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Retranslocation of nutrients and zinc sulphate fertilization of banana plants in central Amazon

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Abstract

Banana cultivation is ranked as one of the agricultural activities of greatest economic importance and social significance in Brazil. The area under banana cultivation in Brazil (516,000 ha) is larger than India and Ecuador, leading countires in production, but with rather lower productivity due to lack of adequate crop management, particularly fertilizer application. The objective of this work was to investigate the rate of nutrient retranslocation and the effect of fertilization on the yield and uniformity of banana bunches cultivated in central Amazon region. Two field experiments were conducted in a xanthic Ferralsol (dystrophic Yellow Latosol) - predominant soil of the region, examining: a) the nutrient translocation rate in twelve plants; and b) the efficiency of zinc use, in a completely randomized blocks in split plot design with four rates of $ZnSO_4$ (0, 30, 60 and 120 g $plant^{-1}$ $cycle^{-1}$) and two application times (in the hole together with the seedling or applied in the fifth month after planting), with four replicates. Under the local edaphoclimatic conditions, the results show that N, P, K, Mg and Cu have a high retranslocation rate. The plant yield was influenced by the rates of $ZnSO_4$, with the most efficient application method being in the planting hole. Results indicated that at high concentrations, zinc had mobility in the phloem from the leaves to the fruits. The proposed critical leaf zinc concentration at the start of inflorescence was 12.9 mg kg^{-1} for the third leaf.

Key words: Critical leaf zinc concentration, foliar nutrients, nutrient mobility, *Musa* spp.

Introduction

The dynamics of nutrients in fruit-bearing plants is very important in the various processes, such as ripening, growth, senescence and development of physiological disorders (Johnson *et al.*, 1987; Fergunson *et al.*, 1999). Studies on the transport and distribution of zinc in the plants can help in understanding for improved and efficient translocation of the applied nutrients. Still, these studies have received relatively little attention, although some have shown that deficiency at an early growth stages results in significant productivity losses (Pearson and Rengel, 1994; Martinez *et al.*, 2005).

The mineral composition of the leaves is a consequence of factors that influence the absorption, long-distance transport and translocation of mineral elements (Malavolta *et al.*, 1997; Epstein and Bloom, 2005). Leaf diagnosis can be used both as a way to recommend fertilizer rates and to adjust application timing, mainly in perennials. The remobilization of nutrients is important particularly during the reproductive phase, when seeds, fruits and storage organs are formed. At this stage, the root activity usually declines as a result of the decrease in supply of carbohydrates (sink competition) (Marschner, 1995). In plants, zinc is absorbed by the roots and quickly transported to the aerial part. It is partially mobile within the plant and its transport occurs passively through transpiration flow (Epstein and Bloom, 2005). Nevertheless, the transport mechanisms of sap in the xylem are subject of considerable debate (Longnecker and Robson, 1993).

Zinc deficiency in banana plants stunts their growth and causes their leaves to become lanceolate, narrow and yellowed, with chlorotic striping between the secondary veins and yellow coloration on the underleaf surface, mainly in the primary vein. The symptoms are more evident in the fruits, with reduced length and diameter, as a rule in the top and bottom thirds of the bunches. Besides the reduced size, the fruits have a cigar-shape, with green tips (Moreira *et al.*, 2007), and the distance between hands is reduced, giving the bunches a compact appearance (Brown *et al.*, 1993; Borges *et al.*, 1999).

In places where there is a deficiency of Zn, the amount and physiological timing of correct application of the nutrient may be essential to increase the yield, with a greater number of marketable fruits. The objective of this work was to verify the nutrient remobilization rate and establish a suitable critical zinc level in leaf under the edaphoclimatic conditions of the Central Amazon, and to define the best physiological stage for the application of nutrients in the soil.

Material and methods

Study site: The experiments were conducted in clayey texture (719 g kg^{-1}) and kaolinthic dystrophic Yellow Latosol (Brazilian classification– Embrapa, 1999) – Xanthic Ferralsol (FAO, 1990), with low natural fertility (Table 1), located at the Embrapa Western Amazon experimental station, at coordinates 3°8' S and 59°52' W, in the municipality of Manaus, Amazonas State, Brazil. The natural vegetation is a tropical rainforest. The region's predominant climate is humid tropical, classified as Afi by the Köppen system, with relatively abundant rainfall throughout the year (mean of 2,250 mm). The amount of rainfall in the driest

months (July to September) is always above 60 mm, and the wettest months are February to April. The average temperature is about 26ºC (Vieira and Santos, 1987).

Experiments

The field experiments were established in January on an upland area ('terra firme') of about 0.5 ha which first had been cleared for a rubber plantation in 1978, but this had been abandoned with development into secondary forest.

Experiment (a): The experiment was set up with twelve banana plants (cv FHIA 18), grown from tissue culture, in a non-irrigated regime, with periodic defoliations and pruning to determine the rate of nutrient remobilization. At the start of inflorescence, central part of the leaf blade of the third leaf was removed (Malavolta, 1992 – diagnostic leaf) counted from the apex (phase called F_1), and symmetrically from this same leaf another part at the time of harvesting the bunches (phase called F_2). After collection, the leaf parts were dried at ± 65ºC until they reached constant weight and then were ground and sieved through 0.40-mm mesh. The total N was extracted by sulfur digestion and determined by the micro-Kjeldahl method (Nelson and Sommers, 1972), while the P, K, Ca, Mg, S, Cu, Fe, Mn and Zn were extracted by nitropercloric digestion, with the P and S determined by spectrophotometry (blue molybdenum photometry) and turbidimetry methods, respectively (Novozamsky *et al.*, 1983). The other nutrients were analyzed by atomic absorption spectrophotometry according to the method described by Malavolta *et al.* (1997).

To determine the internal retranslocation rate of the nutrients, we calculated the fraction of nutrient retranslocated (FNR), using the following equation, adapted from Ares *et al.* (2003).

$$FNR = 1 - \left(\frac{\frac{NF_1}{\text{Ca or B in } NF_1}}{\frac{NF_2}{\text{Ca or B in } NF_2}} \right)$$

where,

NF_1 = nutrient obtained in the leaf part at the start of flowering;

NF_2 = nutrient obtained in the leaf part at the time of harvesting.

The use of the Ca content (g kg^{-1}) for the macronutrients and B (mg kg^{-1}) for the micronutrients as a comparison variable was due to the low mobility within the plant (Epstein and Bloom, 2005; Malavolta, 2006).

Experiment (b): An experiment was set up with the Thap Maeo cultivar, employing random complete blocks in a 4x2 factorial scheme in split-plot design, with four replicates. The plots were treated with four rates of $ZnSO_4$ (0, 30, 60, 120 g $plant^{-1}$ $cycle^{-1}$ - 20% of Zn), while the subplots were the two application times (in the hole at the time of planting the seedling and in the fifth month after planting). The treatments consisted of the average of the data collected from the five central plants of each replicate.

Fertilization: In both the experiments, the spacing was three meters between rows and two meters between plants (1, 667 plants ha^{-1}). Forty-five days before planting, we fertilized the holes (60 x 60 x 60cm) with five liters of chicken manure and 500 g of dolomitic limestone [Effective Calcium Carbonate (ECC) = 90%]. At the time of planting in the remobilization experiment, 60 g of P_2O_5 (single superphosphate – 20% of P_2O_5) and 50 g of fritted trace elements (FTE BR12® - B, 1.8, Cu, 0.8, Fe, 3.0, Mn, 2.0, Mo, 0.1 and Zn, 9.0%) was applied together with the seedlings while in the $ZnSO_4$ rate experiment (b), the amount of FTE BR12® was 10 g, only to supply the plants development requirements. The broadcast fertilization consisted of 256 g $plant^{-1}$ of urea (44% of N) and 1,600 g $plant^{-1}$ of potassium chloride (58% of K_2O), distributed in four applications: in the second, fourth, seventh and tenth month after planting (Moreira *et al.*, 2005). The first three fertilizations were done around the plant in a range of 50 cm, and the last in a semicircle beside the daughter plant.

Table 1. Characteristics and nutrient availability in a xanthic Ferralsol (dystrophic Yellow Latosol) located in the municipality of Manaus, Amazonas State, Brazil

Characteristics[(1)]	Depth (cm)		
	0-10	10-20	20-40
pH in $CaCl_2$	3.43	3.50	3.65
P (mg dm^{-3})	2.94	2.28	2.02
K (mg dm^{-3})	25.60	17.67	14.33
Ca ($cmol_c$ dm^{-3})	0.19	0.11	0.10
Mg ($cmol_c$ dm^{-3})	0.19	0.15	0.15
H+Al ($cmol_c$ dm^{-3})	8.80	10.08	8.30
Al ($cmol_c$ dm^{-3})	2.12	1.58	1.09
S (mg dm^{-3})	19.52	19.55	32.03
SOM (g kg^{-1})	42.77	31.04	23.50
B (mg dm^{-3})	0.34	0.30	0.25
Cu (mg dm^{-3})	0.11	0.10	0.08
Fe (mg dm^{-3})	170.07	166.67	144.57
Mn (mg dm^{-3})	1.90	1.25	1.26
Zn (mg dm^{-3})	0.67	0.47	0.35

[(1)] available P, K, Cu, Fe, Mn and Zn was extracted with Mehlich 1; exchangeable Ca, Mg, Al was determined after extraction with KCl 1.0 mol L^{-1}; exchangeable H+Al was with calcium acetate 0.01 mol L^{-1}; SOM (soil organic matter) = C x 1.724 – Walkley Black method (Embrapa, 1997).

In the fourth month after planting, mulch containing 100 g of magnesium sulfate (9% Mg), 20 g of copper sulfate (13% Cu), 20 g of iron sulfate (19% Fe), 10 g of manganese sulfate (26% of Mn) and 30 g of boric acid (17% of B), and 30 g of zinc sulfate (20% of Zn) was applied (Moreira *et al.*, 2005) in the remobilization experiment.

Just as in the remobilization experiment, the Zn content was determined in the leaves in phases F_1 and F_2. At the time of harvesting the bunches, fruit of hands 2, 6 and 10 were sampled to measure the length, diameter and Zn content of the fruit. In phase F_1, the foliar critical concentration was established according to the methodology described by Cate Junior and Nelson (1971) .

Statistical analyses: The data were subjeted to Analysis of Variance (ANOVA). Comparison of least significant differences between means (Tukey test, $P \leq 0.05$), and regression analysis at 5% significance (Pimentel Gomes and Garcia, 2002) was performed.

Results and discussion

Based on the chemical analyses and soil critical levels established by Moreira *et al.* (2005) for cultivating banana plants in Amazonas State, it was inferred that independent of the depth, the concentrations of P, K, Ca, Mg, B, Fe, Mn and Zn in the

soil were within the classes considered very low, while the Al and exchangeable H+Al were very high (Table 1). Lehmann *et al.* (2001) reported 90% of soils in the Amazonian area had poor fertility. These characteristics were ideal for the study of nutrient translocation in banana plants and their response to fertilization.

The analysis of variance of the yield per hectare and the leaf content of Zn in phases F_1 and F_2 indicate a significant effect of the $ZnSO_4$ rates ($P \leq 0.05$), time of application and interaction of rates versus timing of application (Table 2). The plant productivity was greater with fertilization in the planting hole, even with application of 10 g of FTE BR12® in all treatments to maintain the minimum level of nutrients required for initial development of the seedlings (Table 3). The interactions indicated that even with the incremental responses following a quadratic equation, the application timing did not show the same behaviour as a function of the $ZnSO_4$ rates. To obtain better estimated yield in the local edaphoclimatic conditions, it would be necessary to apply 100.8 kg ha^{-1} in the hole to obtain 48.3 t ha^{-1} $cycle^{-1}$, while in broadcast method the quantity applied would be 129.2 kg ha^{-1}, with estimated yield of 47.0 t ha^{-1} $cycle^{-1}$.

These results show the superiority of zinc sulfate placed in the planting hole. Besides acting on the formation of the fruits (Borges *et al.*, 1999), with elongation of the cells caused by the synthesis of tryptophane, a precursor of indoleacetic acid (Marschner, 1995), zinc is also important during the initial root formation and vegetative growth stages (Malavolta, 2006). In addition to the nutritional aspects, the application in the hole is less expensive, requiring less fertilizer and fewer crop treatments.

Based on the studies of Moreira *et al.* (2005), on the concentrations of Ca and B (Epstein and Bloom, 2005) and on the rate of increment, we found that the retranslocated fractions of Ca, Fe and Mn have low remobilization to the fruits, with most being retained in the leaves (Table 3), while B, Zn and S had an intermediate retranslocation rate. Regarding the macronutrients, the high rates found for Mg, N, P and K, which agree with the results obtained by Turner and Barkus (1983) and Ares *et al.* (2003), and confirm the trend of their mobility within the plants described in the literature.

The time of application and the rates of Zn significantly influenced the concentration of the element in the leaves, and there was also an interaction between these two variables (Table 2). Despite being significant in the two sampling times, the collection done at the start of flowering with incremental rates of $ZnSO_4$ provided a better response than those obtained at the harvest of the bunches. The highest concentrations of Zn were obtained in the estimated rates of 111.3 g $plant^{-1}$ $cycle^{-1}$ and 120 g $plant^{-1}$ $cycle^{-1}$, with application in the hole and in broadcast, respectively (Fig. 1a).

The concentrations of Zn obtained in the leaves collected together with the bunches exhibited a negative interaction with increasing rates (Fig. 1b). Despite the significant effect of the concentrations at rates of 30, 60 and 120 g $plant^{-1}$ $cycle^{-1}$ in broadcast method showed similarities, differing only with the control. The efficiency of utilization of zinc, defined by the quantity of the element absorbed to increase yield (Tyney and Webb, 1946; Malavolta, 2006), showed that increasing rates diminished the utilization factor in the two application periods. However, this efficiency

Table 2. Analysis of variance of banana yield and foliar Zn concentration obtained at the initiation of inflorescence (F1) and at harvest of bunches (F2)[1]

Sources of variation	Degree of freedom	Yield	Foliar Zn concentration	
			F_1	F_2
Blocks	3	–	–	–
Rates - A	3	13.41***	71.89***	4.74**
Residue (a)	9	–	–	–
Plots	15	–	–	–
Application time - B	1	5.18*	3.36*	4.90**
A x B	3	5.66**	5.60**	2.69*
Residue (b)	12	–	–	–
Total	31	–	–	–
CV % (a)		6.52	3.26	14.07
CV % (b)		7.67	4.59	17.13

[1] *, ** and ***: significant at P=0.1, P=0.05, P=0.01, respectively. CV – coefficient of variation.

Table 3. Foliar concentration, increment and fraction of retranslocation of nutrients in banana plants cultivated in Central Amazon, Amazonas State, Brazil[1]

Nutrient	F_1	F_2	Δ	FNR
	g kg^{-1}		%	
N	26.1	21.7	-16.9	-0.73
P	1.7	1.4	-17.6	-0.72
K	27.5	22.5	-18.2	-0.74
Ca	4.7	6.6	40.4	-
Mg	2.9	2.2	-24.1	-0.83
S	1.7	1.9	11.8	-0.24
	mg kg^{-1}			
B	19.3	17.5	-9.3	-
Cu	5.5	3.4	-38.2	-0.56
Fe	52.4	92.6	76.7	0.45
Mn	280.0	540.1	92.9	0.50
Zn	13.0	14.0	7.7	0.05

[1]F_1 – Foliar concentration at the initiation of inflorescence. F_2 – foliar concentration at harvest of bunches. Δ - increment of foliar level. FNR – fraction of nutrient retranslocated.

was highest when the fertilizer was applied in the planting hole (Fig. 1c and 4), indicating that even though there was nutrient mobilization from "mother" to "daughter" plant (Lahav, 1995), it is more advantageous to accumulate the nutrient in the tissue (redistribution) than to fertilize the plant at the start of its metabolism to form bunches (Malavolta, *et al.*, 1997).

In plants grown in Zn deficient conditions, the fruits are stronger sinks, creating a greater demand for the nutrient. These observations corroborate with the results obtained by Longnecker and Robson (1993) and Martinez *et al.* (2005), that tissues undergoing growth, in the case of fruits (Webb and Loneragan, 1990), are preferred zinc sinks as compared to mature tissues. The authors reported that in plants grown with the highest Zn rates, the sink effects of the growing tissues are not strong. Although our preliminary results showed a low retranslocation rate of zinc to the growing organs (Table 3), the higher concentration observed in the leaves collected at phase F_2 in the control, along with the greater Zn content in the central fruit of hand 2, the next-to-last hand produced (Fig. 4), also indicates the mobility pattern of zinc in banana plants.

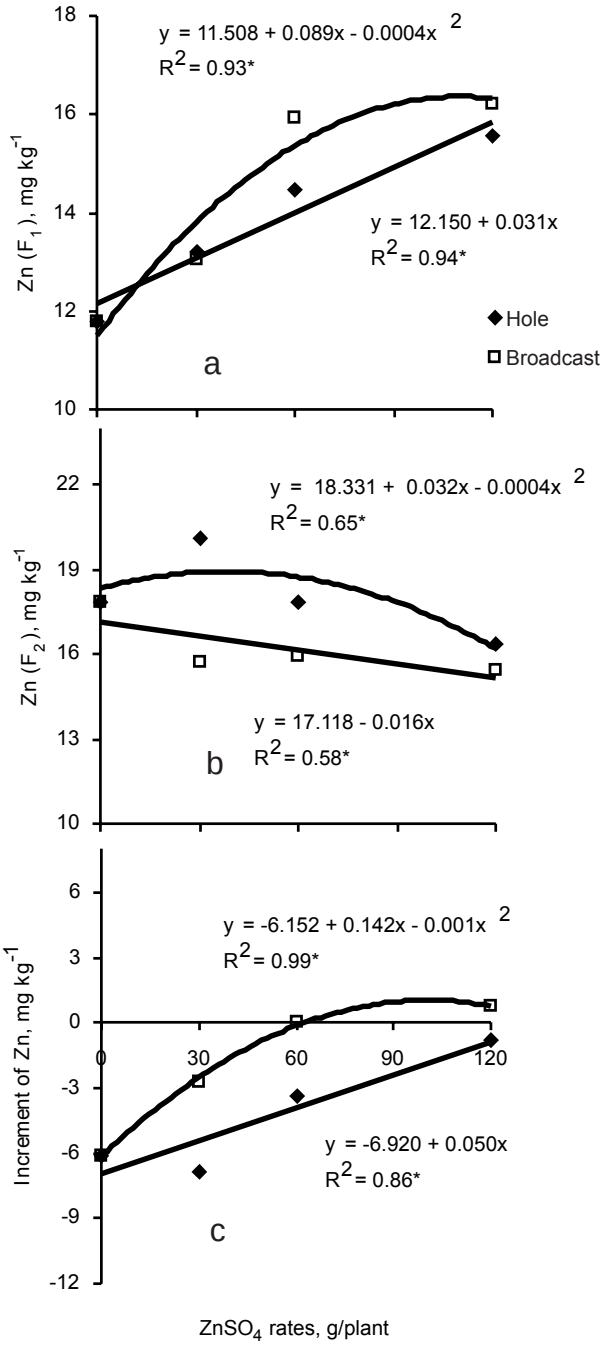

Fig. 1. Effect of zinc sulfate on foliar Zn concentration at intiation of the inflorescence (F_1), harvest of bunches (F_2) and increment of nutrient in leaf, as a function of sampling times. *Significant at P=0.05.

Regarding the application time, it was observed that except for the rate of 60 g plant^{-1}, the quantities of Zn in the tissue were similar. Moreira *et al.* (2005) suggested 16 to 22 mg kg^{-1} as the critical range for zinc in banana plants grown in Amazonas State. Using this index as a reference, it was found that only the treatments with 120 g plant^{-1} of $ZnSO_4$ at the two application times were within this sufficiency range (Fig. 1).

The relationship between relative yield and Zn leaf concentration (Fig. 3) indicates that the critical level obtained for banana plants, using the procedure proposed by Cate Junior and Nelson (1971), was 12.9 mg kg^{-1}, a concentration below the 16 to 19 mg kg^{-1} and 15 to 23 mg kg^{-1} suggested by Moreira *et al.* (2005) and Borges *et al.* (2006), respectively, for the same cultivar. However, taking the high yield obtained, above 35 t ha^{-1} cycle^{-1} as a base (Fig.1),

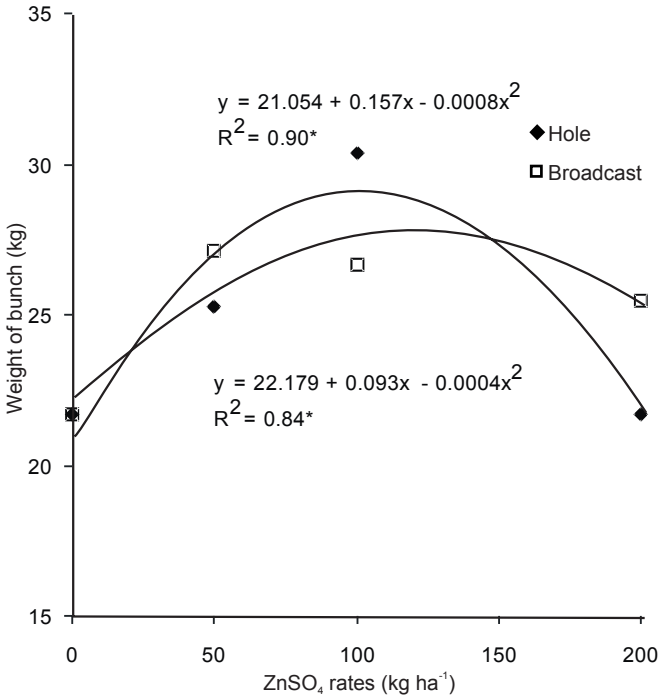

Fig. 2. The effect of zinc sulphate rates on the banana yield cultivated in xanthic Ferralsol (dystrophic Yellow Latosol), as a function of time and fertilization. *Significant at P=0.05.

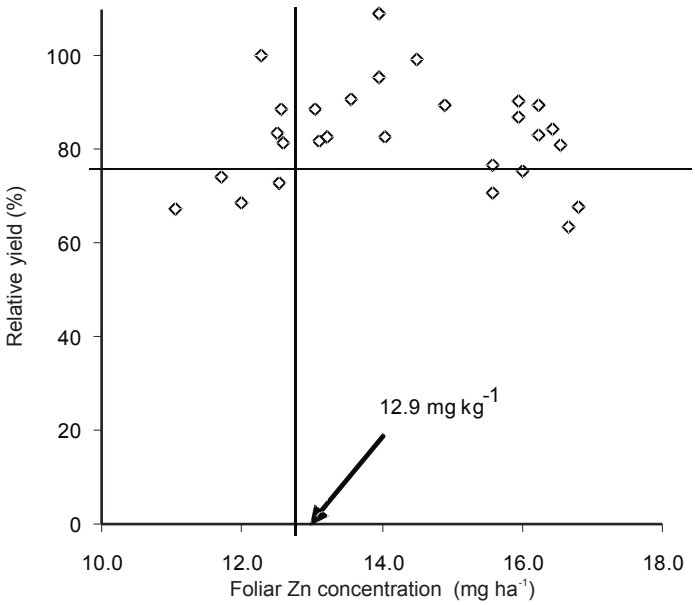

Fig. 3. Relationship between relative yield and foliar zinc concentration in diagnostic leaf at intiation of the inflorescence (F_1) in banana plants cultivated in a xanthic Ferralsol (dystrophic Yellow Latosol), as a function of zinc sulfate rates and time of fertilization.

only the control remained below this sufficiency level. Besides dilution effect (Marschner, 1995) caused by the high productivity, different climatic conditions at the time of collection of the leaves for foliar diagnosis could also have influenced the result.

According to the hands produced (2, 6 and 10), there was a significant decrease in fruit length and diameter (Fig. 4). Extrapolating the classification of the 'Prata' subgroup (ABANORTE, 1998) to the Thap Maeo subgroup, the bananas of hand 10 were on average within the second-grade banana

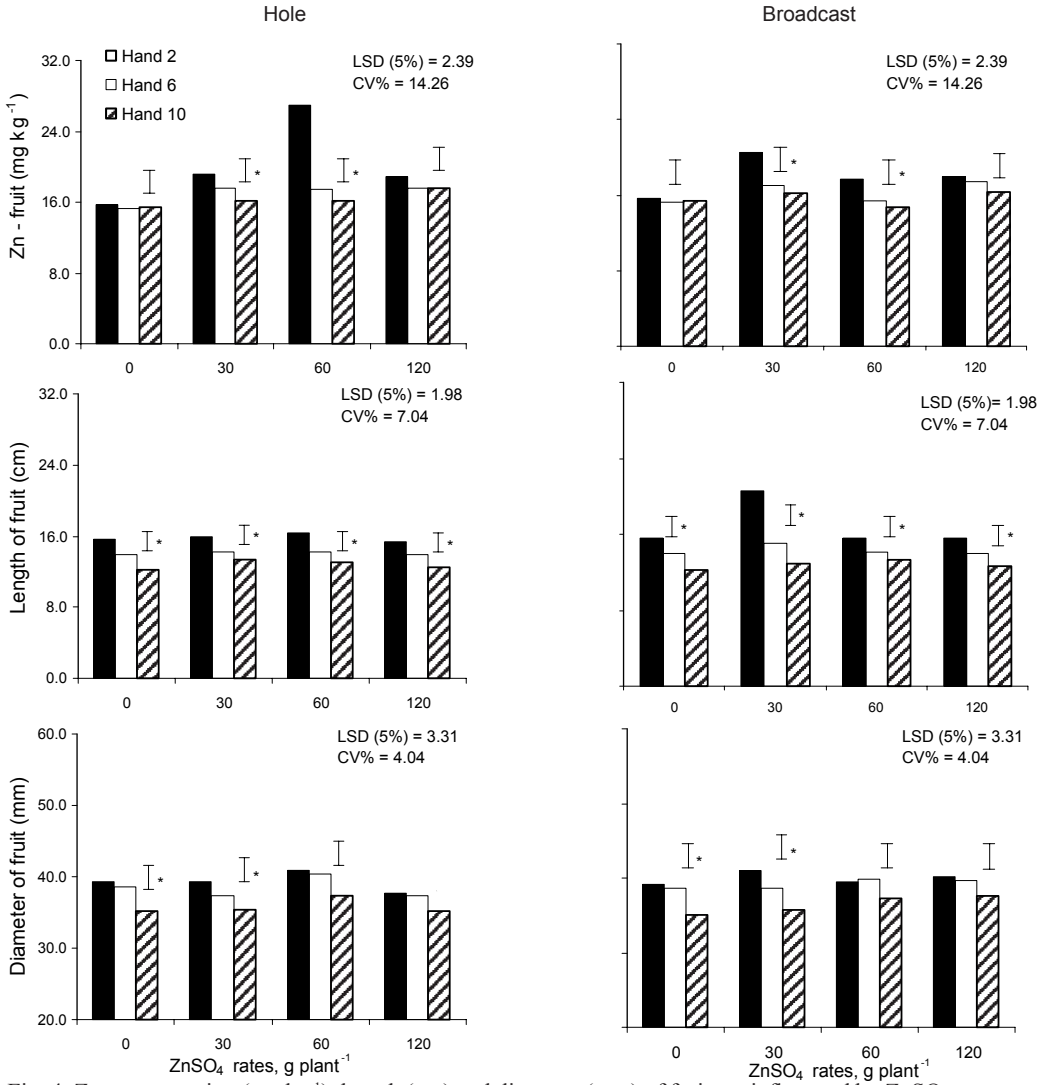

Fig. 4. Zn concentration (mg kg^{-1}), length (cm) and diameter (mm) of fruits as influenced by $ZnSO_4$ rates (0, 30, 60 and 120 g $plant^{-1}$). *Significant at P=0.05.

classification (length < 14 cm), while for those from hands 2 and 6 the classification was first-grade and export-grade (length > 14 cm). Based on the diameter, the bananas from all three hands analyzed were within the first- and export-grade classification (diameter > 32 mm). The application times had similar results, with the length of the fruits presenting significant statistical differences for the four rates of $ZnSO_4$, while for the diameter the differences were only significant for the rates of 0 and 30 g $plant^{-1}$ $cycle^{-1}$ (Fig. 4). This shows that the zinc was retranslocated from the older fruits, located in the tenth and sixth hands, to the second hand (the penultimate hand produced). Regarding the rates, they did not influence the diameter and length of the fruits.

These results reveal that banana plant yield can be boosted through administration of $ZnSO_4$, in increasing rates according to the market and sales pattern (weight, unit or bunch), For example, unlike in the Center-South region of Brazil, where the fruit is sold in boxes of detached groups, in the North nearly all are sold by bunch.

The results show that in banana plants, the retranslocated fractions of Ca, Fe and Mn have low remobilization, while B, Zn and S were intermediate. The high rates were found for Mg, N, P and K. The productivity of the banana plant is influenced by the rates of zinc sulfate. In the first cycle, the application of $ZnSO_4$ in the planting hole is more efficient than broadcast application after planting in mulch. In the local edaphoclimatic conditions, the proposed critical foliar concentration of zinc in the banana leaf is 12.9 mg kg^{-1}. In banana plant, zinc mobility in the phloem from the leaves to the fruits and from the older to the younger fruits is indicated.

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Response of tomato plants to deficit irrigation under surface or subsurface drip irrigation

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Abstract

Field studies were conducted to compare the yield and fruit quality of processing tomatoes in surface and subsurface drip irrigation, with 100 and 50% of crop evapotranspiration (ETc). The results showed that when irrigation was reduced by 50% ETc the subsurface treatment showed higher water content at root depth compared with the on-surface treatment. At 50% ETc subsurface irrigation yield increased by 66.5% compared with the surface treatment. However at 100% ETc no significant difference in total fruit yield was observed between irrigation methods. The superficial and water-stressed treatment increased the pH and the acidity of the fruits but the subsurface treatment did not show differences with respect to the full-irrigation treatments. Our results show that the subsurface drip irrigation method could be reasonably applied for processing tomato when water resources are limited.

Key words: Yield, crop quality, total soluble solids (TSS), drought, stress, *Solanum lycopersicon* L.

Introduction

The scarcity of water in arid and semi-arid regions has increased the search for technology with improved water use efficiency. Drip irrigation is widely used in processing tomato cultivation in areas with dry and warm summers and high evapotranspiration rates throughout the growing season. Subsurface drip irrigation has evolved into an irrigation method with high potential for efficient and economical productivity and its use has progressed from being a novelty employed by researchers to an accepted method of irrigation for both perennial and annual crops (Ayars *et al.,* 1999). It has been found that subsurface drip irrigation reduced evaporation from the soil and increased the wetted soil volume and surface area more than surface systems allowing a deeper rooting pattern (Oliveira *et al.,* 1996; Phene, 1995).

Tomato plants are sensitive to water stress and show high correlation between evapotranspiration (ET) and crop yield (Nuruddin *et al.*, 2003). Thus yield reductions can be expected if ET is reduced due to insufficient soil moisture. Though, growth being impaired due to water stress (Kamgar, 1980; Wolf and Rudich, 1988), fruit quality parameters like colour or total soluble solids usually improve (Shinohara, *et al.,* 1995) and, therefore, establishment of methods to control the extent of stress, based on the focused yield and Brix value is important. The aim of this study was to evaluate subsurface drip irrigation as an efficient water-saving irrigation technique versus the surface system and to analyse the response of tomato fruit when water stress was imposed under different irrigation techniques.

Materials and methods

Experimental site: The experiment was conducted in Calcaric Fluvisol (FAO-UNESCO system) with a high permeability. Soil properties and meteorological data (absolute maximum and minimum values) during the experiment are summarised in Tables 1 and 2, respectively. Soil electrical conductivity was measured in the saturated paste extract. The soil showed a low nutrient level (Marx *et al.*, 1999)

Experimental design and treatments: Four treatments: two depths (surface and subsurface at 40 cm depth) and two irrigation treatments at 100 and 50% ETc (crop evapotranspiration) were arranged in a spit-plot experimental design with three replications. Total water applied in the 100% ETc treatment was 7967 m^3 ha^{-1}. Initially, from May 16 (transplanting date) to June 14 a total of 871 m^3 ha^{-1} was applied in order to guarantee plant establishment, especially for the subsurface treatment. Each treatment had 18 lines of plants, with two lines of plants per line of drip irrigation. A total of 1770 tomato (*Solanum lycopersicon* L.) cv. Hypeel 244A seedlings was transplanted at the experimental site. RAM emitters (2.3 L h^{-1}) were placed 30 cm apart.

Soil moisture determination: Soil water content was calculated with a neutron probe (Troxler Electronic Laboratories Inc. model 3332). Eight aluminium tubes were placed in the experimental

Table 1. Soil physical and chemical characteristics of the experimental site

Parameter	Depth (cm)	
	0-50	51-100
EC (dSm^{-1})	0.61	0.52
pH (H_2O)	7.79	7.88
Organic matter (%)	1.41	1.67
N (%)	0.75	1.02
K (mmol kg^{-1})	7.20	4.80
Ca (mmol kg^{-1})	111.60	132.80
Mg (mmol kg^{-1})	29.00	37.30
Na (mmol kg^{-1})	1.20	1.10
Sand (%)	22.00	7.70
Silt (%)	41.00	50.90
Clay (%)	37.00	41.40

Table 2. Monthly rainfall and air temperature (absolute values) during the crop season

Month	Rainfall	Temperature (ºC)	
	(mm)	Maximum	Mininimum
May	1	32.5	8.0
June	38	32.8	10.2
July	3	37.4	11.8
August	31	40.4	15.1
September	4	33.5	-1.1

site. One-meter-long tubes were placed at 15 cm from the dripper in each replicate. The neutron probe was calibrated and readings were compared with water content in the soil at 20, 40, 60 and 80 cm depth. Soil samples were dried in an oven for 24 h at 110ºC and water content determined by weighing.

Crop yield and quality: The central line of each replicate was selected for determination of yield and fruit quality. 16 plants per treatment were hand-harvested and 30 fruits per treatment were taken for quality analysis. Fruit quality parameters determined in the homogenised juice samples were pH, total soluble solids (TSS) content and acidity. TSS was determined by an Atago N-1E refractometer and expressed as ºBrix at 20ºC. Titratable acidity (% citric acid equivalent) was analysed by potentiometric titration with 0.1 N NaOH to pH 8.5, using 10 mL of juice. Fruit dry matter was determined after drying for 72 h at 65ºC in an oven. The data were subjected to analysis of variance and means were separated using Duncan's multiple range test at P=0.05 (SPSS, version 7.5).

Results and discussion

Moisture distribution in the soil profile initially showed higher water content in all the treatments due to the starting irrigation dosage for the transplanting stage (Fig. 1). One of the greatest challenges faced by growers using subsurface drip irrigation (SB) is crop establishment. Establishment with SB relies on unsaturated water movement from the buried source to the seed or seedling. Establishment is therefore affected by distance from source, soil texture, structure and antecedent water content (Charlesworth and Muirhead, 2003). Based on soils characteristic, climate and emitter depth, a total of 871 m^3 ha^{-1} was applied to this crop stage. Moisture was directly correlated with the amount of water applied at full or half-irrigated treatments. At 20 cm depth, the superficial and fully-irrigated treatment (SP100) had the higher soil moisture followed by the subsurface treatment (SB100). But, from 40 cm onwards, SB100 showed higher water content than the surface treatment. With respect to the water-stressed treatments, the subsurface method (SB50) showed higher water content in the soil profile especially at 40 cm depth and kept this difference until the end of the crop cycle. This difference in water content could affect the rooting pattern among irrigation methods. Phene *et al.* (1989) and Ben-Asher and Phene (1993) have reported that specific characteristics of the subsurface system allowed a spherical and larger volume of a wetted soil. This could result in a large concentration of roots at the depth of the irrigation emitter as found for several crops like tomato (Bar-Yosef *et al.*,1991), maize (Mitchell, 1981; Phene, 1991) or cotton (Plaut *et al.,* 1996). Our result agreed with those findings in the wetting patterns especially at limited irrigation dosage.

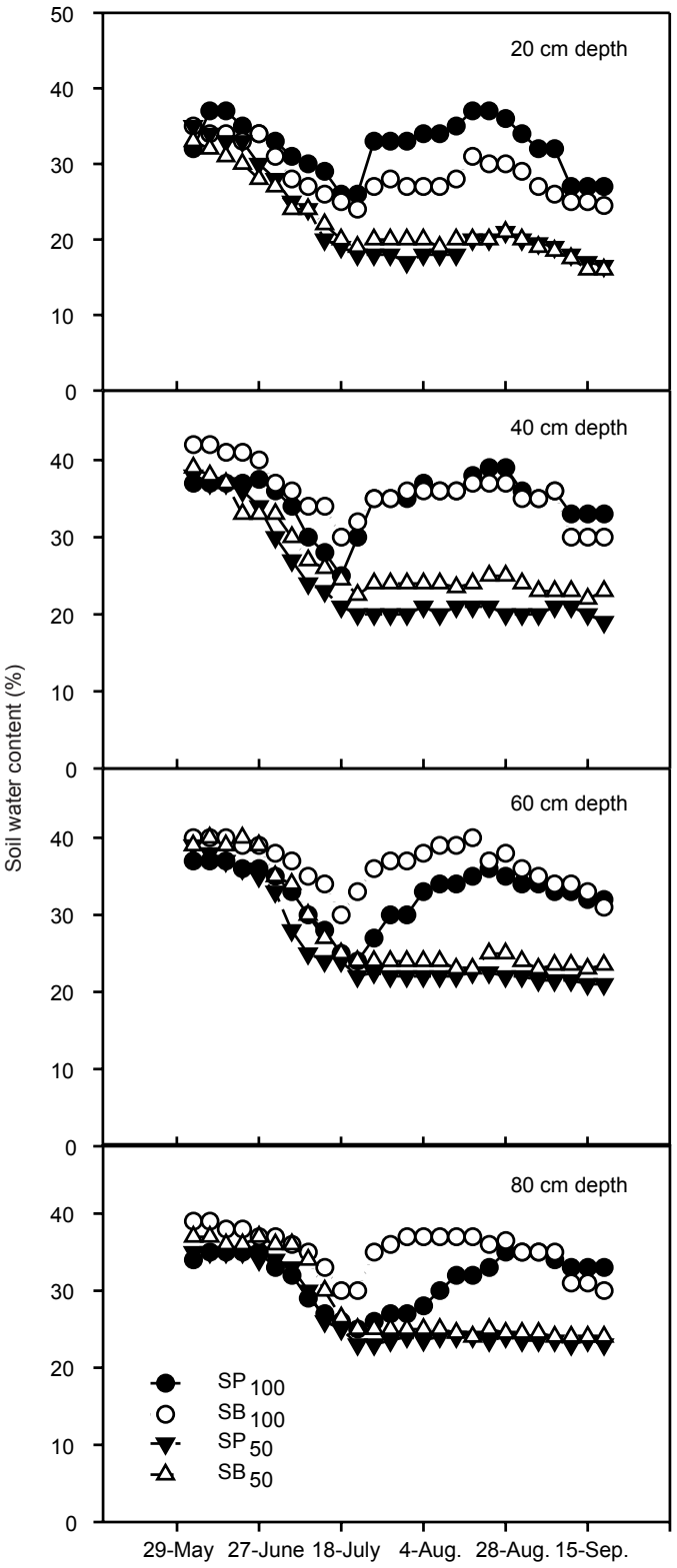

Fig. 1. Soil water content at 20, 40, 60 and 80 cm depth, 15 cm from the dripper. Treatments: Superficial (SP) or subsurface (SB) drip irrigation at two irrigation regimes (100 and 50% Etc).

Subsurface irrigation increased total yield compared with the superficial method (Fig. 2) but statistical significance was observed only for the water-stressed treatments. Thus, at 100% ETc, subsurface drip irrigation only increased yield by 8.3% but at 50% ETc this method increased yield by 66.5% with respect to the superficial treatment under the similar conditions, indicating the higher water use efficiency of this irrigation system. Other

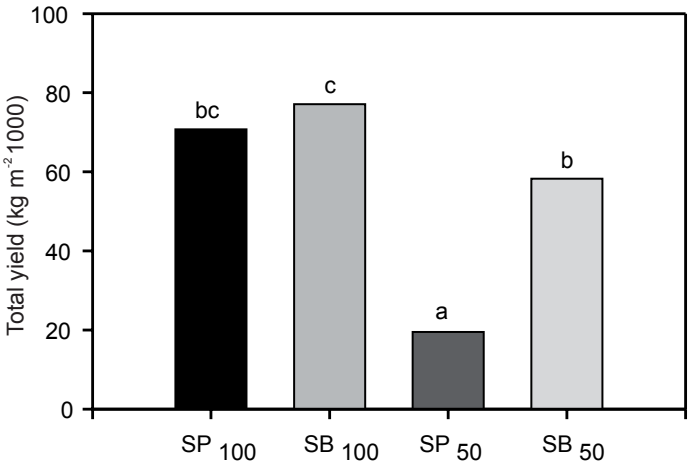

Fig. 2. Effect of the irrigation methods, superficial (SP) or subsurface (SB) drip irrigation at two irrigation regimes (100 and 50%Etc), on total fruit yield. Treatments with the same letter are not significantly different (Duncan test, $P<0.05$).

studies of irrigation and fertilisation management demonstrated significant yield and water use efficiency increase for the subsurface method in vegetable crops (Ayars *et al.,* 1999; Enciso-Medina *et al.,* 2002). Machado *et al.* (2003) found slightly higher commercial yield of processing tomatoes when the subsurface method was used, compared with surface irrigation, under non-stressed conditions.

As a result of the lower water availability for the roots in the surface treatment at 50% ETc (SP50), with respect to the subsurface treatment (SB50), fruit quality especially pH and acidity were significantly affected (Fig. 3). The surface and water-stress treatment increased TSS by 18.9%, pH by 13.2% and fruit dry matter by 17.2% but reduced acidity by 30% compared to the subsurface treatment at the same water regime. Variations in the concentration of dry matter have been coupled with the conditions of climate and root medium (Guichard *et al.,* 2001). For processing tomatoes, higher solids content in fruits is a target characteristic as this would reduce the cost for processing. The dry matter content of the ripe fruit is generally inversely related to the fruit size (Davies and Hobson, 1981). Furthermore, the dry mater content is positively related to the total sugar content of the fruit (Ho, 1988). The dry matter content as a percentage is also determined by the balance of the accumulation of assimilates and water (Marschner, 1995). Thus, while the import of assimilates depends on the effect of light on canopy photosynthesis and of temperature on fruit metabolism, the import of water is affected by plant water relations, which are affected by root water absorption and leaf transpiration. Water stress relatively imposed in the superficial treatment could promote the translocation of photosynthates into fruit and improve the fruit quality, whereas it inhibits photosynthesis and transpiration (Shinohara *et al.,* 1995) and total fruit yield is reduced.

Many southern areas of Europe and US are facing a dramatic decrease of water resources for agriculture due to both an increase of long-lasting drought periods and a considerable competition for water from new residential areas. Saving of water is a constant concern and new methods and irrigation strategies are foreseen. This study shows that, when water is strongly reduced throughout the crop season, total yield reduction could be reasonably overcome using subsurface irrigation due to its higher water use efficiency. Further work and continuous monitoring is still required to find a proper equilibrium between yield and quality, using the special advantages of this irrigation system.

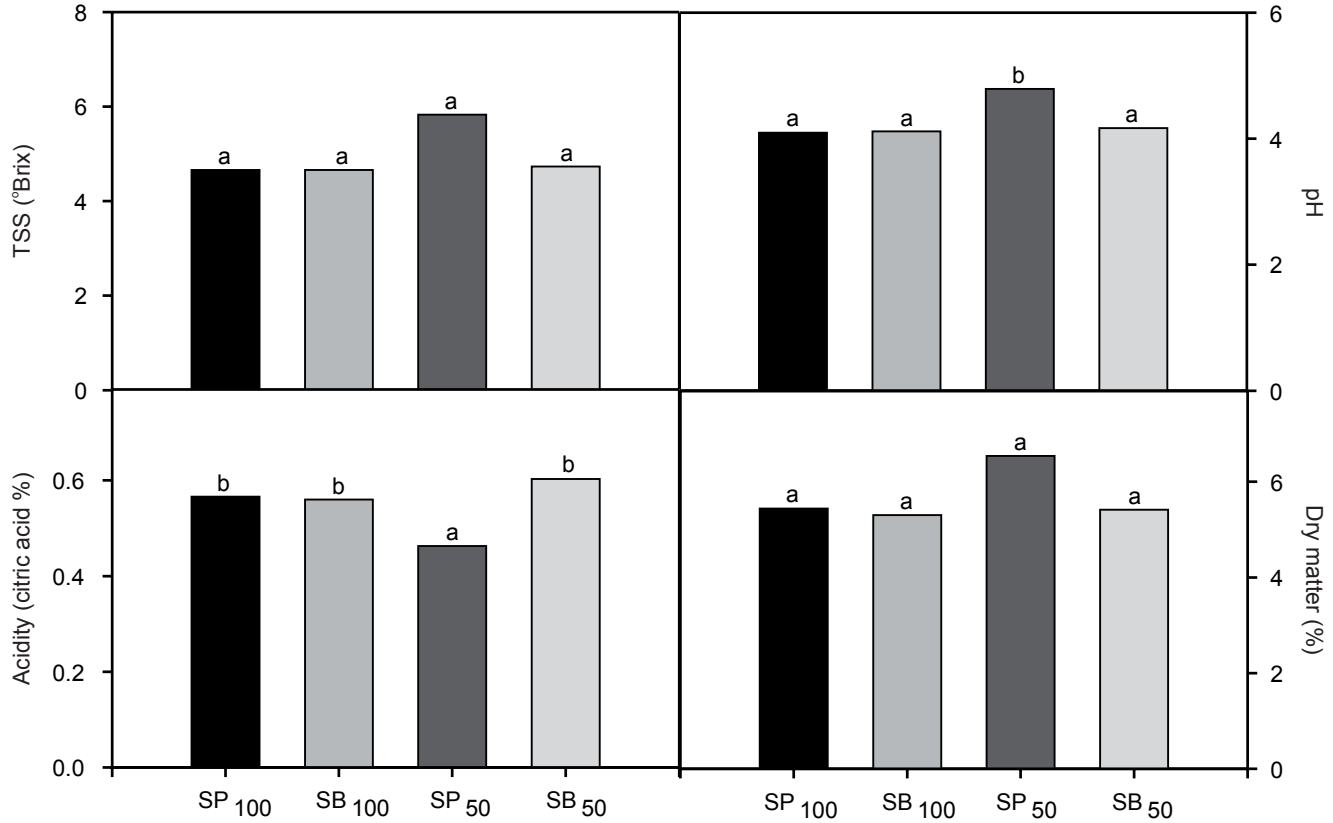

Fig. 3. Effect of the irrigation methods, superficial (SP) or subsurface (SB) drip irrigation at two irrigation regimes (100 and 50%ETc), on tomato fruit quality parameters. Treatments with the same letter are not significantly different ($P\leq0.05$).

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Seasonal changes in texture, sugar and organic acid contents and activities of some ammonia-assimilating enzymes in lettuce

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Abstract

As a cool weather crop, lettuce (*Lactuca sativa* L.) is very sensitive to the changes in temperature during growth. This study investigated the textural, compositional and some biochemical changes in the outer and inner leaf tissues of two crisphead lettuce cultivars ('Bittsu' and 'Cisco') harvested in different seasons. The result demonstrated that in colder months, the crispiness of lettuce leaves reduced significantly and higher amount of sugars, organic acids and ammonia were accumulated. In general, between the two cultivars, 'Bittsu' contained higher amount of sugars and organic acids, while 'Cisco' contained higher amount of ammonia. However, inner leaf tissues contained higher amount of ammonia than outer leaf tissues in both cultivars. The level of fructose was found to be higher than glucose and sucrose in all cases while malic acid was the main component in organic acid fraction. The activities of ammonia-assimilating enzymes such as glutamine synthetase (GS; EC 6.3.1.2) and asparagine synthetase (AS; EC 6.3.5.4) either decreased or nearly remain constant depending on the tissue types during the colder months. Outer leaf portion showed higher GS activity than inner leaf tissues. However, both of aminating and deaminating activity of glutamate dehydrogenase (GDH, EC 1.4.1.2) decreased in the outer leaves whereas deamination activity slightly increased in the inner leaf tissues during warmer harvest months.

Key words: Ammonia, amination, asparagine synthetase, crispness, deamination, glutamine synthetase, *Lactuca sativa,* sugar.

Introduction

Climate limits the agriculture production capacity of any country and nearly every element in planning, producing, harvesting and postharvest operations such as marketing of the products are directly or indirectly linked to the variations of weather. It is commonly accepted that seasonal factors such as temperature influences the composition of plant tissues during growth and development. Total available heat and the extent of low and high temperature are the most important factors in determining growth rate and chemical composition of horticultural crops (Lee and Kader, 2000). For instance, in iceberg lettuce, maximum head weight was found when crops experienced a mean temperature lift of about 2ºC from transplanting to maturity, corresponding to a mean temperature of about 12ºC, while the time to maturity steadily decreased with increased temperature (Wurr *et al.*, 1996). Head density, diameter and solid heading are also affected by fluctuations in temperature during different growth stages of crisphead lettuce (Wurr *et al.*, 1992). High temperature during the summer rainy season is associated with bolting (Glenn, 1984; Wurr *et al.*, 1992) and some physiological disorder like tipburn. Low temperature, on the other hand, slackens the growth of leaf lettuce (Knight and Mitchell, 1983).

Iceberg or crisphead lettuce is more popular mainly due to its crisp texture than others such as butterhead, leaf or romaine. Textural changes are among the main causes of quality loss for lettuce and soft or limp product may be rejected by the consumer. Since temperature has a direct influence on metabolism, it also indirectly affects the cellular structure and other components which determine texture (Sams, 1999). Hence, it is important to know the textural and some compositional properties of the products when it is cut and ready for consumption. Especially, in lettuce, sugar and nitrate contents greatly vary with the seasonal temperature (Behr and Wiebe, 1992).

Recently, Tosun and Ustun (2004) summarized that raw consumed vegetables like lettuce contain high nitrate nitrogen which may be detrimental to human health. The incorporation of inorganic nitrogen, nitrate and ammonium into the carbon skeleton is an important biochemical feature of land plant which might be influenced by the environmental conditions (Inokuchi *et al.*, 2002). Ammonium is a preferred source of nitrogen and a key metabolite situated at the junction between carbon metabolism and nitrogen assimilation, because nitrogen compound can choose an alternative pathway according to the stage of crop growth and environmental conditions (Inokuchi *et al.*, 2002). Therefore, it is desirable to study the enzymes responsible for ammonia assimilation in relation to seasonal environmental variation. The incorporation of ammonium into the pool of N-containing molecules is first catalysed by the enzyme glutamine synthetase (GS; EC 6.3.1.2). Glutamine and glutamate, which are formed through the action of GS from ammonia, serve as nitrogen transport compounds and nitrogen donors in the biosynthesis of many compounds in plant including amino acids and chlorophyll. Nitrogen may also be channeled from glutamine and glutamate to asparagines by another enzyme asparagine synthetase (AS; EC 6.3.5.4) (Suarez *et al.*, 2002). In addition to GS and AS, glutamate dehydrogenase (GDH; EC 1.4.1.2) also plays key role among other enzymes which maintain the balance of carbon and nitrogen

(Miflin and Habash, 2002). GDH is induced by high levels of ammonia (Cammaerts and Jacobs, 1985) and capable of releasing amino nitrogen from amino acids to give keto-acid and ammonia that can be separately recycled to be used in respiration and amide formation, respectively (Miflin and Habash, 2002).

Studies on lettuce have generally been limited to sensory attributes, general appearance, wilting, enzymatic browning, decay and physiological disorders during packaging and storage (Alsadon, 1993; Artes and Martinez, 1996; Toole *et al.*, 2000; Murata *et al.*, 2004). Hence, this study was conducted to measure the changes in texture, sugar, organic acid and ammonia contents as influenced by seasonal temperature, tissue type and cultivar. The activities of enzymes related to ammonia assimilation which have been reported to influence the overall quality and shelf life of lettuce after harvest are also discussed.

Materials and methods

Plant materials: Heads of two crisphead lettuce (*Lactuca sativa* L.) cultivars 'Bittsu' and 'Cisco' grown in field condition were harvested from Kagawa Prefectural Agricultural Experiment Station, Busshouzan, Kagawa, Japan. Harvesting was done monthly intervals from December, 2005 to April, 2006 when the head reached at commercial maturity. Temperature was recorded daily in respect of maximum, minimum and a-day average. Then the values were averaged individually on the calendar month basis (Fig. 1). The harvested heads were packed in a box with crushed ice and immediately transported to the laboratory. Outer (green) and inner (white) leaves were separated from the head, cut into small pieces (ca. 2 × 2 cm) and immediately stored at -30ºC until needed for analysis.

Texture measurement: Texture was measured rheologically based on the measurement of breaking force to puncture the leaf tissue. Breaking force was determined with a creep meter (Yamaden Rheoner RE-33005) equipped with software ver. 2.0 for automatic analysis. With a running load cell of 20 N, the rheometer was mounted with a cylinder like plunger (adapter no. 5) of 5 mm diameter and 18 mm in length. The flat base containing a 12 mm high and 12 mm diameter hole, on which the sample was horizontally placed and tightened the sample with clips, moves upward to the plunger at a speed of 1 mm s^{-1} to measure the puncture or breaking force as an index of crispness of the sample. Lower breaking force indicates higher crispness and conversely higher breaking force is needed to puncture the soft or limp tissues. For each sample, measurements were taken from each of five pieces (approx. 3 × 5 cm) of outer and inner leaf segments separately and the average of 15 measured values was expressed as breaking force of the sample.

Determination of soluble sugars and organic acids: Approximately 4 g of lettuce tissue (for each portion) was mixed with 1 g sea sand and homogenized in a cool mortar and pestle. To make a total volume of 10 mL of the homogenate, required amount of distilled water was added to the homogenate and centrifuged at 11,000 × *g* at 2ºC for 10 min. The supernatant was filtered through a cellulose nitrate membrane filter (0.45µm pore size). Soluble sugars were analyzed using a high performance liquid chromatography (HPLC) containing a stainless steel column (10.7 mm ID × 30 cm) packed with silica gel (gel pack C 610). The mobile phase, filtered air free distilled water, was pumped through the column at a flow rate of 1.0 mL min^{-1}. The pressure was adjusted to 28-30 kg cm^{-2} and the column temperature was maintained at 60ºC. A refractive index (RI) monitor (Hitachi L-3300) was used to record the peak heights. Sucrose, glucose and fructose were identified by their retention times and were quantified according to standard. On the other hand, organic acids were also analyzed by HPLC using a stainless steel column (10.7 mm ID × 30 cm) packed with silica gel (gel pack GL-C610H-S). The mobile phase was 0.1% phosphoric acid adjusted to a flow rate of 0.5 mL min^{-1}. The pressure was adjusted to15-20 kg cm^{-2} and the column temperature was maintained at 60ºC. The ultraviolet (UV) detector (Hitachi L-4200) set at 210 nm was used to record the peak heights. Citric and malic acids were identified by their retention times and were quantified according to standard.

Ammonia assay: To assess ammonia content, 2 g sample from each portion of lettuce tissue was extracted with 10% trichloroacetic acid at 1:10 ratio(w/v) in an ice bath (0- 4ºC) and centrifuged at 11, 000×*g* at 2ºC for 10 min. Ammonia content was assayed as described by Kun and Kearney (1974), where 1 mL assay mixture contained 200 µL 0.5 M tris-HCl buffer (pH 8.0), 100 µL 0.1 M 2-oxoglutarate solution (pH 7.4), 30 µL 8 mM β-NADH solution, 20 µL G*l*DH (10mg mL^{-1}), 150 µL distilled water and 500 µL of neutral extract sample. The decrease in NADH, as determined by the change of extinction at 365 nm was used as a measure of the reaction.

Enzyme extraction: Approximately 5 g lettuce sample from outer and inner leaf portion was homogenized in ice cold condition (ca. 0-4ºC) with 1% polyvinylpolypyrrolidone (PVPP) proportional to the sample weight, 1 g sea sand and 5 mL buffer solution using a mortar and pestle. Extraction was performed as described by Hurst and Clark (1993), in which buffer A contained 50 mM tris-HCl (pH 7.6), 10 mM $MgSO_4.7H_2O$, 1 mM EDTA, 1 mM dithiothreitol (DTT), 12 mM 2-mercaptoethanol, 5 mM L-glutamate and 100 mL glycerol per liter and buffer B contained 50 mM KH_2PO_4 (pH 8.0), 50 mM KCl, 1 mM EDTA, 1mM EGTA, 1 mM DTT, 12 mM 2-mercaptoethanol, and 100 mL glycerol per liter. Buffer A was used for the extraction of GS and GDH while buffer B was used for AS. The homogenate was squeezed through four layers of cotton cloth. The residual tissues were re-extracted with an additional 5 mL of the same buffer and the filtrate was centrifuged at 11,000×*g* at 2ºC for 10 min. The resulting supernatant was used for enzyme assay.

Enzyme assay: The enzymatic activities were assayed in a total volume of 1 mL assay mixture. The activity of GS was determined with 80 mM Na-L glutamate, 100 mM tricine-KOH buffer (pH 7.0), 6 mM hydroxylammonium chloride ($HONH_3Cl$), 20 mM $MgSO_4.7H_2O$, 1 mM diethylenetriamine pentaacetic acid (DTPA), 8 mM ATP and 8 mM mercaptoethanol. For AS, activity was assayed with a mixture of 20 mM Na-L-aspartate monohydrate, 100 mM tris-HCl (pH 7.0), 12mM $MgCl_2.6\ H_2O$, 10 mM ATP-2Na and 800 mM hydroxylammonium chloride ($HONH_3Cl$). After incubating the assay mixture at 35ºC for 8 min and at 30ºC for 10 min for GS and AS, respectively, the reaction was stopped by the addition of 1 mL ferric chloride reagent that contains 0.37 M $FeCl_3$, 0.67 N HCl and 0.2 M trichloroacetic acid (TCAA). Both of GS and AS activity was measured using

a double beam spectrophotometer (Shimadzu model UV-150-02) at 540 nm and soluble protein contents was measured following the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Both aminating and deaminating activities of GDH were determined spectrophotometrically (Shimadzu model UV-150-02) at 350 nm according to NADH oxidation or NAD^+ reduction maintaining a temperature of 30ºC. For GDH amination, a total volume of 1.0 mL assay mixture contained 10 mM α-ketoglutaric acid, 100 mM tris-HCl (pH 8.0), 200 mM NH_4Cl, 1mM $CaCl_2$ and 0.2 mM NAD(P)H. The reaction was started by adding 200 µL crude extract. Likewise, the 1.0 mL assay mixture for GDH deamination consisted of 100 mM L-glutamate, 100 mM tris-HCl (pH 9.3), 1 mM $NAD(P)^+$ and 0.5 mM $CaCl_2$. The reaction was started with the addition of 200 µL crude extract. Blank controls were performed omitting individual substrates. One unit of GDH activity is defined as the reduction or oxidation of one micromole of coenzyme (NADPH/ NADP, respectively) per min at 30ºC.

Statistical analysis: A randomized complete block design was used with three replications. Following ANOVA, the level of significance between the means were calculated using the Duncan's multiple range test (DMRT). Linear correlation was used to evaluate the relationship between enzyme activities and ammonia contents.

Results

Textural changes: The textural measurement showed that breaking force changed significantly in the outer and inner leaf of two lettuce cultivars harvested at different months. Higher ($P <$ 0.05) breaking force was recorded during the colder months (Figs. 1 and 2). Both the tissues followed almost the same pattern in changes, except the outer leaves of both cultivars, which showed the maximum breaking force in February.

Changes in sugar and organic acid contents: Significant changes in soluble sugar contents in both portions of lettuce head were observed at different months (Fig. 3). Comparatively higher amount of sugars (sucrose, glucose and fructose) contents were observed in the cultivar 'Bittsu' than that in 'Cisco'. Generally, inner leaf contained higher amounts of glucose and fructose than outer leaf, while an inverse relation was found for sucrose content. Except in January, sugar content in outer leaf tissues declined (P <0.05) with the increase in temperature. In inner leaf tissues, the same change was observed only for sucrose content. Among the three sugars, the level of fructose was found to be higher than glucose and sucrose in all cases. The content of malic acid, on the other hand, was substantially higher than citric acid in both tissue types (Fig. 4). However, outer leaf tissues contained higher amount of citric acid than the inner leaf tissues. The content of malic acid was significantly higher in 'Bittsu' than that in 'Cisco'. Generally, the quantities decreased (P <0.05) during the warmer harvest period except in January where the content of malic acid declined rapidly. However, malic acid content in 'Bittsu' outer leaf was almost constant during warmer harvest months.

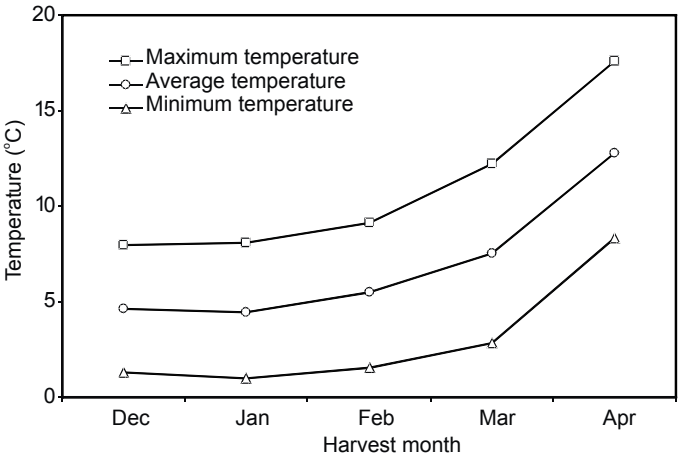

Fig. 1. Temperature of harvest months from December to April.

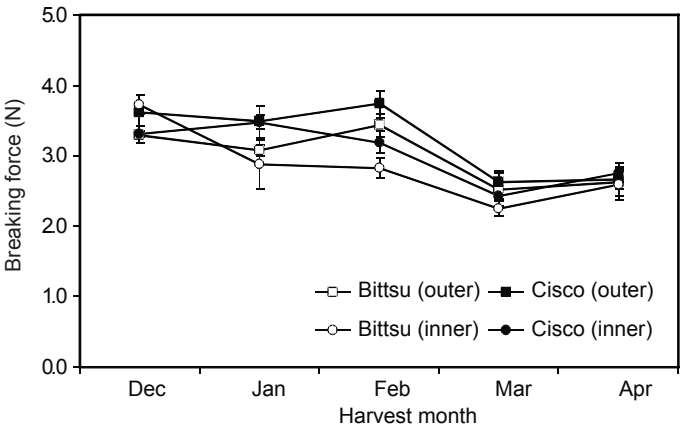

Fig. 2. Changes in breaking force to puncture the leaf tissues (outer and inner) of two lettuce cultivars harvested in different months. Data are the means of three replications. Vertical bars represent SE.

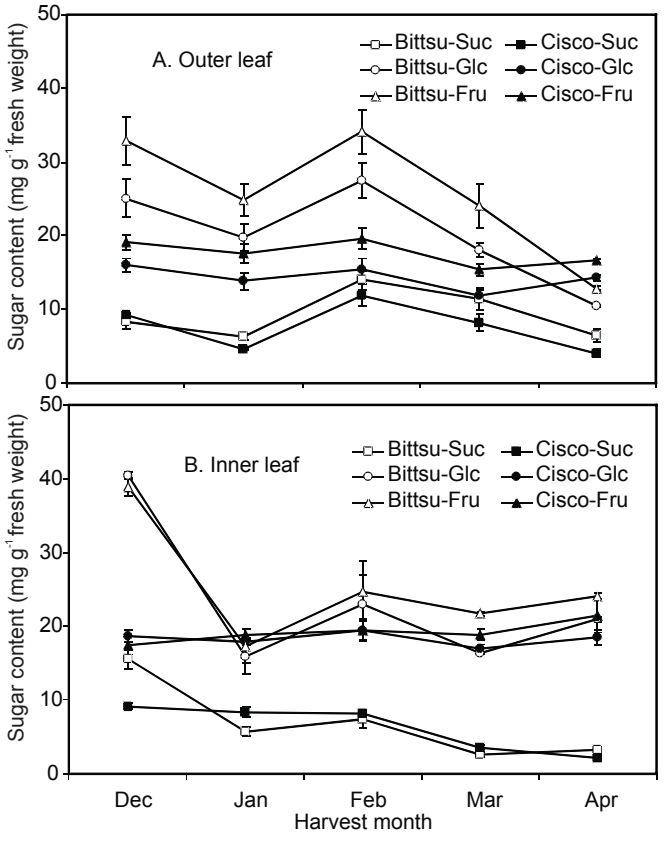

Fig. 3. Seasonal changes in soluble sugar contents in the outer leaf (A) and inner leaf (B) of two lettuce cultivars harvested in different months. Data are the means of three replications. Vertical bars represent SE. Suc= sucrose, Glc=glucose, Fru= fructose.

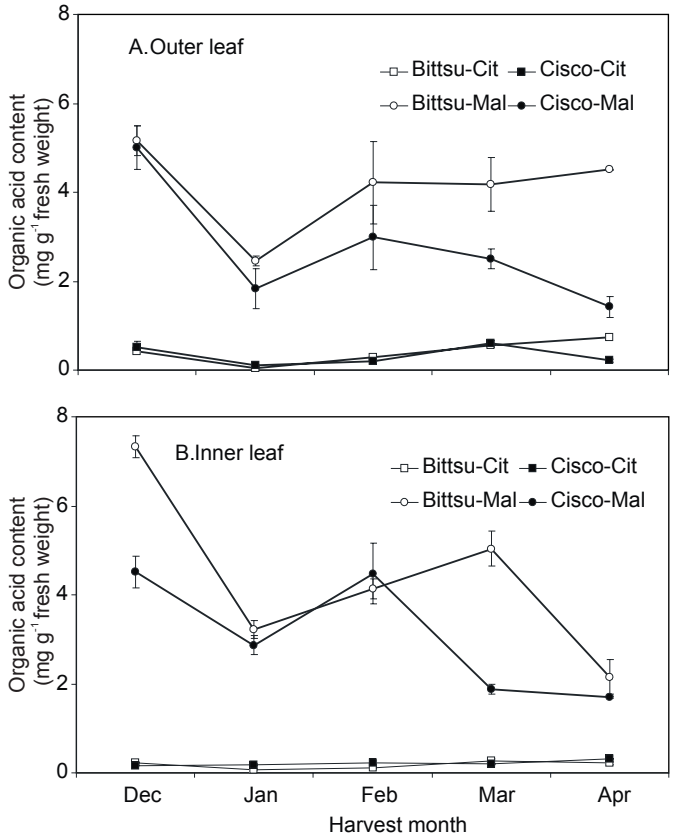

Fig. 4. Seasonal changes in organic acid contents in the outer leaf (A) and inner leaf (B) of two lettuce cultivars harvested in different months. Data are the means of three replications. Vertical bars represent SE. Cit = citric acid, Mal = malic acid

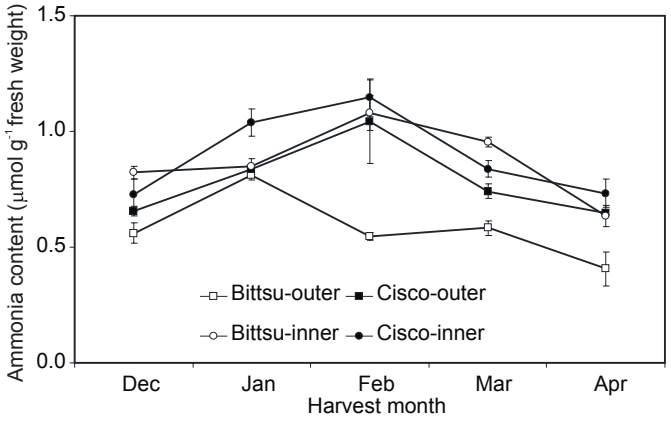

Fig. 5. Ammonia content in the outer leaf and inner leaf of two lettuce cultivars harvested in different months. Data are the means of three replications. Vertical bars represent SE.

Ammonia content: Ammonia content of lettuce leaves changed noticeably throughout the harvest months. Higher ($P < 0.05$) ammonia was found in colder months with a maximum level in February, except the outer leaf tissues of 'Bittsu' (Fig. 5). Between the two cultivars, 'Cisco' contained higher amount of ammonia than 'Bittsu'. On the other hand, inner leaf tissues of both cultivars contained higher ammonia than that of outer tissues.

Glutamine synthetase activity: The GS activity in the outer leaf tissues decreased in January and remained unchanged in the following month and slightly increased at the last two harvest months (Fig. 6). In both the cultivars, outer leaf tissues showed considerably higher activity than inner tissues. The highest GS activity of outer tissues was measured in December and April for

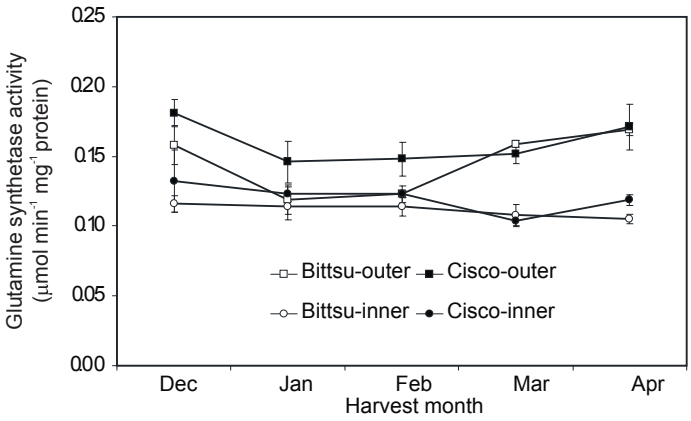

Fig. 6. Changes in the activities of glutamine synthetase in the outer leaf and inner leaf of two lettuce cultivars harvested in different seasons. Data are the means of three replications. Vertical bars represent SE.

'Cisco' and 'Bittsu', respectively. No specific trend in changes of GS activity was found in inner tissues.

Asparagine synthetase activity: The highest AS activities of leaf tissues were obtained when lettuce was harvested in December (Fig. 7). After that the activity of the outer leaf tissues decreased ($P < 0.05$) gradually in the following months and again increased with the rise in temperature. However, in the inner leaf tissues AS activities fluctuated with changing temperature during the harvest months. Cultivar 'Bittsu' showed comparatively higher enzyme activity than 'Cisco'.

Glutamate dehydrogenase activity: GDH-amination activity was higher than deamination activity in both tissue types and cultivars. In outer leaf tissues, GDH-aminating activity declined ($P < 0.05$) with the increase in temperature for both cultivars while inner leaf tissues did not show any trend (Fig. 8). Outer leaf tissues showed noticeably higher aminating activity than inner leaf while an opposite trend was found for GDH-deaminating activity. The deamination activity of inner leaf tissues slightly increased in the warmer harvest month. On the other hand, the deamination activity of 'Bittsu' outer leaf decreased significantly ($P < 0.05$) with the increases in temperature throughout the harvest period.

Correlation between enzyme activities and ammonia content: A significant negative correlation was found between GS activity and ammonia content in outer leaf portion of 'Bittsu', while in

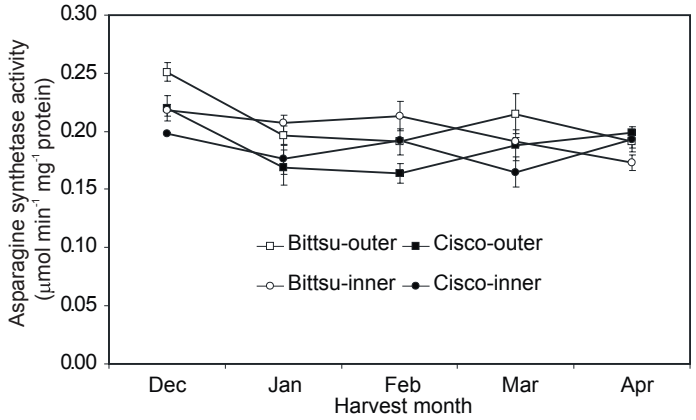

Fig. 7. Changes in the activities of asparagine synthetase in the outer leaf and inner leaf of two lettuce cultivars harvested in different months. Data are the means of three replications. Vertical bars represent SE.

other cases, the correlations were poor (Table 1). On the other hand, significantly negative correlation was observed between AS activity and ammonia content in the outer leaf portion of 'Cisco'. However, this relationship was significantly positive in the inner leaf portion of 'Bittsu'. No significant correlation was observed between GDH-amination/ deamination activities and ammonia content, except in the outer leaf portion of 'Bittsu'.

Table 1. Correlation coefficient (r) values computed from linear regression analyses between enzymes' activities and ammonia content in outer and inner leaf tissues of lettuce head harvested in different months

Cultivar	Portion	Enzyme	Correlation coefficient (r)
'Bittsu'	Outer	Glutamine synthetase	-0.592*
		Asparagine synthetase	0.068
		GDH-amination	0.536*
		GDH-deamination	0.637*
	Inner	Glutamine synthetase	0.276
		Asparagine synthetase	0.640*
		GDH-amination	0.041
		GDH-deamination	-0.461
'Cisco'	Outer	Glutamine synthetase	-0.188
		Asparagine synthetase	-0.519*
		GDH-amination	0.204
		GDH-deamination	0.107
	Inner	Glutamine synthetase	-0.086
		Asparagine synthetase	0.033
		GDH-amination	-0.375
		GDH-deamination	-0.307

* Significant at $P \leq 0.05$

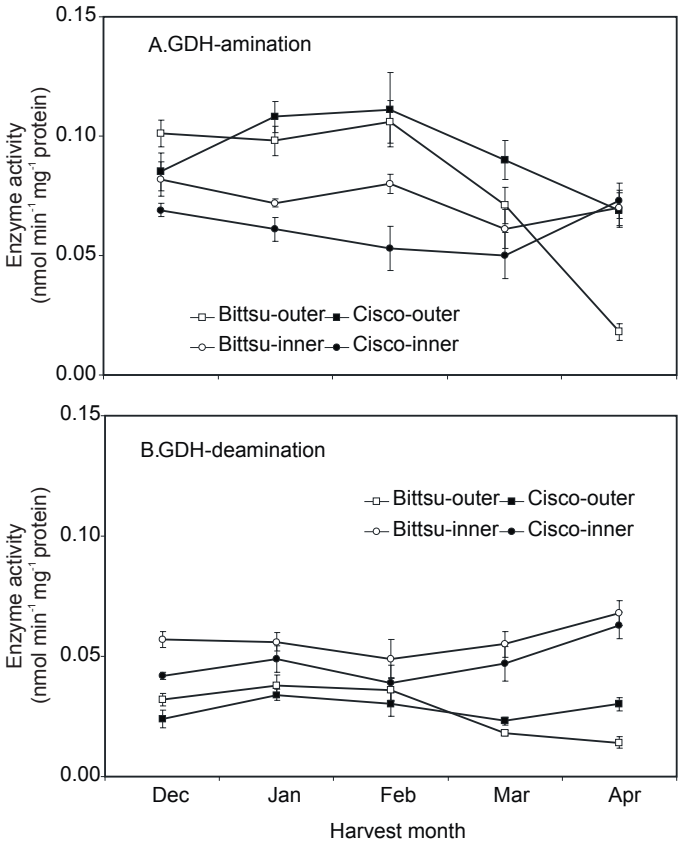

Fig. 8. Changes in the activities of (A) GDH-amination and (B) GDH-deamination in the outer leaf and inner leaf of two lettuce cultivars harvested in different months. Data are the means of three replications. Vertical bars represent SE.

Discussion

Crisphead lettuce has been described as a plant of temperate zone, cool weather crop (Whitaker *et al.*, 1974), so the production and quality of head is most dependent on ambient temperature. This study illustrates some significant qualitative and biochemical changes that occurred in lettuce harvested from winter to spring season. Since lettuce is consumed as raw, mainly for salad, its textural quality is critically important to meet the consumer demand. However, the texture evaluation of lettuce is relatively difficult due to the heterogeneity of the product (Martin-Diana *et al.*, 2006). In this study, the textural properties of outer/ green (photosynthetic) and inner/ white (vascular) leaf tissues showed that winter season's crops are less crispy than spring crops. The maximum breaking force to puncture the leaf tissues was recorded in February and December in outer and inner leaf tissues of both cultivars, respectively (Fig. 2). The higher breaking force which indicates a lower crispness of the tissues might be due to the slow growth during the cold weather as reported in other vegetables like asparagus spears (Bhowmik *et al.*, 2002). Moreover, calcium is associated with maintaining the cell wall structure of vegetables by interacting with pectin to form calcium pectate (Martin-Diana *et al.*, 2006). Due to the action of the enzyme pectin methyl esterase (PME), calcium diffusion into tissues increases at higher temperature (Bartolome and Hoff, 1972) thus reveals a firmer structure of the tissues.

Higher accumulations of soluble sugars were found in the colder months. Specially, sucrose content of both lettuce cultivars decreased gradually in warmer months except with some fluctuations in the outer leaf tissues. This result is an agreement with other results of green vegetables like asparagus spears (Bhowmik *et al.*, 2001) and broccoli (Pramanik *et al.*, 2004). In spinach, leafy vegetable, Guy *et al.* (1992) reported that accumulation of sugars increased 10 to 20 fold at low temperature. It could be argued that sucrose is a storage carbohydrate that can be rapidly mobilized as metabolic needs, and at low temperature photosynthetic energy capture is reduced but to a lesser degree than the metabolic utilization processes. Moreover, active growth is almost always reduced or suspended at low temperature resulting in decreased demand of photosynthate which leads to reserve the excess photosynthate in the form of carbohydrate (Guy *et al.*, 1992). However, all the sugar contents decreased steeply in January without almost any changes in temperature (Figs.1 and 3). The reason might be the foggy weather and less hours of photoperiod prevailing that time. In some previous studies it was reported that lettuce growth is significantly affected by the interaction of solar radiation and temperature (Glenn, 1984; Koontz and Prince, 1986; Wurr and Fellows, 1991). Furthermore, organic acid content followed almost the same trend with sugar and showed similarity with the result of Pramanik *et al.* (2004) in broccoli. In most cases, the immediate precursor of an organic acid is sugar or another organic acid from which organic acid is formed or synthesized (Kays, 1991). However, unlike Pramanik *et al.* (2004) the organic acid fraction consisted of only two acids, where malic acid was the main component and oxalic acid could not be detected (Fig. 4). Blom-Zandstra and Lampe (1985) also reported that the main component of the organic acids in lettuce

is malate and oxalic acid can not be detected either by G.L.C. or other analytical techniques.

Except in December, higher ammonia content was found in the colder harvest months. The reason could be the higher ammonium uptake of the crop at low temperature as reported in barley (Macduff and Jackson, 1991) and in broccoli (Baclayon *et al.*, 2006). The lower ammonia content in December could be explained as a consequence of higher temperature in the previous months. However, significantly higher ammonia contents were observed in the inner tissues compared with the outer tissues. Marsic and Osvald (2002) also reported that inner tissues accumulate considerably higher NH_4^+ than outer leaf tissues. The possible reason might be the inner leaf contains more growing tissues than outer leaf, and growing tissues accumulate higher amount of nutrients like ammonium.

The activity of GS was almost constant in the inner leaf tissues during the harvest period (Fig. 6). However, the activity in outer leaf tissues changed with the changes in temperature. The inverse relationship between ammonia content and GS activity in the outer leaf portion ($r = -0.592$ and -0.188 for 'Bittsu' and 'Cisco', respectively) showed an agreement with Peeters and Van Laere (1992), where they concluded that the accumulation of ammonium coincided with the disappearance of GS activity. The variation of the GS activity between two types of tissues might be due to the possiblity that the inner leaf tissues contain higher GS_1 (cytosolic) and outer tissues contain higher GS_2 (chloroplastic), in which the later one is highly contributing in the total GS activity (Chandra *et al.*, 2006). The activities of AS in the outer leaf of both cultivars followed almost the same trend as GS except in April for the cultivar 'Bittsu', while in inner leaf tissues AS activity fluctuated over the harvest months (Fig. 7). It was suggested that there is a functional GS_1/ AS cycle, where the induction of AS has been observed in parallel with the induction of GS_1 expression (Avila *et al.*, 2001). However, significantly negative correlation was found between ammonia content and AS activity only in the outer leaf portion of 'Cisco' (Table 1). It is evident that AS is capable to use glutamine more efficiently than NH_4^+ in the conversion of aspartate to asparagine (Rognes, 1975).

In both aminating and deaminating directions, GDH activity was measured as this enzyme provides an assimilatory pathway for ammonium under various stress conditions (Srivastava and Singh, 1987). In both directions, the activity of GDH in outer leaf decreased whereas deaminating activity of inner leaf slightly increased in the warmer harvest months. The higher activity of GDH amination in colder months may be a consequence of higher ammonia content during that period. It was reported that, GDH is induced at high levels of ammonia (Cammaerts and Jacobs, 1985). The positive relations between ammonia content and GDH activity in the outer leaf portion suggest that GDH is playing more vital role in ammonia assimilation in this portion (Table 1). However, such relation was stronger in 'Bittsu' than that in 'Cisco'. As deamination activity operates in energy generation (Cammaerts and Jacobs, 1985), very little changes in deamination could be explained in such a way that the tissues contained higher amount of sugar which is supplying energy. Sugar appears to play a more central role in the regulation of GDH than ammonia and other nitrogenous sources (Lea *et al.*, 1990). Moreover, the decrease in GS activity might be compensated by the increased GDH activity during the colder months as the antagonistic relation between GS and GDH have already been reported (Ratajczak *et al.*, 1981).

In conclusion, during colder months the crispness of lettuce leaf reduced and accumulated higher amount of sugars, organic acids and ammonia. In general, between the two cultivars, 'Bittsu' contained higher amount of sugars and organic acids, while 'Cisco' contained higher amount of ammonia. The activities of GS and AS either decreased or remained nearly constant depending on the tissue type during the colder months. However, GDH activity in the outer leaf and GDH deaminating activity in the inner leaf tissues changed, with the changes in temperature throughout the harvest period. Detailed study is needed to confirm the effects of environmental factors such as temperature, photoperiod and light intensity on the textural and compositional quality, and the activities of ammonia assimilating enzymes in lettuce harvested monthly for a longer period. The effect of such growing conditions on the shelf life and storability along with textural and compositional quality would provide an idea for the growers to select the suitable growing season to achieve longer shelf life of this perishable commodity.

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Rooting and growth response of grapevine nurslings to inoculation with arbuscular mycorrhizal fungi and irrigation intervals

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Abstract

This study was conducted during two successive seasons (2005 and 2006); in the experimental farm of Faculty of Agriculture, Kafr El Sheikh University; with the aim to investigate the influence of arbuscular mycorrhizal fungi (AMF) inoculation and irrigation intervals on growth of grapevine nurslings cv. Ruby King. Two mix mycorrhizal fungi including *Glomus fasiculatum* and *Glomus mosseae* were used for inoculation. The AMF inoculated and non-AMF nurslings were irrigated at 3, 6 and 9 days interval. The results showed that a combined treatment of AMF inoculation and irrigation at 3 days intervals recorded the highest values in terms of length of main root, total root length, root volume, root dry weight (%), top/root ratio, number of fine roots (< 2 mm), number of small roots (2-5 mm), number of leaves and leaf area per nursling. These results are of practical importance, as they highlight the potential of using mycorrhizal fungi inoculation for root development and growth improvement in grapevine nurslings and hence increases its adaptability upon transfer from the nursery to the open field.

Key words: Arbuscular mycorrhizal fungi, grape, irrigation, nurslings

Introduction

Most of the major plant families are able to form mycorrhiza naturally, the arbuscular mycorrhizal (AM) associations being the commonest mycorrhizal type involved in agricultural systems (Berta *et al.*, 1993). Arbuscular mycorrhiza (AM) is a mutualistic symbiosis between AM fungi and the roots of terrestrial plants. The fungus biotrophically colonizes the root cortex and develops an extra-matrical mycelium that helps the plant to acquire mineral nutrients from soil (Harley and Smith, 1983). It has been recognized that mycorrhizal symbioses play a key role in nutrient cycling in the ecosystem and also protect plants against environmental and cultural stress (Barea and Jeffries, 1995).

The primary effect of AM symbiosis is the increase in the supply of mineral nutrients to the plant, particularly those whose ionic forms have a poor mobility rate, or those which are present in low concentration in the soil solution. This mainly concerns phosphate, ammonium, zinc and copper (Barea and Azcon-Aguilar, 1982). Mycorrhizal fungi also enable plants to cope with abiotic stress by means of alleviating nutrient deficiencies, improving drought tolerance, overcoming the detrimental effects of salinity and enhancing tolerance to pollution (Berta *et al.*, 1993).

Since AM symbiosis can benefit plant growth and health, there is an increasing interest in ascertaining their effectiveness in particular plant production situations and, consequently, in manipulating them so that they can be incorporated into production practices when feasible. Evidence is accumulating to show that indigenous and/or introduced AM fungi (AMF) are involved in the development of different plant production systems including both field sown and plantation crops and transplantable horticultural crops. Fruit crops are one of the target systems in which AM biotechnology can express its potential. This is due to the characteristics of the components and the plant production practices, which make it necessary and easy to inoculate fruit crop plants with AMF. Considering the high returns from fruit crops, it seems that the application of AM inoculation may represent only a small contribution to the input costs. In the present study, we investigated the influences of mycorrhizal inoculation and irrigation intervals on rooting and growth of grapevine nurslings.

Materials and methods

Plant material: Grapevine nurslings cv. Ruby King were transplanted individually in clay pots (40 cm in diameter) containing clay loamy soil. The nurslings were first pruned to two eyes and were allowed to grow without fertilizers. Then, two shoots were left on the nurslings and the other shoots were removed. These nurslings were used as initial plant material. The experiments werer conducted during two successive years (2005-2006), from February through September of each year. All experiments were carried out at the experimental farm of the Horticulture Department, Faculty of Agriculture, Kafr El-Sheikh University, Egypt.

Mycorrhizal fungi and irrigation treatments: Two mix mycorrhizal fungi including *G. fasiculatum* and *G. mosseae* were used to inoculate the nurslings with the same inoculation density according to the method described by Gerdemann and Nicolson (1963) and all mycorrhizal and non-mycorrhizal nurslings were irrigated at the day of AMF inoculation. To investigate the effects of irrigation intervals, irrigation was done at 3, 6 or 9 days interval that gives six treatments as follows: (1) Mycorrhiza inoculation + irrigation at 3 days (AMF + 3 d), (2) No AMF + 3 d, (3) AMF + 6 d, (4) No AMF + 6 d, (5) AMF + 9 d, (6) No AMF + 9 d.

Measurements of growth parameters: Total root volume (cm^3) was measured by water displacement according to Kolesnikov (1971). Total root length was determined according to methods of Newman (1966), Marsh (1971) and Tennant (1975). Dry weight was measured after drying the stems, roots and tops for 48 h at 70°C and top/root ratio and dry weight percentage was calculated. Total leaf area was measured using an area meter (LI-3100, Li-Cor, Lincoln, NE) and average leaf area was calculated using 10 individual leaves per nursling.

Experimental design and statistical analysis: The experiments were set up in a randomized complete block design and each treatment was represented by three replicates, each replicate contained 4 pots. Data were subjected to Duncan's Multiple Range Test for mean comparison (Snedecor and Cochran, 1980).

Results and discussion

Effect of AMF inoculation and irrigation regime on root growth, root thickness and root branching: The results presented in Table 1 indicate that AMF inoculation treatments had the greatest root growth in terms of length of main root, total root length, root volume, root dry weight (%) and top/root ratio compared to non-AMF. The best results were obtained when AMF inoculation was combined with irrigation at 3 days interval. Root volume tended to decrease with increasing irrigation intervals in the non-inoculated plants. However, the highest root volume in the AMF inoculated plants was obtained in the 6 days irrigation intervals. Although, there was a gradual increase in top/root ratio with increasing irrigation interval, it was clear that AMF inoculation and 9th day irrigation had a better root system compared to non-AMF and 9th day irrigation (Table 1). It could also be noticed that top/root increased as the irrigation intervals increased.The heighest value in this concern was obtained in the AMF inoculated plants at 9 days irrigation interval.

AMF inoculation and irrigation intervals also influenced root thickness and root branching (Table 2). AMF nurslings had the highest number of fine roots (<2 mm) and number of small roots (2-5 mm) compared to non-AMF nurslings and best results were recorded at 3 days irrigation treatment. However, number of medium roots (5-10 mm) was highest when AMF inoculation treatment was combined with irrigation at 6 days in both seasons and recorded 2.5 and 3.0, respectively.

Arbuscular mycorrhiza (AM) fungi can induce morphological modifications in the host plant root system (Berta *et al.,* 1993; Atkinson *et al.,* 1994). A more branched root system has been observed in mycorrhzal plants of different herbaceous and woody species (Amijee *et al.*, 1989; Berta *et al.*, 1990; Schellenbaum *et al.*, 1991; Tisserant *et al.,* 1992). Different AM fungal species can induce different effects on root system morphology (Berta *et al.* 1995), which could be related to the extent of root colonization (Hooker *et al.,* 1992). Although root system morphology is genetically determined (Harper *et al.,* 1991), many environmental factors can influence root development, including mineral nutrition (Drew and Saker, 1978). In addition, differences between mycorrhizal and non-mycorrhizal plants may decrease at high external phosphorus levels (Hetrick *et al.,* 1998). Vidal *et al.* (1992) reported that the inoculation of plants of Avocado with vescular–arbuscular mycorrhiza fungus improved root growth and increased the top/root ratio. Inoculation of citrus seedling with vescular–arbuscular mycorrhiza fungus increased growth and dry weight (Dixon, 1989; Nawar *et al.*, 1998).

Aguin *et al.* (2004) indicated that inoculation with vescular–arbuscular mycorrhiza fungus (VMF) *G. aggregatum* in rooting beds of grapevine cuttings changed root morphology by increasing branching of first-order lateral roots. When rooted cuttings were transplanted to pots, a significant growth enhancement was found in two of inoculated rootstocks. *G. aggregatum* alone or in synergy with the indigenous AM fungi seemed to have a higher affinity for 161-49 Couderc, the roots of which were more extensively colonized and exhibited a greater positive growth response. Marschner *et al.* (1997) reported that mycorrhizal infection had a little effect on the physiological status compared to the non-mycorrhizal plants. Dell' Amico *et al.* (2002) found that mycorrhizal infection improved physiological activity in non–stressed and stressed plant. This improvement was accompanied by higher root hydraulic conductivity values, indicating enhanced

Table 1. Effect of mycorrhiza inoculation and irrigation intervals on length of main root, total root length, root volume (cm^3), root dry weight (%) and top /root ratio of grape nurslings during 2005 and 2006 seasons

Treatment	Length of main root		Total root length		Root volume (cm L^{-3})		Root dry weight (%)		Top / Root ratio	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
AMF+ 3 d	72.33a	80.50a	1400.00a	1341.67a	315.00c	520.00a	96.07b	52.38a	0.67c	0.60d
No AMF+ 3 d	58.33c	62.50b	898.33b	1048.33b	430.00a	530.00a	50.85a	48.21c	0.74c	0.75cd
AMF+ 6 d	58.50bc	59.83b	765.00b	858.33c	525.00a	448.00b	47.77ab	52.41a	0.80c	0.83bc
No AMF+ 6 d	55.83c	58.50bc	181.67c	781.67c	337.33b	410.67b	50.67a	46.71d	0.85c	0.67d
AMF+ 9 d	60.67b	34.50d	276.67c	461.67d	271.67d	310.00c	44.24c	50.18b	1.00b	0.94b
No AMF+ 9 d	44.67b	50.17c	131.67c	34.67e	261.67c	230.00d	43.46c	47.64cd	1.34a	1.28a

Table 2. Effect of mycorrhiza inoculation and irrigation intervals on root thickness and number of roots in grape nurslings during 2005 and 2006 seasons

Treatment	Root thickness						Number of roots			
	Number of medium roots (5-10 mm)		Number of small roots (2-5mm)		Number of fine roots (< 2mm)		2nd order		3rd order	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
AMF+ 3 d	1.17b	2.50ab	14.50a	18.50a	17.33a	18.83a	34.00a	33.67a	34.33a	48.67a
No AMF+ 3 d	1.50b	1.17c	9.50c	11.50b	14.33ab	18.00a	34.33a	28.67b	18.33c	38.00b
AMF+ 6 d	2.50b	3.00a	12.50b	10.50bc	9.50cd	9.00b	32.00a	24.00c	22.17b	23.67c
No AMF+ 6 d	1.17b	1.67bc	8.17d	9.50c	8.50cd	6.50c	19.33c	19.67d	20.00bc	19.50d
AMF+ 9 d	1.67ab	1.17c	5.17e	10.00bc	11.17bc	9.00b	24.67b	19.50d	19.67bc	18.00d
No AMF+ 9 d	1.67ab	2.50ab	5.50e	5.50d	6.00d	8.00bc	12.50d	16.00e	13.33d	14.00e

Table 3. Effect of mycorrhiza inoculation and irrigation intervals on leaf number/nursling, leaf area (cm^2), total leaf area /nursling, stem dry weight (%) of grape vine nurslings during 2005 and 2006 seasons

Treatments/nursling	Leaf number /nursling		Leaf area (cm^2)		Total leaf area		Stem dry weight (%)	
	2005	2006	2005	2006	2005	2006	2005	2006
AMF+3 d	95.33a	115.00a	83.20a	95.44a	8706.53a	9748.14a	54.97cd	54.12b
No AMF+3 d	87.33b	69.67c	72.34b	83.71c	6546.11b	5758.57b	60.23b	57.46a
AMF+6 d	76.33d	81.33b	80.37a	90.35b	5776.74b	5612.68b	56.46c	53.80b
No AMF+6 d	72.67d	53.33d	68.31c	74.83d	5301.14bc	4268.97c	62.22ab	50.48c
AMF+9 d	81.33c	42.00e	55.76d	72.61d	4326.77c	3040.60d	64.47a	54.10b
No AMF+9 d	48.67e	33.00f	50.50e	63.67e	1868.43d	1738.41e	53.48d	53.30b

water uptake in drought conditions. The beneficial effect of the mycorrhizal symbiosis on water status of tomato plants stimulated plant growth. On the other hand, Caravaca *et al.* (2005) also reported that the mycorrhizal inoculation and the irrigation of plants had a strong effect on the growth parameters.

Effect of AMF inoculation and irrigation intervals on shoot growth: Table 3 shows that both AMF inoculation and irrigation treatments significantly influenced shoot growth of grapevine nurslings. The greatest number of leaves, leaf area and total leaf area per nursling were obtained in both seasons when nurslings were inoculated with AMF and irrigated at 3 days interval. The lowest values were recorded with AMF+9 days treatment. The percentage of stem dry weight recorded the highest value (64.47) in both seasons when nurslings were inoculated with AMF and irrigated at 9 days interval. Al-Karaki (1998) reported that shoot and root dry matters were higher for water stressed AM wheat than those for corresponding non-AM plants. Effect of AM fungi on shoot behaviors was not often closely linked to the extent of AM colonization of roots (Fitter and Merryweather, 1992; Smith and Read, 1997).

The results obtained strongly indicate that inoculation of grapevine nurslings with mycorrhizal fungi increased root growth of young nurslings. The improved growth could be due to direct effects of mycorrhizal fungi on plant water and nutrient uptake and also indirect effects via mycorrhizal-induced changes in the bacterial community composition. These results are of practical importance, as they highlight the potential of using mycorrhizal fungi inoculation for root development and growth improvement in grapevine nurslings and hence increase its adaptability upon transfer from the nursery to the open field.

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Influence of lysophosphatidylethanolamine application on fruit quality of Thompson Seedless grapes

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Abstract

The effect of foliar applications of lysophosphatidylethanolamine (LPE) on 'Thompson Seedless' (*Vitis vinifera* L.) was evaluated to determine the suitability of this plant amendment aid as a management tool in table grape production. LPE at 10 mg L^{-1} was sprayed on vines at two different stages of berry growth and development. Treatments were: 1) 4 weeks after fruit set; 2) 6 weeks after fruit set; and 3) 4 and 6 weeks after fruit set. Soluble solids content (SSC) of berries at all harvest dates was significantly higher for vines treated with LPE compared to the control. Titratable acidity (TA) gradually decreased during ripening, and by the third harvest, TA of berries from vines treated with LPE was lower than that of control. All the treatments resulted in higher fruit firmness when compared to control. However, there were no significant differences in firmness of berries from vines given LPE treatment at different stages of growth. LPE treatment increased berry size, although no significant difference in size between single and sequential applications of LPE was observed. These results indicate that LPE may play a role in plant hormone-associated regulation of berry growth and development.

Key words: Firmness, plant growth regulators, size, soluble solids content, titratable acidity

Introduction

Lipids are known to play important roles in membrane structure and energy reserves. It is now evident that lipids and their metabolites play important roles in other critical cellular functions particularly as mediators in signal transduction, cell activation, and cell proliferation (Cowan, 2006; Divecha and Irvine, 1995; Ryu *et al.*, 1997). Lysophospholipids are present in biological membranes in trace amounts but their concentration changes during exposure of plants to freezing (Welti *et al.*, 2002), in response to wounding (Lee *et al.*, 1997), and during cell expansion (Lee *et al.*, 2003; Scherer, 2002). Lysophosphatidylethanolamine (LPE) is a minor glycerolipid present in all extra-chloroplastic membranes and is formed from the parent phospholipid, phosphatidylethanolamine (PE) by the action of phospholipase A_2 (PLA_2).

Previous studies showed that LPE, a naturally occurring phospholipid, can retard senescence in attached and detached leaves and fruits of tomato (Farag and Palta, 1993a). LPE-treated tomatoes displayed a longer shelf life whether they were harvested at the breaker, pink, or red stages of maturity (Farag and Palta, 1993b). LPE treatment has also been found to reduce senescence of leaves, fruits and cut-flowers (Kaur and Palta, 1997; Ozgen *et al.*, 2005). It was observed that LPE increased marketable yield, stimulated ripening and extended shelf life of green pepper (Hong and Chung, 2006). Hong (2006) further reported that the influence of LPE on fruit tissue was dependent on the stage of ripening. Thus, in a mature fruit (ready to ripen), LPE stimulated ripening while in a ripened fruit, it inhibited ethylene production and maintained fruit firmness thereby prolonging shelf life.

As mentioned above, LPE has been shown to affect various physiological processes in plants and it was therefore of interest to study the effect of this lysophospholipid on fruit quality of table grapes. 'Thompson Seedless' is one of the most important table grape cultivars grown worldwide, which is marketed nearly year-round throughout the world. Fruit quality parameters are most important for growers and contribute directly to on-farm income and success of the business. Therefore, in the present study the effects of foliar applied LPE on quality parameters, including soluble solids content (SSC), titratable acidity (TA), firmness, and berry size of 'Thompson Seedless' table grapes were investigated. In preliminary small scale experiments, we observed an increase in SSC and firmness of grapes indicating that foliar application of LPE might be an ideal management tool to enhance berry quality of 'Thompson seedless' grapes.

Materials and methods

The study was performed on 8-years old vineyards cv. Thompson Seedless, located at Coltauco, Rancagua, Chile. The experimental design consisted of a randomized block with three replicates of five vines each. Two buffer vines were used between different experimental vines to limit drifting of spray material from adjacent treatments. For single application studies, vines were foliar-sprayed with 10 mg L^{-1} LPE either 4 or 6 weeks after fruit set (December 29, 2005 and January 11, 2006). For sequential treatment, foliar application with 10 mg L^{-1} LPE was carried out at 4 weeks after fruit set, followed by 10 mg L^{-1} LPE at 6 weeks after fruit-set.

Clusters for analysis of SSC and TA were harvested on three occasions and at weekly intervals. Berry firmness and size were measured only at the third harvest. The first sampling was on February 7, 2006. Three clusters were selected at random from each vine and ten berries were collected from each cluster.

For SSC and TA analyses, ten berries without peel were first

homogenized in a Waring blender. A refractometer (PR-101, Ataog Co., Ltd., Japan) and an autotitrator (DL 50 Grphix, Mettler-Toledo GmbH, Switzerland) were used to estimate SSC and TA, respectively. TA was measured by titrating the samples to pH 8.2 using 0.1 mol L^{-1} NaOH. Acidity was expressed as an acid factor of 0.075 (tartaric acid).

Fruit firmness and size were determined for berries from the middle part of each cluster harvested at random. For each treatment, 180 berries were analyzed for firmness by digital Durometer (Durofel 25, Agro Technologie, Tarascon, France) and for fruit diameter by caliper without detachment from the vines.

Data were evaluated using GLM procedures in the SAS 6.12 statistical package (SAS Institute, Inc. Cary, N.C.). Differences between treatment means were determined using Fisher's least significant difference (LSD), $P < 0.05$.

Results and discussion

For the first and third harvests, SSC of berries at all harvest dates was significantly higher for vines treated with LPE when compared to control (Table 1). However, there were no significant differences in SSC among the LPE treatments. In the second harvest, SSC of berries was highest for vines sprayed with LPE 4 weeks after fruit set.

The TA for the first harvest did not differ significantly among treatments (Table 1). In the second harvest, TA of berries was lowest for vine sprayed with LPE at 4 weeks after fruit set. For the third harvest, all LPE treatments, regardless of spray timing and frequency, resulted in higher SSC, compared to control. As expected, TA was lower for each treatment in the third harvest compared to the first harvest.

The importance of harvesting grapes at the best stage of maturity is well recognized. Legal minimum standards for maturity based on SSC and TA of berries have been established, but these standards vary considerably for different varieties, locations, and whether the fruit is destined for export or domestic markets. In many countries such as Chile, USA, and Australia, the export regulations require that 'Thompson Seedless' table grape has a minimum of 18°Brix. In early varieties including 'Thompson Seedless' grape, there is a strong desire by growers to harvest as early as possible in the expectation of receiving higher prices for new season fruit. Therefore, it is not unreasonable to suggest that the LPE-induced increase in SSC could contribute to earlier harvests which would be of considerable economic benefit to the grape growers.

All LPE treatments increased berry firmness. There were, however, no significant difference in firmness of berries from vines given LPE treatments at the different stages of growth (Table 2). Fruit firmness is one of the most important commercial characteristics of grapes, especially where fruit is destined for exportation. A problem that sometimes arises with 'Thompson Seedless' table grapes is the occurrence of soft berries. Softer berries reduce the attractiveness of table fruit, and do not store well or as long as firmer berries. Hong and Chung (2006) reported that LPE increased the marketable yield, stimulated ripening, and extended shelf life by increasing firmness in green pepper. It has been found that in a ripened fruit, LPE inhibited ethylene production to prolong shelf life and maintain fruit firmness (Hong, 2006).

Table 1. Effect of foliar-applied LPE on soluble solids content (SSC) and titratable acidity (TA) of 'Thompson Seedless' grape berries at first, second, and third harvest

Treatment[z]	SSC (°Brix)	TA (%)
First harvest (Feb. 7, 2006)		
T1	15.7 c[y]	0.84 a
T2	16.2 a	0.79 ab
T3	15.9 abc	0.84 a
T4	16.0 ab	0.80 ab
Second harvest (Feb. 14, 2006)		
T1	16.2 d	0.73 a
T2	17.4 a	0.65 b
T3	17.0 b	0.69 ab
T4	16.8 bc	0.70 ab
Third harvest (Feb. 21, 2006)		
T1	16.8 b	0.58 a
T2	17.7 a	0.54 b
T3	17.9 a	0.53 bc
T4	18.0 a	0.55 b

[z]Treatment
T1: Control
T2: LPE (10 mg L^{-1}) at 4 weeks after fruit set (December 29, 2005)
T3: LPE (10 mg L^{-1}) at 6 weeks after fruit set (January 11, 2006)
T4: LPE (10 mg L^{-1}) at 4 and 6 weeks after fruit set
[y]Across harvest time, means within columns with different letters are significantly different at $P < 0.05$ (n=45) using Fisher's LSD.

Singh *et al.* (1978) reported that in Thompson Seedless grape, gibberellic acid (GA_3) applied at veraison resulted in higher firmness compared to control, while application at 4 weeks after fruit set had no effect on firmness. In the present study, LPE application at 4 or 6 weeks after fruit set significantly increased fruit firmness.

All LPE treatments increased berry size. However, unlike the situation reported for GA_3 there was no significant difference in size of berries from vines treated with either single or sequential applications of LPE (Table 2). In general, the more applications of GA_3 have resulted bigger berry size at harvest (Gowda *et al.*, 2006; Singh *et al.*, 1978).

In seedless grapes, particularly in cv. Thompson Seedless, GA_3 increased berry size (Ben-Tal, 1990) by enhancing cell division, or cell enlargement, or both (Sachs and Weaver, 1968) which results in increased sugar and water uptake by the berry. It has been reported that the timing of GA_3 application for increasing berry size coincided with that for invertase stimulation and berry size correlates positively with increased invertase activity, indicating

Table 2. Effect of foliar-applied lysophosphatidylethanolamine (LPE) on berry firmness and diameter of 'Thompson Seedless' grapes at third harvest (February 21, 2006)

Treatment[z]	Firmness (Durofel Unit)	Diameter (mm)
T1	10.1 b[y]	17.9 b
T2	11.1 a	18.3 ab
T3	10.8 a	18.5 a
T4	11.1 a	18.5 a

[z]Treatment
T1: control
T2: LPE (10 mg L^{-1}) at 4 weeks after fruit set (December 29, 2005)
T3: LPE (10 mg L^{-1}) at 6 weeks after fruit set (January 11, 2006)
T4: LPE (10 mg L^{-1}) at 4 and 6 weeks after fruit set
[y]Means within columns with different letters are significantly different at $P < 0.05$ (n=180) using Fisher's LSD.

that upregulation of activity and/or synthesis of this enzyme is associated with increased berry size (Perez and Gomes, 2000). We have found that exogenous LPE increased invertase activity in expanding cotyledons of radish and that LPE-induced senescence delay correlates with changes in activity of extracellular acid invertase (data not shown).

In conclusion, results from this study confirm that foliar applications of LPE to 'Thompson seedless' vines increase SSC, fruit firmness, and berry size of table grapes. These results, together with the recent demonstration of the growth regulating activity of LPE (Cowan *et al.*, 2006), suggest that it may play an important role in plant hormone-coordinated regulation of berry growth and development. However, the underlying mechanisms by which exogenous LPE is able to effect physiological change remains to be elucidated. We hope future studies will provide more insight on this important and yet unresolved question.

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Thidiazuron effects on physiochemical characteristics of carnation during pre and postharvest periods

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Abstract

Experiments were conducted to determine the effects of Thidiazuron (TDZ) applied at preharvest stage under glasshouse conditions on *Dianthus caryophyllus* 'Lunetta'. Thidiazuron at 0, 1, 10, 100, and 1000 µM was applied as a foliar spray arranged in completely randomized design. Time to flowering was recorded, and relative stem length, total nitrogen and tissue water content were measured at harvest. Postharvest vase life, relative fresh weight changes, and solution uptake were also measured. TDZ treatments decreased relative stem length compared to the control (0 µM). TDZ treatment tended to decrease total nitrogen and water content of tissues slightly, but not significantly ($P > 0.05$). TDZ at 100 µM significantly increased the vase life of cut carnation flowers compared to the control. TDZ treated flowers tended to maintain higher relative fresh weight, with positive differences for the 100 µM TDZ treatment being apparent at day 5, 7 and 9 of vase life. Solution uptake was higher in TDZ treated flowers.

Key words: *Dianthus caryophyllus* 'Lunetta', preharvest, postharvest, Thidiazuron, vase life.

Introduction

Various studies have demonstrated improvement of the postharvest life of cut flowers as a result of cytokinin treatments (Lukaszewska *et al.,* 1994; Paull and Chantrachit, 2001). However, the effects of preharvest treatment on postharvest characteristics of cut flowers are largely unexplored. Preharvest variables can be an important determinant of the quality and longevity of cut flowers and foliage (Celikel and Karaaly, 1995). For example, mean relative humidity in the greenhouse is an important variable accounting for vase life differences in the one cultivar produced in different greenhouses (Marissen and Benninga, 2001).

Thidiazuron (N-phenyl-N-1, 2, 3-thidiazol-5-ylurea; TDZ) is a relatively novel urea-type compound having plant growth regulator-like activity. It has been used commercially at high concentrations as a cotton defoliant (Malik *et al.,* 2002). At low concentrations, it has been used for regeneration in tissue culture (Singh and Syamal, 2001). TDZ appears to be stable in plant tissues (Ferrante *et al.,* 2002a; Mok *et al.,* 2000). Although the exact mode of TDZ action is not known, it has been reported to modulate cytokinin biosynthesis and / or metabolism (Mok *et al.,* 2000), influencing ancillary endogenous hormones, proteins, and enzymes (Murthy *et al.,* 1998). TDZ seems to mimick both auxins and cytokinins (Murthy *et al.,* 1998). It is around 50 - 100 times more active in inducing cytokinin-like effects than common cytokinins (Genkov and Iordanka, 1995).

Bosse and Van Staden (1989) reported that DHZ (dihydrozeatin) used as a pulse treatment for 6-24 h at a concentration of 2×10^{-4} M significantly delayed carnation flower senescence. An increase in the longevity of carnation flowers by 15 d was recorded at 4×10^{-6} M. Lukaszewska *et al.* (1994) showed that exogenous application of zeatin, zeatin ribiside, 2iP, and 2iPA in holding solutions delayed rose senescence by 34-56 %, and thereby prolonged longevity. Zeatin and zeatin ribiside were most effective on roses at 1×10^{-7} M, and prolonged rose longevity by 13 d. Treatments with TDZ effectively prevented leaf yellowing in *Alstroemeria* (Ferrante *et al.,* 2002a). It was most effective as a 10 µM pulse treatment or at 1 µM as a continuous treatment. TDZ treatment was also useful on phlox inflorescences at < 50 µM (Sankhala *et al.,* 2003). Treatment with TDZ greatly reduced flower shedding and induced additional flower buds during vase life. The efficacy of TDZ treatments depends upon the genotype and the concentration. TDZ treatment of chrysanthemum and tulip inhibited leaf yellowing, but did not enhance the quality of the flowers (Ferrante *et al.,* 2003). TDZ in comparison with BA had little effect on vase life of cut *Eucalyptus parvifolia* (Ferrante *et al.*, 2002b). Pulse treatments with 10 µM TDZ decreased the vase lives of 'Champagne', 'Laser', 'Magnum' and 'Neon' roses, but slightly increased that of 'Tresor 2000' (Chamani *et al.*, 2006). A 10 µM TDZ pulse treatment increased the vase life of 'First Red' rose, and TDZ in combination with sucrose had a greater positive effect on vase life than either TDZ or sucrose alone.

The present study was conducted to examine effects of preharvest TDZ treatments on the physiochemical characteristics of *Dianthus caryophyllus* 'Lunetta' during the subsequent pre- and postharvest periods.

Materials and methods

D. caryophyllus 'Lunetta' plantlets were obtained from a commercial greenhouse (Pakdasht, Tehran) and transported to the research greenhouse of Mohaghegh Ardabili University, Ardabil. Plants were potted-up into media comprised of 25% sand, 25% cattle manure, and 50% farm soil. 3 weeks after planting, the plants were pinched-back to encourage uniformity of flowering. 6 weeks after pinching, plants were treated with foliar sprays of TDZ. TDZ (Sigma Chemical Co.) was dissolved in 2 mL of 1 M KOH and prepared at 0, 1, 10, 100, and 1000 µM concentrations. pH was neutralized with 2 mL 1 M HCL. The experiment was

duplicated, and 15 plants for each TDZ concentration were used in both experiments. Experiments were arranged in completely randomized designs. The traits measured were: time to flower, relative stem length (%), total nitrogen, tissue water content at harvest, vase life, relative fresh weight changes (%), and solution uptake changes after harvest. Flowers were harvested at the fully open stage (Eisinger, 1977). Postharvest experiments were carried out under vase life evaluation room conditions of 22 ± 2 °C, 60 - 70 % relative humidity (RH) and 12 h photoperiod with cool white florescent lamps. The harvested flowers were kept into vases containing distilled water and 10 µl L^{-1} chlorine (a.i.).

Time to flowering was recorded as days from TDZ application to full flower opening (harvest). Relative stem length percentage (RLP, %) for stems was calculated using the formula: $\{(L_t - L_0) / L_0\} \times 100$; where, L_t = stem length (cm) at time t (week) and L_0 = stem length (cm) at time 0. Time 0 was the treatment day. Water content of tissues (WCT %) was calculated using the formula: $\{(W_t - W_d / W_t\} \times 100$; where, W_t = fresh weight of the stem (g) on day 0 (day 0 was harvest day) and W_d = dry weight of the stem (g) at the end of vase life. Total N was determined in dried tissue samples of 1 g dw by the Kjeldhal method using concentrated H_2SO_4, K_2SO_4 and selenium to digest the sample.

Vase life was recorded as the time (days) after harvest (day 0), when flowers started showing petal in-rolling, which was taken as the first sign of flower senescence (Bosse and Van Staden, 1989). Relative fresh weight (RFW, %) for stems was calculated using the formula: $(W_t / W_{t=0}) \times 100$; where W_t = weights of stems (g) on days 2, 4, 6, etc. and $W_{t=0}$ = weight of the same stem (g) on day 0. Vase solution usage (mL day^{-1} g^{-1} fw) was determined using the formula: $(S_{t-1} - S_t) / W_{t=0}$; where, S_t = solution weight (g) at t = days 1, 2, 3, etc. S_{t-1} = solution weight (g) on the previous day, and $W_{t=0}$ = fresh weight of the stem (g) on day 0.

Statistical analyses: Data were analyzed with Minitab Release 13.1 for Windows (Minitab Inc.). The Duncans Multiple Range Test (DMRT; $P = 0.05$) was used for comparisons of treatment means. Least significant difference (LSD; $P = 0.05$) values were also calculated for mean comparision.

Results and discussion

Treatment with TDZ at 1000 µM significantly ($P \leq 0.05$) delayed flowering compared to 10 and 100 µM TDZ treatments (Fig. 1). Thus, flowers were produced earliest with treatments of 10 and 100 µM TDZ. TDZ treated flowers generally had proportionally lower stem lengths compared to the control (Fig. 2). Significant differences were initially apparent at 2 and 3 weeks after treatment; *e.g.* control vs. 100 µM TDZ treated flowers. The lowest stem length percentage in the end was for plants treated with 1000 µM. TDZ treatments tended to slightly decrease total carnation stem N and tissue water contents at harvest, but these effects were not significant (Table 1).

TDZ applied at 10 and 100 µM increased the vase life of cut carnation flowers compared to the 0 µM control (Fig. 3). TDZ treated flowers sometimes maintained a higher relative fresh weight, with significant differences at 5, 7, and 9 days after treatment between 100 µM TDZ treated flowers and the control (Fig. 4). Highest relative fresh weight was specifically associated with plants treated with 100 µM TDZ. Vase solution usage tended to be higher in TDZ treated flowers, although no significant difference was found between TDZ treated flowers and control (data not shown).

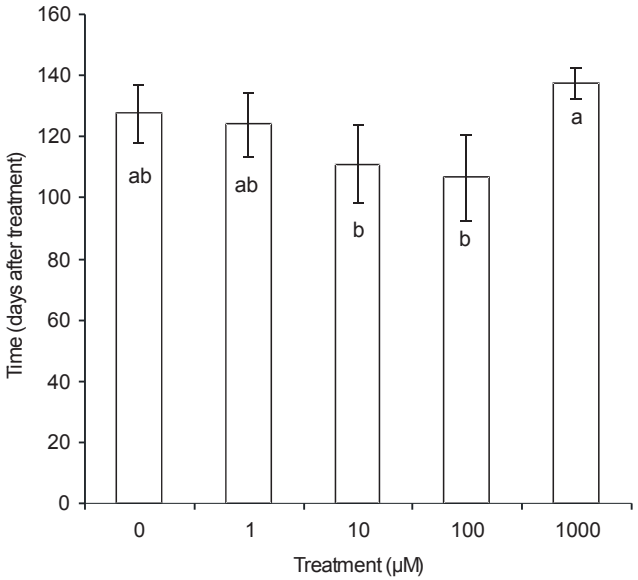

Fig. 1. Effects of different TDZ concentrations on the time to flowering of cut 'Lunetta' carnation plants.

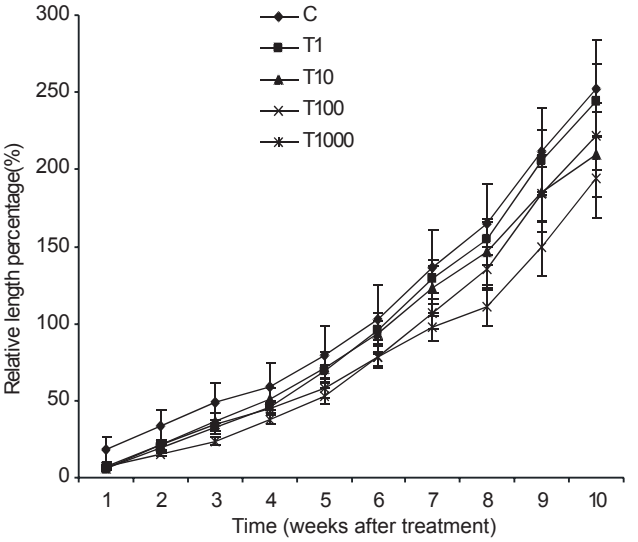

Fig. 2. Effects of different TDZ concentrations on preharvest changes in relative stem length percentage of cut 'Lunetta' carnation flowers

The vase life of cut Lunetta carnation flower could be increased by preharvest application of 10-100 µM TDZ. Increase in the postharvest life of carnations by the application of cytokinins has been reported previously (Shibuya *et al.*, 2000; Eisinger, 1977). Bosse and Van Staden (1989) used DHZ as a pulse treatment for 6 - 24 h at 2×10^{-4} M to delay carnation flower senescence. Similarly, a 10 µM TDZ pulse treatment increased the vase life of 'First Red' rose, which exhibited greater water uptake and higher

Table 1. Effect of different TDZ concentrations on the N and water content of 'Lunetta' carnation flower stems at harvest

Treatment	Total Nitrogen (%)	Water content (%)
Control	2.28	82.7
TDZ (1 µM)	2.04	82.5
TDZ (10 µM)	2.05	81.9
TDZ (100 µM)	2.06	81.7
TDZ (1000 µM)	2.15	82.2
LSD (P=0.05)	0.21	Non significant

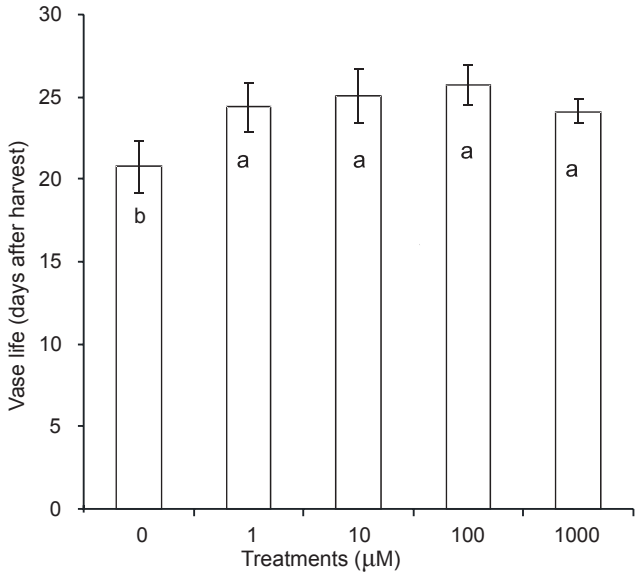

Fig. 3. Effect of different TDZ concentrations on the vase life of cut 'Lunetta' carnation flowers.

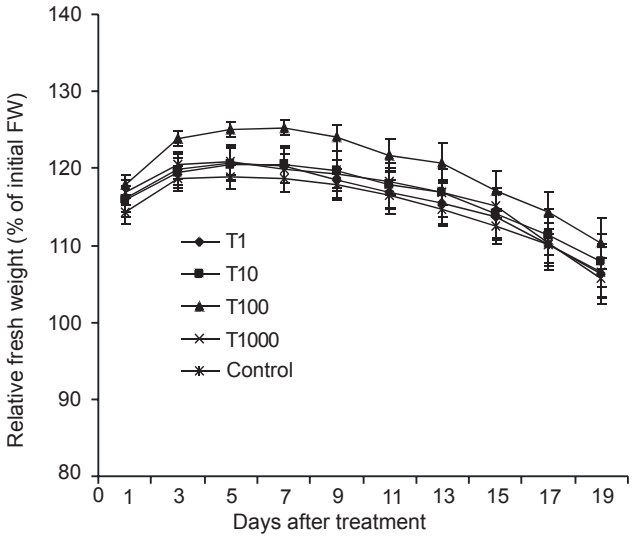

Fig. 4. Effect of different TDZ concentrations on postharvest changes in relative fresh weight of cut 'Lunetta' carnation flowers (experiment 2).

relative fresh weight (Chamani *et al.,* 2006). Increased vase life of cut Lunetta carnations may be associated with solution uptake maintenance by TDZ and / or inherently low levels of cytokinins in their tissues. BA treatments can maintain water uptake (Paull and Chantarachit, 2001). In anthurium, a decline in water uptake was related to reduced vase life (Paull and Goo, 1985). Paull and Chantrachit (2001) reported that various *Anthurium* cultivars showed different vase life responses to BA treatment, from a 20 % reduction to a 2.5-fold increase. They suggested that a lack of response to BA may be due to high natural levels of cytokinins already in the tissue.

The study revealed that TDZ treatments decreased relative stem length compared to the control. TDZ at 100 µM significantly increased the vase life of cut carnation flowers compared to the control. TDZ treated flowers tended to maintain higher relative fresh weight.

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Comparative morphology and RAPD analysis of some turfgrass cultivars grown in Saudi Arabia

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Abstract

With the increasing number of turfgrass cultivars, development and use of reliable identification methods is becoming important. Random amplified polymorphic DNA (RAPD) markers along with morphological markers proved useful for cultivar identification. Seven turfgrass cultivars encompassing four bermudagrass and three zoysiagrasses were grown under uniform greenhouse conditions and their key diagnostic features were described. Bulk samples of leaves were collected from each cultivar and subjected to RAPD analysis using standard protocols. Out of the 35 Operon primers used, 20 detected polymorphism among the cultivars. 'Nagissa' and 'Miyako' zoysiagrasses showed close genetic relationship as compared to the rest of the cultivars. They had the highest value in the similarity matrix for Nei and Li's coefficient (0.802) while one variant of Miyako clustered with Bermuda-1. Tifgreen Bermuda and Bermuda-2 also clustered together while 'Tifway' stood apart. Analysis of the morphological data showed that the variant of ' Miyako' belonged to the *Zoysia* genus but its genetic affinity with Bermudagrass needs to be explained. Within and between species, the cultivars having similar leaf-texture showed a tendency to cluster together.

Key words: Miyako, Nagissa, Tifgreen, Tifway, Zoysiagrass, Bermudagrass, RAPD, morphology

Introduction

Turfgrasses have been the subject of conventional systematic studies using comparative morphological and ecological characters. However some turfgrasses have similar or narrow distinguishing morphological characters that complicate taxonomical classification and demand genetic evidence to prove phylogenetic relationships at the inter specific level (Caetano-Anolles,1998). Since morphological characters of the turfgrass, such as growth, leaf-colour, size, texture, internodal length etc., are highly variable depending on the climatic and edaphic changes, cultivars within some species are difficult to distinguish. In a desert country like Saudi Arabia, the environmental conditions are very harsh and extremely flexible during different seasons, and differs significantly from the other locations in which these turfgrasses are cultivated. Phenotypic expressions of some turfgrass cultivars in response to these harsh conditions complicate identification within the species and rarely between the cultivars of allied species. Okawara *et al*. (2002) reported various responses of some Zoysiagrasses and other grasses to the environmental conditions of Saudi Arabia. One of the solutions to overcome this complication is to grow all the cultivars under uniform environmental conditions and then describe the morphological characters.

Recent advances in technology have shown that amplification of DNA using single arbitrary primers generates an almost infinite number of polymorphisms (Williams *et al.*,1990; Caetano-Anolles *et al*.,1991). RAPD techniques were successfully utilized in identifying the cultivars of perennial ryegrass (Sweeny and Danneberger, 1994;1997), *Zoysia japonica* Steud (Caetano-Anolles *et al*.,1991), *Eremochloa ophiuroides* (Munro.) Hack.(Weaver *et al*.,1995), Kentucky Bluegrass (Ohumura *et al*.,1997), *Paspalum vaginatum* O.Swartz (Chen *et al*., 2005) and should prove useful for identifying cultivars in other turfgrass species.

The objective of this study was to identify the key diagnostic morphological features of 3 cultivars of zoysiagrass and 4 cultivars of bermudagrass grown under uniform greenhouse conditions and to apply RAPD techniques for the identification and determination of their phylogenetic relationships.

Materials and methods

Four cultivars of bermudagrass and three cultivars of zoysiagrass were procured from various sources (Table 1). Tillers of the cultivars were planted in polystyrene pots of 27cm diameter and 30cm height filled with peat moss, perlite and coarse sand in the ratio 2:1:1. All pots were nurtured in a greenhouse with temperature ranging between 28°C in day and 15°C during night and having a photoperiod of 14 hours. The pots were arranged in a randomized complete block design (RCBD) with three pots per cultivar as one replicate and four replicates were used.

After 3 months of growth, 10 randomly selected shoots were carefully removed from each replicate pot and all the replicates were represented. Three leaf samples were collected from the apical portion of each shoot, after leaving the terminal rolled ones. Length and maximum width of leaf samples were measured using a millimetre scale. The formula used to calculate leaf area was: leaf area = length x maximum width x 0.84 as reported by Shabana and Antoun (1980). Internodal length of each sample collected from each cultivar was also measured from the detached shoots, after leaving the terminal long ones. Measurements of leaf-length, width, internodal length and leaf-area are provided

Table 1. Name and source of turf grass cultivars

Sl. No	Name	Scientific name	Source
1	Miyako	*Zoysia matrella* (L.) Merr.	Saudi-Japan Research Project.
2	Nagissa	*Zoysia matrella* (L.) Merr.	Saudi-Japan Research Project.
3	Variant of Miyako	*Zoysia matrella* (L.) Merr.	Saudi-Japan Research Project.
4	Tifgreen	*Cynodon dactylon* (L.)Pers. x *C. transvaalensis* Burtt.-Davy	Southern Turf Nurseries, Georgia.
5	Tifway	*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt.-Davy	
6	Bermudagrass (1)	*Cynodon dactylon* (L.) Pers.	Local farms (cultivated)
7	Bermudagrass (2)	*Cynodon dactylon* (L.) Pers.	Central part of Saudi Arabia (wild)

in Table 2. Inflorescences were collected during flowering and preserved in FAA and then studied under a stereomicroscope. Colour of the inflorescence, stamen, and stigma was recorded before preservation and measurements of diagnostic characters were taken. Leaf texture was evaluated visually on a 1-9 scale where 1-4=coarse, 5-7=medium and 8-9= fine textured.

Data were analyzed statistically by analysis of variance and least significant differences (LSD) according to Snedecor and Cochran (1973). Samples collected from three pots of each cultivar were treated as one replicate.

Composite samples of leaves were collected from each replicate pot of the seven cultivars for RAPD analysis. Total genomic DNA was extracted from the young leaves of each cultivar. The leaves were ground into a fine powder in liquid nitrogen using pestle and mortar and DNA was extracted following the protocol of Dellaporta *et al*. (1983). The quantity and quality of the DNA extracted was determined using a flourometer (Hoefer DyNA Quant 200; Pharmacia Biotech, Piscatawaym, N.J.). The stock DNA samples were diluted with sterilized distilled water to make a working solution of 10 ηg μL^{-1} for use in PCR analysis.

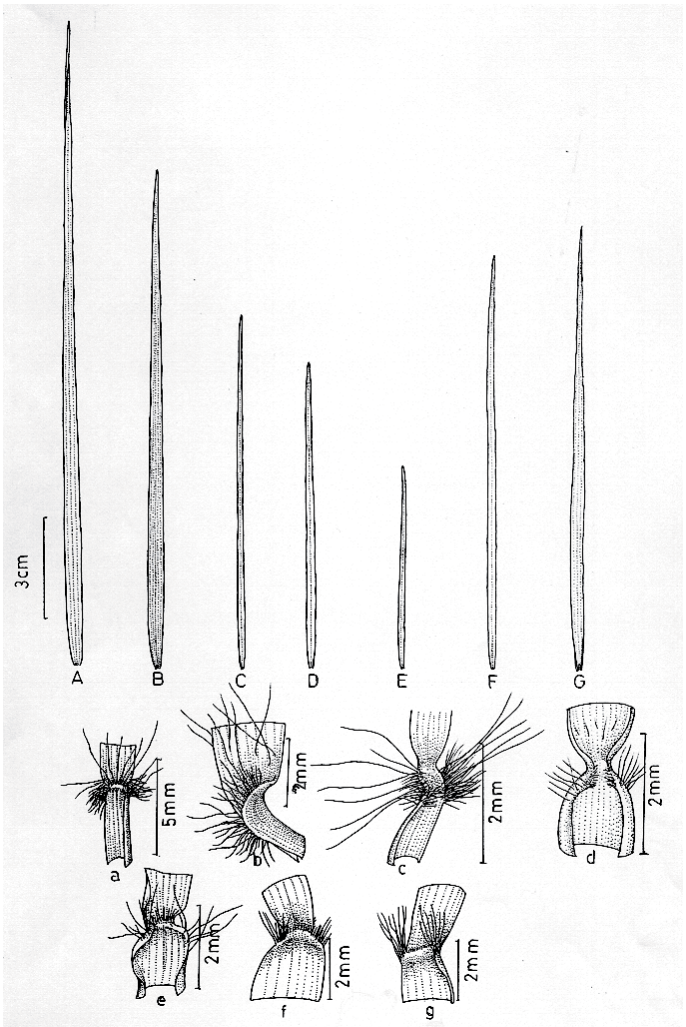

Fig.1. Leaves(A-G) and collar regions(a-g) of seven cultivars of turfgrass. A,a Miyako; B,b Variant of Miyako; C,c Nagissa; D,d Tifgreen; E,e Tifway; F,f Bermuda-1; G,g Bermuda-2.

Thirty five random 10-mer RAPD primers (OPERON Technologies, Alameda, Calif.) of A, B and C series were used for PCR amplification of the 7 DNA samples. The primers were diluted in TE buffer at a concentration of 10 ηg μL^{-1}.

PCR amplification reactions were performed in a Flexigene Thermal Cycler (Techne Inc.) in volumes of 25 µL containing 1 unit of *Taq* DNA polymerase (Amersham Pharmacia Biotech) per reaction. The amplified fragments were separated by electrophoresis according to their molecular weight in 1.4 % (w/w) agarose gels horizontally submerged in 1x TBE buffer. The gels were then stained with ethidium bromide (10 µg mL^{-1}) solution for 20 min. The RAPD products were observed on a UV transilluminator and documented by the Gel Documentation System of Bio Rad. (Hercules, Calif.). To estimate the molecular weight of the fragments λDNA Ladder (Sigma) was run in the gels as standard size marker.

The 20 selected amplification profiles of the 7 different turfgrass samples were compared with each other using 'Diversity Data Base' (Bio Rad) software. The data were applied to estimate the similarity on the basis of the number of shared amplified fragments (Nei,1978; Nei and Li, 1979). Cluster analysis by the unweighted pair group method of arithmetic means (UPGMA) was also performed using the 'Diversity Data Base' (Bio Rad) software.

Results and discussion

Morphology

Zoysiagrass: The Zoysiagrass samples were more compact in appearance with uniformly short internodes. Leaves are rolled in bud-shoot; ligules are a fringe of hairs longer than in the bermudagrasses. Inflorescence is a terminal unbranched spike-like raceme with many spikelets.

Miyako is a sod-forming cultivar of Zoysiagrass that possess both stolons and rhizomes. Leaf blades are 19 x 0.39 cm, smooth with occasional hairs near the base, margins entire and pointed at apex. Collar is 1.5mm wide with tuft of hairs at the margins (Fig. 1: A, a). Racemes 5 x 0.3 cm, with pedicelled, adpressed spikelets. Spikelets small, ovate-acuminate. Stamens yellow. Stigma long, brown and feathery.

Variant of Miyako is very much similar to Miyako except for its

short stature and very soft texture. Leaf blades are 14.5 x 0.38 cm, smooth, more hairy towards the base. Collar constricted, tuft of hairs present at the margins (Fig. 1: B, b). Racemes 4 x 0.4 cm; spikelets small, ovate-acute; stamens and stigma yellow.

Nagissa is a coarse textured, dark green cultivar of Zoysiagrass. Leaf blades are 10.3 x 0.17 cm, glabrous, entire, and pointed at apex. Collar narrow, not constricted (Fig. 1: C, c). Racemes 1.5 x 0.3 cm, brownish-pink. Spike let ovate-acute, rounded at base. Stamens yellow; stigma pink.

Analysis of the measurements of leaves and internodes showed that among Zoysiagrasses there were very distinct variations in the leaf-length and leaf-area but only Nagissa showed distinctiveness in leaf-width (Table 2). The leaf-area of Nagissa was significantly low but their internodal length showed no significant variations with the other zoysiagrass cultivars. Nagissa produced pink coloured short inflorescence while the other two cultivars produced yellow coloured, almost three times longer inflorescence than Nagissa. Regarding leaf-texture Miyako and Nagissa were coarse-textured while the variant of Miyako was soft-textured.

Bermudagrass: In the Bermudagrass samples, leaves are borne on stems with long internodes alternating with one or more very short internodes. Leaves are folded in bud-shoot; ligules are a fringe of short hairs. Inflorescence is 3-7 digitately arranged spikes from the top of a terminal stalk. Spikelets are similar in two rows on the abaxial side of a flattened axis.

Tifgreen is a low growing, rapid spreading cultivar of bermudagrass that develops a dense turf by producing stolons. Leaf blades are 9.05 x 0.27 cm, smooth, sparsely ciliate on the ventral side, glabrous on the dorsal side and sharply-pointed. Collar is continuous and has long, tufted hairs on the margins (Fig. 1: D, d). Inflorescence with 3 to 4 spikes, up to 2.8 cm. Stamens yellow. Stigma dark pink, giving pinkish appearance to the inflorescence.

Tifway is very similar to Tifgreen except for its greater stiffness of leaf blades and darker green colour. Persisting scales are very prominent at the nodes. Leaf blades are 5.6 x 0.14 cm, sharply pointed, ciliate on the ventral side, dorsal glabrous. Collar narrow, tuft of hairs up to 2 mm long (Fig. 1: E, e). Flowering is observed very rarely. Spikes, when present, 2-3 only.

Leaf blades of Bermudagrass-1 are 12 x 0.3 cm, pubescent, minutely serrulate on the margins, sharply pointed. Collar is constricted, narrow, glabrous and hairy on margins with a tuft of hairs of 2-5 mm long (Fig. 1: F, f). Inflorescence with 5 to 7 spikes. Spikelets 3 x 1.25 mm, ovate-acute and greenish-white in appearance. Stamens light yellow; stigma greenish-white, much branched.

Leaf blades of Bermudagrass-2 are 13x0.34 cm, smooth to sparsely pubescent and sharply-pointed. Collar is continuous with tuft of hairs at the margins (Fig. 1: G, g). The inflorescence consists of 3-5 spikes. Spikelets 2.5 x 1 mm, ovate-acute and dark pink in appearance.

Among bermudagrass cultivars Bermudagrass-1 was tall with significantly longer internodes and produced inflorescence with 5-7 spikes while the Bermudagrass-2 was short, and produced inflorescence with 3-5 spikes. Tifgreen and Tifway were quite distinguishable by their leaf-length, leaf-width, area and texture but they were not significantly different in their internodal length. Regarding leaf-texture, Tifgreen and Bermudagrass-2 were medium-textured while Tifway was coarse and Bermudagrass-1 was fine-textured.

RAPD analysis: Out of the 35 RAPD primers screened, 20 primers detected clear polymorphism between the genotypes and were found reproducible in repeated trials. All the seven cultivars revealed a unique profile with the 20 primers and thus selected for DNA-fingerprinting. Different levels of polymorphism were detected among the 7 cultivars with different primers (Fig. 2).

The similarity matrix based on Nei and Li's (1979) coefficient showed genetic distance ranged from 0.45 to 0.80 (Table 3). A dendrogram constructed by using unweighted paired group method of arithmetic means (UPGMA) showed maximum

Table 2. Measurements of leaf, internodes and texture of seven cultivars of turfgrass (Mean ± SD)

Name of cultivar	Leaf-length (cm)	Leaf-width (cm)	Area (cm^2)	Internodal length (cm)	Leaf-texture*
Miyako	19.16 ± 3.09	0.39 ± 0.02	6.33	5.16 ± 0.92	3.7
Variant of Miyako	14.65 ± 1.82	0.38 ± 0.06	4.73	4.61±1.01	8.0
Nagissa	10.38 ± 0.17	0.17 ± 0.01	1.49	2.80 ± 0.69	3.0
Tifgreen	9.05 ± 0.12	0.27 ± 0.01	2.10	5.87 ± 0.85	6.7
Tifway	5.62 ± 0.22	0.14 ± 0.01	0.67	3.67 ± 0.79	2.2
Bermudagrass-1	12.20 ± 0.51	0.29 ± 0.04	3.02	11.22 ± 1.21	8.0
Bermudagrass-2	13.09 ± 1.67	0.34 ± 0.02	3.73	6.97 ± 1.79	5.5
LSD (P<0.05)	2.58	0.04	0.62	3.18	1.3

*Leaf texture was determined visually with a scale of 1-4=coarse, 5-7=medium, 8-9=fine.

Table 3. Similarity matrix (Nei and Li's coefficients) of 7 turfgrass cultivars obtained from RAPD markers

		1	2	3	4	5	6	7	8
Bermudagrass 1	1	100.0							
Bermudagrass 2	2	50.3	100.0						
Miyako	3	59.6	52.3	100.0					
Nagissa	4	55.2	47.6	80.2	100.0				
Tifgreen	6	59.3	65.3	52.6	49.1	53.3	100.0		
Tifway	7	64.5	49.7	55.9	53.6	59.1	51.3	100.0	
V. Miyako	8	73.2	45.0	57.8	58.2	63.2	48.8	69.0	100.0

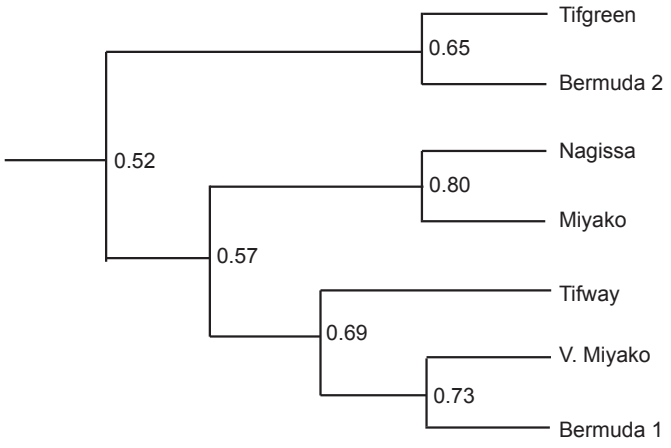

Fig. 3. A dendrogram of genetic relationship among seven cultivars of turfgrass based on the RAPD analysis.

similarity between the cultivars Nagissa and Miyako (Fig. 3). But variant of Miyako clustered with Bermuda-1, with the second highest value in the similarity matrix (0.73). Variant of Miyako showed 0.57 similarity matrix values with its other two Zoysiagrasses. The morphological analysis clearly indicated that the variant of Miyako, with rolled leaves in bud shoots; ligules with a fringe of long hairs and terminal unbranched spike like raceme belong to the *Zoysia* species. The unusual

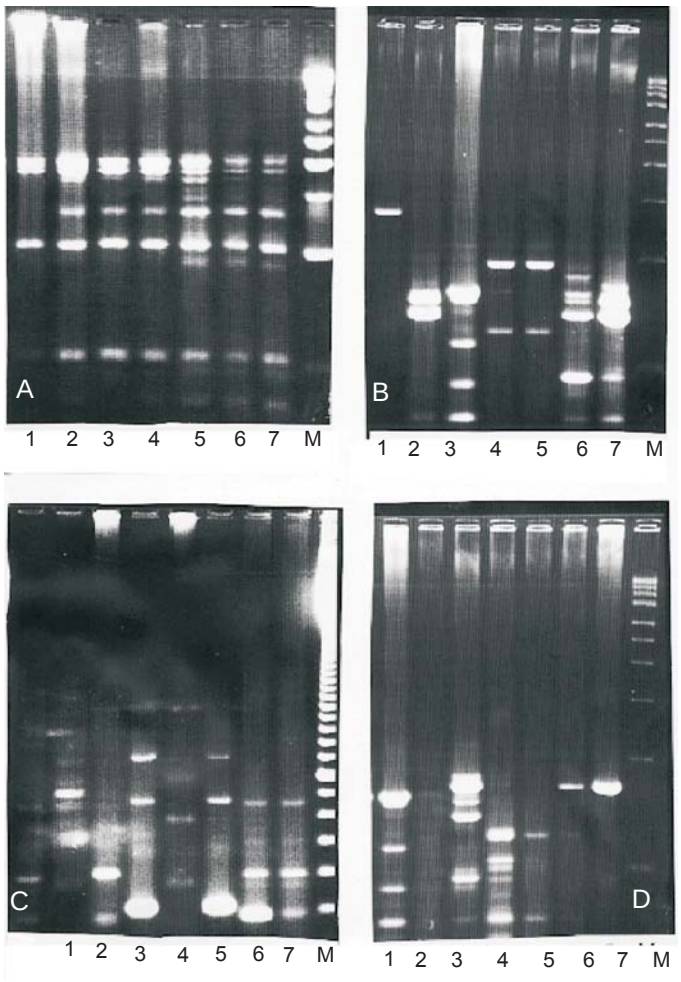

Fig. 2. (A-D) RAPD profiles of seven turfgrass cultivars using OPA11, OPA4, OPA6 and OPA14 primers, respectively. Bermudagrass 1, Bermudagrass 2, Miyako, Nagissa, Tifgreen, Tifway V. Miyako, molecular markers represent 1, 2, 3, 4, 5, 6, 7 and M lane, respectively

behaviour of the variant of Miyako, clustering with Bermudagrass cultivars needs to be explained. Stammers *et al*. (1995) analyzed phylogenetic relationships in the *Lolium/Festuca* complex using RAPD technique faced a similar problem in *Festuca pratensis* which, clustered with *Lolium multiflorum* and *L.perenne*. They assumed that the reason for this unusual behaviour of *Festuca pratensis* could be homoplasy or convergence effect. Tifgreen and Bermudagrass-2 were also closely related with the similarity matrix value (0.65). The affinity of Tifgreen with Bermudagrass-2 indicates that the hybrid is sharing major characters of *Cynodon dactylon* than *C.transvaalensis*. From the low similarity value between Tifgreen and Tifway (0.52) it is assumed that latter is holding major characters donated by its parent, *C. transvaalensis*. Burton (1966) reported that Tifway appeared as a chance hybrid in a seed lot of *Cynodon transvaalensis* Burtt.-Davy from Johannesburg, South Africa, in 1954.

Bermudagrass-1 did not show close similarity with Bermudagrass-2 (0.50). The former may be a selection made by the farmers from the spontaneous mutant originated in the wild forms of *Cynodon dactylon.* This mutant showed more relationship with Tifway (0.64), a hybrid bermudagrass than its wild counterpart. Within species the cultivars showed another tendency of clustering together based on its leaf texture. Miyako and Nagissa, the cultivars of zoysiagrass having coarse texture clustered together while their relative, variant of Miyako with fine texture stood apart. Medium textured cultivars of Bermudagrass like Tifgreen and Bermuda-2 clustered together while the coarse textured Tifway stood apart. Between the species, where there was an anomaly of inter specific clustering of cultivars, the fine textured Bermudagrass-1 formed cluster with fine textured zoysiagrass cultivar.

In general RAPD results of cultivar identification were in agreement with the conventional taxonomic identification using morphologic markers but in some cases discrepancies were found which needs to be explained.

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Occurrence and detection of sweet potato virus disease (SPVD) in West Bengal

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Abstract

The natural occurrence of sweet potato virus disease (SPVD) in 26 Indian sweet potato cultivars was evaluated at Horticultural Experimental Field of the B.C.K.V. University, West Bengal during 2004-2005 seasons based on the possible symptoms and serology. The leaves from virus suspected plants were indexed for viruses by nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) and coat protein study. Disease incidence was highest in Pol-4-9 during 2004 (12.87%) and 2005 (25.19%). Results were confirmed in several seropositive plants with higher incidence and diversity of viruses**.** Sweet potato feathery mottle virus (SPFMV), sweet potato cauliflower mosaic like virus (SPCaLV), Sweet potato mild speck virus (SPMSV) and C-6 virus were detected serologically in single or mixed infections in many leaf samples of the cultivars. The frequency of C-6 virus was very high (73.07%) followed by SPCaLV (34.61%), SPFMV (26.92%) and SPMSV (23.07%). Attempt was made to characterize the virus coat protein of the partially purified virus from the leaves with most frequently observed symptoms. Protein analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed a major protein band of 65 kDa, and 38 kDa which were assumed to be the viral coat proteins of associated virus. Minor protein bands of 24 kDa were also observed. The viral protein degraded upon storage at 4°C over time to yield a protein band of 22 kDa.

Key words: Sweet potato, viruses, symptoms, NCM-ELISA, coat protein

Introduction

Sweet potato (*Ipomoea batatas*) is considered to be the world's most important subsistence crop and is ranked seventh in global production. This crop is best suited to address the growing world concerns for food availability, nutrition and sustainable agricultural system. This crop is widely grown in tropical and sub-tropical regions with low input regimes. The crop is extremely important for developing countries, which produce 98% of the global production. Several biotic factors limit the productivity of this crop worldwide. Among these, viral diseases pose significant loss of the crop in terms of yield and quality of tuber as well as planting materials. Sweet potato virus disease (SPVD) is the main disease of this crop in Africa (Geddes, 1990) and elsewhere (Carey *et al.*, 1999). Despite the apparent broad meaning of the name, SPVD has become associated closely with symptoms caused by a combination of two viruses: Sweet potato chlorotic stunt virus (SPCSV) (Crinivirus; Closteroviridae) and Sweet potato feathery mottle virus (SPFMV) (Potyvirus; Potyviridae) (Gibson *et al.*, 1998; Karyeija *et al.,* 2000). In most cases these viruses occur as mixed infections and tend to be specific to members of the Convolvulaceae family (Moyer and Salazar, 1989). Moreover, it has been shown that these viral mixtures or interactions may lead to the occurrence of a synergistic effect which results in more severe damage to the crop than would be expected if an individual virus was present (Gutierrez *et al.,* 2003). The symptoms include severe stunting of the plant, distortion either a chlorotic mottle or vein clearing of leaves. The tuberous root yield of affected plants is also less.

Very little efforts have been made so far on the distribution, occurrence and characterization of viruses in India. Considering this fact, our preliminary work was devoted to investigate the natural incidence of the SPVD in some Indian cultivars and detection of viruses, if any, associated with SPVD using immunoenzymatic assay and virus coat protein characters.

Materials and methods

Field trial: 26 sweet potato cultivars were grown at the Horticultural Experimental Field in randomised block design in two consecutive years from 2004-2005. The natural incidence of SPVD among the cultivars was recorded based on the symptoms expression pattern. The intensity of the foliar symptoms among the cultivars was recorded in each replicated plot at 60 and 90 days after planting and analysed statistically.

Virus indexing by nitrocellulose membrane enzymed linked immunosorbent assay (NCM-ELISA): Detection of sweet potato viruses were carried out by immunoenzymatic method using nitrocellulose membrane enzymed linked immunosorbent assay (NCM-ELISA) following the protocol standardized by the International Potato Centre (CIP), Virology Laboratory, Lima, Peru. For this, sample preparation was carried out by cutting leaf disc of 1 cm diameter and ground in to 3 mL of Tris base (TBS) buffer of pH 7.5. After 30-45 minutes, 15µl of clear supernatant for each macerated leaf disc was dropped at the centre of the individual nitrocellulose membrane with the micropipette and membranes were kept 15-30 minutes for air drying. Then blocking of the membranes was done by TBS buffer of pH 7.5 and Triton X-100 followed by adding of antibody and then T-TBS buffer

solution and colour development solution of NBT and BCIP as by the protocols.

Coat protein: Partial purification of the virus and analysis of the coat protein was performed as per the methods described by Dougherty and Hilbert (1980) with slight modification by Gorsane *et al.* (1999). The leaves showing chlorotic feathery discolouration and vein clearing symptoms were harvested from the plants maintained in net house and immediately after harvest 5 g of diseased leaves were ground in liquid nitrogen and homogenized at a ratio of 1:3 (w/v) in 20 mM HEPES buffer of pH 7.6, containing 200mM urea and 0.1% sodium sulphite. The homogenate was filtered through two layers of cheese cloth and butanol-1 was added to make final concentration of 8%. The mixture was stirred over night at 4ºC and then clarified by centrifugation at 6000 rpm for 20 mins at 4ºC. After centrifugation, Triton X-100, Polyethylene glycol (6000 mol wt.) and NaCl were added to the supernatant separately for the final concentration of 2%, 4% and 0.1M, respectively and stirred at 4ºC for 90 min. This solution was centrifuged at 13000 rpm for 30 mins at 4ºC and pellet was resuspended in 10 mL of 20 mM HEPES buffer. After stirring over night at 4ºC the solution was spined at 8000 rpm for 10 mins. The supernatant was layered on a 20% sucrose cushion and again centrifuged at 21000 rpm for 15 min. The virus pellet was suspended in 300:1 Laemmli buffer and virus coat protein was detected by performing Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (SDS-PAGE). In the SDS-PAGE, a broad range protein molecular weight marker (Promega Cat#V 8491) was used as marker protein.

Results and discussion

Disease incidence: Wide ranges of symptoms were noticed in sweet potato cultivars in both the years where chlorotic spot, feathery mottle with purple ring and mosaic symptoms were dominated on the plants. The results on the average percentage of incidence of SPVD for both the years (Table 1) revealed that intensity of the virus disease gradually increased with age of the plants in most of the cultivars. The overall intensity of the disease was high at 90 DAP (days after planting) in both the years. A differential symptom was noticed in the test cultivars. Moderately high incidence of the SPVD was observed in the local cultivars BCSP-10 and Kamala Sundari in the year 2004. It was also observed that SPVD symptoms increased to 9.44 and 7.78% in BCSP-10 and Kamala Sundari, respectively at 60DAP during the year 2005. The cultivar Pol-21-1 showed very high incidence of SPVD with 12.87 and 25.19% at 60 and 90 DAP, respectively, during 2005. However, it was observed that SPVD is consistently associated with the cultivars in both the seasons. Infection rate of viral diseases of sweet potato was 40% in China, (Shang *et al.,* 1999), 54 % in Uganda and 60% in Indonesia (Carey *et al.,* 1999). Studies indicate that there are five major poty viruses

Table1. Incidence of sweet potato virus disease (SPVD) during 2004-2005

Cultivars	Percent infection at two different stage of plant growth					
	60 DAP		Pooled data 2004 -2005	90 DAP		Pooled data 2004-2005
	2004	2005		2004	2005	
Tripty	2.22 (8.56)*	4.44 (12.16)	3.34 (10.53)	5.46 (13.51)	7.50 (15.89)	6.48 (14.74)
IBM-95-229	1.67 (7.42)	2.90 (9.80)	2.28 (8.68)	0.74 (4.93)	3.24 (10.37)	1.99 (8.10)
WBSP-4	2.22 (8.56)	2.96 (9.90)	2.59 (9.26)	4.91 (12.80)	3.35 (10.54)	4.13 (11.72)
IBM-95-220	2.59 (9.26)	3.89 (11.37)	3.24 (10.37)	2.47 (9.04)	8.15 (16.58)	5.31 (13.32)
Pol-20-6-2	5.01 (12.93)	6.66 (14.95)	5.83 (13.97)	4.31 (11.98)	7.59(15.99)	5.95 (14.11)
Pol-4-4-2	2.59 (9.26)	3.89 (11.37)	3.24 (10.37)	8.36 (16.80)	9.63 (18.07)	8.99 (17.44)
BCSP-14	3.14 (10.21)	4.46 (12.19)	3.8 (11.24)	3.33 (10.51)	4.81 (12.66)	4.07 (11.63)
Pol-4-9	2.22 (8.56)	4.26 (11.91)	3.24 (10.37)	2.22 (8.56)	7.13 (15.48)	4.67 (12.48)
BCSP-5	0.77 (5.03)	1.30 (6.54)	1.05 (5.88)	1.46 (6.94)	4.63 (12.42)	3.04 (10.04)
IBM-95-206	3.33 (10.51)	12.43 (4.64)	3.98 (11.50)	3.97 (11.49)	5.93 (14.09)	4.95 (12.85)
Pol-13-4	1.83 (7.77)	3.33 (10.51)	2.58 (9.24)	2.41 (8.93)	7.41 (15.79)	4.91 12.80)
Pol-21-1	2.41(8.93)	3.71 (11.10)	3.06 (10.07)	12.87 (21.02)	25.19 (30.12)	19.03 (25.86)
IGSP-7	1.11 (6.04)	2.41 (8.93)	1.76 (7.62)	0.56 (4.29)	1.67 (7.42)	1.12 (6.07)
NDSP-9	1.66 (7.40)	2.59 (9.26)	2.12 (8.37)	1.11 (6.04)	1.67 (7.42)	1.32 (6.59)
CO-3	2.41 (8.93)	4.26 (11.91)	3.33 (10.51)	3.39 (10.61)	1.11 (6.04)	3.77 (11.19)
IGSP-6	2.59 (9.26)	4.26 (11.91)	3.42 (10.65)	0.56 (4.29)	4.16 (11.76)	0.83 (5.22)
NDSP-10	4.26 (11.19)	6.48 (14.74)	5.37 (13.39)	3.49 (10.76)	6.48 (14.74)	4.98 (12.89)
S-1221	1.66 (7.44)	2.96 (9.90)	2.31 (8.74)	3.71 (11.10)	5.74 (13.86)	4.72 (12.54)
RNSP-1	2.89 (9.78)	5.37 (13.39)	4.13 (11.72)	1.11 (6.04)	5.93 (14.09)	3.52 (10.81)
IGSP-8	2.78 (9.59)	4.16 (11.76)	3.47 (10.73)	2.41 (8.95)	5.01 (13.93)	3.71 (11.10)
IGSP-9	1.67 (7.42)	3.15 (10.22)	2.41 (8.39)	0.74 (4.93)	2.96 (9.90)	1.85 (7.81)
BCSP-7	3.33 (10.51)	3.89 (11.37)	3.61 (10.95)	0.74 (4.93)	3.33 (10.51)	2.03 (8.19)
RNSP-2	0.92 (5.50)	1.85 (7.81)	1.38 (6.71)	3.15 (10.22)	4.91 (12.90)	4.03 (11.58)
RNSP-3	4.25 (11.89)	6.30 (14.55)	5.27 (13.27)	4.26 (11.91)	6.48 (14.74)	5.37 (13.39)
BCSP-10	6.11 (14.31)	9.44 (17.89)	7.77 (15.27)	2.01 (8.15)	3.34 (10.53)	2.67 (9.40)
Kamala Sundari	6.11 (14.31)	7.78 (16.19)	6.94 (15.27)	6.29 (14.52)	11.11 (19.47)	8.70 (17.15)
SEd±	0.03	0.09	1.76	0.11	0.03	0.33
CD(*P*=0.05)	0.05	0.13	2.49	0.14	0.05	0.46

* Figures in the parenthesis are angular transformed values

that could affect sweet potato production: sweet potato feathery mottle virus (SPFMV), sweet potato mild mottle virus (SPMMV), sweet potato latent virus (SPLV), sweet potato vein mosaic virus (SPVMV) and sweet potato yellow dwarf virus (SPYVD) (Moyer and Salazar, 1989).

Table 2. Detection of sweet potato viruses by NCM-ELISA in some Indian sweet potato cultivars

Cultivars	Sweet potato viruses					
	SPFMV	SPMMV	SwPLV	SPCFV	C-6	SPMSV
Tripty	+		+++	+++	++	
IBM – 95 – 229	+				+	
WBSP – 4	+				++	
IBM – 95 – 220	+				+	+
Pol – 20 – 6 – 2						
Pol – 4 – 4 – 5		+			+	
BCSP – 14			+		+++	
Pol – 4 – 9			+	++	+	
BCSP – 5		+++		+	+	+
IBM – 95 – 206				++	+++	+++
Pol – 13 – 4					+++	
Pol – 21 – 1	+++				++	
IGSP – 7		+				
NDSP – 9		+	+++		++	
CO – 3		+++	+		++	++
IGSP – 6				+++	+	+
NDSP – 10						
S – 1 – 221						
RNSP – 1					+	+
IGSP – 8						
IGSP – 9		+	+++	++	++	+++
BCSP – 7						
RNSP – 2	++	+			++	
RNSP – 3					+	

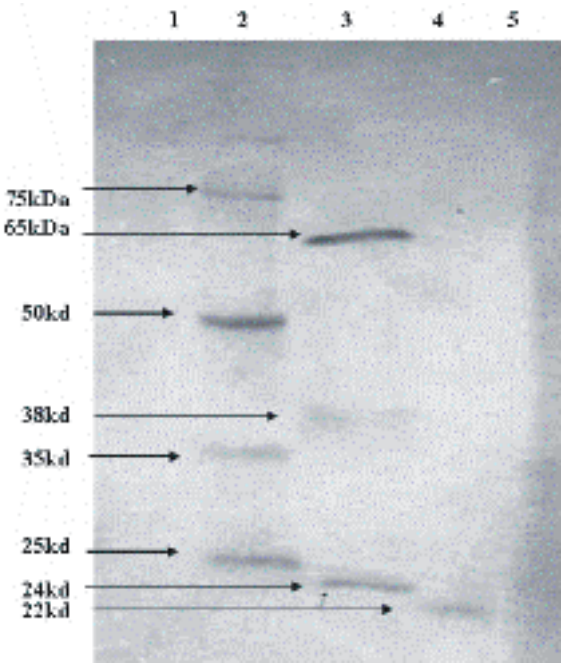

Fig. 1. SDS-PAGE of partially purified virus coat protein (Sample taken from SPVD infected plant)

Nitrocellulose membrane enzymed linked immunosorbent assay (NCM-ELISA): The results on indexing of eight viruses infecting 26 promising sweet potato cultivars are presented in Table 2. The serological tests by NCM-ELISA confirmed the viruses present in the cultivars and many of the samples which showed virus like symptoms did not show the positive reaction to the test. All the cultivars except Pol-20-6-2, NDSP-10, S-1221, IGSP-8, BCSP-7 and BCSP-10 showed positive indication for all the viruses alone or mixed infection. Among the cultivars IBM-95-220, Pol-4-4-5, IGSP-6, RNSP-1 and Kamala Sundari resulted in light purple colour for SPFMV, C-6, SPMSV and SPCaLV. Leaf samples (73.07%) gave positive results to C-6 virus followed by SPCaV (34.61%), SPFMV and SPMSV (26.92%) and SPMMV (23.07%). BCSP-5, RNSP-2, IGSP-9 and CO-3 showed moderate to high infection to different viruses in NCM-ELISA test. NCM-ELISA is found to be the most effective method for virus detection in sweet potato among NCM-ELISA, ELISA and ISEM which have also been tested by Yang *et al.* (1991).

Coat protein: Major protein bands of 65 and 38 kDa (Fig.1) were obtained which are assumed to be viral coat protein and a 24 kDa of minor protein band was observed. Total degeneration of coat protein of the isolated virus particle was observed upon exposure to high temperature (boiling temperature) whereas storage at low temperature (4ºC) gave apparently single 22kDa protein band. The results indicated that the isolated virus particle from infected plant would have the mixed infection with SPFMV and sweet potato ring spot nepo virus (SPRV) which has high sensitivity to freezing and thawing. Similar observations of three major coat proteins of 32, 30 and 29 kDa have been reported in *Ipomoea* sp infected with SPFMV (Usugi *et al.,* 1994).

A detailed description of the SPRV has been given by Brunt *et al.* (1996) and it suggested that SPRV had four different major protein subunits and the second largest version protein was identified as 65 kDa, which was not glycosylated or phosphorylated. However, our present investigations on coat protein of sweet potato feathrery mottle and purple ring producing agent have the affinity to other similar strains of SPFMV reported so far. It is also predicted that the occurrence of SPRV might have the component virus in producing SPVD syndrome on sweet potato plants infected in this area and similar observation was also reported by Usugi (1994).

The present study has clearly indicated the relative abundance and magnitude of virus diseases infection among the sweet potato varieties in West Bengal, which would be useful in further investigations on sweet potato viruses.

Acknowledgement

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Effects of UV-C and salicylic acid on quality of 'Muskule' table grapes during cold storage

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Abstract

Muskule grape variety which has table and late maturing attributes, was used for this study. Storage of table grapes requires stringent control of gray mold, which is caused by *Botrytis cinerea* Pers. In spite of the fact that the use of sulfur dioxide (SO_2) in controlling gray mould is common practice, it has some advantages and disadvantages. Thus, physical, natural organic elicitors and biological methods have been used for delaying decays. In this study, UV-C (0.25kJ m^{-2}), salicylic acid (1, 2, 3mM) and $Na_2S_2O_5$ (0.4g powdered sodium metabisulfate pads) treatments were used to reduce quality losses during the cold storage of Muskule grape. Treated clusters were placed into polyethylene container and packaged with polyethylene bags having 10.5 µ thicknesses and stored at 0±1 °C and 90±5% relative humidity throughout 100 day. At the end of 100 day, weight loss (%), soluble solids content (%), titratable acidity (g 100 mL^{-1}), pH of fruit juice, sensory evaluation, view of cluster skeleton and decay rate (%) were determined at 20 days interval. SA (3mM) + UV-C combined treatment and SA (3mM) treatment were found to be effective depending on examined criterion.

Key words: Grape, UV-C treatment, salicylic acid, storage, sensory evaluation

Introduction

Grape is a non-climacteric fruit with low physiological activity and is sensitive to water loss and fungal infection that is mainly caused by *Botrytis cinerea* Pers. during postharvest handling. Sulfur dioxide is highly corrosive to metals, injurious to most of the fresh fruits and causes injury to rachis and berries if used excessively (Nelson, 1985). Fumigation with SO_2 is the most common method to control decay during cold storage of table grape clusters (Luvisi *et al.,* 1992; Crisosto *et al.*, 1994).

In recent years, there has been increasing consumer pressure to eliminate or decrease the use of synthetic fungicides on fresh products. As table grape consumers are becoming increasingly cautious about SO_2 residues, alternative methods to control decay on grapes are of increasing interest to the fresh produce sector. One approach has been the use of controlled atmosphere to suppress the development of *Botrytis cinerea* Pers. on table grapes (Yahia *et al.,* 1983). An alternative method is the use of heat and in recent years, there has been renewed interest in the potential of heat treatments (Lurie, 1998) although its commercial application is still limited.

Salicylic acid is not only a well known natural inducer of disease resistance in plants (Sticher *et al.,* 1997), but also a simple phenolic compound involved in the regulation of many processes in plant growth and development, including stomatal movement, seed germination, ion absorption, sex polarization. It can also interfere with the biosynthesis and action of ethylene in plants (Raskin, 1992). Yalpani *et al.* (1994) and Kang *et al.* (2003) observed that application of salicylic acid could significantly induce resistance against a variety of biotic and abiotic stress.

Some researchers reported that exogenous application of salicylic acid or methyl-salicylic acid could also induce the expression of many defense genes in tobacco (Fraissinet-Tachet *et al.,* 1998), tomatoes (Ding *et al.,* 2002) and parsley (Thulke and Conrath, 1998). Zainuri Joyce *et al.* (2001) reported that preharvest and postharvest applications of 2.0 mg salicylic acid mL^{-1} tended to suppress postharvest anthracnose disease severity caused by *Colletotrichum gloeosporioides* in fruits of mango cv. Kensington Pride.

Studies about effects of salicylic acid treatments on fruits of different species such as *Actinidia deliciosa* (kiwifruit) (Poole and McLeod, 1994), *Citrus paradisi* (grapefruit) (Droby *et al.*, 1999), *Cucumis melo* (rock and hami melon) (Huang *et al.,* 2000), *Passiflora edulis* (passionfruit) (Willingham *et al.,* 2002), *Mangifera indica* (mango) (Zainuri Joyce *et al.*, 2001), *Prunus persica* (peach) (Han *et al.*, 2003) and *Prunus avium* (sweet cherry) (Yao and Tian, 2005) have been performed until recent times.

Induction of natural disease resistance in horticultural crops using physical elicitors has received increasing attention over recent years (Wilson *et al.*, 1994; 1997). In spite of the fact that fungal spores and mycelia infections on and in the outer cell layers of fruit or vegetables are removed or destroyed by using physical treatments like low temperature storage, wounding (Ismail and Brown, 1979), CO_2 treatment (Prusky *et al.*, 1993), heat treatment (Schirra *et al.*, 2000), ionizing irradiation (McDonald *et al.,* 2000) and UV-C irradiation (Wilson *et al.*, 1997), they also enhance natural disease resistance.

Wilson *et al.* (1997) reported that non-ionizing radiation had considerable potential amongst physical methods for controlling postharvest diseases. Terry and Joyce (2004) reported that low doses of short-wave ultraviolet light (UV-C) (190–280 nm wavelengths) can control many storage rots of fruit and vegetables by targeting the DNA of micro-organisms. In addition to being a

germicidal or mutagenic agent, UV-C irradiation can modulate induced defense in plants.

Using appropriate wavelength and dose, UV-C irradiation can stimulate accumulation of stress-induced phenylpropanoids, many of which have been associated with induced disease resistance (Ben-Yehoshua *et al.,* 1998) and pathogenesis-related proteins (Porat *et al.,* 2000).

Liu *et al.* (1993) reported that the responsiveness of harvested horticultural product to UV-C treatment reduced with ripening process and was also influenced by harvest time (D'hallewin *et al.*, 1999). The aim of present work was to determine effects of salicylic acid and UV-C treatments, which are accepted as new methods against conventional SO_2 application during storage of Muskule grape by using modified atmosphere packaging.

Materials and methods

This study was conducted under the laboratory conditions of the Horticulture Department, Agricultural Faculty, Namik Kemal University, Turkey in 2005.

Muskule grape, which is known as late-maturing table variety in Turkey was used in the study. Some average values of the quality characteristics in this variety were as follows: Soluble solids content: 16.4%, titratable acidity: 0.4 g 100mL^{-1} and pH: 3.69.

After discarding decayed and badly viewed grapes from harvested clusters, following treatments were performed

Code	Treatments
$Na_2S_2O_5$	Treatment of 0.4 g powdered sodium metabisulfate pads
UV-C	According to Akbudak and Karabulut (2002), UV-C treatment from 100 cm distance (0.25 kJm^{-2}) for 4 min on clusters which were put in a special cabin designed by Nigro *et al.* (1998)
SA	Dipping of clusters into 1, 2 and 3 mM of salicylic acid solutions for 2 min, drying with an electric fan and packaging (Sigma-Aldrich, Germany)
SA+UV-C	1, 2 and 3 mM of salicylic acid treatment plus UV-C treatment

During UV-C treatments, radiation was provided with fluorescent germicidal lamps (Osram12 HNS OFR, GE 30 W) with a peak emission at 254 nm.

After all treatments were performed, clusters were put into polyethylene containers carrying paper towels at their bases to absorb moisture and these polyethylene containers were placed in polyethylene bags of 10.5 µ thicknesses. Afterwards, all these packages were stored in cool air store (at 0±1^{0}C, 90±5% relative humidity) throughout 100 days.

Analyses and measurements such as weight loss (%), soluble solids content (%), titratable acidity (as tartaric acid) (g 100mL^{-1}), pH of fruit juice, sensorial evaluation performed by 5 panelist according to 1-9 scale (1: extremely poor or soft in texture; 3: poor or soft; 5: moderate; 7: good; 9: excellent) based on Kader *et al.* (1973) and Lipton (1980), view of cluster skeleton according to 0-5 scale (0: bright green; 1: green; 2: matt green. 3: green-light brown; 4: brown; 5: dried grey brown) based on Harvey *et al.* (1988), decay rate (%) were carried out at 20 day intervals throughout 100 days.

In the study, split parcels in randomized complete blocks design were used as experimental model with three replicates (Turan, 1995) and each replicate was consisted of 3 polyethylene containers. LSD (P=0.05) values were used to indicate the differences among mean values.

Results and discussion

Weight loss is most important factor limiting storage and increase in weight losses are related with treatments which were especially found out towards storage period in this study. Results on different species about UV-C radiation performed by Taira *et al.* (1997), Maharaj *et al.* (1999), Akbudak and Karabulut (2002) and SA treatments carried out by Zheng and Zhang (2004) showed that weight losses were lower in treated fruits than control. In a similar way, in our study weight losses were found to be lower in SA and UV-C treatments than control. As regards weight loss, the highest value was 4.32 g for control on 100th day. On the 20th day there was no significant difference in weight loss among the treatments (Table 1).

Soylemezoglu and Agaoglu (1992) and Turkben and Eris (1990) stated difference in soluble solids content depending on weight loss in grapes. Fluctuations in soluble solids content of grapes were apparent during storage period. While the lowest value of soluble solids content was 17.91% for $Na_2S_2O_5$ treatment at the end of 100th day, the highest value was 18.64% for SA 3mM +UV-C combined treatment (Table 2).

Increase in acidity of grapes during storage period was explained by respiration (Turkben and Eris, 1990). There are some studies about effects of UV-C radiation on decreasing of titratable acidity in literatures such as Ozer and Akbudak (2003) on grape and Kim (1997) on apple. When the values of titratable acidity were examined, it was seen that averages generally declined with storage period. Among the treatments, the lowest value was 0.35 g 100 mL^{-1} for control, the highest value was 0.40 g 100 mL^{-1} for SA 3mM treatment (Table 3).

Table 1. Effect of different treatments on weight loss (%) in Muskule grape throughout storage period

Storage period	Control	$Na_2S_2O_5$	UV-C	SA 1mM	SA 2mM	SA 3mM	SA 1mM +UV-C	SA 2mM +UV-C	SA 3mM +UV-C	Time effect
20th day	0.50a-d	0.44a-c	0.34a	0.35a	0.39ab	0.36a	0.48a-d	0.43abc	0.38a	0.41a
40th day	1.37g-k	0.90b-g	0.87b-f	0.90c-g	0.71a-e	0.95d-h	1.16e-ı	0.87b-f	1.21f-j	0.99b
60th day	2.70o	1.82klm	1.64jkl	1.58ı-k	1.42h-k	1.22f-j	1.27f-j	1.44ıjk	1.37g-k	1.61c
80th day	3.29p	2.05lmn	2.28mno	2.28mno	2.15mn	2.38no	2.41no	2.39no	2.13mn	2.37d
100th day	4.32r	3.59pq	3.27p	3.55pq	3.85qr	3.59pq	3.34p	3.36p	3.34p	3.58e
Treatment effect	2.44b	1.76a	1.68a	1.73a	1.70a	1.70a	1.73a	1.70a	1.69a	
LSD (P=0.05)	Treatment x Storage period : 0.481;			Treatment :0.215;		Storage period :0.160				

Table 2. Effect of different treatments on soluble solids content (%) in Muskule grape throughout storage period

Storage period	Control	$Na_2S_2O_5$	UV-C	SA 1mM	SA 2mM	SA 3mM	SA 1mM +UV-C	SA 2mM +UV-C	SA 3mM +UV-C	Time effect
20th day	16.73h-q	16.97f-q	15.90q	16.84g-q	17.12e-p	17.62a-j	16.63ı-q	17.64a-ı	17.11f-p	16.95c
40th day	16.09opq	17.15e-p	17.63a-j	16.18n-q	16.17n-q	17.75a-ı	16.31l-q	16.67h-q	16.47j-q	16.71c
60th day	17.02f-q	16.04pq	17.23d-o	17.49a-k	16.27m-q	16.88g-q	16.33k-q	16.93f-q	17.29c-n	16.83c
80th day	17.69a-ı	17.46b-l	17.80a-h	18.08a-f	17.56a-j	17.41b-m	17.67a-ı	18.08a-f	17.95a-g	17.74b
100th day	18.52ab	17.91a-g	18.49ab	18.28a-e	18.09a-f	18.36a-d	18.49ab	18.42abc	18.64a	18.35a
Treatment effect	17.21	17.10	17.41	17.37	17.04	17.60	17.09	17.55	17.49	
LSD (P=0.05)	Treatment x Storage period: 1.164;			Storage period: 0.387						

Table 3. Effect of different treatments on titratable acidity (g 100 mL^{-1}) in Muskule grape throughout storage period

Storage period	Control	$Na_2S_2O_5$	UV-C	SA 1mM	SA 2mM	SA 3mM	SA 1mM +UV-C	SA 2mM +UV-C	SA 3mM +UV-C	Time effect
20th day	0.39	0.40	0.38	0.40	0.41	0.42	0.42	0.41	0.41	0.41c
40th day	0.36	0.43	0.39	0.41	0.41	0.42	0.39	0.43	0.41	0.41c
60th day	0.38	0.40	0.39	0.39	0.42	0.42	0.40	0.39	0.40	0.40c
80th day	0.32	0.39	0.36	0.37	0.39	0.39	0.37	0.37	0.38	0.37b
100th day	0.30	0.34	0.32	0.33	0.33	0.34	0.31	0.31	0.34	0.32a
Treatment effect	0.35a	0.39d	0.37b	0.38c	0.39d	0.40e	0.38c	0.39d	0.39d	
LSD (P=0.05)	Treatment :0.014	Storage period: 0.01								

Table 4. Effect of different treatments on pH of grape juice in Muskule grape throughout storage period

Storage period	Control	$Na_2S_2O_5$	UV-C	SA 1mM	SA 2mM	SA 3mM	SA 1mM +UV-C	SA 2mM +UV-C	SA 3mM +UV-C	Time effect
20th day	3.71m-q	3.68o-q	3.81d-j	3.71k-q	3.67q	3.67q	3.70n-q	3.74ı-q	3.68o-q	3.71c
40th day	3.87c-f	3.77g-o	3.87c-f	3.73j-q	3.72k-q	3.67q	3.80e-l	3.73j-q	3.75h-q	3.77b
60th day	3.97ab	3.80e-k	3.85c-g	3.76h-p	3.72k-q	3.70n-q	3.75h-q	3.78f-n	3.79f-m	3.79b
80th day	4.01a	3.80e-k	3.92abc	3.82d-ı	3.85c-g	3.82d-ı	3.87c-f	3.88cde	3.89bcd	3.88a
100th day	3.99a	3.88cde	3.88cde	3.88cde	3.82d-ı	3.82d-ı	3.87c-f	3.84c-h	3.84c-h	3.87a
Treatment effect	3.91a	3.79bc	3.87a	3.78bc	3.75cd	3.73d	3.80b	3.79bc	3.79bc	
LSD (P=0.05)	Treatment x Storage period: 8.887			Treatment: 0.042		Storage period: 0.031				

Table 5. Effect of different treatments on sensory evaluation scores in Muskule grape throughout storage period

Storage period	Control	$Na_2S_2O_5$	UV-C	SA 1mM	SA 2mM	SA 3mM	SA 1mM +UV-C	SA 2mM +UV-C	SA 3mM +UV-C	Time effect
20th day	7.80c-f	8.60abc	8.60abc	8.46abc	8.73ab	8.46abc	9.00a	8.46abc	8.20a-d	8.48a
40th day	8.33a-d	8.46abc	8.33a-d	8.86ab	8.60abc	7.80c-f	8.60abc	8.60abc	9.00a	8.51a
60th day	6.73g-j	7.53d-g	7.26e-h	7.53d-g	7.26e-h	7.80c-f	8.06b-e	8.20a-d	8.06b-e	7.60b
80th day	5.53lm	6.73g-j	6.20ı-l	6.46h-k	6.86ghı	7.13fgh	6.60hıj	6.73g-j	7.13fgh	6.60c
100th day	3.40n	5.40lm	5.10m	5.26m	5.13m	5.66klm	5.46lm	5.66klm	5.86j-m	5.21d
Treatment effect	6.36c	7.34ab	7.10b	7.32ab	7.32ab	7.37ab	7.54a	7.53a	7.65a	
LSD (P=0.05)	Treatment x Storage period: 0.879;			Treatment :0.389;		Storage period: 0.290				

Data showed that treatments had different effects on pH of grape juice and values increased depending on the storage period. As shown in Table 4, the highest pH values were 4.01 in 80th day, 3.99 in 100th day and 3.97 in 60th day for control.

Studies on UV-C treated grape (Akbudak and Karabulut, 2002) and SA treated cherry (Yao and Tian, 2005) have demonstrated that better fruit quality was obtained in UV-C treated grapes and SA treated fruits than control. It was seen that scores of sensory evaluation given by sensory evaluation panelists were low towards the end of storage period. At the end of 100th day, only control grapes were unmarketable (3.40). While the highest scores of sensory evaluation was 9.00 for SA 1mM + UV-C combined treatment in 20th day and for SA 3mM + UV-C combined treatment in 40th day; towards the end of 100th day, the highest values became 5.66 for SA 3mM and SA 2mM + UV-C combined treatment and 5.86 for SA 3mM + UV-C combined treatment (Table 5). The present study indicated that UV-C treatments had positive effect on preserving flavour and there were no negative effect of SA on grape.

Variations derived from withering on cluster skeleton of stored grapes were examined and it was observed that treatments slowed down color variation of cluster skeleton at different rates. The best score among the treatments about scale of cluster skeleton was 0.40 for $Na_2S_2O_5$ treatment (20th day) and SA 3mM treatment followed it as 0.80 (20th day). At the end of 100th day, excessive color variation on cluster skeleton was observed in control (Table 6).

It is thought that salicylic acid has direct toxicity to different fungi species responsible for decay and it also prevents spore germination of pathogens (Yao and Tian, 2005). Throughout the storage period of Muskule grape, decays were observed first in control (3.36%) and UV-C treatment (1.24%) at the end of 60th

Table 6. Effect of different treatments on view of cluster skeleton of Muskule grape variety throughout storage period

Storage period	Control	$Na_2S_2O_5$	UV-C	SA 1mM	SA 2mM	SA 3mM	SA 1mM +UV-C	SA 2mM +UV-C	SA 3mM +UV-C	Time effect
20th day	1.40def	0.40a	0.93bc	0.93bc	1.06bcd	0.80ab	0.86bc	0.93bc	1.13bcd	0.94a
40th day	1.40def	0.93bc	1.00bcd	0.93bc	1.13bcd	0.83b	1.00bcd	1.26cde	1.13bcd	1.07ab
60th day	1.80fg	1.00bcd	0.86bc	0.93bc	1.06bcd	1.00bcd	1.20b-e	1.60efg	1.26cde	1.19b
80th day	2.86j	1.60efg	1.80fg	1.93gh	1.93gh	2.00gh	1.93gh	2.26hı	2.00gh	2.03c
100th day	3.33k	2.66ıj	2.66ıj	2.53ıj	2.46ıj	2.46ıj	2.60ıj	2.60ıj	2.60ıj	2.65d
Treatment effect	2.16e	1.32a	1.45abc	1.45abc	1.53bc	1.42ab	1.52bc	1.73d	1.62cd	
LSD (P=0.05)	Treatment x Storage period : 0.432; Treatment :0.192; Storage period: 0.143									

Table 7. Effect of different treatments on decay rates (%) in berry of Muskule grape variety throughout storage period

Storage period	Control	$Na_2S_2O_5$	UV-C	SA 1mM	SA 2mM	SA 3mM	SA 1mM +UV-C	SA 2mM +UV-C	SA 3mM +UV-C	Time effect
20th day	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
40th day	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
60th day	3.36c-g	0.00a	1.24abc	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.52a
80th day	11.46k	1.60a-d	2.43b-e	2.70b-e	0.57ab	0.00a	3.53d-g	1.44a-d	0.00a	2.64b
100th day	17.33l	7.03hıj	8.23j	7.63ıj	4.50efg	2.97c-f	5.50ghı	5.16fgh	4.43efg	6.97c
Treatment effect	6.43e	1.73bcd	2.38d	2.07cd	1.02ab	0.60a	1.81bcd	1.32abc	0.89ab	
LSD (P=0.05)	Treatment x Storage period: 2.221; Treatment: 0.987; Storage period: 0.735									

day. While no decay symptom was detected for SA 3mM and SA 3mM + UV-C combined treatment at the end of 80th day; the highest decay rate was 17.33% for control and the lowest value was obtained from SA 3mM treatment (2.97%). Hence, SA treatment and SA + UV-C combined treatments were effectively protective as $Na_2S_2O_5$ treatment (Table 7).

Akbudak and Karabulut (2002) suggested that UV-C treatments could be used for prevention of decays caused by *B. cinerea* during the cold storage of grapes. Besides, Nigro *et al.* (1998) also stated that UV-C treatment stimulated resistance to *B. cinera* in grapes. Findings of our study were also consistent with these results.

In conclusion, UV-C treatment, which is one of the physical controls and different doses of salicylic acid, which is natural organic elicitor were found to be effective in maintaining quality attributes of Muskule grape during cold storage. In the course of storage, while titratable acidity was slightly decreased, soluble solids content and pH values generally increased. It was determined that weight loss remained to be lower in all the treatments than control. As far as view of cluster skeleton, sensory evaluation and prevention of decay, which are some of the important quality attributes in grapes, were concerned, SA 3mM + UV-C combined treatment and SA 3mM treatment gave best results and these may be used as alternative to $Na_2S_2O_5$ during the cold storage of Muskule grape.

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Characterization of new apricot cultivars by RAPD markers

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Abstract

Molecular markers are the most widely used tools in cultivar and species identification. The objective of this study was to characterize some Turkish and European cultivars and new apricot cultivars derived by hybridization between Turkish and European apricot cultivars using RAPD markers. Five new, two local cultivars, and four promising hybrids from Turkey, and 13 cultivars from Europe, North America, South Africa were characterized. Sixty RAPD primers produced 57 polymorphic and 79 monomorphic markers, totaling 136. All the 136 markers were used to construct a dendogram based on UPGMA. All cultivars were distinguished from each other with the similarity value ranging from 0.90 to 0.96. Known hybrids were grouped between or close to either one of parental genotypes. This study may imply narrow genetic diversity among the most widely grown apricot cultivars in the world.

Key words: Apricot, *Prunus armeniaca,* RAPD, molecular markers

Introduction

The apricot is a fruit tree originated in Central Asia and China. It was introduced into Europe through Greece (400 BC), and also later (100 BC) by the Romans. It is accepted that there are four different species and one naturally occurring interspecific hybrid under the generic term of apricot. These are: *P. armeniaca*, the cultivated apricot; *P. sibirica* L., the Siberian apricot; *P. mandshurica* (Maxim.) Koehne, the Manchurian apricot; *P. mume* (Sieb.) Sieb. et Zucc., the Japanese apricot; and *Prunus* x *dasaycarpa* Ehrh., the black or purple apricot. All diploid species include eight pairs of chromosomes (2n=16). The most cultivated apricots belong to the species *P. armeniaca* (Hormaza, 2002).

In *P. armeniaca,* six eco- geographical groups have been proposed, the European group being the most recent and also the least variable of all. This group is characterized by being mostly self-compatible, with a relatively short dormant period, early budbreak and low vigour trees. Genotypes of this group are also grown in North America, Australia and South Africa (De Vicente, 1998). Despite its lower variability, most of the progress in apricot breeding has been carried out through hybridization and selection within the European group (Hormaza, 2002).

Traditional methods to characterize and identify cultivars and rootstocks in fruit tree species are based on phenotypic observations, but this approach is slow and subject to environmental modifications (Staub and Serquen, 1996). Consequently, new methods based on studies at the DNA level must be incorporated into fruit breeding programme to accelerate and optimize genotype fingerprinting and study genetic relationships among cultivars (Hormaza, 2002). For example, De Vicente *et al.* (1998), determined genetic variability in apricot using RFLP analysis. The RAPD procedure is a fast, sensitive method that avoids use of radioactive isotopes and is well suited for studies of many samples. Therefore, RAPD analysis can be used to identify polymorphisms. A number of apple, red raspberry and rose cultivars have been fingerprinted recently using RAPD markers (Lu *et al.*, 1996). Besides, RAPD markers have been used to identify genetic variability in *Prunus* species. Peach rootstock cultivars (Lu *et al.*, 1996), peach (Warburton *et al.*, 1996; Raddova *et al.*, 2003), almond (Bartolozzı *et al.*, 1998; Mirali and Nabulsı, 2003), cherry (Hormaza, 1999; Aka-Kacar and Cetiner, 2001), plum (Heinkel *et al.*, 2000) and apricot (Zhebentyayeva and Sivolap, 2000; Hormaza, 2001; Mariniello *et al.*, 2002) have been identified by RAPD markers. On the other hand, Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) markers have also been used for molecular characterization of *Prunus* species. SSR markers have been used in determination of molecular diversity in apricot (Hormaza, 2001; Hormaza, 2002; Zhebentyayeva *et al*., 2003), *Prunus* rootstock (Serrano *et al.*, 2002), peach (Sosinski *et al.*, 2000), peach and almond (Martinez-Gomez *et al.*, 2003) and genetic diversity in apricot (Hagen *et al.*, 2002; Salava *et al.*, 2002) was revealed by AFLP markers.

Total world apricot production has reached 2.68 million tons. Turkey account 16% of that production (Faostat, 2004). In Turkey, new apricot cultivars for fresh consumption have been developed by hybridization. The objective of this study was to characterize some Turkish and European apricot cultivars and new apricot cultivars derived through hybridization between Turkish and European apricot cultivars using RAPD markers.

Materials and methods

Plant materials: A total of 24 genotypes were used in this study. Plant materials were obtained from Alata Horticultural Research Institute located in Mersin, Turkey. The twenty-four genotypes included 5 new cultivars, 2 local cultivars, 13 European cultivars, and 4 new promising hybrid genotypes (Table 1). Hybrid genotypes have desirable traits such as early maturing and coloring. These all cultivars and genotypes are

Table 1. List of the 24 apricot cultivars used in the study and their geographic or genetic origin

Cultivars	Origin	Parents
Errani	Italy	Chance seedling
Bebeco	Greece	Chance seedling
Bulida	Spain	Chance seedling
Canino	Spain	Chance seedling
Feriana	France	Hamidi x Canino
Fracasso	Italy	Chance seedling
Priana	France	Hamidi x Canino
Goldrich	USA	Chance seedling
Harcot	Canada	Chance seedling
Palstein	South Africa	Chance seedling
Precoce de Tyrinthe	Greece	Chance seedling
Precoce de Colomer	France	Chance seedling
Sakıt-2	Turkey	Chance seedling
Sakıt-6	Turkey	Chance seedling
Alatayildizi	Hybrid	Sakıt-6 x P. de Colomer
Cagataybey	Hybrid	Sakıt-2 x P. de Colomer
Cagribey	Hybrid	Sakıt-6 x P. de Colomer
Dr. Kaska	Hybrid	P. de Colomer x 07-K-11
Sahinbey	Hybrid	Sakıt-6 x J. Foulon
2-89	Hybrid	P. de Colomer x 07-K-11
28-89	Hybrid	Sakıt-1 x Fracasso
33-89	Hybrid	Sakıt-1 x Cafano
15-90	Hybrid	J. Foulon x Sakıt-1
7-89	Hybrid	Sakıt-6 x J. Foulon

Table 2. List of the 14 RAPD primers amplifying fragments, number of markers scored, number of polymorphic markers, and estimated size of the polymorphic markers

RAPD primers	Number of fragments scored	Number of polymorphic fragments	Polymorphic fragment sizes (bp)
OPAA 01	10	4	700, 750, 900, 1000
OPAA 02	10	7	370, 800, 850, 1050, 1350, 1400, 1500
OPAA 09	6	2	730, 740
OPAA 14	5	3	600, 650, 1200
OPAA 17	10	8	900, 1200, 1300, 1350, 1400, 1450, 1600, 2000
OPAA 18	4	1	740
OPAA 19	5	3	500, 900, 1350
OPAH 06	5	5	800, 900, 1250, 1400, 1500
OPAH 09	3	2	500, 1700
OPAH 14	3	2	700, 750
OPAH 16	11	4	350, 550, 1000, 1500
OPAH 17	10	6	300, 600, 650, 800, 1450, 1500
OPAH 18	8	5	1400, 1600, 2000, 2100, 2200
OPAH 20	10	7	300, 500, 550, 600, 700, 800, 1500

commercially important.

DNA extraction: Genomic DNA was isolated from leaf samples using DNA extraction procedure described by Dellaporta *et al.* (1983) with some modifications. Three gram of young leaves were crushed in liquid nitrogen and were ground in centrifuge tube with 30 µL mercaptoethanol solution. Tubes were set for few minutes at room temperature. Then, 15 mL extraction buffer [for 800 mL extraction buffer, 200 mL stock (4x), 400 mL of pure water 40 g PVP, 80 mL SDS (2%)] was added and incubated at 65°C for 30 min. Extraction solution was kept in ice for 2 min, 6 mL 5M KAc (potassium acetate) was added and kept on ice for 5 min. The samples was centrifuged (Suprafuge 22, Heraeus-Sepatech) at 13,475 rpm for 15 min at 4°C and aqueous phase was transferred into clean tube containing 15 mL of cold isopropanol. It was kept at room temperature for 15-30 min and centrifuged at 5000 rpm for 20 min. Liquid phase was removed from tube. Five mL of washing buffer (76% ETOH, 10 mM NH_4Ac) was added on pellet. After 20 min, it was centrifuged at 5,000 rpm for 10 min at 4°C, liquid phase was removed, 1.5 mL TE (50 mM Tris-HCl, 10 mM EDTA, pH 7.4) was added. Shaking, it was incubated at 37°C for 1 h. One-hundred and fifty µL of 3 M NaAc was added and waited on ice for 20 min. After centrifuged at 12,000 rpm for 15 min, aqueous phase was transferred to new tube and mixed with 15 mL of cold isopropanol. After incubation for 30-60 min, sample was centrifuged at 6,000 rpm for 10 min. Upper phase was removed and pellet was mixed with 5 mL of washing buffer (76% ETOH, 10 mM NH_4Ac). Centrifuged at 10,000 rpm for 10 min and upper phase was removed from tube. Pellet was air-dried at 37°C for 30 min, then mixed with 200 µL TE until completely dissolved. RNAs were removed from pellet by adding RNAse at a final concentration of 10 µg/mL, and incubation at 37°C for 30 min. Then 200 µL of TE and 3 M of NH_4Ac at final concentration of 2.5 M (pH 7.7), 500 µL of cold ethanol was added. DNA pellet was recovered by centrifuging at 14000 rpm for 2 min. After air-drying, DNA pellet was dissolved in 200 uL of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.4). DNA concentrations were measured using spectrophotometer.

PCR Amplification: Sixty RAPD primers were used for amplification of isolated apricot DNA. 20 RAPD primers were described by Hormaza (2001), 40 primers were Operon KIT AA and KIT AH primers (Operon Technologies Inc., Alameda, CA, USA). Primers and other PCR components (in 0.5 mL eppendorf tube were as follows: 20 ng of genomic DNA, 12.5 µL of 2x PCR master mix (Promega Cat.no: M7502) (Promega Corp., Madison, WI, USA), 1 µL of 25 mM $MgCl_2$, 20 ng primer, 0.2 unit of Taq DNA polymerase and ddH_2O). PCRs were carried out in Perkin Elmer Cetus 480. Cycling parameters were as described by Hormaza (1999).

Amplified DNA fragments were analyzed by gel electrophoresis in 1.5% agarose (Sigma, A5093) at 70 V for 3 h. After electrophoresis gels were stained with 0.1% ethidium bromide for 30 min. Then visualized under UV with an Polaroid type 667 black-white film (Polaroid MP 4+ Instant Camera System). A 1 kb ladder (GeneRulerTM DNA Ladder Mix, Fermentas) was used to estimate the approximate molecular weight of the amplified products.

Data Analysis: Each band was scored as present (1) or absent

(0) and data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) version 1.8 software package (Exeter Software, Setauket, N.Y., USA) (Rohlf, 1993). A similarity matrix was constructed based on Dice`s coefficient (Dice, 1945), which considers only one to one matches between two taxa for similarity. The similarity matrix was used to construct a dendogram using the unweighted pair group method arithmetic average (UPGMA) to determine genetic relationships among the germplasm studied. To provide a goodness-of-fit test for the similarity matrix to cluster analysis, first, COPH module was used to transform the tree matrix to a matrix of ultrametric similarities (a matrix of similarities implied by the cluster analysis) and then, MXCOMP module was used to compare this ultrametric similarities to the similarity matrix produced.

Results and discussion

Out of 60 primers tested, 39 gave non-scorable bands, 7 produced non-polymorphic fragments. The remaining 14 primers amplified from 1 to 8 polymorphic fragments ranged 300 to 2200 bp. OPAA 17 primer amplified the highest number of fragments, 8 bands followed by OPAH 20 and OPAA 2 (7 fragments), OPAH 17 (6 fragments), OPAH 5 and OPAH 18 (5 fragments) primers (Table 2). The number of polymorphic markers was 59 out of 136 (43.4%). On an average, each primer gave 6.47 scorable markers and 4.21 polymorphic markers per amplification. Cophenetic correlation between ultrametric similarities of tree and similarity matrix was found to be high ($r = 0.70$, $P<0.01$). No two apricot cultivars were found to be identical. However, the similarity level was high among the cultivars studied, ranging from 0.90 and 0.96 (Fig.1). There were two groups at the similarity level of 0.90. The lower branch included three cultivars (Priana, Feriana, and Canino) and one promising genotype (28-89). Feriana and Canino were found to be the most closely related genotypes among all cultivars with the similarity level of 0.96. Cultivar Feriana and Priana are progeny of Canino (Yilmaz, 2002). This indicates the strength of RAPD markers in detecting relationships and diversity among germplasm. The upper branch contained twenty cultivars including five newly developed apricot cultivars and four other promising hybrids. These genotypes were derived from known parental combinations (Table 1). They were placed between or close to either one of the parental genotypes. This finding may indicate that RAPD markers can successfully determine genetic relationships among genotypes, and identify cultivars.

The similarity value among 24 genotypes from diverse geographic regions ranged from 0.90 to 0.96. This may imply low genetic variability among the 24 apricot cultivars studied. This narrow variability is a drawback from the point of view of breeders, because they need high genetic variability to improve agronomic traits and the cultivars are selected only based on very few agronomic traits such as maturation time, coloring, and yield.

In general, cultivar breeding programmes do not involve resistance to biotic and abiotic factors. Hence, outbreaks of disease and other pests destroy whole tree or crop in many instances. Increasing genetic variability is crucial to breeding

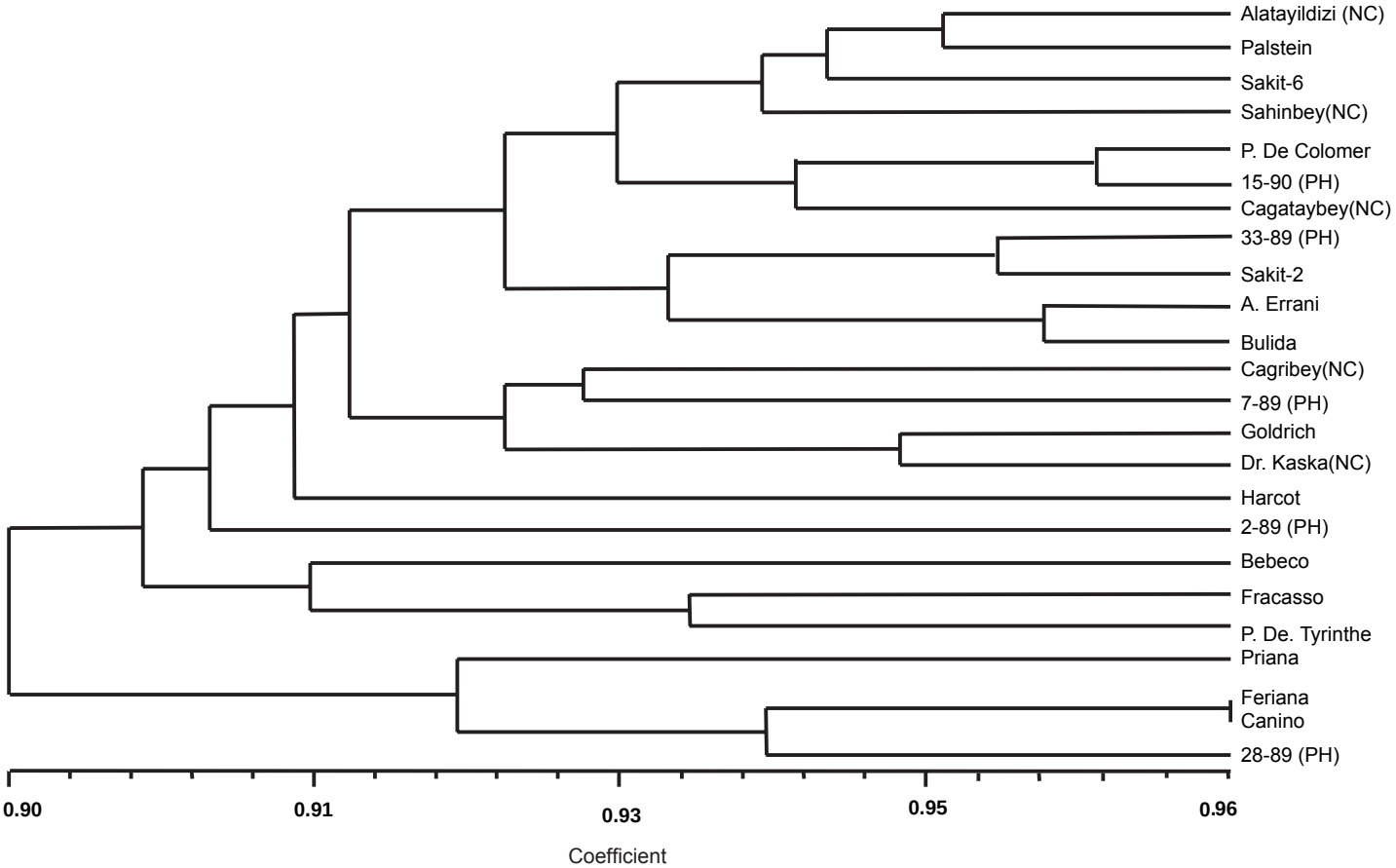

Fig. 1. Dendrogram based on the shared polymorphic amplification product resultant from the use of 21 primers on 24 apricot cultivars and genotypes using UPGMA. Abbreviations are as follows: NC, new cultivar; PH, promising hybrid.

programs. For this aim, wild apricots or the closely related species could be the useful source of variation for plant breeders because within the genus interspecific crosses are usually feasible and efficient in terms of selection pressure. Studies evaluating the disease resistance and fruit quality characteristics of germplasm collections are essential for future breeding programme (Hagen *et al.,* 2002). In conclusion, using genotypes from different ecogeographical groups in breeding programme will allow widening of genetic base in apricot.

The RAPD markers efficiently discriminated all cultivars in this study. Hence, they may be readily used in understanding relationship level, establishing germplams collections, and integrating markers into genetic linkage maps establishing germplasm core collections. As cheap and quick markers, RAPD markers can be used for genetic map of apricots, and used in marker aided selection. RAPDs may have potential in developing resistant cultivars via marker- aided selection, which would further enhance apricot improvement opportunities.

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Telfairia production: Consideration for alleviating rural poverty among Nigerian women

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Abstract

Gender roles in telfairia leaf production were investigated in Makurdi using a survey based questionnaire administered to 50 farmers to identify gender-disaggregated roles in telfairia production. The survey showed that women have major role as producers and marketers of telfairia leaves. Women and girls provided 80.0% of labour requirements for hole digging, sowing, irrigation, weeding, harvesting and marketing. The men cleared land and dug holes while girls and boys in primary and secondary schools assisted in weeding and hole digging. The results also revealed that a typical telfairia farm using ₦10,650.00 (US$84.5 at ₦126/dollar) worth of seeds produced 16.5 t/ha of leaves valued at ₦212,400.00 (US$1,685.7) with 85.0% profit. Seed accounted for 60.7% of total cost of production, while irrigation cost was 20.3%. A minimum take-off fund of ₦210,572 (US$1671.2) was needed to give revenue of ₦386,000 (US$2920.6) and a gain of 83% per hectare. Total fruit equivalent of fruits/shoots produced 2,056 fruits and the price of fruit equivalent of fruits/shoots produced ₦514,000 (US$4079.4) with a gain of 144%. Two major constraints to leaf production were high cost of quality seeds (36.1% of respondents) and water pumps (13.9% of respondents). Women participation in telfairia vegetable production, marketing and utilization in Makurdi can provide a means of livelihood and appreciable income for women in rural and urban areas, which is capable of sustaining the running of the home and enhancing the living standards of women.

Key words: *Telfairia occidentalis* Hook. F., fluted pumpkin, gender, production, constraints, poverty, profit, sustainability, Nigeria.

Introduction

Fluted pumpkin (*Telfairia occidentalis* Hook. F.), a member of the family Cucurbitaceae, contains high vitamin A and iron which can take care of vitamin A deficiency in children and pregnant women. In Nigeria, it is generally referred as a "woman's" crop (Akoroda, 1990; Lewis, 1997) with folktales that refer to women's pregnancy. The juice squeezed out of the leaves, serves as blood tonic for anemic patients and pregnant women whose Packed Cell Volume (PCV) is low, which also means low blood level (E. Anujuem, personal communication). In Benue State of Nigeria, however, minimal attention was given to telfairia production and consumption, until recently, when increased awareness of the nutritional value of the leaves (Ifon and Bassir, 1980) and seeds which contain 53% fat and 27% crude protein (Longe *et al.,* 1983) encouraged its consumption. However, it is believed that enhanced production and consumption can play a catalyst role in the income generation and employment, alleviation of rural poverty, improved health status, enhancement of resource use efficiency and overall socio-economic development of the people (Bahar, 1988; Bennett Lartey and Akromah, 1996; Illo, 1988; Ozkan *et al.,* 2000; Roy, 1990; Siddiqui *et al.,* 1999; Wijerante, 1992).

Recognizing that one of the fundamental organizing principles of human society, which affects almost every aspect of what an individual thinks and does, is gender (Illo, 1988), this study was carried out to analyse the respective roles of men and women in telfairia production and to explore how they relate to each other and the profitability of production.

Materials and methods

A questionnaire was designed to cover farmers' activities on their telfairia plots, farm-size, production inputs and techniques and demographic data. Primary data was collected using a structured questionnaire, which was distributed once to each respondent. The study areas in Makurdi were the three major sites for dry season vegetable production, namely the north and south banks of River Benue and the lower Benue. Homestead farms, a common feature in Makurdi area were also observed. Subjective questions were asked to telfairia producers who were target population for this study. A forty four item questionnaire data was administered, and quantitative data were generated for statistical analyses. Hundred (100) telfairia producers were interviewed on their farm sites. Uncompleted questionnaires totaling fifty were disregarded and the remaining ones were used for analysis.

Profitability efficiency of the enterprise was analysed using gross margin analysis with the equation below.

Gross Margin (GM) = Total Revenue (TR) – Total Cost (TC)

Gain(%) = GM / TC x 100

Gross Margin of fruit equivalent of shoots/fruits produced was also calculated.

Results

Socio-economic characteristics of telfairia growers in Makurdi: Most females (92%) were educated. Among the elderly, 70% were females, while 30% were males, and within the middle-

aged group, 72% were females, while 28% were males. The main sources of funding for the female producers were personal savings, friends and cooperatives, while the males depended on personal savings and moneylenders. The size of plots cultivated and source of farmland differed according to gender (Table 1). Acquisition of this land for production depended on existing interpersonal/family relationships (71% of the respondents). Out of those who leased or hired, 76% were females while 24% were males. The women owned most of the telfairia plots. For instance; out of the 50 respondents who produced on less than 0.2ha, 40 were women while 10 were men.

Table 1. Source of farmland and farm size of responding telfairia leaf producing farmers in Makurdi

Variables	Male	Female	Total Number
Source of farm land			
Family	5 (10.0)	2 (4.0)	7 (14.0)
Leased/hired	6 (12.0)	36 (72.0)	42(84.0)
Friends	1 (2.0)	0 (0.0)	1 (2.0)
Initial plot size			
<0.2ha	5 (10.0)	26 (52.0)	31 (62.0)
0.2-0.4ha	6 (12.0)	7 (14.0)	13 (26.0)
0.4-0.6ha	0 (0.0)	5 (10.0)	5 (10.0)
0.6-0.8ha	0 (0.0)	0 (0.0)	0 (0.0)
>0.8ha	0 (0.0)	1 (2.0)	1 (2.0)
Present plot size			
<0.2ha	8 (16.0)	8 (16.0)	16 (32.0)
0.2-0.4ha	6 (12.0)	10 (20.0)	16 (32.0)
0.4-0.6ha	5 (10.0)	8 (16.0)	13 (26.0)
0.6-0.8ha	0 (0.0)	0 (0.0)	0 (0.0)
>0.8ha	2 (4.0)	3 (6.0)	5 (10.0)

*Figures in parenthesis are percentage

Gender roles in telfairia shoot/fruit production in Makurdi: The activities practised in the production of telfairia were distributed according to gender and enumerated in Table 2. Most of the respondents (96%) agreed that the major activity done by men (including boys) is land clearing, while, another 72% considered hole digging as the major activity of men. The major activities performed by women (including girls), were harvesting and marketing although they were also engaged in planting, watering and weeding. It was clear from the data collected that men did not practise harvesting at all, but the boys assisted their mothers in weeding. The female contribution to the total labour was 24% higher than the males when valued (Table 3).

Table 3. Percent contribution to total labour by gender among 50 responding telfairia leaf producing farmers in Makurdi

Gender	Value of total % labour (₦)	Male to female ratio	Male to female (%)
Male	2,465.3	12.7	35.2
Female	6,855.8	35.3	57.8
Both	8,756.6	45.0/2 = 22.5	
Total	19,444		

Gender roles in marketing of telfairia in Makurdi: Marketing of telfairia leaves was at the site of production by both retailers and middlemen from Makurdi, Jos, Abuja (middle belt of Nigeria), Kano and Kaduna (far north of Nigeria) during the dry seasons. The quantity of leaves demanded determines the transportation pattern. The middlemen, who came to purchase large quantities of telfairia shoot, used J5 motor vans with carrying capacity of 9.435m^3 for old model and 7.57m^3 for new model. The J5 motor vans were loaded after the harvested leaves had been graded. To minimize the risks associated with long distance marketing, most women prefered to sell their vegetables to middlemen at the production sites as seen in the transportation chain. Seventy percent (70%) of the respondents concluded that women were more prominent in the sale of the leaves. All the respondents (100%) sold telfairia shoots directly to (a) consumers, (b) retailers and (c) middlemen. Only 24% of the respondent produced and sold telfairia fruit, to generate additional income for the family. Telfairia fruits were often graded according to size and arranged in-groups of 100, 50, 20, 10 and 5. Generally, 82% of the respondents used the income generated from the sale of telfairia products for family upkeep and for future telfairia production, while others used their income for personal upkeep.

Shoot yield, cost and returns of telfairia in Makurdi: The yields of telfairia shoot harvested from the respondents' farms differed with the size of farm. Harvested shoots were heaped, graded into bunches called 'heads' (35kg/head) and tied with a jute bag. The quantities produced at the end of the season ranged from 8 to 600 'heads' (280-21,000kg). From an average farm size of 0.25ha, a mean of 118 'heads' (4,130kg) of telfairia shoots was harvested. This showed that 472 'heads' (16,520kg) of telfairia shoots were produced in a hectare. The cost of input in

Table 2. Gender roles in total value and percentage of labour on in the production of 0.25ha telfairia plot among 50 responding telfairia leaf producing farmers in Makurdi

Activity	Male (%)	Value (₦)	Female (%)	Value (₦)	Both (%)	Value (₦)	Total (%)	(₦)
Land clearing	96	936.0	2	19.5	2	19.5	100	979
Hole digging	72	842.4	4	46.8	24	280.8	100	1170
Planting	8	46.8	48	280.8	44	257.4	100	585
Watering	6	640.1	28	2987.0	66	7040.9	100	10668
Weeding	20	200.0	50	600.0	30	550.0	100	1365
Fertilizer application	0	0.0	23	89.7	77	300.0	100	390
Harvesting	0	0.0	80	3432.0	20	858.0	100	4290
Marketing	4		80		16			
Total	12.7	2465.3	35.3	6855.8	45	8756.6		19444

Actual size of plot without removing pathway	71,684.54ha	Mean total number of seeds/fruit	74 seeds
Actual size of plot after removing pathway	70,802.87ha	Total number of fruits needed to plant/ha	957 fruits
Total number of seeds needed for a hectare	70,803 seeds		

Table 4. Average variable cost of inputs in telfairia production for a 0.25 ha plot size among 50 responding telfairia leaf producing farmers in Makurdi

Cost of items	Mean number of man days required	Cost of labour/ man day (₦)	Total amount paid for labour for a 0.25ha (₦)	Percent labour cost	Percent of each input cost to total cost
a. Variable costs					
Labour					
Land clearing	5	195	975	5.0	2.0
Hole digging	6	195	1,170	6.0	2.0
Planting	3	195	585	3.0	1.0
Fertilizer application	2	195	390	2.0	1.0
Watering	28	195	10,668	55.0	20.0
Weeding	7	381	1,365	7.0	3.0
Harvesting	22	195	4,290	22.0	8.0
Total labour	73	195	19,443	100.0	
Seed			31,950		61.0
Total variable cost			51,393		
b. Fixed cost					
Land rent			1,250		2.0
Total			52,643		100.0

Table 5. Gross Margin Analysis of telfairia production per ha estimated from 50 responding telfairia leaf producing farmers in Makurdi

Items	Value of production per hectare		
	₦ 0.25 ha^{-1}	₦ ha^{-1}	$ ha^{-1} (@₦110/$)
Total variable cost	51,393	205,572	1,868.9
Total fixed cost	1,250	5,000	45.5
Total cost	52,643	210,572	1,914.3
a. Revenue			
i. shoot sale	88,500	354,000	3,218.2
ii. fruit/seeds	6,750	27,000	245.5
Total revenue	95,250	381,000	3,463.6
b. Variable cost			
i. labour	19,443	77,772	707.1
ii. seed	31,950	127,800	1,161.8
Total variable cost	51,393	205,572	1,868.8
Gross margin (a-b) =	43,857	175,428	1,594.8
% Gain	85.3%	85.3%	85.3%

the production of telfairia leaves is shown in Table 4, with a mean cost of labour per man-day of ₦195. The total cost of labour in producing telfairia leaves was ₦19,443 for a plot size of 0.25ha (₦ 77,772/ha) and the cost of seed was ₦31,950 for a plot size of 0.25ha (₦ 12,7800/ha). Sixty one percent (61%) of the total cost of production was allocated to seed input while 37% was allocated to labour (Table 4). Fifty five percent (55%) of the total cost of labour goes to watering alone. The price/head of telfairia shoot ranged from ₦450 to ₦1200.00 with an average price of ₦750.00. The total number of vines (92cm average length) in a bunch ('head') was 485 with a mean of 12 leaves/vine. At retail market, a vine sells for ₦10, which indicates that a leaf sells for ₦1: 2kobo. The fruit yield ranges from 14 to 50 fruits per 0.25ha with a mean of 27 fruits/0.25ha (108 fruits/ha). The price range of telfairia fruits is between ₦100 to ₦500 with a mean of ₦250 per fruit. This shows that ₦27,000/ha can be derived from the fruits. The gross margin (₦175,428 /ha) made by the respondents in Makurdi on telfairia shoot/fruit production had a gain of 83% as shown in Table 5. The price of fruit equivalent of fruits/shoots produced in Makurdi by respondents is ₦514,000 with a gain of ₦303,428 (144%).

Discussion

Among other things, there was need to identify role of gender in telfairia seed/shoot production in Makurdi area. From the survey results, division of labour by sex and age was evident, and family members jointly carried out most of the farming activities. The few males that accompanied the females in such activities as land clearing, which was tedious, were often their spouses. The women were highly involved in harvesting, marketing, planting and watering operations. In each of these practices, the female contribution, which was more than 80% of the total labour requirement, made it a predominantly female enterprise as reported by Siddiqui *et al.* (1999) and Ozkan *et al.* (2000) for some other crops. Thus it is evident that in Makurdi, as in the rest of Nigeria and Africa, women play vital roles not only in the management and maintenance of telfairia production, but also ensure that seeds are always available for planting for the next season. This also confirms the findings of Roy (1990) on the pivotal roles of women in agriculture. It has been shown in the current study that while men and women were involved in wholesale marketing, women were more into retailing. The marketing of telfairia shoot by women significantly contributed to their income for family upkeep and future telfairia production.

Women are therefore the major producers of telfairia in Makurdi. This finding agrees with the report of Ekwe (1996), that the production of vegetables among other crops, was primarily the work of women. These female producers find it easy to go into telfairia commercial production because of the minimal take-off fund, the high returns, accessibility to their spouses land, opportunities to rent land at a very low price and also because of the very small piece of land they start off with the information on telfairia market structure revealed both wholesale and retail markets from within and outside Makurdi. The demand of telfairia leaves and the means of transportation confirmed that the demand of telfairia shoot from the northern state had increased.

It might be necessary to involve the Benue State Agricultural Development Authority (BNARDA) to use its extension service system to educate telfairia producers and encourage them to join co-operative societies. This will guarantee sustained loyalty, provide take-off loans or facilitate bank loans, and ensure easy access to land and also standardization of produce and prices. NGO's promoting the participation of women in vegetable production, conservation and utilization as reported by Karim, 1996) should promote telfairia production and encourage producers to identify with such organizations.

Commercial production of telfairia shoot can be started on 0.002 hectares and on an average of 0.25 hectares with ₦500.00 and ₦10,650, respectively, which was enough to buy seeds. This initial take-off fund can come from savings from other enterprises. These enterprises are necessary to avoid idleness because telfairia production in Makurdi is seasonal. Seed and irrigation are major inputs and they constitute the major constraints in the production of telfairia in Makurdi. This was so because other inputs such

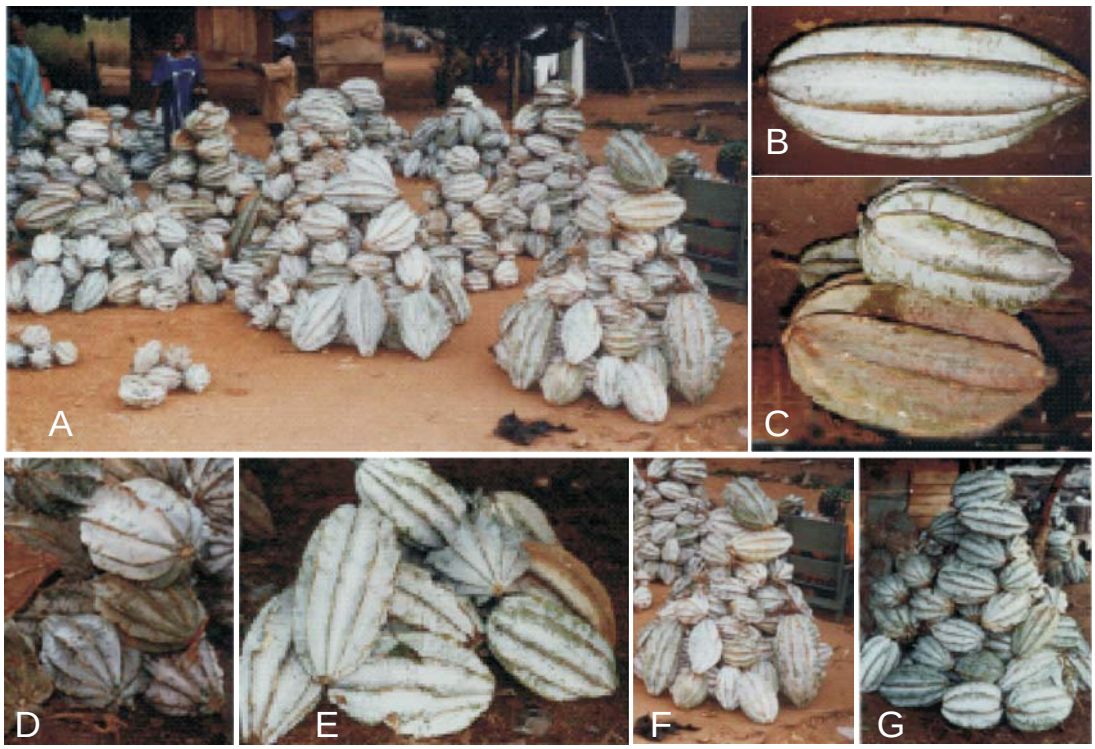

Fig. 1. Graded telfairia fruits for sale (A) A typical telfairia market, (B) A telfairia fruit sells for ₦700, (C) A group of three fruits sells for ₦1,500, (D) A group of four fruits sells for ₦2,000, (E) A group of ten fruits sells for ₦7,500, (F) A group of one hundred mixed fruits sells for ₦13,500, (G) A group of one hundred big fruits sells for ₦17,500.

as land could be leased, labour could be from the family and no chemicals such as fertilizer or insecticide were needed. Acquisition of pumps by farmers could be a cheaper means of managing farms. If only female producers could take decision in the kind of input to buy as suggested by Ozkan *et al.* (2000) they could buy these pumping machines with money saved from previous harvest. If these constraints are redressed, farmers could increase their farm size, to meet the ever-increasing demand for telfairia shoot and fruit, within and outside Makurdi.

The study revealed that for one ha commercial production, a minimum take off fund of ₦210,572 is needed to give revenue of ₦386,000 and a gain of 83%. Total fruit equivalent of fruits/shoots produced 2,056 fruits and the price of fruit equivalent of fruits/shoots produced ₦514,000 with a gain of 144%. Women were actively involved in the production of telfairia leaves. It was found that women contributed 80% of total labour requirement regarding planting, watering, weeding, harvesting and marketing of the crop. Although men also market their produce, their main activities were land clearing and hole digging. Mainly women carry out retail activities. Children assist their mothers in weeding and hole digging. The cash generated contributes significantly to food security at the household level and enables women to attain a degree of financial independence within the family budget.

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Minerals in pericarp of tomato (*Solanum lycopersicon L.*) fruit and its ripening behaviour

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Abstract

Two contrasting varieties of tomato (*Solanum lycopersicon* L.) fruits *i.e*. 'Pusa Gaurav' (slow ripening type) and 'Pusa Ruby' (fast ripening type) were examined for Ca, P, K, Zn, Cu and Mn contents in the fruit's pericarp portion. Fruits were examined either at different ripening stages during their maturation on the plant itself or at different intervals during storage when harvested at green mature stage. Ca was found to be higher in 'Pusa Ruby'. 'Pusa Gaurav', on the other hand, showed higher content of P, Zn, Cu, Mn but low K in comparison to 'Pusa Ruby'. The roles of these minerals were explained towards their stabilizing effect on plasma membrane and cell wall along with their involvement in the antioxidative system and thereby determining the rate of ripening.

Key words: *Solanum lycopersicon,* minerals, pericarp, ripening, tomato, fruit

Introduction

Inorganic solutes are known to play structural and regulatory roles in physiological processes. Ripening, storage behaviour and quality of fruit are influenced by mineral nutrition and mineral content within the fruit (Wills and Tirmazi, 1979; Marcelle, 1990; 1995). Among postharvest physiological events, membrane damage is the key event leading to a cascade of biochemical reactions (Marangoni *et al.,* 1996). Enhanced ion leakage due to loss of membrane integrity and increased free radical mediated damage are characteristics of senescing plant tissue and fruit ripening (Stanley, 1991; Ferrie *et al.,* 1994; Palma *et al.,* 1995) along with textural change at cell wall level (Powell and Bennett, 2002).

Ca is reported to maintain the integrity of membranes (Morre and Bracker, 1976) and delay senescence and ripening process (Poovaiah and Leopold, 1973; Tingwa and Young, 1974). P is a component and also acts as a bridge for the phospholipids (Marschner, 1998). Zn, like Ca and P, is also reported to be required for maintenance of integrity of biomembranes (Marschner, 1998). It might bind to phospholipids and sulfhydryl groups of membrane constituents or form tetrahedral complexes with cysteine residue of polypeptide chains (Vallee and Falchuk, 1993). This thereby protects membrane lipids and proteins against oxidative damage. Zn controls the generation of toxic oxygen radicals by interfering with the oxidation of NADPH as well as by scavenging $O_2^{-\cdot}$ in its function as a metal component in Cu-Zn superoxide dismutase (SOD) (Cakmak and Marschner, 1988a; 1988b). Zn deficiency is therefore, reported to cause increase in membrane permeability (Cakmak and Marschner, 1988c; 1990).

Process of ripening and senescence are characterized by peroxidation of membrane lipids through elevated levels of free oxygen radicals (Marangoni *et al.,* 1996). Plants, therefore, possess a range of defense systems for detoxification of oxygen radicals and hydrogen peroxide including SOD ($O_2^{-\cdot} \rightarrow H_2O_2$) and peroxidase/catalase ($H_2O_2 \rightarrow H_2O$). As a component of detoxifying enzyme like SOD, nutrients such as; Zn, Cu, and Mn play critical role (Elstner, 1982; Cakmak and Marschner 1988a; b; Bowler *et al.,* 1991). K, on the other hand, reduced the fruit loss due to decay in storage (Zhu and Shu, 1991) and it also has a positive effect on tomato (*Solanum lycopersicon* L.) quality besides fruit yield and fruit size (Forster, 1973).

Besides the effect of nutrients on membrane stability, the dynamic changes in the cell wall of ripening fruits are also anticipated to be under tight control by ionic conditions (Ricard and Noat, 1986; Huber and O'Donoghue, 1993; Almeida and Huber, 1999). Further, varietal variation was noticed for nutrients in pericarp and locular portions of tomato (Stevens *et al.,* 1977). It is in this context, the present study was carried out to quantify the contents of Ca, P, K, Zn, Cu and Mn in the pericarp portion of the fruits in two contrasting varieties *viz.,* 'Pusa Ruby' (fast ripening) and 'Pusa Gaurav' (relatively slow ripening) at different ripening stages of fruits directly harvested from the plants as well as at intervals during storage of fruits that were harvested at green mature stage.

Materials and methods

Seeds of tomato varieties, *viz.,* 'Pusa Ruby' and 'Pusa Gaurav' obtained from the Division of Vegetable Crops, Indian Agricultural Research Institute, New Delhi were treated with fungicide (0.5 % mercuric chloride solution) for 5 minutes and rinsed with distilled water thoroughly and sown on raised soil bed during the end of October, 2004. Grown up seedlings at five leaves stage (height 10 ±2 cm) were transplanted in experimental field already supplied with 20 tonnes ha^{-1} of organic manure in the form of farm yard manure at spacing of 75 x 45 cm. Fertilizers @ 100 kg N, 80 kg P_2O_5 and 80 kg K_2O ha^{-1} were applied. Half dose of N (urea) along with full dose of P_2O_5 and K_2O were applied to the soil at the time of transplanting and rest of N was applied as top dressing after 5th week of transplanting. Uniform irrigation was given whenever required. Other cultural practices, were followed as per recommendations. Healthy tomato fruits having comparable

size and weight in a range of 60-70 g were harvested manually at required ripening stage/s at the end of March, 2005. For ripening stages description as given by United Fresh Fruit and Vegetable Association (UFFVA, 1975) was followed. Fruits were gently and properly washed under running tap water followed by three times rinsing in double-distilled (DD) water and then air dried for further experimental use.

As required, harvested fruits were either directly subjected to estimations or they were stored in well-ventilated plastic baskets under room conditions. For storage purpose, three replications, each represented by 30 tomatoes, at green mature stage, were used. From each replications, 15 fruits were used to assess the ripening index (RI %), percentage for red ripe tomato (RRT %) and shelf life (days). Rest of 15 fruits were divided randomly in five equal lots, each having 3 fruits. They were marked for their respective sampling at 0, 5, 8, 10 and 14 days after harvest (DAH). These marked fruits were used for sample preparation at their respective DAH towards the analysis of nutrients. Likewise, 3 fruits at specific ripening stages, as attained on the plant, were also analyzed for the content of nutrients.

RI (%): This measures the extent of ripening for a given lot of tomato fruits. Methodology as described by Wang and Morris (1993) was followed.

RRT (%): It was calculated as per the method of Wills and Ku (2002). The number of tomato fruits reached to red ripe stage [whole fruit became red ripe in colour as per UFFVA (1975)] out of total number of fruits at required days intervals were counted and expressed in percentage. It also indicated the extent of ripening.

Shelf life: It was calculated as per the method of Wills and Ku (2002). Ripe tomatoes were examined routinely to assess for external appearance. The end of shelf life in days *i.e.*, DAH was the time when fruit showed either a moderate level of shriveling or an extent of black spots that could make fruit unacceptable for marketing.

Nutrient analysis

Procedures for sample preparation, digestion and estimations of nutrients as described by Tandon (1998) and Jones (2001) were followed as such or with minor modifications as described below:

Sample preparation: Composite samples of three fruits (selected randomly) from each of the replications were collected for both the varieties at different ripening stages and at required DAH. Fruits were washed gently under running tap water followed by rinsing thrice with DD water. The pericarp tissue was separated from the jelly and the seeds. It was again rinsed twice with DD water and blotted dry and kept in oven at 75 ^{o}C till the samples dried properly. Dried pieces of pericarp tissues were then grinded thoroughly in motor and pestle (made up of glass) to a fine power. Sample wise, powder was stored in butter paper bag inside the desiccator till further use.

Digestion: Just before the use, the powder was again heated for 2-3 h at 75 ^{o}C to make it free from any moisture and then 1.0 g of dry powder was transferred into the digestion tube. Tissue samples were predigested by adding 10 mL of concentrated HNO_3 in digestion tubes for overnight. Next day, additional 15 mL of concentrated HNO_3 was added followed by 5 mL of $HClO_4$ for its complete digestion in diacid mixture of HNO_3 and $HClO_4$ in a ratio of 5:1(v/v). Samples were then heated at 60 ^{o}C for 30 minutes, 120 ^{o}C for 30 minutes and 255 ^{o}C for 2 h or till the digestion mixture became transparent on digestion block. On complete digestion, only 3-4 mL of digestion mixture had been left in the tube. Tubes were then removed from the block and allowed to cool down to room temperature. The content was diluted to 25 mL by adding DD water and then it was filtered through Whatman filter paper (number 42). For each sample, final volume was made up to 50 mL in volumetric flasks. Now samples along with control in replicates were used for the estimation of Ca, P, K, Zn, Cu and Mn. Standard solutions for each nutrient elements were also prepared to make standard curves for respective element.

Ca and K: Ca and K were analysed using flame photometer of Elico make, India with facility of internal calibration. Air pressure was kept constant at 10 psi. Characteristic emission of K and Ca was recorded at optical wavelength of 768 nm and 622 nm, respectively.

P: P was estimated by colorimetric method by developing vanadomolybdo phosphoric acid yellow coloured complex with aliquot of diacid digest. Actual P was then calculated from the standard curve prepared for P.

Mn, Zn and Cu: These were determined using atomic absorption spectrophotometer (Electronic Corporation of India Limited). Absorptions were recorded at 279.5, 213.9 and 324.8 nm for Mn, Zn and Cu, respectively using standard specifications and their respective standard curves. Content of nutrients were expressed either in mg g^{-1} dry weight (dw) or in µg g^{-1} dw.

Statistical analysis: The obtained replicated data were statistically analysed using two factor complete randomized design. Mean values were then ranked using Duncan's Multiple Range Test using MSTAT-C statistical software. Statistical procedures as described by Gomez and Gomez (1984) were followed.

Results

Ripening: Variety 'Pusa Ruby' showed faster ripening rate in comparison to 'Pusa Gaurav' (Table 1). At 14 DAH, fruits of 'Pusa Ruby' attained 86 and 45% while 'Pusa Gaurav' had the values of 55 and 8% for RI and RRT, respectively. The shelf life of 'Pusa Ruby' was 12-15 days in comparison with 20-22 days for 'Pusa Gaurav' when fruits were harvested at green mature stage and stored at room temperature (31.2 ±6 ^{0}C) and relative humidity (36.1 ±5 %). (data not presented).

Ca: Progress of ripening of fruits attached with plants, in both the varieties, had no effect on the Ca content (Table 2A). Further the Ca content was also comparable in these two contrasting varieties. Storage duration also could not affect the levels of Ca significantly (Table 3A) but, on an average, higher Ca content was recorded in 'Pusa Ruby' (538.83 µg) in comparison with 'Pusa Gaurav' (388.18 µg) during storage (Table 3A). 'Pusa Gaurav', inspite of being relatively slow ripening variety, maintained lower total Ca content than 'Pusa Ruby'.

P: No fixed pattern of change was observed with the progress in ripening of tomato fruit on the plant (Table 2B). Comparison of

Table 1. Effect of storage duration on ripening of tomato fruits harvested at green mature stage in two varieties

Days after harvest (DAH)	Ripening index (RI %)			Red ripe tomatoes (RRT %)		
	'Pusa Ruby'	'Pusa Gaurav'	Mean (DAH)	'Pusa Ruby'	'Pusa Gaurav'	Mean (DAH)
5	35.3^{ef}	24.2^{f}	29.7^{d}	0.0^{b}	0.0^{b}	0.0^{b}
8	50.0^{cd}	39.2^{de}	41.0^{c}	4.0^{b}	0.0^{b}	2.0^{b}
10	70.9^{b}	47.3^{cde}	59.1^{b}	14.2^{b}	0.0^{b}	5.1^{b}
14	86.2^{a}	55.1^{c}	70.6^{a}	45.4^{a}	8.2^{b}	26.8^{a}
Mean (V)	60.6^{a}	41.4^{b}		14.9^{a}	2.0^{b}	
LSD (*P*= 0.01)	V = 6.25, DAH = 9.28, V x DAH = 12.62			V = 9.12, DAH = 13.20, V x DAH = 18.25		

Values followed by different alphabetic letter/s are significant over one another.
Tomato fruits were stored at temperature of 31.2 ±1 ^{0}C and RH 34.5 ±5 %.

two varieties, irrespective of their ripening stages, indicated that 'Pusa Gaurav' (1240.14 µg) had 17.5 % more of P content than the 'Pusa Ruby' (1055.49 µg) (Table 2B). Unlike ripening of fruit on plant itself, storage of fruits harvested at green mature stage showed gradual increase in P content in both the varieties (Table 3B). Again higher P content in 'Pusa Gaurav' (1738.54 µg) than the 'Pusa Ruby' (1203.60 µg) was recorded (Table 3B).

K: Comparable trend for K was observed in fruits undergoing ripening either on the plant (Table 2C) or during storage (Table 3C). With progress of ripening, no significant change was recorded but 'Pusa Ruby' always maintained higher K content than the 'Pusa Gaurav'. Like the Ca, K was also less in slow ripening ('Pusa Gaurav') than the fast ripening variety ('Pusa Ruby').

Zn: Turning and pink stages for attached fruits showed maximum content of Zn and both the varieties had at par level of Zn during ripening (Table 2D). Storage duration had no significant effect on Zn level but, 'Pusa Gaurav' did show higher Zn level (28.62 µg) than the 'Pusa Ruby' (20.81 µg) (Table 3D).

Cu: Fruits of 'Pusa Gaurav' undergoing ripening on plant (5.72 µg) or during storage (6.03 µg) had higher Cu content than the 'Pusa Ruby' under similar conditions with values of 3.69 µg and 4.33 µg, respectively (Table 2E and 3E). Neither ripening stage nor the DAH had any significant effect on Cu content.

Mn: Comparison revealed significant fluctuations in Mn content for attached fruits during the course of ripening (Table 2F) than for the fruits harvested and stored (Table 3F). Further, significantly higher values were recorded for Mn in 'Pusa Gaurav' (5.37 and 9.08 µg) than the 'Pusa Ruby' (1.93 and 4.68 µg) for ripening of fruits under attached and detached conditions respectively.

Discussion

Varietal variation was noted in ripening behaviour of tomato fruit (Stevens and Rick, 1986). Results presented in Table 1 revealed that 'Pusa Gaurav' was comparatively slow ripening variety than 'Pusa Ruby'. Therefore, these two contrasting varieties were selected for evaluation of nutrients' content in the pericarp region of the fruit during ripening on the plant as well as during storage of fruits that were harvested at green mature stage.

Attached and detached conditions for ripening of tomato fruits differentially affected the Ca content. Differences were observed only during storage where inspite of being a slow ripening type, 'Pusa Gaurav' showed lower Ca although; DAH had no effect on Ca content (Table 2A and 3A). During storage, Almeida and Huber (1999) also reported relatively constant levels of Ca during the ripening in pericarp tissues of tomato fruits. Low fruit Ca levels have been associated with reduced postharvest life and increased rate of softening (Wills *et al.,* 1977; Poovaiah *et al.,* 1988). Delayed ripening response with increase in fruit Ca levels has been reported in tomato (Wills and Tirmazi, 1982). Detailed studies on the changes in soluble and bound Ca (Suwwan and Poovaiah, 1978; Rigney and Wills, 1981) revealed that it was the bound Ca content rather than total along with the process of Ca solubilization that played detrimental role for ripening related changes in tomato fruit. Increasing the Ca content of fruits by spraying or postharvest dip of fruits in $CaCl_2$ solution was also reported to increase the firmness of the fruit (Cooper and Bangerth, 1976) and delayed or even prevented the fruit ripening (Wills *et al.,* 1977). However, correlations between the decrease in bound Ca and fruit ripening might not always occur. For example, during degradation of the middle lamella by methyl esterase new binding sites for Ca were reported to be formed (Burns and Pressey, 1987). Likewise in mango fruit also, increasing the Ca content delayed the ripening and extended the shelf life (Wills *et al.,* 1988; Singh *et al.,* 2000). But, recently contradictory report by Joyce *et al.* (2001) showed that the Ca treated mango fruits exhibited no difference when compared with control fruits since Ca did not extended the shelf life for any of the four cultivars of mango.

Slower rate of ripening for fruits of 'Pusa Gaurav' in comparison to 'Pusa Ruby' inspite of lower total Ca content emphasized the role and importance of bound and soluble levels of Ca in determining the rate of ripening and extent of storability as demonstrated in tomato by Suwwan and Poovaiah (1978) and Rigney and Wills (1981) besides the alternation of Ca binding sites on the cell walls by enzymatic action of methyl esterase (Burns and Pressey, 1987), genotypical differences (Marschner, 1998) and other reported factors (Huber, 1983; Kumar *et al.,* 1998).

At different ripening stages, no fixed pattern was noticed for P content (Table 2B). But, during storage, higher P content was recorded at 8, 10 and 14 DAH when compared to 0 DAH (green mature stage) (Table 3B). Almeida and Huber (1999) too found the increase in P content with ripening when compared with green mature tomato fruits that were harvested and stored. This therefore, indicated the mobilization of P into the pericarp tissue from other parts of the fruit during storage. P provides the stability to membranes as it is component of phospholipids and also bridges adjoining phospholipids of membranes (Ratnayake *et al.,* 1978; Marschner, 1998). So, the higher P content in 'Pusa

Table 2. Ca, P, K, Zn, Cu and Mn in the pericarp tissue of tomato fruits in relatively fast ('Pusa Ruby') and slow ('Pusa Gaurav') ripening varieties. Fruits, at different ripening stages, were harvested directly from the plant on the same date

Ripening stage (RS)	'Pusa Ruby'	'Pusa Gaurav'	Mean (RS)
A.		Ca (µg g^{-1} dw)	
Green mature	573.45	430.09	501.77
Breaker	622.96	359.13	491.05
Turning	456.41	473.65	465.03
Pink	424.10	491.60	457.85
Light red	390.96	282.13	336.54
Red	446.10	453.80	449.95
Mean (V)	485.66	415.06	
LSD	V = NS, RS = NS, V x RS = NS		
B.		P (µg g^{-1} dw)	
Green mature	1071.49	1304.67	1188.08b
Breaker	1001.36	989.10	995.23c
Turning	944.18	1230.47	1087.32bc
Pink	896.89	1038.99	967.92c
Light red	1221.77	1530.06	1375.92a
Red	1197.25	1347.52	1272.43ab
Mean (V)	1055.49^{b}	1240.14^{a}	
LSD	V = 103.73**, RS = 179.70**, V x RS = NS		
C.		K (mg g^{-1} dw)	
Green mature	22.15	18.81	20.48
Breaker	28.34	15.99	22.16
Turning	19.39	18.21	18.80
Pink	25.64	22.06	23.85
Light red	27.91	12.13	20.02
Red	24.65	12.81	18.73
Mean (V)	24.68^{a}	16.67^{b}	
LSD	V = 5.18**, RS = NS, V x RS = NS		
D.		Zn (µg g^{-1} dw)	
Green mature	16.24	25.17	20.71^{b}
Breaker	24.10	29.27	26.68^{ab}
Turning	38.88	31.18	35.03^{a}
Pink	35.49	34.09	34.79^{a}
Light red	20.59	30.34	25.46^{ab}
Red	22.32	25.37	23.85^{b}
Mean (V)	26.28	29.24	
LSD	V = NS, RS = 9.60*, V x RS = NS		
E.		Cu (µg g^{-1} dw)	
Green mature	3.63	5.08	4.36
Breaker	4.46	5.18	4.82
Turning	2.91	6.08	4.50
Pink	2.38	6.07	4.23
Light red	3.89	5.68	4.78
Red	4.91	6.25	5.58
Mean (V)	3.69^{b}	5.72^{a}	
LSD	V = 1.07*, RS = NS, V x RS = NS		
F.		Mn (µg g^{-1} dw)	
Green mature	5.16	7.65	6.40^{a}
Breaker	1.65	6.02	3.83^{ab}
Turning	0.57	4.99	2.78^{b}
Pink	0.29	2.96	1.62^{b}
Light red	1.89	4.09	2.99^{b}
Red	2.02	6.50	4.26^{ab}
Mean (V)	1.93^{b}	5.37^{a}	
LSD	V = 2.03**, RS = 2.59*, V x RS = NS		

Values followed by different alphabetic letter/s are significant over one another. LSD at P = 0.05 (*) or P = 0.01 (**)

Table 3. Ca, P, K, Zn, Cu and Mn in the pericarp tissue of tomato fruits in relatively fast ('Pusa Ruby') and slow ('Pusa Gaurav') ripening varieties. Fruits were harvested at green mature stage and stored for 14 days at room conditions

Days after harvest (DAH)	'Pusa Ruby'	'Pusa Gaurav'	Mean (DAH)
A.		Ca (µg g^{-1} dw)	
0	573.45	430.08	501.77
5	499.54	393.04	446.29
8	485.98	332.77	409.38
10	610.58	309.00	459.79
14	524.62	476.00	500.31
Mean (V)	538.83^{a}	388.18^{b}	
LSD	V = 116.77**, DAH = NS, V x DAH = NS		
B.		P (µg g^{-1} dw)	
0	1071.49	1304.67	1188.08^{c}
5	1175.62	1463.78	1319.69^{bc}
8	1202.45	1807.96	1505.20^{b}
10	1137.30	1910.66	1523.98^{ab}
14	1431.15	2138.54	1784.84^{a}
Mean (V)	1203.60^{b}	1738.54^{a}	
LSD	V = 225.35**, DAH = 2261.30*, V x DAH = NS		
C.		K (mg g^{-1} dw)	
0	22.15	18.81	20.48
5	21.69	12.62	17.16
8	21.75	16.04	18.89
10	25.62	17.20	21.41
14	27.19	20.77	23.98
Mean (V)	23.68^{a}	17.76^{b}	
LSD	V = 4.36*, DAH = NS, V x DAH = NS		
D.		Zn (µg g^{-1} dw)	
0	16.24	25.17	20.71
5	14.11	22.35	18.23
8	27.19	32.01	29.60
10	28.16	36.79	32.48
14	18.33	26.75	22.54
Mean (V)	20.81^{b}	28.62^{a}	
LSD	V = 7.79*, DAH = NS, V x DAH = NS		
E.		Cu (µg g^{-1} dw)	
0	3.63	6.42	4.36
5	5.08	4.43	4.80
8	4.67	6.99	5.47
10	4.94	4.41	5.72
14	4.52	6.71	5.56
Mean (V)	4.33^{b}	6.03^{a}	
LSD	V = 1.41*, DAH = NS, V x DAH = NS		
F.		Mn (µg g^{-1} dw)	
0	5.16	7.65	6.40
5	3.82	7.01	5.41
8	2.65	10.14	6.39
10	5.13	8.07	6.60
14	6.64	12.55	9.59
Mean (V)	4.68^{b}	9.08^{a}	
LSD	V = 3.10**, DAH = NS, V x DAH = NS		

Values followed by different alphabetic letter/s are significant over one another. Tomato fruits were stored at temperature of 31.2 ±1 ^{0}C and RH 34.5 ±5 %. LSD at P = 0.05 (*) or P = 0.01 (**)

Gaurav', irrespective of attached or detached conditions of fruits, could be associated with its slow ripening behaviour.

For individual variety, ripening of tomato fruits either on plant or during storage had no effect on the level of K (Table 2C and 3C). Level of K in the bulk sap of pericarp was also reported to

remain relatively constant during ripening (Almeida and Huber, 1999). Comparison of two varieties, on the other hand, revealed significantly lower K content for 'Pusa Gaurav' than the 'Pusa Ruby'. A comparative study of non-ripening mutant (*rin*) and normal tomato fruit of variety 'Rutgers' revealed that mutant (non-ripening type) had lower K content in the pericarp tissue especially late during the developmental stages of fruits (Suwwan and Poovaiah, 1978). Further, Chun and Huber (1998) reported that there was need for optimum amount of K for realization of maximum polyglacturonase activity in tomato. Therefore, relatively lower content of K in 'Pusa Gaurav' might have also contributed for slower rate of ripening in this variety.

Ripening of fruit under attached (Table 2D) and detached (Table 3D) conditions showed differential effect on Zn content. The Zn was found to be higher in 'Pusa Gaurav' only for detached fruits. Zn is not only involved in maintenance of integrity of biomembranes (Cakmak and Marschner, 1988c; Pinton *et al.,* 1993) but it also protects membrane lipids and proteins against oxidative damage (Cakmak and Marschner, 1988a; 1988b). Since, loss of membrane integrity and enhanced free radical mediated damage are associated with senescing plant tissue or ripening of fruits (Marangoni *et al.,* 1996) therefore counter action of Zn might assist in delaying the damage done to membranes and free radical production. Significantly higher levels of Zn in fruits of 'Pusa Gaurav' during storage period in comparison with 'Pusa Ruby', could have delayed the ripening process.

Higher levels of Cu in fruits of 'Pusa Gaurav' for attached as well as detached conditions (Table 2E and 3E) and its slow ripening feature in comparison with 'Pusa Ruby' could be due to its antioxidative role through Cu-Zn SOD enzyme. The enzyme is directly involved in the detoxification of superoxide radicals (Elstner, 1982). It has role in protecting membrane lipids from the peroxidation and thereby delaying the process of senescence (Sandalio and del Rio, 1987).

Mn is a metal component of Mn SOD enzyme, which scavenge superoxide radicals (Bowler *et al.,* 1991). In transgenic plants of tobacco, higher Mn SOD caused lower solute leakage from mitochondria and chloroplast in comparison with control plants with low levels of Mn SOD (Bowler *et al.,* 1991). Low Mn content was also responsible for higher peroxidase activity in tomato and cucumber (Valenzuela *et al.,* 1993). It has been proposed that conditions such as ageing or stress that contributed to the loss of photosynthetic output below a certain threshold levels or loss in the integrity of chloroplast membrane might produce signal that initiate senescence process (Smart, 1994; Quirino *et al.,* 2000). Deficiency or low Mn causes decrease in chlorophyll content, glycolipids and polyunsaturated fatty acids (all are typical constituents of thylakoid membranes) (Constantopoulus, 1970), change in ultrastructure of thylakoid membrne due to loss of PS II associated with the stacked region of thylakoid membranes (Simpson and Robinson, 1984) and alternation in O_2 evolution (as Mn is also an essential part of water splitting system associated with PSII of photosynthetic machinery) (Kriedemann *et al.,* 1985). In view of above-mentioned roles of Mn, higher Mn content in 'Pusa Gaurav' (Tables 2F and 3F) appeared to contribute for its slow ripening behaviour along with Zn, Cu and P in comparison with fruits of 'Pusa Ruby'.

The study indicated the differences in the levels of some of the mineral elements in the pericarp tissue of the tomato fruits in two varieties with contrasting ripening behaviour. As these nutrients influence the ripening or ripening associated changes therefore obtained differences in the level of these nutrients could also be attributed as one of the causes for varietal differences in ripening behaviour. Data indicated that possibly the higher bound form, rather than total content of Ca and more of P, Zn, Cu and Mn along with low of K contributed for slower ripening for the fruits of 'Pusa Gaurav' in comparison to 'Pusa Ruby' possibly by delaying the ripening associated deterioration of cell wall and membranes along with the potential damaging effects due to enhanced oxidative system during the course of ripening.

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Screening of cultivated okra, related species and their inter specific hybrid derivatives for resistance to powdery mildew (*Erysiphe cichoracearum* DC)

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Abstract

Okra germplasm, consisting of 85 accessions, which included cultivars, related species and their inter specific hybrids was screened for two seasons, while their amphidiploids, backcrosses and F_3 generations were screened for one season for powdery mildew resistance (*Erysiphe cichoracearum* DC) under severe field epiphytotic conditions. Only the wild species *A. caillei*-2 and *A. moschatus*-1 were found immune while two biotypes of *A. tetraphyllus*, *A. manihot* spp. *manihot*, *A. manihot* spp. *tetraphyllus*, *A. manihot* (L.) Medikus and *A. angulosus* were found highly resistant to powdery mildew in both seasons. *A. tuberculatus*-1, *A. caillei*-1, *A. ficulneus* and cultivars of *A. esculentus* were susceptible. Reaction of inter specific hybrids, backcrosses and amphidiploids revealed that the resistance in *A. caille*i-2, *A. angulosus* and *A. manihot* spp. tetraphyllus were partially dominant. Further, it was observed that in F_3 generations, only the lines of *A. caillei* -2 inter specific hybrid derivatives (lines derived from hybrids having *A. caillei*-2 as one parent) were found highly resistant to powdery mildew.

Key words: Okra, powdery mildew resistance, inter-specific hybrids, amphidiploids

Introduction

Powdery mildew (*Erysiphe cichoracearum* DC) is a serious disease of okra in India (Joi and Shende, 1979; Raj *et al.*, 1993 and Neeraja *et al.*, 2004). The literature reveals that there is no source of resistance to powdery mildew in *A. esculentus* and a search for resistance should be invariably shifted to related species. Jambhale and Nerkar (1992) reported *A. manihot*, *A. tetraphyllus*, *A. manihot* spp. *manihot* and *A. moschatus* were immune to powdery mildew. Considering the above facts, an experiment was conducted to screen okra cultivars, related wild species and their inter-specific hybrids, amphidiploids, backcrosses and F_3's for field resistance to powdery mildew.

Materials and methods

A programme to transfer resistance to yellow vein mosaic virus (YVMV) disease from wild *Abelmoschus* to cultivated okra was undertaken in this department during 2002-2005 (Prabu, 2005). The same experimental material consisting of *A. esculentus* cultivars, related wild species (*viz.*, *A. tetraphyllus*-1 and 2, *A. manihot* spp *manihot*, *A. moschatus*, *A. manihot* spp. *tetraphyllus*, *A. manihot* (L.) Medikus, *A. angulosus, A. tuberculatus*-1, *A. caillei*-1 and 2 and *A. ficulneus*-1) and their inter-specific hybrids were screened for two seasons (Kharif, 2004 and summer, 2005) while their amphidiploids, backcrosses and F_3's were screened for one season (summer, 2005) for powdery mildew resistance. In summer, humidity in the field was maintained by giving frequent irrigations. A highly susceptible Pusa Sawani was grown around the experimental field for providing uniform powdery mildew inoculums. No control measures were done. However, all the other agronomical practices were carried out as per recommendations. The observations on the disease severity and intensity were recorded at 30 days interval on ten randomly selected plants of each genotype. The disease reaction was recorded by following 0-4 scale:

Scale for screening okra for powdery mildew resistance

Scale	Symptoms	Disease rating
Grade 0	No infection	Immune (I)
Grade 1	Powdery mildew specks having less than 10 % leaf area affected	Highly Resistant (HR)
Grade 2	Large patches but no coalescing with 11-25 % leaf area affected	Moderately resistant (MR)
Grade 3	Coalescing large patches covering 26-50 % leaf area affected	Moderately susceptible (MS)
Grade 4	More than 51 % leaf area affected, coupled with defoliation of leaves	Highly susceptible (HS)

Results and discussion

In the present findings (Table 1), it was observed that the wild species *A. moschatus*-1 and *A. caillei*-2 were immune to powdery mildew disease while *A. tetraphyllus* lines (1 and 2), *A. angulosus*, *A. manihot* (L.) Medikus, *A. manihot* spp. manihot and *A. manihot* spp. tetraphyllus were found highly resistant. Further, it was observed that *A. ficulneus*-1 was found moderately susceptible, while *A. caillei*-1 and *A. tuberculatus* were highly susceptible to powdery mildew disease. Prabhu *et al.* (1971) observed *A. ficulneus*, *A. tetraphyllus* and *A. tuberculatus* to be highly susceptible while *A. manihot* var. pungens, *A. moschatus* and *A. manihot* as resistant. Joi and Shende (1979) observed *A. manihot* and *A. tetraphyllus* to be immune to powdery mildew. Jambhale and Nerkar (1992) reported that *A. tetraphyllus*, *A. manihot*, *A. manihot* spp. *manihot* and *A. moschatus* were immune

Table 1. Screening cultivated okra, related species and their inter specific hybrid derivatives for resistance to powdery mildew

Sr No.	Material	Source	Powdery mildew incidence (%)						Disease reaction
			Kharif 2004		Summer 2005		Mean		
				Disease score		Disease Score		Disease score	
	Okra cultivars (*Abelmoschus esculentus* (L.) Moench)								
1	Pusa Sawani	A.I.C.V.I.P, Rahuri	70.63	4	70.00	4	70.32	4	HS
2	Red Bhendi	A.I.C.V.I.P, Rahuri	61.88	4	50.00	3	55.94	4	HS
3	Arka Abhay	A.I.C.V.I.P, Rahuri	48.33	3	60.00	4	54.17	4	HS
4	Phule Utkarsha	A.I.C.V.I.P, Rahuri	50.50	3	75.00	4	62.75	4	HS
5	Arka Anamika	A.I.C.V.I.P, Rahuri	60.00	4	50.00	3	55.00	4	HS
6	Parbhani Kranti	A.I.C.V.I.P, Rahuri	48.00	3	80.00	4	64.00	4	HS
7	Varsha Uphar	A.I.C.V.I.P, Rahuri	59.38	4	70.00	4	64.69	4	HS
8	P7	A.I.C.V.I.P, Rahuri	51.50	4	45.00	3	48.25	3	MS
	Wild *Abelmoschus* spp.								
9	*A. tuberculatus* - 1	N.B.P.G.R., Akola	51.00	4	55.00	4	53.00	4	HS
10	*A. tetraphyllus* - 1	I.I.V.R., Varanasi	8.50	1	4.50	1	6.50	1	HR
11	*A. tetraphyllus* - 2	N.B.P.G.R., Akola	5.25	1	3.00	1	4.13	1	HR
12	*A. ficulneus* - 1	N.B.P.G.R., Akola	52.50	4	25.00	2	38.75	3	MS
13	*A. moschatus* - 1	I.I.V.R., Varanasi	0.00	0	0.00	0	0.00	0	I
14	*A. caillei* - 1	N.B.P.G.R., Thrissur	57.50	4	50.00	3	53.75	4	HS
15	*A. caillei* - 2	K.A.U., Thrissur	0.00	0	0.00	0	0.00	0	I
16	*A. manihot* spp.*manihot*	N.B.P.G.R., Thrissur	6.75	1	5.50	1	6.13	1	HR
17	*A. manihot* spp.*tetraphyllus*	N.B.P.G.R., Thrissur	7.50	1	3.50	1	5.50	1	HR
18	*A. angulosus*	N.B.P.G.R., Thrissur	7.00	1	11.00	2	9.00	1	HR
19	*A. manihot* (L.) Medikus	N.B.P.G.R., Thrissur	8.50	1	6.50	1	7.50	1	HR
20	Pusa Sawani x *A. tetraphyllus* - 2	F_1	32.00	3	35.00	3	33.50	3	MS
21	Pusa Sawani x *A. caillei* - 2	F_1	10.00	1	10.00	1	10.00	1	HR
22	*A. caillei* - 2 x Pusa Sawani	F_1	10.50	2	7.00	1	8.75	1	HR
23	Pusa Sawani x *A. manihot* spp. *manihot*	F_1	35.00	3	25.00	2	30.00	3	MS
24	Pusa Sawani x *A. manihot* spp. *tetraphyllus*	F_1	30.00	3	20.00	2	25.00	2	MR
25	Pusa Sawani x *A. manihot* (L.) Medikus	F_1	30.00	3	15.00	2	22.50	2	MR
26	Red Bhendi x *A. tetraphyllus* -2	F_1	40.00	3	25.00	2	32.50	3	MS
27	Red Bhendi x *A. caillei* -2	F_1	9.00	1	6.00	1	7.50	1	HR
28	*A. caillei* – 2 x Red Bhendi	F_1	5.00	1	10.00	1	7.50	1	HR
29	Red Bhendi x *A. manihot* spp. *manihot*	F_1	26.25	3	25.00	2	25.63	3	MS
30	Red Bhendi x *A. manihot* spp. *tetraphyllus*	F_1	11.00	2	20.00	2	15.50	2	MR
31	Arka Abhay x *A. tetraphyllus* -2	F_1	27.50	3	25.00	2	26.25	3	MS
32	Phule Utkarsha x *A. tuberculatus* - 1	F_1	36.00	3	70.00	4	53.00	4	HS
33	Phule Utkarsha x *A. tetraphyllus* - 1	F_1	37.50	3	34.00	3	35.75	3	MS
34	Phule Utkarsha x *A. tetraphyllus* -2	F_1	46.00	3	29.00	3	37.50	3	MS
35	Phule Utkarsha x *A. caillei* -1	F_1	49.00	3	37.50	3	43.25	3	MS
36	Phule Utkarsha x *A. caillei* -2	F_1	8.00	1	12.00	2	10.00	1	HR
37	*A. caillei* –2 x Phule Utkarsha	F_1	7.00	1	12.00	2	9.50	1	HR
38	Phule Utkarsha x *A. manihot* spp. *manihot*	F_1	25.00	2	17.50	2	21.25	2	MR
39	Phule Utkarsha x *A. manihot* spp. *tetraphyllus*	F_1	20.00	2	10.00	1	15.00	2	MR
40	Phule Utkarsha x *A. angulosus*	F_1	26.50	3	15.00	2	20.75	2	MR
41	Phule Utkarsha x *A. manihot* (L.) Medikus	F_1	30.00	3	15.00	2	22.50	2	MR
42	Arka Anamika x *A. tetraphyllus* - 1	F_1	30.00	3	25.00	2	27.50	3	MS
43	Arka Anamika x *A. tetraphyllus* -2	F_1	23.00	3	20.00	2	21.50	2	MR
44	Arka Anamika x *A. caillei* -2	F_1	11.00	2	7.50	1	9.25	1	HR
45	*A. caillei* –2 x Arka Anamika	F_1	9.50	1	8.50	1	9.00	1	HR
46	Arka Anamika x *A. manihot* spp.*manihot*	F_1	25.00	2	25.00	2	25.00	2	MR
47	Arka Anamika x *A. manihot* spp.*tetraphyllus*	F_1	13.00	2	25.00	2	19.00	2	MR
48	Arka Anamika x *A. manihot* (L.) Medikus	F_1	32.00	3	21.50	3	26.75	3	MS
49	Parbhani Kranti *x A. tuberculatus* -1	F_1	28.75	3	62.50	4	45.63	3	MS
50	Parbhani Kranti x *A. tetraphyllus* -1	F_1	25.50	3	50.00	3	37.75	3	MS
51	Parbhani Kranti x *A. tetraphyllus* -2	F_1	24.50	2	35.00	3	29.75	3	MS
52	Parbhani Kranti x *A. caillei* - 1	F_1	26.50	3	50.00	3	38.25	3	MS
53	Parbhani Kranti x *A. caillei* - 2	F_1	9.50	1	10.00	1	9.75	1	HR
54	*A. caillei*-2 x Parbhani Kranti	F_1	9.00	1	10.00	1	9.50	1	HR

Sr No.	Material	Source	Powdery mildew incidence (%)						Disease reaction
			Kharif 2004		Summer 2005		Mean		
				Disease score		Disease Score		Disease score	
55	Parbhani Kranti *x A. manihot* spp. manihot	F_1	19.25	2	30.00	3	24.63	2	MR
56	Parbhani Kranti x *A. manihot* spp.*tetraphyllus*	F_1	19.00	2	15.00	2	17.00	2	MR
57	Parbhani Kranti x *A. angulosus*	F_1	28.00	3	12.50	2	20.25	2	MR
58	Parbhani Kranti x *A. manihot* (L.) Medikus	F_1	40.00	3	30.00	3	35.00	3	MS
59	Varsha Uphar x *A. tuberculatus* - 1	F_1	26.50	3	55.00	4	40.75	3	MS
60	Varsha Uphar x *A. tetraphyllus* - 1	F_1	27.00	3	37.50	3	32.25	3	MS
61	Varsha Uphar x *A. tetraphyllus* - 2	F_1	24.00	2	35.00	3	29.50	3	MS
62	Varsha Uphar x *A. caillei* - 1	F_1	35.00	3	60.00	4	47.50	3	MS
63	Varsha Uphar x *A. caillei* - 2	F_1	8.00	1	11.00	2	9.50	1	HR
64	*A. caillei*-2 x Varsha Uphar	F_1	11.00	2	7.50	1	9.25	1	HR
65	Varsha Uphar x *A. manihot* spp.*manihot*	F_1	27.00	3	37.50	3	32.25	3	MS
66	Varsha Uphar x *A. manihot* spp.*tetraphyllus*	F_1	17.25	2	18.75	2	18.00	2	MR
67	Varsha Uphar x *A. angulosus*	F_1	25.00	2	16.50	2	20.75	2	MR
68	Varsha Uphar x *A. manihot* (L.) Medikus	F_1	30.00	3	20.00	2	25.00	2	MR
69	P7 x *A. tetraphyllus* - 1	F_1	26.00	3	30.00	3	28.00	3	MS
70	P7 x *A. tetraphyllus* - 2	F_1	31.50	3	45.00	3	38.25	3	MS
	Amphidiploids								
71	Phule utkarsha x *A.tetraphyllus*-1	Amphidiploids	-	-	25..50	2	25..50	3	MS
72	Phule utkarsha x *A.tetraphyllus*-2	Amphidiploids	-	-	27..00	2	27.00	2	MS
73	Phule utkarsha x *A. manihot* spp.*tetraphyllus*	Amphidiploids	-	-	13..50	2	13..50	2	MR
74	Phule utkarsha x *A. manihot* spp. *manihot*	Amphidiploids	-	-	12.00	2	12.00	2	MR
	Backcrosses		-	-					
75	Varsha Uphar x (Varsha Uphar x *A. manihot* spp *tetraphyllus*)	Backcrosses	-	-	15..00	2	15..00	2	MR
76	Parbhani Kranti x (Parbhani ranti x *A. manihot* spp. *tetraphyllus*)	Backcrosses	-	-	19.50.	2	19.50.	2	MR
77	Varsha Uphar x (*A. caillei*-2 x Varsha Uphar)	Backcrosses	-	-	7.50	1	7.50	1	HR
78	(*A. caillei*-2 x Arka Anamika) x Arka Anamika	Backcrosses	-	-	6.50	1	6.50	1	HR
79	(*A. caillei*-2 x Varsha Uphar) x Varsha Uphar	Backcrosses	-	-	7.25	1	7.25	1	HR
	F_3 generations		-	-					
80	Phule Utkarsha x *A. tetraphyllus* - 2	F_2 -37	-	-	24.5	2	14.5	2	MR
81	*A. caillei*-2 x Varsha Uphar	F_2- 71	-	-	5.5	1	5.5	1	HR
82	*A. caillei*-2 x Varsha Uphar	F_2- 52	-	-	6.5	1	6.5	1	HR
83	*A. caillei*-2 x Varsha Uphar	F_2 - 15	-	-	3.5	1	3.5	1	HR
84	*A. caillei*-2 x Arka Anamika	F_2 -5	-	-	9.0	1	9.0	1	HR
85	*A. caillei*-2 x Arka Anamika	F_2 -18	-	-	7.0	1	7.0	1	HR

while *A. ficulneus*, *A. tuberculatus* and *A. esculentus* cultivars were found susceptible to powdery mildew. The present results are in agreement with the earlier workers only for *A. moschatus*, *A. tuberculatus* and *A. esculentus*. The present study however revealed that *A. manihot* (L.) Medikus, *A. tetraphyllus* and *A. manihot* spp. *manihot* are highly resistant to the disease. This is in line with Dhankar (1998) and Fageria *et al.* (2001). Prabhu *et al.* (1971) and Jambhale and Nerkar (1992) reported *A. ficulneus* to be susceptible to powdery mildew. In the present study, *A. ficulneus*-1 was found highly susceptible during kharif season but expressed moderate resistance to the disease during summer season, while *A. angulosus* was found highly resistant during kharif season but showed only moderate resistance during summer season. This might be due to the different seed material and environmental conditions under which the material was grown. None of the *A. esculentus* cultivars, screened for two seasons, were found resistant to powdery mildew. These findings are in consonance with those of Premnath (1975), Joi and Shende (1979), Jambhale and Nerkar (1992) and Neeraja *et al.* (2004).

Among the inter-specific hybrids, hybrids having *A. caillei*-2 as one of its parents were found highly resistant to powdery mildew incidence in both the seasons. Nerkar (1990) reported *A. caillei* to be highly resistant to powdery mildew. None of the other inter-specific hybrids screened were found highly resistant to powdery mildew. However, sixteen inter-specific hybrids showed moderate resistance to powdery mildew either in one or in both the seasons. The hybrids between *A. esculentus* and *A. manihot* spp. *tetraphyllus* and *A. esculentus* and *A. angulosus* were found to be moderately resistant to powdery mildew. Thus in above inter-specific hybrids, resistance seems to be partially dominant. Among the amphidiploids, only Phule Utkarsha x *A. manihot* spp. *tetraphyllus* and Phule Utkarsha x *A. manihot* spp. *manihot* showed moderate resistance to powdery mildew. From the present study it was observed that the backcrosses and F_3 of *A. caillei*-2 hybrids (hybrids having *A. caillei*-2 as one of its parents) were found highly resistant to powdery mildew. These hybrids can be exploited in the future breeding programme by backcrossing them continuously to their respective *A. esculentus*

cultivars followed by selection in segregating generations in order to develop a new okra cultivar having desirable recombination of both powdery mildew resistance and *A. esculentus* with consumer preference characters.

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Low-temperature threshold and growth degree day (GDD) for two pistachio cultivars

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Abstract

Chilling threshold and growth degree day (GDD) of two main pistachio pistillate cultivars were determined. Layout was factorial based on a complete randomized design with three factors, two cultivars (Qazvini and Ouhedi), 5 thermal levels (+2, 0, -2, -4 and -6 ^oC) and three developmental stages including dormant bud, swelling bud and fully bloomed flowers for chilling studies. Critical temperature for reversible tissue colour change was determined as -4^oC at bud stage, -2^oC at blooming bud and +2^oC at flower. Decreasing temperature down to two more degrees (*e.g.* -6 ^oC at bud) could shift the damage into the irreversible browning injury. For GDD measurements, three factors, including cultivar, thermal accumulation (calculation based on +4.5^oC) and phenological stages were considered. Kernel filling period varied in two cultivars; Ouhedi's bigger kernel required more time to grow fully and more growth degrees day. Qazvini needed 2561.044 GDD and 138.5 days for total bearing period (flowering to harvest), and 623.363 GDD and 30 days for kernel filling period. Ouhedi needed 2917.823 GDD and 160 days for total period, and 730.61 GDD and 33.5 days for kernel filling.

Key words: Pistachio, chilling injury, growth degree day, flowering, fruit development

Introduction

Spring chilling is a major environmental stress in some years causing serious injuries to pistillate organs of pistachio. Spring chilling also adversely affects fruit set and development processes such as pollination rate and fertilization (Faust, 1997; Quamme, 1978). Temperature and flowering-fruiting phenomenon are closely related (Baskerville and Emin, 1969). Pistachio pistillates experience some significant phenological stages in which some commercially-important events including nut shell (endocarp) hardening, resuming embryo development, nut kernel filling (seed growth) happen. Determination of the incidence time of each stage can significantly help the cultural and developmental practices directly leading to quantitative and qualitative yield improvement. The tree needs a hot and dry season to develop seed (kernel) expressed partly by growth degree-day.

Amount of growth degree-day (GDD) needed for fruit ripening processes, particularly "kernel filling", accompanying with spring chilling injury threshold are regarded as two main criteria to select an area to develop pistachio cultivation.

"Ouhedi" is a globally well-known cultivar because of its market-favorable size and tasteful kernel. "Qazvini" is a small-sized nut; it is, however, famous and diversely used in confectionary industries because of its dark green and fragrant kernel.

A part of this study was carried out to determine critical temperatures for incidence of the strain in different low-temperature levels, on females of two commercial cultivars.

Materials and methods

Experiment plots were located at 35° 8' N, 49° 45' E and 1260 m altitude in a well-managed orchard.

Chilling injury: Shoots containing flower buds were collected from selected trees at different phenological stages (bud, blooming and full-bloomed inflorescence) and transferred to a low-temperature chamber. Temperature was reduced from +2^oC, down to -6 at a rate of 2^oC per hour. At the end of each one-hour phase, some buds and/or bloomed inflorescence samples were removed from chamber and assessed for macro- (naked-eye visible) and microscopic injuries. The critical temperatures for incidence of different injury levels were recorded and visual tissue browning was considered as the first serious (probably irreversible) injury (Quamme, 1978). Data were statistically analyzed.

Growth degree-day: Flowering and fruiting phenology of two main commercial cultivars of pistachio ("Ouhedi" and "Qazvini") were screened and the date of 50% blooming, start and finish of fast kernel filling period, and harvest were recorded. Experiment layout was based on a complete randomized block design in three replications. Each replication included three trees on which three branches in different directions were labeled for sampling.

Temperature data was received from the nearest synoptic climatology station.

GDD was calculated using the following equation considering +4.5^oC (Faust, 1997) as the baseline temperature (Tb).

$GDD= \sum n (T_{max}+T_{min})/2-T_b)$

where,

n: the number of days of mentioned period

T_{max}: maximum daily temperature

T_{min}: minimum daily temperature

Data was analyzed using Microsoft Excel® and SAS® software. Cultivars were compared statistically using Hoteling-Lawley Trace T-test.

Results and discussion

Chilling could adversely affect the reproductive structures at different morphological-anatomical levels varying with temperature; injury from higher temperatures to lower included deterioration or necrosis of stigma, style, inflorescence, current spring shoot, and whole flower buds, respectively.

Critical injury temperatures are presented in Table 1. Injuries were categorized into four levels including healthy, tissue color change (reversible injury), browning (serious and probably irreversible), and complete necrosis. No significant difference was revealed between two cultivars.

Based on the results, critical temperatures for occurance of reversible (tissue color change) and irreversible (tissue browning) injuries are summarized in Table 2. Flower buds' chilling resistance was the highest among all reproductive structures. Their resistance, however, decreased as they finished their dormancy, especially just before blooming. Decrease in temperature down to about -4 °C for about 2h could lead to their delayed bloom with some blooming and flowering abnormalities. Lower temperatures (-4 to -6 for about 6 hours) resulted in the necrosis and deterioration of very young pistils in closed buds (Fig. 1).

In the first visible injury level, the vegetative tip growth (on the inflorescence shoot) became dark green in colour. At this stage, the fertilization rate and subsequent fruit set can decrease, possibly because of negative effect of the lower temperature on the pollen tube growth and shortening of the physiologically effective pollen-reception age of the pistil. With more temperature reduction (low to about -2 for 2h), stigma, and then style showed morphological necrosis and anatomical deterioration, which resulted in pollen germination and tube growth inhibition (Fig. 2).

Complete necrosis of flowers, and then, inflorescence, was caused by about -4°C for about 2h (Fig. 3).

Growth degree-day: Table 3 summarizes phenology of main flowering and fruiting phenomena of two pistachio cultivars. Obviously, in Qazvin area, Ouhedi is an early-flowering and late-ripening and Qazvini a late flowering and early ripening cultivar. As apparent from the table, flowering to start of kernel filling period is the longest. It partly includes the period of fertilization, primarily embryo and mainly the full growth of endocarp.

Kernel filling period varied in two cultivars; Ouhedi's bigger

Table 2. Critical temperatures for chilling injury incidence at two levels in three developmental stages

Stage	Color change	Browning
Bud	-4 °C	-6
Blooming bud	-2	-4
Bloomed inflorescence	+2	0

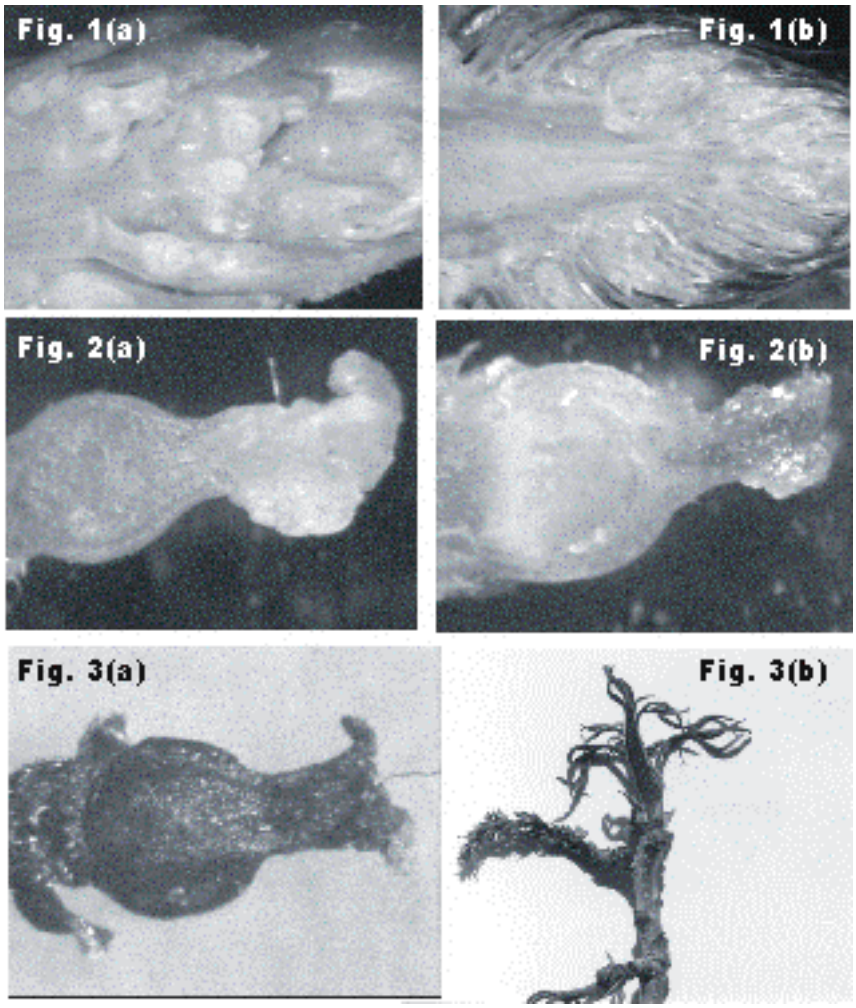

Fig. 1. Healthy (a) and browned (b) bud
Fig. 2. Healthy (a) and necrotic (b) stigma and style
Fig. 3. Complete flower (a) inflorescence (b) necrosis

Table 1. Mean comparison for different injury levels in five temperatures through Duncan method (P<0.01). In each column, bold letters are presenting critical injury incidence for two important levels (tissue color change and browning)*

Temperature (°C)	Bud				Blooming bud				Bloomed inflorescence			
	Healthy	Color change	Browning	Necrosis	Healthy	Color change	Browning	Necrosis	Healthy	Color change	Browning	Necrosis
+2	*20.0a	0.0b	0.0c	0.0b	16.6a	3.33b	0.0d	0.0c	2.16b	**11.0**a	6.3b	0.5b
0	19.6a	0.33b	0.0c	0.0b	15.8a	4.16b	0.0d	0.0c	0.0b	1.83b	14.0a	4.16c
-2	18.6a	1.33b	0.0c	0.0b	2.83b	**13.5**a	3.3b	0.0c	0.0b	0.16b	6.83b	13.0b
-4	0.16b	**11.5**a	7.16b	1.6b	0.33b	1.8b	**14.5**a	3.6b	0.0b	0.0b	1.0c	19.0a
-6	0.0b	1.5b	**15.8**a	2.6a	0.0b	0.0b	1.66c	17.8a	0.0b	0.0b	0.0c	20.0a

*Numbers are based on 20 samples each including 5 buds

Table 3. Phenology of important events of flowering and fruiting of two pistachio cultivars

Cultivar	Stage			
	Flowering	Kernel filling start	Kernel filling finish	Harvest
Ouhedi	16-26 April	13-18 July	15-29 Aug.	20-26 Sept.
Qazvini	20-27 April	3-9 July	4-30 Aug.	1-6 Sept.

Table 4. Length of period and GDD required for each important periods of pistachio cultivars flowering and fruiting in Qazvin area

Cultivar		Periods			
		Flowering to start of kernel filling	Kernel filling	Finish of kernel filling to harvest	Total (flowering to harvest)
Ouhedi	Length (day)	87.0	33.5	39.5	160.0
	GDD (degree)	1406.4	730.6	780.8	2917.8
Qazvini	Length (day)	74.5	30.0	34.0	138.5
	GDD (degree)	1178.7	632.4	750.0	2561.1

kernel took more time to grow fully and needed more growth degree days. The period is horticulturally important as the yield (total kernel weight) and market value (based on split and full nut percent) of the crop depends highly on the proper growth of kernel. It lasts about one month; some cultural practices (hormonal and chemical treatments) are aimed to help the embryo growth in that period (Gholipour, 2005). GDD requirements for various growth and development process of Ouhedi were higher than Qazvini (Table 4). Proper GDD accumulation during the kernel-filling period is one of main criteria when evaluating a new area for pistachio cultivation.

The study revealed that chilling threshold and growth degree day requirements of the two main pistachio pistillate cultivars are different. Critical temperature for reversible tissue colour change was -4°C at bud, -2°C at blooming bud and +2°C at flower stage. Decreasing temperature down to two more degrees could shift the damage into the irreversible injury. Cultivar, Ouhedi's with bigger kernel required more GDD for fully developed kernel.

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High frequency *in vitro* shoot regeneration of *Capparis deciduas* from shoot tip culture

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Abstract

Capparis deciduas is an important constituent of desert ecosystem, however, due to population pressure on the land their stands are reducing at an alarming rate. Establishment through root suckers and seeds being very slow and cumbersome, remains the major bottleneck for their reestablishment in the area. Thus, realizing the constraint, a rapid and efficient micropropagation protocol for multiple shoot regeneration employing shoot tip explant was developed. The protocol is more efficient and reproducible than reported earlier. Maximum number of explants (100%) responded on Murashige and Skoog (MS) medium supplemented with 6-benzyl amino purine (BAP) 7 mg L^{-1} and naphthaline acetic acid (NAA) 0.1 mg L^{-1} while number of shoots per explant were maximum (8.5) on alone BAP 7 mg L^{-1}. Regenerated shoots could be rooted best on MS medium supplemented with indole butyric acid (IBA) 1 mg L^{-1}. Rooted shoots could be hardened and transplanted in to the field.

Key words: *Capparis deciduas,* shoot tip culture, plant growth regulators, micropropagation, 6-benzyl amino purine, naphthaline acetic acid, indole butyric acid, arid horticulture

Introduction

Ker [*Capparis deciduas* (forsk) Edgew], an important indigenous shrub, belongs to family Capparidaceae. There are 234 species of *Capparis*, of which, 26 are known to occur in India (Hooker, 1960). Among these, *Capparis deciduas* is an important constituent of desert ecosystem and plays an important role in the rural economy of western Rajasthan by providing food, feed and fuel. Ker fruits are rich in carbohydrates and minerals and can be used to make pickle and vegetable curry. The stem and root bark extracts contain isocodonocarpine and other alkaloids, which are effective in treating asthma, inflammation, cough etc. (Ahemad *et al.,* 1989). Its termite resistant wood is used by rural people for making handles and cart wheels (Gupta *et al.,* 1989). However, due to anthropogenic activities such as deforestation for agriculture, its natural population is declining at an alarming rate. Hence, there is a need for large scale plantation of this shrub of economic, medicinal and ecological significance on marginal and unproductive lands of arid region.

Ker is naturally propagated through root suckers with extremely slow rate of multiplication and through seeds. The seeds of ker remain viable for a short period and establishment of saplings is difficult under hostile environment of Thar Desert. The vegetative propagation of *C. deciduas* through stem cutting though has been reported (Vashishtha, 1987), however, with negligible success rate (Bhargava *et al.*, 2000). Alternatively plant tissue culture techniques provide a means for mass clonal multiplication of selected trees/genotypes (Haissig *et al.,* 1987; Cheliak and Rogers, 1990; Hammatt, 1992).

Attempts have been made for micropropagation of *C. deciduas* using nodal explants by Deora and Shekhawat (1995) and Tyagi and Kothari (1997). In present communication we report a high frequency regeneration protocol using shoot tip explant. Advantage of shoot tip culture method for rapid multiplication and propagation is well accepted (Soniya and Das, 2002). The present investigation also reports successful rooting and hardening of the regenerated plants.

Materials and methods

Ripe fruits from elite shrub of *C. deciduas* were collected during the months of November and December 2005. The seeds were extracted from freshly harvested fruits by washing out the pulp with distilled water and dried for three days at room temperature. Dried seeds were treated with 70% ethanol for 30-40 seconds followed by surface sterilization with 0.1% solution of $HgCl_2$ (W/V) for 10 minutes. Surface sterilized seeds were washed with sterilized distilled water for 6-8 times and placed in test tube on filter paper whose lower half was dipped in ½ MS salts solution. All operations were carried out in a laminar airflow cabinet. Shoot tip explants measuring 1.0-1.5 cm were taken from 30 -35 days old *in vitro* grown seedlings and inoculated vertically on MS medium (Murashige and Skoog, 1962) supplemented with different cytokinins (BAP and Kn) at varying concentration (1.0 -7.0 mg L^{-1}) either singly or in combination of auxin (NAA, 0.1-0.5 mg L^{-1}) for multiple shoot induction. The pH of the culture medium was adjusted to 5.8 before autoclaving at 121°C for 18 minutes. The cultures were incubated at 27±0.5°C with a light intensity of 38 µ mol m^{-2} S^{-1} and 14/10 hrs photoperiod. Observations were recorded on percent response, days to multiple shoot induction, number of shoots per explant and average shoot length. All treatments were replicated 10 times and data were analyzed using CRD.

The regenerated shoots measuring 3-4 cm were excised and transferred to MS medium supplemented with or without auxins (NAA and IBA, 0.1-1.0 mg L^{-1}) for root induction. Plantlets each with 2-5 roots were hardened using pre acclimation chambers (PAC) as described by Bhargava *et al.* (2003). PAC hardened plants were transferred to pots and placed in protected and shaded area for 30-45 days before final transplanting in the field.

Results and discussion

The shoot tip explants cultured on hormone free medium remained green but failed to produce shoots even after 3 weeks of incubation. Incorporation of cytokinins both BAP and Kn stimulated multiple shoot induction within 2 weeks of incubation thus indicating that shoot induction is a function of cytokinins activity. Time taken in multiple shoot induction (11.10 and 11.80 days) was less on lower levels of cytokinins (1mg L^{-1}). Percent explants responding were more (86%) on higher levels of cytokinins (7 mg L^{-1}, Table 1). The cultured explants showed their response by increase in length and formation of callus at basal cut end of the shoot tips. Later from this callus clump, new shoot buds were induced within 4-5 weeks of incubation. Number of shoots per explant was significantly more (8.50 and 6.00) on higher levels of cytokinins *i.e.* 7 mg L^{-1} either BAP or Kn, respectively (Fig. 1A) as compared to lower levels of Kn (1 and 3 mg L^{-1}) in the medium (2-4 shoots per explant, Table 1). A concomitant decline in shoot length was observed as the concentration of cytokinins increased in the medium. Moreover, increased levels of cytokinins in the medium resulted in excessive cell division, and produced a clump of shoot. Those shoots having normal mode of regeneration developed into healthy plantlets. Similar observations in various tree species are also recorded by Ahuja *et al.* (2002) and Pattnaik and Debata (1996). However, many of the stunted shoots when placed on plant growth regulator free medium, developed into normal shoots (Fig. 1B). The superiority of BAP over other cytokinins as observed in the present investigation has been well documented for several tree species (Bonga and Von Aderkas, 1990). BAP is also the least expensive source of cytokinin.

Table 1. Effect of plant growth regulators on shoot regeneration from shoot tip explant of *Capparis deciduas*

Treatment (mg L^{-1})	Response (%)	Time taken in multiple shoot induction (MSI)	Number of shoots per explant (30 DAI)	Average length of shoots (cm) (30 DAI)
MS plain + BAP	0.00	0.00	0.00	0.00
1	66.60	11.10	6.80	3.40
3	80.00	12.60	7.20	3.20
5	80.00	13.10	8.20	2.00
7	86.00	15.30	8.50	1.00
Kn				
1	73.30	11.80	2.10	3.10
3	73.30	14.40	2.10	1.60
5	80.00	14.70	4.40	0.60
7	86.00	15.10	6.00	0.40
CD (*P*=0.05)	-	0.65	0.45	0.16
BAP+NAA				
3+0.1	73.30	7.20	8.80	4.10
5+0.1	80.00	8.20	8.10	3.30
7+0.1	100.00	10.10	7.90	3.10
3+0.5	66.60	7.20	7.90	4.90
5+0.5	80.00	7.30	5.60	3.70
7+0.5	93.30	9.10	2.60	3.50
Kn+NAA				
3+0.1	86.60	8.20	5.50	4.40
5+0.1	80.00	9.10	7.80	3.10
7+0.1	86.00	10.40	7.90	2.10
3+0.5	66.60	9.20	7.90	4.50
5+0.5	73.30	9.40	6.40	3.10
7+0.5	86.00	10.20	5.60	1.80
CD (*P*=0.05)	-	0.72	0.40	0.14

In addition to the two cytokinins (BAP and Kn) alone, their combinations with auxin (NAA, 0.1 mg L^{-1}) were also tested for their effect on proliferation and elongation of shoots (Table 1). Incorporation of NAA in the medium with either of the cytokinins significantly reduced the number of days required for multiple shoot induction. The inhibitory effect of alone BAP on shoot elongation was reverted on addition of NAA in the medium. Average of 3.1 cm long shoot was recovered on combination of BAP 7 mg L^{-1} and NAA 0.1 mg L^{-1} as against 1.0 cm on BAP 7 mg L^{-1} alone. This promotory effect was more evident when higher level of NAA (0.5 mg L^{-1}) was supplemented with all the concentration of BAP used (Table 1). Combination of Kn and NAA however produced less pronounced effect on percent response but number of days in multiple shoot induction was reduced considerably as compared to alone Kn treatments. Number of shoots per explant increased when Kn 3 and 5 mg L^{-1} was supplemented with both the concentrations of NAA (0.1 and 0.5 mg L^{-1}). Thus, incorporations of NAA showed marked increase in average shoot length indicating the stimulatory effect of NAA on shoot elongation.

For further multiplication, *in vitro* generated shoots were cut in to nodal segments and placed on MS medium with BAP 5 mg L^{-1} and NAA 0.1 mg L^{-1}. These segments with auxiliary buds and/or shoot tip again produced multiple shoots. However, remaining callus with smaller shoots were not discarded and sub-cultured on lower levels of BAP (1.0 mg L^{-1}) and NAA (0.1 mg L^{-1}) for further proliferation. Repeated transfer of the explants has been suggested as an efficient method for micropropagation of other woody plants (Sharma and Chaturvedi, 1988; Shekhawat *et al.*, 1993).

Well-developed regenerated shoots (3-4 cm long) were excised and transferred to the medium having reduced level of MS salts (½ MS) and different concentration (0.1 - 1.0 mg L^{-1}) of IBA and NAA for root induction. Of the two auxins tested, IBA was more effective in inducing the roots. The highest frequency (86.0 %) of

Table 2. Effect of plant growth regulators on root initiation in regenerated shoots of *Capparis deciduas*

MS medium + PGR (mg L^{-1})	Rooting (%)	Number of days in root induction	Average number of roots per shoot	Root length (cm)
MS plain	53.30	11.10	1.20	1.00
Half MS	53.30	11.00	2.70	1.00
IBA				
0.1	66.60	12.30	5.10	0.90
0.5	73.30	11.20	5.20	1.10
1.0	86.00	11.00	6.10	2.20
NAA				
0.1	CF	-	-	-
0.5	CF	-	-	-
1.0	CF	-	-	-
CD (*P*=0.05)	-	0.69	0.35	0.058

CF- Callus formation

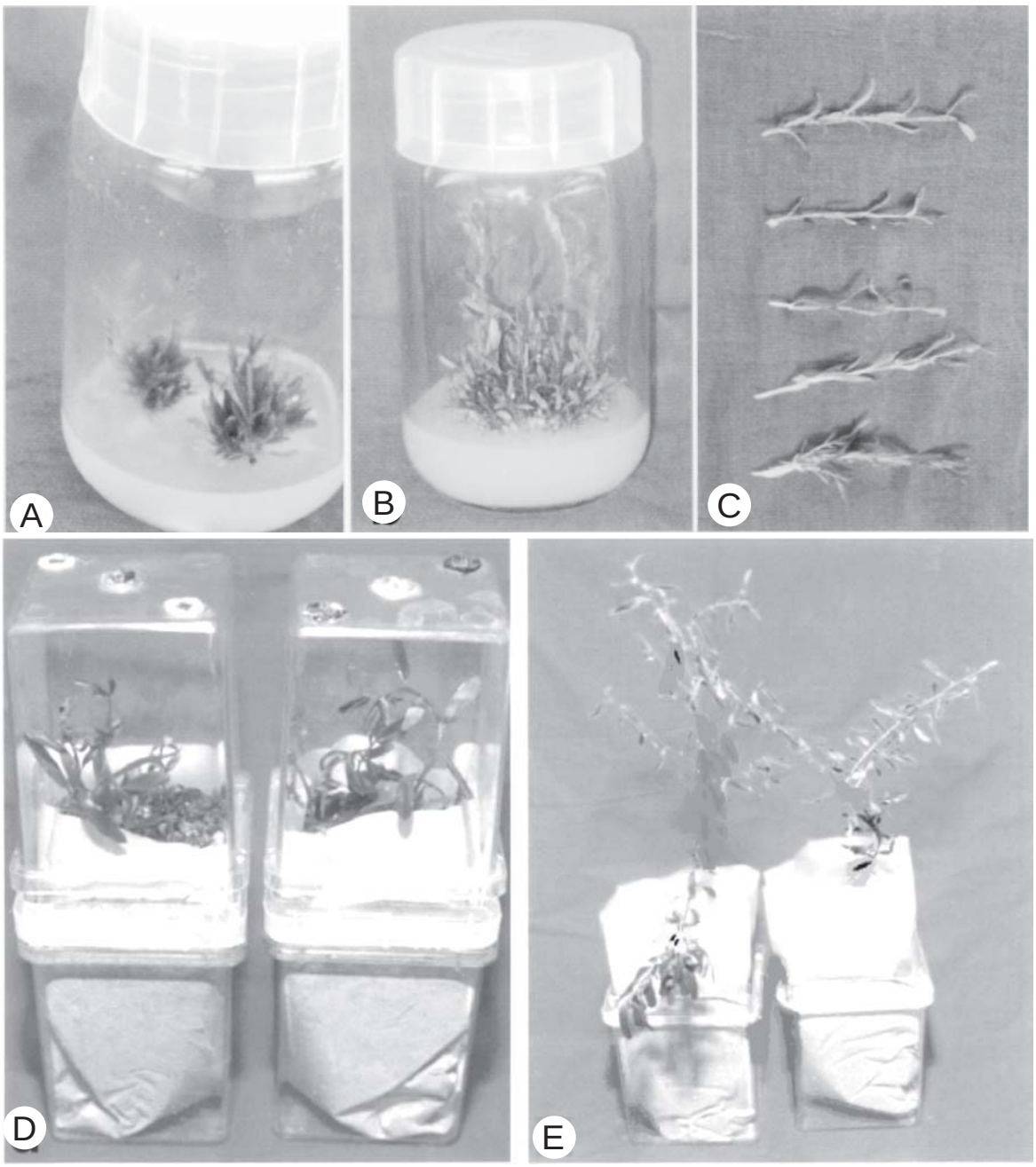

Fig. 1 (A & B) Multiple shoot induction from shoot tip explant (C) Root induction and development in regenerated shoots (D) Hardening of regenerated shoots under pre acclimation chamber (E) Hardened plants ready for field transfer

rooted plantlets with maximum number of roots per plantlet (6.10) was achieved with 1.0 mg L^{-1} IBA (Table 2, Fig 1C). Contrarily, NAA caused callus formation at cut end of shoots rather than roots. IBA has been reported as the most commonly used auxin for *in vitro* root formation from shoots of woody trees (Vander Krieken *et al.,* 1993).

Subsequently, *in vitro* raised plantlets (5-6 cm long) were transferred to Pre Acclimation Chambers (PAC, Fig 1D). Three holes of equal size were made on the top of the chamber to permit free but slow air exchange by plugging them with cotton and removing them one by one as acclimation process proceeded. The chamber ($3/4^{th}$) was filled with 1:1 mixture of sterilized garden soil and FYM. These chambers were watered periodically and supplemented with ¼ MS salt solution occasionally. After four weeks of acclimation, all the plants were shifted to protected and shaded area for further hardening. The PAC hardened plants were maintained there for 2-3 months before transplanting to field (Fig 1D). The plants thus hardened had a very high survival rate (83%).

The present study provides detailed information on the effects of various PGRs on regeneration parameters with high regeneration frequency using shoot tip explant. This protocol reports high survival rate as well during hardening procedure compared to earlier reports (Deora and Shekhawat, 1995; Tyagi and Kothari, 1997). The large number of plants could be regenerated throughout the year ensuring a constant supply of planting material of *C. deciduas.*

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Fruit chemical composition of hazelnut cultivars grown in Portugal

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Abstract

Chemical composition (crude protein, crude fat, starch, neutral detergent fibre – NDF and free α-amino acids) of six hazelnut cultivars (Butler, Ennis, Fertile de Coutard, Grossal, Merveille de Bollwiller and Segorbe) was investigated. Genotype significantly affected fruit nutritive value. Crude protein ranged from 12-17 g 100 g^{-1} dry weight (dw) in cultivar Ennis and Merveille de Bollwiller, respectively; crude fat was 50-62 g 100 g^{-1} dw in cvs. Fertile de Coutard and Butler; starch varied from 1.0 to 2.4 g 100 g^{-1} dw in cvs. Segorbe and Butler; and NDF was 8-14 g 100 g^{-1} dw in cvs. Merveille de Bollwiller and Ennis. Total free α-amino acids content ranged from 144 mg 100 g^{-1} dw (cv. Segorbe) to 413 mg 100 g^{-1} dw (cv. Butler). The essential amino acids content varied between 23 mg 100 g^{-1} dw (cv. Butler) to 55 mg 100 g^{-1} dw (cv. Merveille de Bollwiller). Alanine was the main amino acid found (62% of total amino acids) and methionine was the lowest (0.3%). Based on the available data on the phytochemical content of hazelnuts, including the data presented in this study, there is a high likelihood that this fruit will provide positive health benefits.

Key words: Crude fat, crude protein, free ∝-amino acids, neutral detergent fibre, proximate analysis, starch

Introduction

In recent years, nuts have become more important in human nutrition because of their potential health benefits. Epidemiological studies, in Framingham Massachusetts, USA, have shown that the frequency of nut intake was correlated with a risk reduction of coronary heart disease, atherosclerosis and some types of cancer by up to 50% (Alpan *et al.*, 1997; Richardson, 1997; Brehme, 2002). Moreover, Salas-Salvadó and Megias (2005) considered nuts as a natural functional healthy food. The recognition by the U.S. FDA that nuts must be regarded as "heart-healthy" foods gives a great input for the increase of the consumption of these fruits.

Consumers have become interested in food composition beyond the data usually available in standard composition tables (Souci *et al.*, 1994; Holland *et al.*, 1998). There is also increasing worldwide demand for non-meat protein sources with balanced amino acid profiles. As interest in nuts has been increased, it is important to evaluate the composition of these fruits commonly grown in each country. While it is obvious that hazelnuts have a positive role in human nutrition, it will not be easy to recognize which components have the more significant effects (Savage, 2001). However, Fraser *et al.* (1992) suggested that the health benefits of nuts are due to the synergistic effect between its constituents and enhanced complex biochemical interrelationships working together. Studies carried out by Shahidi *et al.* (2007) suggest that hazelnut and its byproducts, green leafy covers, hard shells and tree leaf, could potentially be considered as an excellent source of natural antioxidants. Most of the studies on the composition of hazelnut kernel have mainly focused on lipid content with little data on amino acid composition, starch, fibre and ash. According to Alasalvar *et al.* (2003), the good nutritional value of hazelnut is its amount of fibre that has an important protective effect against intestinal disorders, cholesterol and hypertension, among other effects. Gu *et al.* (2004) showed that hazelnut kernel contain significant concentration of proanthocyanidins that are known to have positive health effects.

Amino acids are important because they are the precursors of secondary plant metabolites and are involved in the production of compounds which directly or indirectly affect human health (Gomes and Rosa, 2000). Moreover, humans cannot synthesize ten amino acids, and these must be provided by the diet (Anderson *et al.*, 1998). Free amino acids, are essential nonvolatile compounds involved in the overall taste and flavor of many foods having a considerable influence on the sensory characteristics of fruits, bitter, sour and sweet taste, affecting both quality and nutritional value (Fuke and Konosu, 1991).

Several studies indicated that the nut composition of hazelnut is affected by cultivar, harvest year, soil, irrigation and method of cultivation (Parcerisa *et al.*, 1993; Parcerisa *et al.*, 1994; Parcerisa *et al.*, 1995; Amaral *et al.*, 2006).

Therefore, a study was performed to evaluate the fruit quality of six hazelnut cultivars in field grown conditions in Portugal in relation to crude protein, crude fat, starch, neutral detergent fibre and free α-amino acids for better knowledge of their composition *vis-a-vis* nutritional significance.

Materials and methods

Plant material and growth conditions: The study was carried out on 20-year-old plants of hazelnut (*Corylus avellana* L.) in an experimental plot near Vila Real, Northeast Portugal (41º 19' N

and 7º 44' W; altitude 470 m above sea level) in 2005. The climate is characterized as a transition from Csb to Csa (mesothermic climate with a partially dry summer) of Köpen. A plot of 75 plants representing eleven cultivars planted at 5 x 3 m spacing on a Typic Dystrochrept silt loam soil and left unpruned was used for the study. The orchard was fertilized and periodically drip-irrigated (Santos *et al.*, 1998). Nuts from six hazelnut cultivars: Butler, Ennis, Fertile de Coutard, Grossal, Merveille de Bollwiller and Segorbe were hand-picked from the ground at the beginning of the harvest in September, and kept unshelled in a refrigerator (2 ºC) until analyses were carried out.

Physical parameters: Yield per tree was recorded, and individual fruit and kernel weight estimated from 3 samples of 100 fruits. Yield per unit (kg m^{-3}) was calculated. Canopy volume (v) was calculated for a prolate spheroid, a plant taller than wide, by the formula $v = 4/3\pi ab^2$, where a = 1/2 of the tree canopy height and b = 1/2 of the tree canopy width (Lagerstedt and Painter, 1973).

Proximate analysis: Moisture content was determined using the Official Analytical Chemists Methods (AOAC, 1995). Representative samples of each cultivar were removed from the shell and the kernel was finely chopped, and ash content determined by incineration at 550 ºC for 3 h in accordance with the AOAC method (1995). Crude protein (nitrogen x 6.25) was evaluated using the Kjeldahl procedure with selenium as a catalyst (AOAC, 1995). Crude fat was measured by extraction with petroleum ether in a Tecator Soxtec System (model HT1043) according to AOAC (1995). Starch was determined by enzymatic hydrolysis of starch to glucose as described by Salomonson *et al.* (1984). Neutral detergent fibre (NDF) was evaluated after extraction with the neutral detergent solution hydrolysis according to the procedures described by Van Soest *et al.* (1991).

Free α-amino acids: The extraction and purification of free α-amino acids were performed according to Gomes and Rosa (2000). Powdered freeze-dried tissues were extracted twice with boiling methanol (90%) for 2 min under continuous homogenisation, centrifuged for 2 min at 6.25 *g*, and the supernatant poured into a 10 mL volumetric flask. This step was repeated twice using methanol (70%). Combined supernatants were made up to a final volume of 10 mL with methanol (70%) and kept at –18ºC until analysis. Subsequently 2 mL of each extract was evaporated and resuspended in 2 mL of 0.1 M HCl. Mini-columns of 1 mL (Chromabond from Macherey-Nagel) were connected to a solid phase extraction vacuum system (Gilson) and eluted with 0.5 mL of 0.1 M HCl before being filled up to 2 cm with a cation exchange resin, Dowex (H^+) 50WX8-499 (Sigma-Aldrich Chemicals, St Louis, MO, USA). The amino acids were loaded onto the columns and washed with 5 mL of 0.1 M HCl. Free α-amino acids were eluted with 4 x 2.5 mL of 7 M NH_3, pa (Merck, Darmstadt, Germany). After evaporation, the residue was resuspended in 0.3 mL of distilled water, filtered (Spartan 13, 0.2 µm) and kept in vials at –18 ºC until analysis. Amino acids were determined by HPLC using C18 column (Waters, Spherisorb S3ODS, id 4.6 mm) 150 mm length and a UV/VIS detector set at 340 nm, after precolumn derivatisation with o-phthaladehyde/2-mercaptoethanol. The mobile phase was made of two solvents: A – 350 mM Na_2HPO_4. $2H_2O$ and 250 mM propionic acid (1:1), acetonitrile and Milli-Q water (40:8:52); B – acetonitrile / methanol / water (30:30:40). With these solvents, a gradient was set (Table 1). Identification and quantification of detected amino acids were done against external standards after adjustment through regression lines.

Statistical analysis: Data analyses were performed as analysis of variance using the Super ANOVA software (1.1, Abacus Concepts Inc., 1991). Mean separations were made using Fisher's Protected LSD Test ($P = 0.05$), designed to allow all possible linear combinations of group means to be tested. All determinations were performed in triplicate.

Results and discussion

Physical parameters: The physical parameters (fruit and kernel weight, percent kernel and yield) were additionally measured to better characterize the fruits of the six cultivars that showed important visual differences. Physical parameters varied significantly among cultivars ($P < 0.01$) (Table 2). Cv. Ennis presented the heaviest nuts, 64% higher than cv. Segorbe, which presented the lightest ones. The kernel weight followed the same trend, *i.e.*, highest kernel weight was observed in cv. Ennis (1.8 g) and the cv. Segorbe (1.1 g) had the lowest. These values were similar to the average data of the six cultivars recorded during fifteen years (Silva *et al.*, 2005). The percent kernel was high in cvs. Segorbe, Butler, Ennis and Grossal (~45%), and low in cv. Merveille de Bollwiller (39%). Cv. Butler had the highest yield (Table 2).

Proximate analysis: Regarding chemical composition, crude protein, crude fat, starch and neutral detergent fibre (NDF) varied significantly among cultivars (Table 3). Crude protein ranged between 12.3 g 100 g^{-1} (cv. Ennis) and 17.1 g 100 g^{-1} (cv. Merveille de Bollwiller). Hazelnuts, like other nuts, contain high levels of crude protein but few reports are available in literature (Alasalvar *et al.*, 2003). These values were comparable with the levels recorded for six cultivars grown in Tarragona which ranged from 12 to 18 g 100 g^{-1} (Bonvehi, 1995), and slightly lower than the values obtained for Butler, Ennis and Fertile de Coutard grown in New Zealand that showed values of 18, 17 and 15 g100 g^{-1}, respectively (Savage and McNeil, 1998). Also, Alasalvar *et al.* (2003) reported that cv. Tombul contains 15 g 100 $g^{-1}$of protein. However, some cvs. like Yassi and Yuvarlak showed values as low as 7 g 100 g^{-1} (Ayfer *et al.*, 1997).

Generally, crude fat content of the samples was around 50% (50 to 61 g 100 g^{-1} dw in cvs. Butler and Fertile de Coutard, respectively) and was the predominant component of hazelnuts (Table 3). According to Richardson (1997) the oil content varied between 57% dw in cv. Merveille de Bollwiller and 65% dw in cvs. Tombul, Casina and Negret. Fat makes up 60-70% of the kernel, which is responsible for the high source of energy

Table 1. HPLC gradient for free α-amino acid analysis

Time (min)	0.0	9.5	11.0	13.6	20.4	23.4	25.4	32.0	34.0	37.0
Flow (mL min^{-1})	1.3	1.3	1.3	1.3	1.3	1.3	0.8	0.8	1.3	1.3
Solvent B (%)	0.0	11.0	12.0	20.0	45.0	50.0	60.0	100.0	0.0	0.0

Table 2. Physical parameters of the fruits of the six hazelnut cultivars

Cultivar	Fruit weight (g)	Kernel weight (g)	Percent kernel (%)	Yield (kg m^{-3})
Butler	3.55 ± 0.4 c	1.61 ± 0.1 c	45.41 ± 1.2 c	0.15 ± 0.0 c
Ennis	3.86 ± 0.2 d	1.76 ± 0.3 d	45.53 ± 3.1 c	0.05 ± 0.0 a
Fértile de Coutard	3.36 ± 0.2 c	1.45 ± 0.2 b	42.80 ± 3.2 b	0.09 ± 0.0 b
Grossal	2.44 ± 0.2 a	1.12 ± 0.1 a	45.85 ± 2.9 c	0.08 ± 0.0 b
Merveille de Bollwiller	2.81 ± 0.0 b	1.09 ± 0.0 a	38.57 ± 2.5 a	0.14 ± 0.0 c
Segorbe	2.35 ± 0.1 a	1.08 ± 0.1 a	46.09 ± 3.1 c	0.08 ± 0.0 b
P	< 0.001	< 0.01	< 0.01	< 0.01

Different letters within one column denote statistically significant differences ($P < 0.05$) by ANOVA and Fisher's LSD test.
Values are average of three individual samples ± standard deviation.

Table 3. Proximate analysis of the fruits of the six hazelnut cultivars

Cultivar	Crude protein (g 100 g^{-1} dw)	Crude fat (g 100 g^{-1} dw)	Starch (g 100 g^{-1} dw)	NDF (g 100 g^{-1} dw)	Moisture (%)	Ash (g 100 g^{-1} dw)
Butler	14.53 ± 0.6 c	49.90 ± 1.5 a	2.38 ± 0.2 c	12.07 ± 1.3 d	4.03 ± 0.1 c	2.82 ± 0.2 a
Ennis	12.30 ± 0.3 a	54.44 ± 1.6 b	1.90 ± 0.2 b	14.33 ± 0.9 e	6.59 ± 0.2 a	2.93 ± 0.5 a
Fertile de Coutard	13.59 ± 0.5 b	61.15 ± 3.1 d	2.00 ± 0.8 bc	9.60 ± 0.9 b	6.30 ± 0.1 b	2.55 ± 0.5 a
Grossal	12.52 ± 0.2 a	55.00 ± 2.5 c	1.14 ± 0.2 a	11.10 ± 0.9 c	5.52 ± 0.2 a	2.38 ± 0.2 a
Merveille de Bollwiller	17.08 ± 0.9 d	56.18 ± 2.3 c	2.16 ± 0.3 c	8.05 ± 2.0 a	4.58 ± 0.2 d	3.30 ± 0.3 b
Segorbe	14.68 ± 0.9 c	53.75 ± 1.7 b	1.00 ± 0.0 a	9.96 ± 1.9 b	4.88 ± 0.2 c	2.67 ± 0.5 a
P	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001	< 0.01

Different letters within one column denote statistically significant differences ($P < 0.05$) by ANOVA and Fisher's LSD test.
Values are average of three individual samples ± standard deviation.

(Parcerisa *et al.*, 1993; Bota *et al.*, 1997; Pala and Ünal, 1997), where approximately 80% of the calories of nuts are derived from the fat (Salas-Salvadó and Megias, 2005). However, according to Richardson (1997), nuts are low in saturated fatty acids and rich in monounsaturated and polyunsaturated fatty acids and have no cholesterol which is beneficial in reducing the risk of circulatory and coronary diseases.

As referred before, differences among the hazelnut cultivars were observed based on the starch content (Table 3), but all cultivars showed relatively low values of the total starch. Moreover, cv. Butler had two times more starch than cv. Segorbe (2.4 and 1.0 g 100 g^{-1} dw, respectively). Savage and McNeil (1998) also reported similar values of the total starch (1.3 to 2.7 g 100 g^{-1} dw) in the kernels of six cultivars grown in New Zealand. The low starch content (little transformation of starch into sugar during storage) is associated with low state of hydration and when storage conditions are good, is responsible for the long storage period of hazelnuts.

The lowest NDF content (8 g 100 g^{-1} dw) was observed in cv. Merveille de Bollwiller, whilst cv. Ennis showed the highest (14 g 100 g^{-1} dw) (Table 3). These cultivars had higher values than those reported in other hazelnut studies with different cultivars (Lintas and Cappeloni, 1992). Savage and McNeil (1998) and Megias-Rangil *et al.* (2004) indicated one of the human benefits of hazelnuts is their fibre content. Alasalvar *et al.* (2003) assumed that eating ~200 g of Tombul hazelnuts per day is adequate to supply 100% of the total fibre requirement for adults. Although, cv. Ennis nuts seem to be the best for the preparation of fibre-based foods, more data are needed to confirm this.

Ash and moisture were also determined and these parameters varied among cultivars (Table 3). The average ash content was found to be 2.77%, parallel to the results of Pala *et al.* (1996) and Köksal *et al.* (2006). The minimum and maximum values, based on this parameter, ranged between 2.4 g 100 g^{-1} dw (cv. Grossal) and 3.3 g 100 g^{-1} dw (cv. Merveille de Bollwiller). Hazelnuts had very low moisture content (lower than 7%), which is an advantage for adequate storage.

Free α-amino acids: Total free α-amino acids and essential amino acids were significantly ($P = 0.001$) different among cultivars (Table 4). Total amino acid contents ranged from 144 to 413 mg 100 g^{-1} dw in cvs. Segorbe and Butler, respectively. These results are different from those obtained by Silva *et al.* (2005) in the years 2001 and 2002, which emphasises the dependence between the total amino acid and weather. Alasalvar *et al.* (2003) considered that the content of amino acids in hazelnuts varies according to cultivars, growing seasons, environmental factors and maturity. Essential amino acids content ranged from 22 to 55 mg 100 g^{-1} dw in cvs. Butler and Merveille de Bollwiller, respectively, indicating that hazelnuts are a good source of these compounds (Table 4).

Table 4. Amino acid content (mg 100 g^{-1} dw) in the fruits of the six hazelnut cultivars

Cultivar	Total essential amino acids	Total free α-amino acids
Butler	22.23± 0.2 a	413.46± 11.1 e
Ennis	48.94 ± 1.2 d	237.91 ± 10.2 c
Fértile de Coutard	23.13 ± 2.1 a	390.12 ± 15.3 e
Grossal	27.65 ± 0.5 bc	185.26 ± 10.9 b
Merveille de Bollwiller	55.20 ± 1.9 e	330.65 ± 12.6 d
Segorbe	30.91 ± 0.9 c	144.17 ± 10.2 a
P	< 0.001	< 0.001

Different letters within one column denote statistically significant differences ($P < 0.05$) by ANOVA and Fisher's LSD test.

Table 5 depicts content of 16 amino acids identified in the 6 hazelnut cultivars, namely, L-alanine (Ala), L-asparagine (Asn), L-aspartic acid (Asp), glycine (Gly), L-glutamic acid (Glu), L-glutamine (Gln), L-serine (Ser), and the essential amino acids: L-arginine (Arg), L-histidine (His), L-isoleucine (Ile), L-leucine (Leu), L-methionine (Met), L-threonine (Thr), L-phenylalanine (Phe), L-tyrosine (Tyr) and L-valine (Val). Specifically, only the

Table 5. Free α–amino acid content (mg 100 g^{-1} dw) from the fruits of the six hazelnut cultivars

Cultivar	Ala	Asn	Asp	Gly	Glu	Gln	Ser	Arg	His	Ile	Leu	Met	Thr	Phe	Tyr	Val
Butler	315.9±9.9e	11.37±2.5bc	3.91±0.2a	3.04±0.0b	8.37±0.6a	29.92±1.7c	6.25±0.0c	7.40±2.5a	3.50±0.0ab	2.60±0.0b	1.76±0.0a	0.71±0.0a	3.68±0.0b	4.42±0.2bc	4.48±0.2a	6.18±0.1c
Ennis	86.6±4.7b	12.28±0.0c	15.49±1.8b	5.62±0.6c	24.04±1.7b	18.65±0.3b	5.41±0.5bc	15.00±0.3b	3.52±0.0b	4.81±0.6c	14.07±0.8d	0.71±0.0a	17.62±2.0d	4.56±0.3bc	5.88±0.3b	3.66±.0.3b
F. Coutard	311.2±4.3e	8.43±0.1ab	4.38±0.6a	3.14±0.6b	7.29±2.7a	14.19±0.0a	5.37±0.1bc	7.77±0.3a	3.51±0.0ab	1.93±0.3ab	4.79±0.8b	0.71±0.0a	5.87±.0.4c	3.10±0.6a	5.22±0.4ab	3.28±0.8b
Grossal	108.6±8.3c	7.84±0.0ab	2.13±0.1a	0.75±0.0a	9.20±0.3a	13.96±0.0a	4.71±0.0b	5.54±0.3a	3.45±0.0a	1.67±.0.0a	12.91±0.6d	0.71±0.0a	0.01±0.0a	5.43±0.0c	4.90±0.0a	3.49±0.1b
M. Bollwiller	142.1±3.6d	13.23±0.5c	24.08±2.9c	7.77±0.5d	30.50±3.8b	17.31±0.0b	8.02±0.5d	23.74±2.1c	3.47±0.0ab	4.47±0.3c	7.99±0.9c	0.71±0.0a	22.94±0.4e	4.33±0.3b	8.68±0.3c	11.30±0.8d
Segorbe	62.3±0.1a	6.40±0.0a	3.88±1.5a	3.440.1b	10.83±3.1a	14.19±0.0a	2.13±0.0a	5.47±0.2a	3.46±0.0ab	1.42±0.0a	14.05±0.1d	0.71±0.0a	6.99±1.2c	2.61±0.1a	4.60±0.2a	1.69±0.1a
P	< 0.001	< 0.01	< 0.01	< 0.001	< 0.01	< 0.01	< 0.01	< 0.01	NS	< 0.01	< 0.01	NS	< 0.001	< 0.01	< 0.01	< 0.01

Different letters within one column denote statistically significant differences ($P < 0.05$) by ANOVA and Fisher's LSD test.
Values are average of three individual samples ± standard deviation.

amino acids, histidine and methionine were not affected by the cultivar. As in previous studies (Rennie, 1995; Savage and McNeil, 1998; Alasalvar *et al.*, 2003; Köksal *et al.*, 2006) we also did not detect any tryptophan, even though some nutritional databases report this amino acid in hazelnuts (Souci, 1994; Holland *et al.*, 1998).

In all hazelnut cultivars studied Ala was the most common non-essencial amino acid, which was significantly different ($P < 0.001$) among the cultivars, and represented 62% of total free α-amino acids. Mean Ala concentration varied from 62 to 316 mg 100 g^{-1} dw in cvs Segorbe and Butler, respectively. Apart from an important source of energy, Ala, a non-polar amino acid, is responsible for an increase in immune responses and takes part in the metabolism of sugars and organic acids (Rennie, 1995). Other important amino acids included Gln that varied from 13.96 mg 100 g^{-1} dw in cv. Grossal to 29.92 mg 100 g^{-1} dw in cv. Butler, followed by Glu that varied between 7.29 mg 100 g^{-1} dw in cv. Fertile de Coutard and 30.5 mg 100 g^{-1} dw in cv. Merveille de Bollwiller. A group of seven amino acids, five of them essential, had values lower than 10 mg 100 g^{-1} dw: Met, His, Ser, Gly, Phe and Ile. Specifically, Met concentration was the lowest among the amino acids (0.3% of total free α-amino acids) determined (Table 5). Köksal *et al.* (2006) also considered Met the most insignificant amino acid. In general, the fruits of cv. Butler (American origin) had the highest amino acid content and was two times greater than the fruits of cvs. Grossal and Segorbe (Spanish origin). However, the values obtained in diverse studies were different for the amount and the relative proportion of each amino acid (Alasalvar *et al.*, 2003; Köksal *et al.*, 2006).

Our data confirm that hazelnuts are a rich source of a number of important nutrients that can have a very positive effect on human health. The composition of hazelnut kernels, particularly total and individual free α-amino acids, crude protein, crude fat, starch and neutral detergent fibre are strongly affected by cultivar. The major amino acid found was alanine, representing 60% of the total free α-amino acids, and methionine was the lowest (1.5% of the total free α-amino acids).

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Fruit quality characterization of seven clementine cultivars

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Abstract

For a citrus grower to choose the right clementine cultivar for a given region or market, it is very important to know the characteristics of that cultivar particularly in terms of the development of its internal as well as external quality attributes. In particular, it is very important to know when the maturity index is attained along with the rate of color change, sugar accumulation, acid dissipation, firmness loss, etc. This paper describes the results obtained for several quality attributes (rind color, firmness, juice content, juice titratable acidity and soluble solids content) of seven clementine cultivars sampled at different stages of maturity. All of the cultivars reached minimum maturity index (sugar / acid ratio greater than 7.0) by early November. The rate of rind color change is significantly influenced by picking period and is the main attribute that differs among most of the clementine cultivars. In addition, 'Guerdane', the new clementine cultivar, is the only cultivar that matures much later (January-February) and has the characteristics of a late-maturing cultivar both internally (juice quality) and externally (rind color).

Key words: *Citrus clementina* Hort. ex Tan, maturity index, rind color, rind firmness, juice sugar content, juice titratable acidity.

Introduction

Morocco's annual citrus production is ~1.4 million tons, of which the major group is of easy-peeling mandarins, mainly clementine cultivars (*Citrus clementina* Hort. Ex Tan), representing 30% of total production. Morocco is ranked as second largest producer of this specialty fruit after Spain. Consumers of fresh fruit like to have a variety of fruit all the year around. In terms of citrus, preference trends are for a seedless, orange-colored fruit of medium size, with a balanced sugar/acid ratio, and with a relatively firm but easy-to-peel rind. Most of these characteristics are found in the clementine mandarin group (Hodgson, 1967; Saunt, 2000). This group is very diverse in terms of period of maturity, which starts in late September and extends to late January, but the majority of the production occurs in midseason (*i.e.* November-December) (Chapot, 1963; Devaux, 1981). Such great efforts are continuously made in the main clementine-producing countries to find cultivars that mature much earlier (September-October) or much later (January-February) to extend the period of commercial supply (Agustí *et al.*, 2002).

Internal and external quality of fruit are significantly affected by climate (Goldschmidt, 1997; Reuther, 1988). The major factors are temperature, wind and rain. Temperature is the most important environmental factor affecting the citrus crop (Davies, 1997; Goldschmidt, 1997). In general, a climate with low rainfall and plenty of sunshine is good for citrus trees (Goldschmidt, 1997). It promotes good flower differentiation, flower and fruit development, and fruit quality. Wind is a problem to citrus production as both tree and fruit damage can occur (Albrigo, 1988). It is noteworthy that for some varieties such as clementines, although fruit internal maturity can be attained early in the season, appearance of external signs of maturity, such as rind color, is often delayed by high fall temperatures which consequently delay chlorophyll degradation and carotenoid biosynthesis (Ikoma *et al.*, 2001). At commercial level, the development of orange color is often hastened postharvest through the ethylene degreening process which allows the attainment of good prices in the market early in the season (El-Otmani *et al.*, 2000).

Fruit growth and development are associated with morphological, anatomical and physiological changes (Bain, 1958; El-Otmani and Coggins, 1985; El-Otmani *et al.*, 1987). Fruit maturaty is associated with changes in rind texture, juice composition and taste (El-Otmani *et al.*, 1990; El-Otmani and Coggins, 1991). For the citrus grower, and clementine grower in particular, it is very important to have information on the differences in fruit quality among the cultivars and on the changes occurring in quality attributes and their rate of occurrence.

The objective of this paper is to present research findings on the time course of several quality attributes of seven cultivars of clementine mandarin during the period of harvest, in the same environmental conditions, and to evaluate new citrus cultivars for commercial potential.

Materials and methods

The experimental site is located in the Souss Valley of southern Morocco (Latitude 30° 20' N, Longitude 9° 22' W, Altitude 90 m) which has a semi-arid climate with rainfall of ~200 mm

year[1]. Mature trees of seven clementine cultivars included in the study were 'Caffin', 'Bruno', 'Nules', 'Esbal', 'Hernandina', 'Nour' and 'Guerdane', of which 'Caffin', 'Bruno', 'Nour' and 'Guerdane' are of Moroccan origin, and 'Guerdane' which is a mutation of clementine. The trees were budded on 'Carrizo' citrange rootstock, and were healthy and showed no signs of any deficiency. The origin of clementine cultivars are as follows:

'Caffin': cultivar discovered in 1968 in Azemour in Morocco.

'Bruno': cultivar discovered in Le Pontet orchard in Salé Morocco.

'Nour': discovered as bud mutation of 'Cadoux' in the Souss Valley in Morocco.

'Guerdane': previously named as KSN, it was discovered in 1987 as bud mutation of 'Fina' in Abbes Kabbage orchard in the Souss Valley of southern Morocco.

'Esbal': discovered in 1966 as a bud mutation of 'Fina' at Sagunto in Valencia Province.

'Nules': the most popular clementine in Spain, 'Nules' was discovered in 1953 near the town of the same name in Castellón Province as a bud mutation on a 'Fina'.

'Hernandina': discovered in 1966 as a bud mutation of 'Fina' at Picassent in Valencia Province.

To assess fruit quality attributes, fruit were harvested at 2- to 3-week intervals, samples were collected three to six times during ripening (depending on the cultivar) to demonstrate the effect of harvest date on quality, starting on 20 October, 2004, corresponding with the beginning of the maturity season, and ending on 25 January, 2005, corresponding with the last harvest date of the late maturing cultivar. Three single-tree replicates were used with three replications per replicate, each comprising of five fruit. The fruit were sampled from the outer portion of the canopy and from all four quadrants of the tree. At each harvest date, fruit of average size were sampled. The diameter of the sampled fruit was approximately 63 mm for 'Hernandina', 40 mm at the start and 56 mm at the end of the sampling period for 'Guerdane' and between 50 and 60 mm for the other cultivars.

In laboratory, the fruits were weighed and their average diameter was measured. Fruit color index (CI) was determined using a CR300 Minolta chromameter following the method described by Jimenez-Cuesta *et al.* (1981). Parameters "L" (lightness), "a" (greenness to redness) and "b" (blueness to yellowness) were determined at two different spots around the equatorial zone of the fruit and sample averages were calculated. Rind firmness, as measured by puncture resistance force, was determined using a puncture gauge following the method described by El-Otmani and Coggins (1991). Fruits were cut along their equatorial zone, their juice extracted and weighed. Juice pH, titratable acidity (using 0.1 N NaOH titration), and soluble solids content (using a laboratory refractometer expressing the amount of sugars in °Brix) were determined.

Statistical analyses: All analyses were based on 3 replications using SAS software (Statistical Analysis System) and the significance of differences between cultivars and harvest date x cultivars interaction were evaluated using ANOVA, followed by Scheffe's test. Differences between means were considered to be significant at $P \leq 0.05$. The standard deviations of each value was represented on all the figures by vertical lines.

Results

Rind colour index (CI): Harvest date and cultivar had highly significant effect on the rind color index. On the first sample date, overall rind color index (CI) of the clementine cultivars, varied from -3 for 'Bruno' (corresponding to yellow-green with orange patches), -6 for 'Caffin' (corresponding to light green-to-yellow color), -10 to -8 (light green-to-yellow) for 'Nules', 'Esbal' and 'Nour', and -14 to -12 (dark green-to-color break) for 'Hernandina' and 'Guerdane' (Fig. 1). The interaction between cultivar and harvest date was found to be highly significant. Peel color improved with time for all clementine cultivars with intense deep-orange rind colour for 'Caffin' by mid-November. 'Guerdane' was the last cultivar to develop color.

Firmness: Effects of harvest date and cultivar was highly significant on rind firmness and the effect of harvest date x cultivar interaction was highly significant. Rind firmness of all the cultivars declined over time as fruit matured (Fig. 2). Fruit rind firmness was lowest for 'Bruno', significantly higher for 'Guerdane' followed by 'Hernandina' and 'Nour'.

Juice content: On the first harvest date, juice content of all cultivars was >40% (Fig. 3). 'Caffin' and 'Bruno' started drying out by mid-November. The effects of cultivars, harvest date and their interaction were highly significant. The cultivars with the highest juice content were 'Nules' and 'Esbal', whereas 'Caffin'

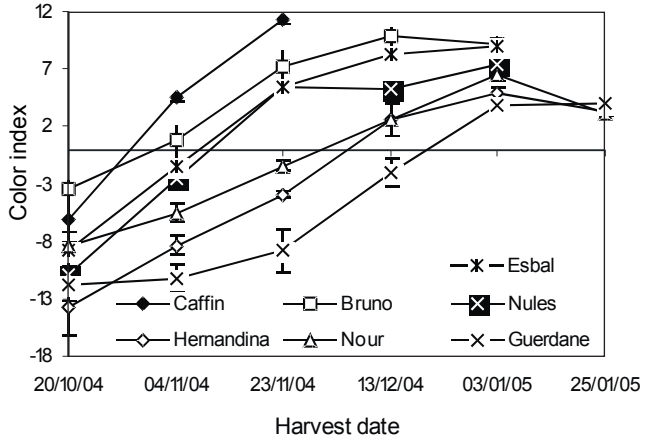

Fig. 1. Time course of the fruit rind color index (CI) of seven clementine selections

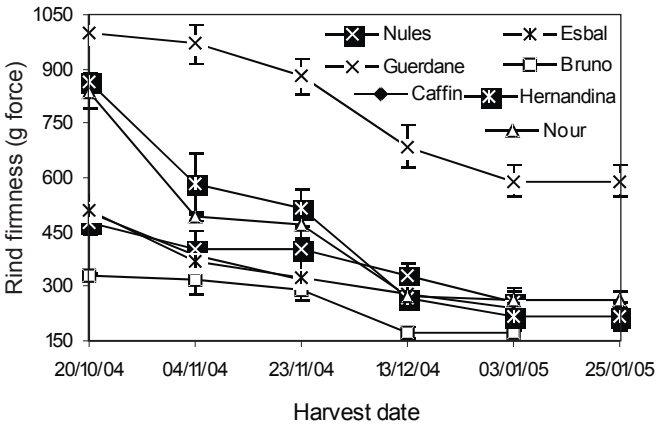

Fig. 2. Time course of rind resistance to puncture for seven clementine selections

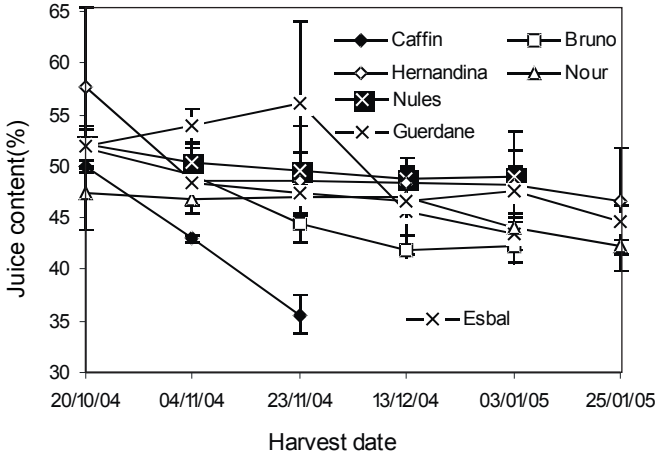

Fig. 3. Seasonal variation of juice content in fruit of seven clementine selections

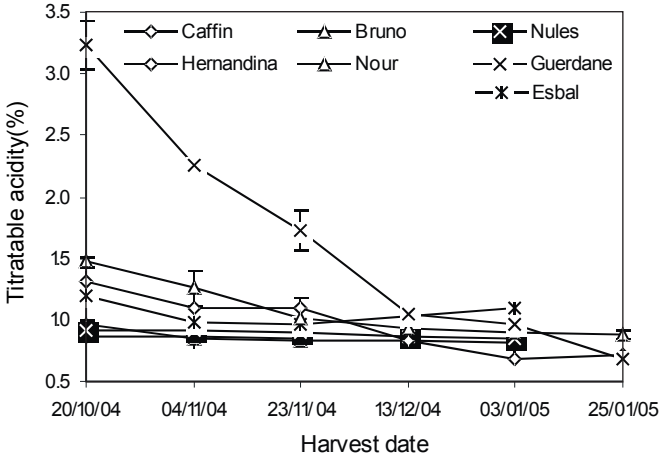

Fig. 4. Seasonal variations of titratable acidity (TA) in fruit juice of seven clementine selections

appears to have the lowest juice content. Juice content of the other cultivars remained more constant.

Titratable acidity (TA): Juice titratable acidity of all cultivars ranged between 0.8 to 1.5 %, except for 'Guerdane', which had the greatest value (3.2%) on the first harvest date (Fig. 4). Titratable acidity declined over time as fruit matured. Harvest date and cultivar had highly significant effects on the measured parameter and harvest date x cultivar interaction was also found to be highly significant.

Soluble solids content (SSC): The effects of cultivars, harvest date and their interaction were highly significant on juice soluble solids content. SSC was significantly lowest in 'Guerdane' (7.9°Brix) on the first harvest date and greatest (14°Brix) in 'Nour' on the last harvest date (Fig. 5), with a general trend to increase from the earliest maturing cultivars to the latest ones.

Maturity index (SSC/TA): Harvest date, cultivar and their interaction had highly significant effects on maturity index. On the first harvest date maturity index was similar for 'Caffin' and 'Bruno' (~12), 'Nules' and 'Esbal' (~10) and 'Hernandina' and 'Nour' (6 to 8). 'Guerdane' had the lowest maturity index (~2). The largest SSC/TA value was obtained for 'Hernandina' (19) on the last sampling date (Fig. 6).

pH: For most cultivars, juice pH was 2.1 to 3.0 on the first sampling date and increased slightly over time to reach 3.0 to

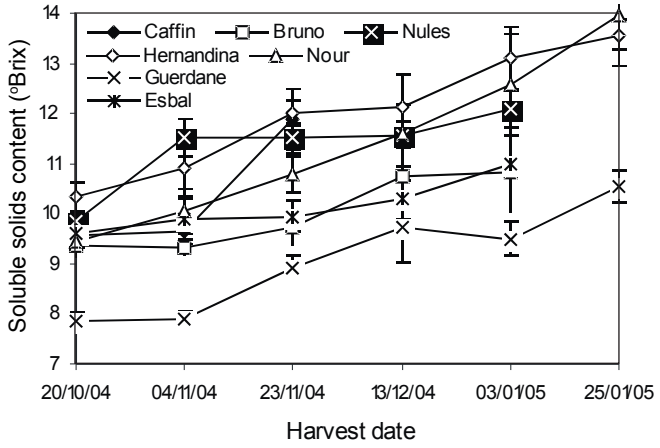

Fig. 5. Seasonal variation of soluble solids content (SSC) in fruit juice of seven clementine selections

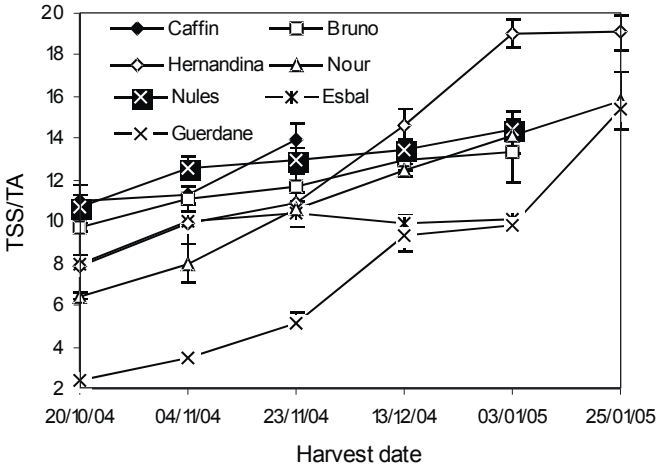

Fig. 6. Seasonal variation of the maturity index (SSC/TA) in fruit juice of seven clementine selections

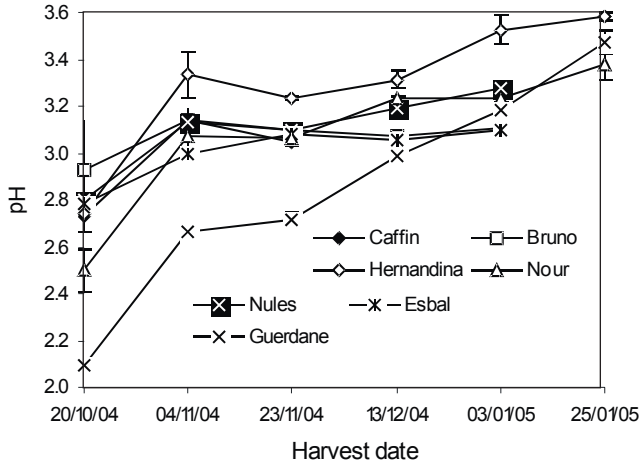

Fig. 7. Seasonal variation of pH in fruit juice of seven clementine selections

3.5. 'Guerdane' is distinguished from other cultivars by the lowest juice pH (Fig. 7) and highest juice titratable acidity (Fig. 4). Effects of harvest date and cultivar and their interaction were highly significant on juice pH.

Discussion

Maturation of citrus fruit, including clementine mandarin, is accompanied by a series of biochemical changes, including

color, texture, accumulation of sugars and reduction of acidity. The minimum values required for each of the quality attributes varies from country to country. For example, the export market for Moroccan clementines requires minimum soluble solids content (SSC) of 9 °Brix, a minimum maturity index value of 7.0, a minimum juice content of 40% and rind color must be typical of the variety on at least one-third of the surface of the fruit (EACCE, 1997). The same values for SSC are required for Corsican clementines (Agostini *et al.*, 1996), whereas for Spain, the minimum required values are 6.5 for the maturity index and 40% for juice content (Agustí, 2000).

Therefore, the seven clementine cultivars assessed in this study easily achieved values for internal quality. Based on the maturity time and fruit quality data, the order of maturity of the different cultivars was established. It is evident that 'Caffin' has the shortest harvest period, limited to only 1 month, and was the earliest maturing cultivar followed by the midseason cultivars 'Bruno', 'Esbal' and 'Nules' (Bono *et al.*, 1981), whereas 'Hernandina' (Tadeo *et al.*, 1981, Continella *et al.*, 1996), 'Nour' and 'Guerdane' had late maturity periods. From the data, only 'Guerdane' can really be considered as a true late maturing clementine cultivar.

Rind color of citrus is considered to be one of the most important external factors of fruit quality, as the appearance of fruit greatly influences consumer choice (Stanley, 1999; Olmo *et al.*, 2000). Moreover, although rind color development is not a limiting factor where fruit are degreened (El-Otmani *et al.*, 2000; Agustí *et al.*, 2002), for all cultivars studied the rind color increased with time. The early cultivars were of an acceptable orange color by mid-October (CI = 0 to +5) but the degreening treatment can enhance the development of orange color (Terblanche, 1999) thus allowing for harvest and marketing of fruit in early- to mid-October. The midseason cultivars developed an orange color naturally by mid-November and the late-maturing cultivars by mid-December to early January, except for 'Guerdane'. It is noteworthy that of all of the cultivars studied, 'Guerdane' had the slowest rate of color change and it reached adequate color 3 weeks later than the other cultivars of its group.

The minimum value of rind firmness (as measured by the puncture resistance force) of the fruit rind for which the fruit is still of good quality to travel over long distances appears to be close to 350 g. Below this value, the fruit is very soft and no longer elastic. This threshold value was attained in mid-October by the early-maturing cultivars, in mid-November by the midseason cultivars and in mid-December by the late maturing cultivars, except for 'Guerdane' which maintained firmness up to late January. The firmness values are therefore effective for evaluating fruit maturity (Olmo *et al.*, 2000), and rind firmness of clementine could be used as a maturity index (Burns and Albrigo, 1997) to determine how late fruit can be harvested and still ensure good quality after transport as reported for peaches by Crisosto (1994). Coggins (1986) also stressed that the rind is somewhat thicker in fruit of the early- and of the late-maturing cultivars and may be looked at as an advantage as thin rinds are more prone to blemishes, injury and decay.

Clementine mandarin loses the juice progressively after fruit color break (Agustí *et al.*, 2002). 'Caffin', the earliest maturing cultivar started to dry out by mid November, while other cultivars showed acceptable values (>40%) during the whole experimentation period (up to the end of January).

The duration of citrus cropping periods are related to the time of onset of rapid physiological changes, principally increases in soluble solids content and decreases in acid during development of citrus fruits (Grierson, 2006)**.** In this study, the soluble solids content minimum value (9%) was reached by mid October for all cultivars, except for 'Guerdane' which accumulated sugars at a much slower rate and did not reach this minimum level until December.

Juice acidity is an attribute often not taken into consideration as such, but it is becoming an important attribute in fruit quality definition. In fact, fruit taste is a balance between acids, sugars and volatile compounds. Holland *et al.* (1999) showed that total soluble solids content increased during maturity of 'Fortune' mandarin fruit, whereas acidity decreased, probably due to the use of the organic acids as respiratory substrates and the dilution of the remaining acids due to increases in size and water content of the fruit. Clementine mandarin fruit that have less than 0.8% acidity are considered of low quality as the sweetness prevails over the sourness and the fruit thus has a somewhat insipid taste and is also more prone to postharvest decay organisms (Coggins, 1986). According to the data presented in this paper, this problem may occur by early-October for the early cultivars, by mid-December for midseason cultivars and by early- to mid-January for the late-maturing cultivars.

It can be concluded that all of the cultivars, except 'Guerdane', reach minimum maturity index (sugar acid ratio >7.0) by early November. 'Guerdane' differed from the other clementine cultivars by a slow decrease in acidity and a slow increase of soluble solids content. In fact, 'Guerdane' is the only cultivar that has the characteristics of a late-maturing cultivar both internally (juice quality) and externally (rind color and firmness), allowing the period of commercial supply to be extended. The other late-maturing cultivars reach minimum maturity approximately 2-3 weeks later than the midseason group and the only attribute that is delayed in these clementine cultivars of the late-maturing group is rind color, which stays green for a longer period of time than that of the midseason group.

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Production of mini-tubers from vine cuttings of white yam (*Dioscorea rotundata*)

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Abstract

The multiplication ratio for seed yam production is very low compared to other tuberous crops. Seven clones of *Dioscorea rotundata* Poir (white yam) were evaluated for production of mini tubers from their vine cuttings. Three to four nodes leafy vine cuttings were prepared from the middle portion of the lateral branches collected from mother plants 127, 134 and 141 days after planting (DAP). The lower portion of these nodes were wounded with a clean razor blade and then dusted with 1.0% Indole-3 butyric acid (IBA) powder in order to promote rooting. The mini tubers were harvested 115 DAP. The developed mini tubers varied in sizes among the tested cultivars from 1.9 to 4.2g. The weights were found to be genotype dependent. The survival rate of the planted vine cuttings ranged from 31.1 to 77.1% while the average total number of roots per vine ranged from 5.1 to 5.8. The average number of tubers per vine was 1.8 ±0.8. If these number and weights of mini tubers can be obtained from propagation of vine cuttings, there will be tremendous increase in propagating material thereby making yam cultivation less expensive and also allowing the ware yam only for consumption and other uses.

Key words: *D. alata, D. rotundata* Poir, cultivars, IBA, mini tuber, root formation, vine cuttings, survival, white yams.

Introduction

Yam thrives successfully from sea level up to 900m. A well-distributed rainfall (or water supply) of 1,500mm per annum is adequate for crop production and plants go dormant during period of extended drought. Short days between 10-11 hours promote tuber formation, while long days (greater than 12 hours) promote vine growth. Compared to other tropical tuber crops, yam requires deep, permeable soil of high fertility. Loose loamy soils are the best as heavy clays tend to be water logged and result in tuber rots and difficult harvesting. Gravel or rock soil tends to hinder tuber formation (O'Hair, 1984).

Limited availability and cost of planting material is a major constraint for yam production in Africa. Planting material account for about 50% of the cost of the production. Large amounts of material (about 10,000 seed yams) are needed to plant 1 hectare. If farmers do not buy new seed yams, they must set aside 30% of their harvest for the next year planting. In addition, seed yams are bulky and perish quickly.

Dioscorea rotundata (white yam) and *D. alata* (water yam) are two of the major food crops grown by small-scale farmers that provide a stable carbohydrate for a population in the humid/sub-humid tropics (Oyetunji *et al*., 2003). It is an ancient crop in central West Africa (Coursey, 1967; Degras, 1993) and provides a promising avenue for alleviating the current food crises.

Despite this great potential, limited research has been carried out on agronomic techniques in the recent past to improve yam cultivation in sub-Saharan Africa (Budelman, 1987; Ekanayeke and Asiedu, 2002; Ikeorju *et al*., 1995; Oyetunji *et al.,* 2003).

In most tropical countries, food yams are propagated vegetatively by planting small whole tubers or pieces cut from large tubers. This method competes with yam availability for human consumption and at the same time makes the cultivation expensive for large scale production. Nevertheless, the propagation of yam through vine cuttings and aerial tubers (bulbils) could offer an even high multiplication rate than the minisett technique (Shiwachi *et al.,* 2002). Successful root development by vine cuttings has been reported but growth was limited and tuber production has been challenging (Shiwachi *et al.,* 2002).

Yam propagation using vine cuttings: The propagation of yam vine cuttings is a very useful technique for rapid multiplication of desirable clonal materials. Successful propagation of yams from vine cuttings was first reported in non-food yams. Using this method, Correll *et al*. (1995) obtained rooted cuttings of *D. floribunda* between 14-21 days. The first detailed and systematic study of propagation of yam vine cuttings was that of Preston and Haun (1962) with the species *D. spiculiflora.* Production of plants occurred most readily within 3-8 weeks on small rosetted shoots of immature plants.

In food yams, the establishment of plants through leafy vine cuttings has been extensively reported (Akoroda and Okonmah, 1982; Cabanillas and Martin, 1978; Njoku, 1963; Okonkwo *et al.,* 1973; van der Zaag and Fox, 1981). Cuttings of several yam species have been rooted in different medium without hormones (Akoroda and Okonmah, 1982; Cabanillas and Martin, 1978; Ferguson, 1971), although hormone treatment may accelerate root, shoot and tuber formation (Cabanillas and Martin, 1978; Kumar and Chacko, 1979).

The retention of leaves is also beneficial for root formation and further development of vine cuttings (Ferguson, 1971; Hartmann *et al.* 1990). Defoliation and heavy shade restrict growth and cause death of vine cuttings. The ease with which the cutting can

root and establish varies with species and cultivars, and is also influenced by physiological factors responsible for plant growth (Cabanillas and Martin, 1978; Hartmann *et al.,* 1990). *D. alata* for example roots more readily than *D. rotundata*, while *D. esculenta* and *D. trifida* are difficult to root (Onwueme, 1984).

The age of a plant also determines the ease with which its cuttings root. Cuttings from young plants (five weeks) have been reported to produce roots, tubers and shoots, while cuttings taken from old plants tend to develop tubers precociously and often fail to produce shoots (Cabanillas and Martin, 1978; Okonkwo *et al*. 1973). The multiplication ratio for seed yam production in the field is very low (less than 1:10), compared for instance to 1:300 for some cereals (IITA, 1999). However, there are indications that yam has great prospects of contributing to the alleviation of the projected food deficit in Africa in the 21st century, if efforts are made to identify and overcome the constraints to its production (Oyetunji *et al*., 2003; Tetteh and Saakwa, 1994). There is, therefore, a current need to find methods of improving the multiplication ratio of yams, in order to increase the amount of seed available for seed and ware yam production, and to shorten the time for developing lines in breeding programs.

Propagation through rooting of vine cuttings and by layering of vines could offer higher multiplication rate than the minisett technique (Okoli *et al*., 1982) and becomes viable alternatives in future. The objective of this study was to develop a method of mini seed tuber production using yam vines, which will reduce yam production cost.

Materials and methods

Field experiment: The study was conducted at the International Institute of Tropical Agriculture (IITA), Ibadan (7° 26'N, 3° 54'E) a transition zone between humid and sub-humid tropics. The soil of the area is derived from basement complex rocks with sandy loam surface texture overlying a layer of angular to sub-angular quartz gravel merging into an argillic horizon (Lal, 1974). Seed setts (100-150g) of seven cultivars of *D. rotundata* Poir, (TDr131, TDr 335, TDr 89/02665, TDr 99-21, TDr 93-49 and TDr 93-31) were planted in the field 50cm apart on ridges separated from each other 1m on the rows. The cultivars were arranged in a randomized complete block design. Sprouting took place averagely 21 days after planting (DAP). Plants were staked individually and thereafter weeding was done at due time.

Preparation of soil for controlled experiments: The soil was prepared by mixing rice husk collected from IITA rice field. The husk was processed using a big drum. The surface was perforated at intervals. The drum was eccentrically placed in a well constructed furnace of size 60 x 60 (cm) using cemented blocks. One side of constructed structure was free of blocks in order to ease the introduction of fire woods into the open side of the drum. The two extreme ends, that is, the top and bottom of the drum remain closed. After fire has been introduced to the fire woods in the drum, a good quantity of rice husk was then poured around the perforated side. The heat that radiated from the drum through the perforated holes carbonized the husk. As wood were continually introduced, the husk was continually turned using a wood stick until they were all homogeneously carbonized. The sterilized carbonized husk (20kg/bed) was then filled into two prepared beds of depth of 9cm and size of 57 x 162.5cm, in the greenhouse The yam cultivars were arranged in a complete randomized design. The beds were covered with a humidity chamber.

First trial: Healthy vines of three cultivars of *D. rotundata* (TDr 131, TDr 335 and TDr 89/02265) were excised from plant 127 days after planting (DAP) using a razor blade. In order to maintain moisture, vines of each clone were placed in a moist transparent polyethylene bag immediately after collection. The collection was done in the morning between 8.30 and 9.30am and taken to the green house for further processing. From the middle portion of each vine 20cm long, cuttings with three nodes each were prepared. Using a sterilized clean razor blade, vines of each cultivar were carefully wounded by scraping to remove the lower epidermis at the cut ends near the nodes.

These vines were wounded with a sterilized clean razor blade to promote root initiation. The cut surfaces were dusted with 1% Indole-3-butyric acid (IBA) (Okishibiran, Sionogi, CO, Japan). Shallow ditches of 2-3cm depths were made on the bed and filled up with sterilized carbonized rice husk. The treated vines with IBA were layered horizontally 1cm apart between the row and 4cm within the row, allowing the lower sides of the nodes dusted with IBA come in contact with the soil. The nodes were then covered with the soil and leaves were left upright to trap sunlight in order to produce more assimilates that will be translocated to the rooting zone. The planted cuttings (Table 1) were watered sparingly. A transparent polythene sheet was used to construct a humidity chamber in order to maintain a higher relative humidity within the chamber. Cheesecloth was used to maintain the shade above the humidity chamber in order to reduce the incidence of sunlight. Air temperature averaged 25.8°C and mean humidity was 85.2% while cuttings were in the rooting chamber.

Second trial: Vine cuttings of seven cultivars of *D. rotundata* (TDr 131, TDr 335, TDr 89/02265, TDr 93-49, TDr 99-12, TDr 99-21 and TDr 93-31)) were planted on carbonized rice husk in the greenhouse on 6 August, 2003 as in the first trial. Healthy vines were collected from plant 134 DAP between 10.30 and 11.30am. They were taken to the green house in a transparent polyethylene bag. To promote root initiation, the cut at lower surface of the nodes were dusted with 1% IBA powder. Cuttings treated with IBA were planted as in the first trial.

Third trial: The above experiments were repeated for the same seven cultivars of *D. rotundata*. Vines were excised from the field 141 DAP between 9.20 and 10.10am. Vines were put into transparent polythene bags as done in the previous trials.

Cuttings were sampled for root initiation at 14 DAP stage. Wetting of the cuttings continued every two days. Plant food supplement (NPK: 14-10-27, phostrogen, UK) at a concentration of 0.52g L^{-1} was applied to the stems avoiding as much as possible the leaves in order to minimize the spread of fungi which may be enhanced by the aid of nitrogen when in contact with the leaves.

Inspection for root formation was done 28 DAP by careful digging out the cutting from the carbonized rice husk. The transparent polyethylene bag used for the construction of the humidity chamber was removed 44 DAP when the roots were formed.

Fungicide (Benlate) was applied to the three trials 15 DAP at

Table 1. Number of cuttings planted for each cultivar in the three trials

Trials	TDr131	TDr335	TDr93-31	TDr93-49	TDr99-12	TDr99-21	TDr89/02665
First	45	36	-*	-	-	-	31
Second	50	36	45	43	15	31	56
Third	24	22	41	30	20	35	25

*Some cultivars did not have branches at the time of first trial.

concentration of 2.5g L^{-1}. Visual observations of the leaves were carried out for any disease occurrence. A fungal disease was observed to affect the plants. Affected leaves were removed to avoid contamination of the healthy ones. The application of Benlate fungicide continued once a week until a week to harvest. The bare soil by the bed side was wetted intermittently to prevent the spread of soil borne fungi.

Statistical Analysis: The data were analyzed using fixed model analysis of variance (ANOVA) for individual experiments using the windows version of Statistical Analysis System (SAS) package (SAS, 1991). The means were separated with t-test for significant variables.

Results

Field experiment: The seed setts planted (100-150g) on 20 March 2003, were harvested on 29 October, 2003. Different sizes and weights of tuber were observed. The average weight of tuber ranged from 1.03 to 1.99kg for TDr 131 and TDr 89/02665 respectively (Table 2). There were significant differences among cultivars, with the highest average weight of 1.99kg for TDr 89/02665 and lowest average weight of 1.03kg for TDr 131 (Table 2). The tuber weight of TDr 335 was similar to that of TDr 89/02665. Also the weight recorded for TDr 99-12 was at par with TDr 131.

Vine cutting survival: It was observed that all the planted vines could not survive (Table 3). The percentage survival was between 31.0% for TDr335 which was the lowest and 77.1% for TDr99-12 as highest. The average percentage vine survival of the 7 cultivars was 56.7% with a deviation of 16.6. It was observed that the survival rates of two cultivars (TDr 335 and TDr 89/02665) were below 50%, while the survival rates of the remaining five cultivars were above average (50%). TDr 99-12 had highest survival rate of 77.1% at par with TDr 93-49 (76.7%) and TDr 99-12. The survival rates of the vines were similar in TDr 335 and TDr 89/02665 and were the lowest. The rest shared similar vines' survival rates (Table 3).

Root formation on cuttings: Root formation on cuttings of the 7 cultivars used in this study showed no genotypic difference. Root initiation on cuttings was visible 2 weeks after planting (WAP). The number of roots per vine ranged between 5.4 and 5.8 for TDr 93-49 and TDr 99-12, respectively and with average rooting per vine of 5.55 (Table 3). The numbers of roots produced from the vine cuttings were higher in TDrs 89/02665, 99-12, and 99-21. The lowest was observed in TDr 335.

Mini tuber development through vine cutting of 7 cultivars of *D. rotundata*: Mature mini tubers were finally harvested from the cuttings of all cultivars at 115DAP. Total number of mini tubers ranged from 31 for TDr335 and 82 for TDr93-49 while the number of mini tubers per vine cutting ranged from 1.2 to 2.1 for TDr93-31 and TDr 99-21, respectively (Table 4).

The data show that each cultivar produced more than one mini tuber per vine. TDr 93-31 produced the least average number (1.2) of mini tubers per vine followed by TDr 93-49 with an average of 1.5 per vine. TDr 99-21 produced the highest number of mini tubers per vine with an average of 2.1 per vine. This was closely followed by TDr 99-12 and TDr 89/02665 with the average of 1.8 per vine. TDr. 335 had an average of 1.7 of mini tubers per vine (Table 4).

The weights of the mini tubers produced varied from one cultivar to another. The least tuber weights were produced by TDr 99-12 and TDr 99-21 with 1.1 and 1.2g per tuber, respectively. The higher tuber weights were recorded among cultivars TDr 93-31 and TDr 93-49 with 4.2g. TDr 131 and Tdr 335 produced mini tubers with average weight of 2.9 tuber each, while TDr 89/02665 produced mini tuber weight of 2.19 (Table 4). These trends of results were also recorded in the field trial. The total average weight was 2.7g whereas the error varied from 1.1g for TDr 99-12 to 3.3g for TDr 93-31 and TDr93-49 (Table 4). The mini tubers had average size of 1.9cm in height, width long of 1.3cm, width short of 0.9cm and error of 0.9, 0.5 and 0.4cm, respectively (Table 4). The moisture content ranged from 67.2% for TDr 93-49 to 72.5% TDr 87/02665 (Table 4).

The vines were observed for vegetative growth. All the cultivars produced shoots profusely with many branches (Table 5). The

Table 2. The fresh weight (kg) of tuber yield per plant of tested cultivars

Cultivar	Tuber weight
TDr 131	1.03e
TDr335	1.76ab
TDr89/02665	1.99a
TDr 93-31	1.54bc
TDr93-49	1.69b
TDr99-12	1.23de
TDr99-21	1.49cd

The means followed by the same letter in a column are not statistically different at P =0.05.

Table 3. Rooting performance and percentage survival of vine cuttings of seven cultivars of *D. rotundata* treated with 1% IBA

Cultivar	Number of planted vines	Average number of roots/vine	Survival (%)
TDr 131	74	5.7b	56.8b
TDr 335	58	5.1c	31.0c
TDr 93-31	86	5.4b	60.5b
TDr 93-49	73	5.4b	76.7a
TDr 89/02665	56	5.8ab	35.7c
TDr 99-21	57	5.8ab	57.9b
TDr 99-12	35	5.9a	77.1a
Mean ± SD	62.7 ± 5.8		

The means followed by the same letter in a column are not statistically different at P = 0.05.

Table 4. Results of mini-tuber development through vine cuttings of seven cultivars of *D. rotundata* harvested at 115 DAP

Cultivar	Tuber characteristics						
	Total number of tubers	Number of tubers per vine	Height (mean±SE)	Width long (mean±SE)	Width short (mean±SE)	Weight/tuber	Moisture content (%)(mean±SE)
TDr 131	69cd	1.6c	1.9±1.1	1.3±0.6	0.9±0.4	2.9b	68.5(0.9)
TDr 335	31f	1.7bc	1.9±0.9	1.3±0.6	0.9±0.4	2.9b	69.8(1.1)
TDr 93-31	62d	1.2d	2.2±0.9	1.5±0.5	1.0±0.4	4.2a	69.2(1.8)
TDr 93-49	82a	1.5c	2.5±0.9	1.6±0.5	1.1±0.4	4.2a	67.2(1.7)
TDr 89/02665	36ef	1.8b	1.5±0.8	1.1±0.6	0.8±0.4	2.1b	72.5(1.8)
TDr 99-21	70b	2.1a	1.5±0.7	1.1±0.5	0.9±0.4	1.2c	72.1(1.9)
TDr 99-12	48e	1.8b	1.5±0.7	0.9±0.4	0.7±0.4	1.4cb	71.4(2.1)

The means followed by the same letter in a column are not statistically different at $P = 0.05$.

Table 5. Average number of branches in 5 randomly selected plants of each cultivar

Cultivar	1^0 branches	2^0 branches
TDr 131	2.2c	3.2b
TDr 335	2.8cb	2.1c
TDr 89/02665	3.6b	4.5a
TDr 99-12	4.0b	4.8a
TDr 99-21	5.6a	4.5a
TDr 93-49	2.2c	2.7b
TDr 93-31	1.9c	3.2b

The means followed by the same letter in a column are not statistically

higher vegetative growth was recorded in TDr 99-12, TDr 99-21 and TDr 335, while the lowest was recorded in TDr 93-31

Discussion

Cultivated food yams are usually propagated vegetatively by planting whole setts or cut pieces of tuber. A considerable proportion of the tuber yield of each yam crop must therefore be reserved for planting. This often competes with supply for human consumption and at the same time makes its cultivation expensive for large scale production. The use of stem cuttings has the advantage of permitting the rapid multiplication of limited quantities of planting materials. The propagation of food yams using stem cuttings represents a departure from the conventional method of propagation using tuber pieces.

Recommendation on sett weights required for planting do not differentiate whole and cut tubers, although whole sett yams are superior planting material compared with cut setts (Onwueme, 1978). Farmers, however, plant whole and cut setts of varying weights. The recommendation of setts does not appear to be based on multiplication ratio of the planted sett. The ratio of sett weight to yield is a measure of the efficiency with which sett yams of a specified weight category produces sett or ware yams. In Our study, the cut setts of *D. rotundata* weighing 100-150g gave an average yield of 1.9kg per cultivar. The yield observed was within the ranges reported in previous studies for African average with setts between 500g to 1kg.

In this study, among the 7 cultivars of *D. rotundata* evaluated, the survival rate of vines planted was between 31.0 and 77.1% with total number of roots per vine between 5.11 and 5.76. These were comparable with the findings of Akoroda and Okonmah (1982) and van der Zaag and Fox (1981) who worked with other varieties of yam. The percentage rooting was within the ranges reported in their previous studies. For good root initiation, the sand or the medium in which the vine cuttings are planted must have good aeration and the root callus on the leafless nodes. This was the main reason why carbonized rice husk was mixed with the soil. The survival rates of the vines were noticeable from the percentage survival recorded. The root production was also very good among all the varieties. Only TDr 335 and 89/02665 had very low survival rates of the vines.

Cuttings from several yam species have been rooted in sand media without hormones (Ferguson, 1972). Application of hormone (IBA) was found to accelerate rooting in this study. It also increased the percentage rooting of the vines compared to the previous reports. This corroborates the findings of Carpenter and Cornell (1992) with *Hibiscus* stem cuttings, Schrader and Graves (2000) with cuttings of *Alnus maritina* and Al-Salem and Karam (2001) with cuttings of *Arbutus andranchne.*

Our study revealed that for the best results, cuttings must be taken between 4 and 14 weeks after sprout emergence from the setts planted and also the position from which the cuttings are collected. However, previous studies have shown that the position of cuttings on a branch determines the ease of root formation (Al-Salem and Karam, 2001; Cabanillas and Martin, 1978). The propagation of these clones using vine cuttings from different positions on a lateral branch is necessary in order to determine the cutting age that most responsive to rooting. The age of vine cuttings determine the ease with which shoots develop on rooted cuttings. With age, branches become woody and meristems show reduced activity, the node is very important in the regeneration of plantlets (Preston and Haun, 1962) and the buried nodes should be healthy and have opposite leaves.

Emphasis has not been laid yet on mini tuber (tubercles) production. Small tubercles, whose size and quantity were not specified, were reported in previous studies of Akoroda and Okonmah (1982). Also, Acha *et al*. (2002) observed the formation of mini tubers when studying the effect of auxins on root development in yam (*D. rotundata*) vine. In this study, the formation or production of min tubers ranged from 1.2 to 2.1 per vine with average size of 1.9 cm in height, 1.3 cm in width (long), 0.9 cm in width (short) and weight of 2.7g. The ease of mini tuber formation on cutting appears to be genotype dependent. The cultivars with higher

vegetative growth (TDr99-21 and TDr 99-12) produced the least tuber weights while those with low vegetative growth (TDr 93-31 and TDr 93-49) produced the highest tuber weights. This suggests that those with low tuber weights converted most of their photo-assimilates for vegetative growth, while those with low vegetative growth diverted more photo-assimilates to the storage tissue (*i.e.* tubers). These attributes help cultivars with high vegetative growth rates to possess higher survival rates than those with low vegetative growth rates as indicated in our results.

The small tuber sett weights produced from this technology can provide suitable materials for research in physiology, botany or entomology where the emphasis is not on commercial tuber yield evaluations *per se*. The small size (compact shape), wholeness and reduced weight of tubercles make them easier to pack and transport, being less prone to injury than tubers, and are therefore suitable specimens for easy transfer of genetic material in tuber form.

Tubercles can be left also to re-grow for one or more seasons to enable the observations of a treatment for several seasons or the production of sizeable tubers. From the mother plant, there is a total average number of 68.7 vines (both primary and secondary branches) per mother plant. If this number of vines can be collected per plant to produce mini-tubers (tubercles), there will be a tremendous increase in number of propagating materials, thereby allowing the ware yam only for consumption and other uses. Also, its cultivation will be less expensive.

The study indicates that vine cutting treated with 1% IBA can survive when propagated in soils with well sterilized carbonized rice husk. This study also suggests that a quantity of quality mini tubers can be produced from vine cuttings when treated with 1% Indole-3-butyric acid powder.

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