<tr>
<td>Partial ringing</td>
<td>203.6±9.2</td>
<td>9.3±2.1</td>
<td>12.25±0.27</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>Partial ringing + liquid nitrogen</td>
<td>186.6±13.9</td>
<td>9.3±1.4</td>
<td>12.63±0.25</td>
<td>0.29±0.02</td>
</tr>
</tbody>
</table>

Values are means ± standard error (n=4).

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)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit weight (g)</th>
<th>Fruit number/tree</th>
<th>Brix (%)</th>
<th>Acid content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>197.0±6.1</td>
<td>12.0±0.8</td>
<td>14.11±0.45</td>
<td>0.37±0.06</td>
</tr>
<tr>
<td>Partial ringing</td>
<td>210.8±13.2</td>
<td>14.0±2.4</td>
<td>14.23±0.58</td>
<td>0.26±0.01</td>
</tr>
<tr>
<td>Partial ringing + liquid nitrogen</td>
<td>213.2±11.1</td>
<td>14.3±4.3</td>
<td>14.29±0.18</td>
<td>0.24±0.01</td>
</tr>
</tbody>
</table>

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 events 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**

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**References**


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 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Average fruit weight (g)</th>
<th>'Salustiana'</th>
<th>'Pineapple'</th>
<th>'Hamlin'</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Cleopatra’ mandarin</td>
<td>167.8 b</td>
<td>163.3 c</td>
<td>154.4 b</td>
<td></td>
</tr>
<tr>
<td>Sour Orange</td>
<td>193.2 a</td>
<td>167.6 bc</td>
<td>155.2 b</td>
<td></td>
</tr>
<tr>
<td>C. macrophylla</td>
<td>213.5 a</td>
<td>178.5 ab</td>
<td>199.7 a</td>
<td></td>
</tr>
<tr>
<td>C. volkameriana</td>
<td>204.8 a</td>
<td>184.1 a</td>
<td>170.1 b</td>
<td></td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Average fruit diameter (mm)</th>
<th>'Salustiana'</th>
<th>'Pineapple'</th>
<th>'Hamlin'</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Cleopatra’ mandarin</td>
<td>70.9 b</td>
<td>69.5 a</td>
<td>65.5 c</td>
<td></td>
</tr>
<tr>
<td>Sour Orange</td>
<td>74.3 ab</td>
<td>70.9 a</td>
<td>67.7 b</td>
<td></td>
</tr>
<tr>
<td>C. macrophylla</td>
<td>73.8 ab</td>
<td>70.1 a</td>
<td>71.5 a</td>
<td></td>
</tr>
<tr>
<td>C. volkameriana</td>
<td>78.4 a</td>
<td>70.5 a</td>
<td>69.1 b</td>
<td></td>
</tr>
</tbody>
</table>

* Mean separation within columns by LSD test, values that don’t share the same letter 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Average seed number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>'Salustiana'</td>
</tr>
<tr>
<td>'Cleopatra’ mandarin</td>
<td>2.7 a</td>
</tr>
<tr>
<td>Sour Orange</td>
<td>2.4 a</td>
</tr>
<tr>
<td>C. macrophylla</td>
<td>3.0 a</td>
</tr>
<tr>
<td>C. volkameriana</td>
<td>2.4 a</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Juice % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>'Salustiana'</td>
</tr>
<tr>
<td>'Cleopatra’ mandarin</td>
<td>42.6 b</td>
</tr>
<tr>
<td>Sour Orange</td>
<td>40.7 c</td>
</tr>
<tr>
<td>C. macrophylla</td>
<td>46.9 a</td>
</tr>
<tr>
<td>C. volkameriana</td>
<td>42.1 bc</td>
</tr>
</tbody>
</table>

* 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. volkameriana gave the highest fruit pH (3.8) (Table 3).

Table 6. Effect of rootstocks on total soluble solids of three sweet orange varieties

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TSS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>'Salustiana'</td>
</tr>
<tr>
<td>'Cleopatra’ mandarin</td>
<td>12.6 a</td>
</tr>
<tr>
<td>Sour Orange</td>
<td>13.1 a</td>
</tr>
<tr>
<td>C. macrophylla</td>
<td>11.6 b</td>
</tr>
<tr>
<td>C. volkameriana</td>
<td>10.8 c</td>
</tr>
</tbody>
</table>

* Mean separation within columns by LSD test, values that don’t share the same letters are significantly different (P<0.05).
macrophylla gave low 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>'Salustiana'</th>
<th>'Pineapple'</th>
<th>'Hamlin'</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Cleopatra' mandarin</td>
<td>4.08 a</td>
<td>3.64 b</td>
<td>3.71 b</td>
</tr>
<tr>
<td>Sour Orange</td>
<td>3.77 a</td>
<td>3.63 b</td>
<td>3.80 b</td>
</tr>
<tr>
<td><em>C. macrophylla</em></td>
<td>3.76 a</td>
<td>3.84 a</td>
<td>4.01 a</td>
</tr>
<tr>
<td><em>C. volkameriana</em></td>
<td>3.94 a</td>
<td>3.75 ab</td>
<td>3.75 b</td>
</tr>
</tbody>
</table>

* 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


Comparison of bananas ripened by two methods for textural sale-grades

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Abstract
This study reflects 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° 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 Loeseecke, 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 peal 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 mean 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.
Comparison of bananas ripened by two methods for textural sale-grades

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 18°C 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 °C- 18 °C under maintained 80 % humidity (with say saturated solution of potassium nitrate). If the initial costs (chamber {size according to the need}, chemicals) 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

<table>
<thead>
<tr>
<th>Changes in parameters</th>
<th>Ripening methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude market</td>
</tr>
<tr>
<td>Reduced mass %</td>
<td>13.80 (± 36.08)</td>
</tr>
<tr>
<td>Moisture (%) in pulp</td>
<td>3.09 (± 4.67)</td>
</tr>
<tr>
<td>Pulp : peel ratio (Fresh)</td>
<td>2.17 (± 0.16)</td>
</tr>
</tbody>
</table>

*Changes from initial raw bananas used.

Table 2. Yield of fermented beverage from day’s available over-ripe
bananas.

<table>
<thead>
<tr>
<th>Total available over-ripe bananas#</th>
<th>5700</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of over-ripe bananas*</td>
<td>460 kg</td>
</tr>
<tr>
<td>%Yield of beverage (alcoholic drink)</td>
<td>69.88</td>
</tr>
<tr>
<td>Beverage from pulp</td>
<td>321 kg</td>
</tr>
</tbody>
</table>

Monetary loss (due to over ripeness)

<table>
<thead>
<tr>
<th>Total available over-ripe bananas#</th>
<th>5700</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of over-ripe bananas*</td>
<td>460 kg</td>
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<td>69.88</td>
</tr>
<tr>
<td>Beverage from pulp</td>
<td>321 kg</td>
</tr>
</tbody>
</table>

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) | Rs. 2375/- |
| Value addition by beverage | 3513 – 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.

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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.

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Fig. 4. Lowering of texture during ripening (4 d period) (observed in
June) by banana ripening methods (Crude market /BIS).

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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 processors/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 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 losing 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, Koshkooeyea 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, Kaleighhoochi 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:

\[ \text{Percent position of each rank} = \frac{100 (R_i - 0.5)}{N_j} \]

\[ R_i = \text{Rank given for } i_{th} \text{ factor by } j_{th} \text{ individual} \]

\[ N_j = \text{Number of factors ranked by } j_{th} \text{ individual} \]

The percentage position of each rank was 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)

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Iran</th>
<th>USA</th>
<th>Turkey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Share in first 2 years (%)</td>
<td>56.5</td>
<td>15</td>
<td>13.00</td>
</tr>
<tr>
<td>Share in last 2 years (%)</td>
<td>46.5</td>
<td>24</td>
<td>10.75</td>
</tr>
<tr>
<td>Change in share (%)</td>
<td>-17.7</td>
<td>60</td>
<td>-17.30</td>
</tr>
<tr>
<td>Harvested area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Share in first 2 years (%)</td>
<td>55</td>
<td>7.5</td>
<td>10.00</td>
</tr>
<tr>
<td>Share in last 2 years (%)</td>
<td>66</td>
<td>9</td>
<td>9.00</td>
</tr>
<tr>
<td>Change in share (%)</td>
<td>20</td>
<td>20</td>
<td>-10.00</td>
</tr>
<tr>
<td>Yield / hectare</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Share in first 2 years (%)</td>
<td>102.5</td>
<td>199</td>
<td>130.25</td>
</tr>
<tr>
<td>Share in last 2 years (%)</td>
<td>71</td>
<td>278</td>
<td>120.50</td>
</tr>
<tr>
<td>Change in share (%)</td>
<td>-30.73</td>
<td>39.7</td>
<td>-7.48</td>
</tr>
<tr>
<td>Export quantity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Share in first 2 years (%)</td>
<td>73</td>
<td>8</td>
<td>0.40</td>
</tr>
<tr>
<td>Share in last 2 years (%)</td>
<td>57.5</td>
<td>12</td>
<td>2.37</td>
</tr>
<tr>
<td>Change in share (%)</td>
<td>-21.23</td>
<td>50</td>
<td>493.75</td>
</tr>
<tr>
<td>Export value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Share in first 2 years (%)</td>
<td>71</td>
<td>7.7</td>
<td>0.67</td>
</tr>
<tr>
<td>Share in last 2 years (%)</td>
<td>61</td>
<td>12</td>
<td>3.36</td>
</tr>
<tr>
<td>Change in share (%)</td>
<td>-14.08</td>
<td>55.84</td>
<td>401.86</td>
</tr>
</tbody>
</table>


**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. Problems faced by producers and traders in Iran

<table>
<thead>
<tr>
<th>Constraints</th>
<th>Score</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of adequate agricultural water</td>
<td>87</td>
<td>1*</td>
</tr>
<tr>
<td>Lack of appropriate pesticides</td>
<td>81</td>
<td>5</td>
</tr>
<tr>
<td>Lack of appropriate chemical fertilizers</td>
<td>83</td>
<td>3*</td>
</tr>
<tr>
<td>Lack of extension and advisory services</td>
<td>79</td>
<td>7</td>
</tr>
<tr>
<td>Inadequate credits for production</td>
<td>82</td>
<td>4</td>
</tr>
<tr>
<td>Inadequate processing plants</td>
<td>78</td>
<td>8</td>
</tr>
<tr>
<td>Unsuitable domestic market structure</td>
<td>85</td>
<td>2*</td>
</tr>
<tr>
<td>Inter and intra year fluctuations of prices</td>
<td>85</td>
<td>2*</td>
</tr>
<tr>
<td>Low price of pistachio in the market</td>
<td>85</td>
<td>2*</td>
</tr>
<tr>
<td>High discount rate in domestic market undertaken by buyers</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>Paying late by buyers</td>
<td>78</td>
<td>8</td>
</tr>
<tr>
<td>High cost of production</td>
<td>82</td>
<td>4</td>
</tr>
<tr>
<td>Others</td>
<td>74</td>
<td>9</td>
</tr>
</tbody>
</table>

*The most important problems

Table 3. Constraints in production, processing and marketing of Pistachio from the Traders’ point of view in Iran

<table>
<thead>
<tr>
<th>Constraints</th>
<th>Score</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of extension services to the producers and processors and low quality</td>
<td>-</td>
<td>Not Ranked</td>
</tr>
<tr>
<td>Lack of modern pistachio processing plants</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>Domestic market structure</td>
<td>47</td>
<td>4</td>
</tr>
<tr>
<td>World market structure</td>
<td>-</td>
<td>Not Ranked</td>
</tr>
<tr>
<td>Inter and intra year fluctuation of prices in world market</td>
<td>-</td>
<td>Not Ranked</td>
</tr>
<tr>
<td>High discount rate in domestic market</td>
<td>44</td>
<td>6</td>
</tr>
<tr>
<td>High and increasing trend for cost of production</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>Low productivity (yield per hectare)</td>
<td>43</td>
<td>7</td>
</tr>
<tr>
<td>Lack of credit to marketing agencies</td>
<td>46</td>
<td>5</td>
</tr>
<tr>
<td>Low and constant trend of prices in world market</td>
<td>-</td>
<td>Not Ranked</td>
</tr>
<tr>
<td>In appropriate and changing Government policies and rules for export</td>
<td>53</td>
<td>2*</td>
</tr>
<tr>
<td>Inadequate transportation system</td>
<td>28</td>
<td>9</td>
</tr>
<tr>
<td>Inadequate packaging industries</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>Irregular supply of product to market during the year</td>
<td>51</td>
<td>3*</td>
</tr>
<tr>
<td>Aflatoxin pollution</td>
<td>60</td>
<td>1*</td>
</tr>
<tr>
<td>Poor quality of product in international market</td>
<td>37</td>
<td>8</td>
</tr>
<tr>
<td>Others</td>
<td>-</td>
<td>Not Ranked</td>
</tr>
</tbody>
</table>

*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 into 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 decades 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
FAO, 2003. FAOSTAT information bank. Food and Agriculture Organization of UN.
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