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Ectopic expression of Mn-SOD in *Lycopersicon esculentum* leads to enhanced tolerance to salt and oxidative stress

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Abstract

Production of reactive oxygen species (ROS) is associated with a number of physiological disorders in plants. Superoxide dismutase (SOD) catalyzes the breakdown of superoxide (O_2^-) into O_2 and H_2O_2 and provides the first line of defense against the toxic effects of elevated levels of ROS. The effect of increased expression of Mn superoxide dismutase (Mn-SOD) on salt stress tolerance was studied using transformed tomato (*Lycopersicon esculentum* cv. Zhongshu No. 5) plants. Northern blots confirmed expression of the heterologous Mn-SOD in transgenic plants. Strong Mn-SOD enzyme activity was detected by native PAGE in transformed plants. Transgenic plants showed resistance to the superoxide-generating herbicide methyl viologen (MV, 10^{-4} M). The total SOD activity was one and one half- to two-fold higher, and APX (ascorbate peroxidase) activity was six to seven fold higher in transgenic, than in wild-type (WT) plant under MV stress. Germination of transgenic tomato seeds at a NaCl concentration of 150 mM was greater than wild-type seeds. When exposed to salt stress, roots of transgenic plants were less stunted and leaf injury was lower than that observed in WT plants. Also, the total APX activity of transgenic plants was 4 to 5 fold higher than that of WT under NaCl (200 mM) stress.

Key words: Superoxide dismutase, oxidative stress, salt stress, transgenic tomato.

Introduction

Salt stress is one of the most common types of abiotic stress that limits the production of agricultural plants around the world (Zhu, 2000). Plants subjected to salt stress also show elevated levels of activated forms of oxygen and free radicals. These elements often are associated with damage to membranes and essential macro-molecules such as protein, DNA, and lipids (Fadzilla *et al.*, 1997; Gueta-Dahan *et al.*, 1997; Hernández *et al.*, 1995).

Superoxide dismutase (SOD) is an important enzyme in a plant's defense against oxidative stress. It catalyzes the conversion of two superoxide anions (O_2^-) into hydrogen peroxide (H_2O_2) and O_2 and alleviates oxidative stress (Bowler *et al.*, 1992). SODs are a group of metal-containing enzymes and are classified into three types according to their metal cofactor requirements: iron SOD (Fe-SOD) is localized in the chloroplast; copper-zinc SOD (Cu/Zn-SOD) is localized in the chloroplast, cytosol, and possibly the extracellular space; and manganese SOD (Mn-SOD) is found mainly in mitochondria and peroxisomes (Alscher *et al.*, 2002).

Antioxidant enzyme activity is found in plants responding to various environmental and chemical stresses (Allen, 1995; Baek *et al.*, 2006), such as freezing (Martinez *et al.*, 2001), chilling (Baek and Skinner, 2005; Iannelli *et al.*, 1999), salt (Gueta-Dahan *et al.*, 1997; Hernández *et al.*, 1995; Rajguru *et al.*, 1999), and methyl viologen (MV) (Bowler *et al.*, 1991; Donahue *et al.*, 1997).

The role of SOD during salt stress has received much attention. Exposure of salt-tolerant pea plants to NaCl resulted in the formation of O_2^- and H_2O_2 and increased the activity of SOD and other antioxidant enzymes, such as ascorbate peroxides (APX). Transcripts levels for Mn-SOD, Cu/Zn-SOD, and APX were strongly induced in the salt-tolerant variety but not in the salt-sensitive one (Hernández *et al.*, 2000). Reports dealing with rice (Dionisio-Sese and Tobita, 1998) and tomato plant (Shalata *et al.*, 2001), have also reported increased SOD activity in salt-tolerant cultivars when exposed to salt stress. Additionally, Tanaka *et al.* (1999) confirmed that overexpression of a yeast *Mn-SOD* gene in rice confers tolerance to salt stress.

Enhanced expression of SODs in transgenic plants has demonstrated tolerance to MV (Allen, 1995; Perl *et al.*, 1993), freezing (McKersie *et al.*, 1999), and salt (Tanaka *et al.*, 1999). In order to further understand the role of APX and SOD in response to oxidative stress induced by abiotic stresses, transgenic tomato plants were produced that overexpress either cAPX (Wang *et al.*, 2005 and 2006) or Mn-SOD and their response to several abiotic stresses was evaluated. The purpose of this study was to evaluate the tolerance of transgenic *Mn-SOD* tomato plants to salt (NaCl) and oxidative (MV) stress.

Materials and methods

Generation and analysis of transgenic tomato plants: Mn-SOD cDNA was synthesized from rubber tissue (*Hevea brasiliensis*) based on primers by Miao and Gaynor (1993). The cDNA was mobilized into the binary vector pDU92.3103 (Tao *et al.*, 1995) between the cauliflower mosaic virus 35S promoter and

terminator regions. The construct, pDU96.2144, contained the β-glucuronidase (GUS) reporter gene and the *NPTII* selectable marker, and was transferred into the tomato (*Lycopersicon esculentum* cv. Zhongshu No. 5) genome via the *Agrobacterium tumefaciens*-mediated transformation method described by Frary and Earle (1996) with modifications by Wang (2005). The kanamycin-resistant transformants were screened by the GUS histochemical staining assay according to the method developed by Jefferson *et al.* (1987). Genomic DNA was isolated from wild-type (WT) and *Mn-SOD*-overexpressing plants (T_1), according to the procedure of Doyle and Doyle (1987).

PCR reaction used the 35S promoter forward primer and rubber *Mn-SOD*-specific reverse primer (Miao *et al.*, 1993). The sequences for the forward and reverse primers for 35S::*Mn-SOD* included 5'– CACGTCTTCAAAGCAAGTGG –3' and 5'– CTAAGAAGAAGGGCATTCTTTGGCAT –3', respectively. About 20 ng DNA was used for the PCR reaction, under the following conditions: 1 min at 94°C, 1 min at 60°C, and 2 min at 72°C for 30 cycles.

Mn-SOD expression was assayed by northern analysis and SOD activity gel assay. Wild-type (WT) and transgenic plants were grown in a greenhouse under natural lighting supplemented with sodium vapor lamps (1000W, Philips, Inc., Eindhoven, Netherlands) for a 16-h photoperiod at approximately 23/21ºC (± 2ºC, day/night temperature). Seedlings were grown in a potting mix containing peat moss (Premier Horticulture, Inc., Quebec, Canada). T_1 seeds obtained by self-pollination of each of T_0 plants were screened for resistance to kanamycin. T_2 seeds were obtained from individual T_1 plants by self-pollination and were used to generate T_2 transgenic progeny. All the transgenic plants were resistant to the kanamycin.

Northern Blot assay: Total RNA was extracted from leaves of transgenic and WT plants using Tri-reagent (Molecular Research, Inc.). 30 µg of total RNA from each sample was used for northern blot assay. The hybridization procedure was as described by Super Hyb kit (Molecular Research, Inc., Cincinnati, OH). The hybridization probe was a ^{32}P-labeled Mn-SOD PCR fragment. RNA hybridization was detected using a PhosphorImager SI (Molecular Dynamics Inc., Sunnyvale, CA).

APX and SOD gel activity assay: About 100 mg of leaf tissue was ground to a fine powder in liquid nitrogen and homogenized in 200 µl of APX activity gel grinding buffer [100 mM $NaPO_4$ (pH 7.0), 5 mM ascorbate, 1 mM EDTA (pH 8.0), 10% glycerol, and 0.001% bromophenol blue] and SOD activity gel grinding buffer [50 mM Tris (pH 6.8), 10% glycerol, and 0.001% bromophenol blue], respectively. The supernatant was collected and protein concentration was determined using a Protein Assay system (Bio-Rad, Hercules, CA). 70 µg of total protein was loaded into each lane of a native PAGE gel. APX gel activity analysis was conducted as described by Mittler and Zilinska (1993). The SOD gel activity assay was described by Payton *et al.* (1997). After staining, the gels were scanned with a Densitometer Scanner I (Molecular Dynamics, Inc., Cincinnati, OH). The bands were analyzed using ImageQuant 5.2 software (Molecular Dynamics, Inc., Cincinnati, OH).

Application of methyl viologen: Shoots were treated with methyl viologen (MV, Sigma) following the procedure described by Perl *et al.* (1993). Shoot cuttings (with three to four leaves) from WT and T_0 transgenic plants were obtained from containerized mature plants grown in the greenhouse. The cut end of the shoots was placed in 10^{-4} M MV. After 16 h, the cut ends were transferred to tap water for an additional two days. Two leaf discs (1.02 cm in diameter) of the third leaf from the apex were excised after the MV treatment, and MV-induced oxidative damage was evaluated using the leaf electrolyte leakage (Wisniewski *et al.*, 1997) with a conductivity meter (Markson Science, Inc., Del Mar, CA). Means for all values were an average of two subsamples in each plant with three replications. The data were subjected to Duncan's multiple range test (NCSS-PASS software, NCSS Inc., Williamsport, PA). Arcsine square root transformations were performed before data analysis; nontransformed means are presented. MV treated leaf discs were also sampled and stored at –80°C prior to APX and SOD analyses.

Salt Stress: To evaluate salt stress, seeds from WT and T_1 plants were placed in petri dishes (≈40 seeds per box) on filter paper (Whatman 3MM) saturated with 150 mM NaCl solution. They were germinated at 23/21°C (± 2°C, day/night temperature) under cool white fluorescent lights (100-150 µmol m^{-2} sec^{-1}) using a 16-h photoperiod. At the end of two weeks, germination was measured. Germination was considered successful when the radicle protruded through the seed coat. Values are means ± SE (three repeats). The data were subjected to Duncan's multiple range test.

To evaluate the tolerance of developing roots to salt stress, shoot cuttings from 10-day-old WT and T_2 seedlings were grown in sterile, solidified Murashige-Skoog (MS; Murashige and Skoog, 1962) medium (Sigma) amended with either 200 mM or 250 mM NaCl. After 5 weeks at 23/21ºC (± 2ºC, day/night temperature), the fresh roots were excised, blotted on filter paper, and weighed. Values are means ± SE (six replicates). The data were subjected to Duncan's multiple range test.

WT and T_2 shoot cuttings were grown in rooting medium for 2 weeks. Healthy seedlings were transferred to 5.8 × 5.8 × 8-cm plastic pots with peat moss soil and watered with tomato fertilizer (9N-4.4P-12.5K, Schultz, Inc., Bridgeton, MO) for one week. Stress was imposed by watering plants with tomato fertilizer and NaCl (200 mM or 250 mM) solution every three days. Controls received only fertilizer. The plants were maintained in a greenhouse with natural lighting supplemented with sodium vapor lamps (1000W, Philips, Inc., Eindhoven, Netherlands) for 16-h photoperiod at approximately 23/21°C (± 2°C, day/night temperature). Height (from the apex to soil) was measured after 10 days (d). The extent of injury was evaluated visually at 10, 20, and 30 d following treatment. The scale was as follows: 0, no injury; 1, slow growth but no obvious damage, ≤20% leaf area exhibited injury; 2, leaves turned yellow, 21 to 40% of the leaf area injured; 3, plants wilted, 41 to 60% leaf area injured; 4, seriously damaged, the plant became soft and could not remain upright, 61 to 80% leaf area injured; 5, 81 to100% leaf area injured or plant died. Mean values of 5 replicates were calculated and the ranks were subjected to the non-parametric Kruskal-Wallis test. The leaf discs from WT and transgenic plants grown for 10-d under NaCl (200 mM) stress were then frozen in liquid nitrogen and stored at –80 ºC for further enzyme activity gel analyses.

Results

Overexpression of the Mn-SOD gene: No detectable GUS activity was seen in WT leaves. Transgenic leaves stained intensely blue, indicating high levels of GUS activity. No PCR products could be amplified from WT plants, whereas products of the expected size (≈800 bp) were amplified from all transgenic lines (date not shown). Northern-blot analysis was performed to assess the mRNA levels in transgenic plants. All the transgenic plants contained transcripts but levels among individual lines varied. WT plants did not exhibit transcript that hybridized to the probe (Fig. 1). Two transgenic lines, S4 and S20, were selected for further study because they showed higher expression levels.

The selected transgenic lines were screened by gel assay for the presence of Mn-SOD activity. Two isoforms were observed in all plants, and may represent chloroplastic (chl) and cytosolic (cyt) Cu/Zn-SOD (Perl *et al.*, 1993). Transgenic plants displayed an additional lower mobility band corresponding to the Mn-SOD enzyme activity (Fig. 2A). Total APX activity was also measured.

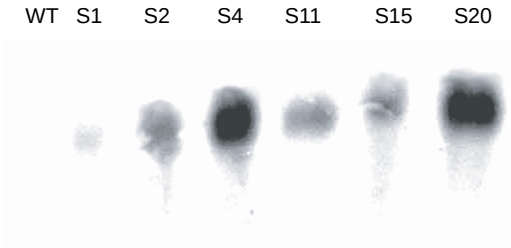

Fig.1. Northern blot analysis of RNA isolated from the T_1 plants. 30 µg of total RNA was used per lane for each blot. Blots were probed with ^{32}P-labeled Mn-SOD PCR products. WT, wild-type plant; S1 to S20, independent transgenic lines.

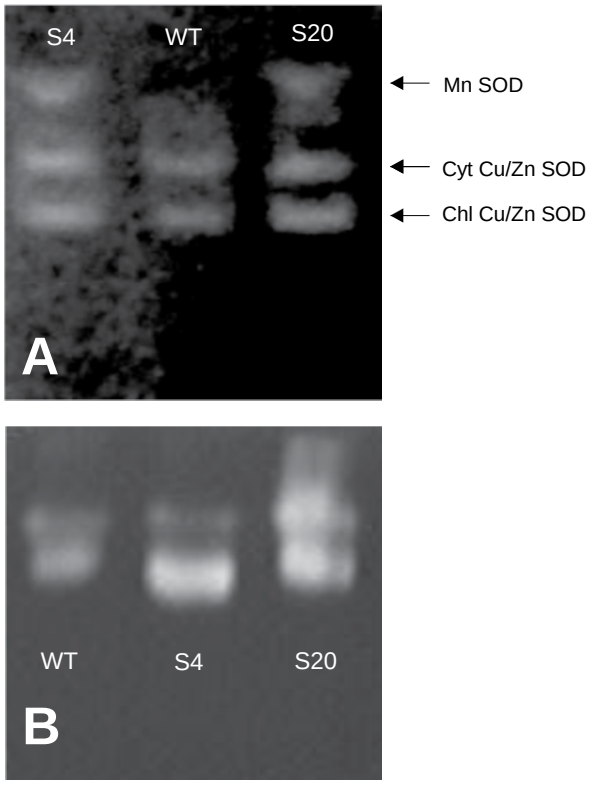

Fig. 2. Detection of (A) SOD and (B) APX activity in leaves of wild-type (WT) and transgenic Mn-SOD (Lines S4, S20) tomato plants. About 70 µg protein was loaded on the non-denaturing polyacrylamide gel.

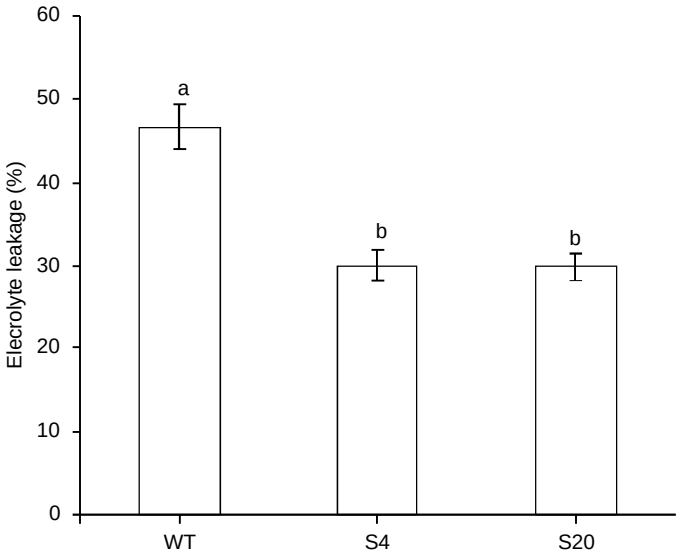

Fig. 3. Effects of methyl viologen (10^{-4} M) stress on electrolyte leakage from transgenic Mn-SOD (Lines S4, S20) and wild-type (WT) tomato plants. Bars represent SE for three replicates. Different letters indicate significant differences (P<0.05) between means (Duncan's multiple range test).

Lines S4 and S20 exhibited 2- to 3-fold higher APX activity than in WT plants (Fig. 2B).

Effects of MV stress: Methyl viologen-induced electrolyte leakage in transgenic plants was significantly less than in WT (P<0.05, Fig. 3). Mean electrolyte leakage in WT leaves was about 47%. In comparison, Mn-SOD-expressing lines (S4, S20) had significantly lower electrolyte leakage (approximately, 30%). After two days under MV treatment, total SOD activity was 1.5- to 2-fold higher in transgenic plants. APX activity of transgenic plants was 6- to 7-fold higher than WT plants following MV treatment (data not shown).

Effects of NaCl stress: The effect of 150 mM NaCl on the percent seed germination at 13 days was 76 and 81% germination for transgenic line S20 and S4, respectively and 39% for WT seeds (Fig. 4). After 5 weeks of treatment, the fresh root weight of transgenic plant (14 g and 8 g for S4; 14 g and 7 g for S20) was significantly (P<0.05) greater than that of WT (8 g and 2 g) tomato plants at 200 mM and 250 mM NaCl, respectively (Fig. 5). Irrigation of transgenic and WT tomato plants with 200mM

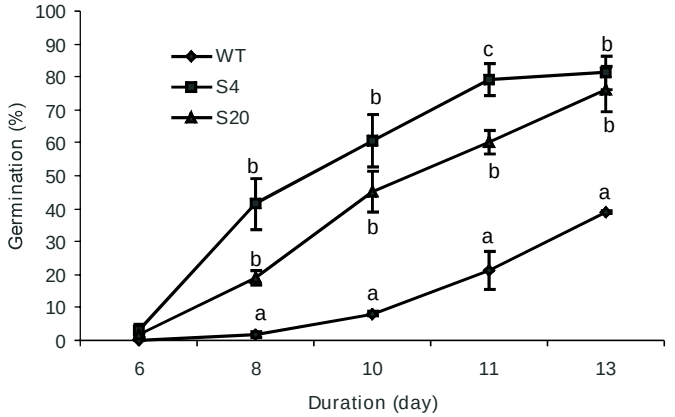

Fig. 4. Germination in T_2 transgenic Mn-SOD (Lines S4, S20) and wild-type tomato seeds treated with NaCl (150 mM) for various periods of time. Values are means ± SE (n=3). Different letters indicate significant differences (P<0.05) between means within each sampling time (Duncan's multiple range test).

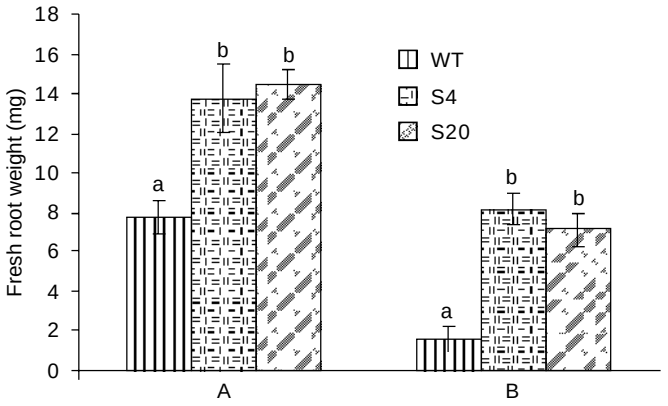

Fig. 5. Effects of 200 mM (A) or 250 mM (B) NaCl treatments on root development of wild-type (WT) and transgenic Mn-SOD (Lines S4, S20) tomato plants. Fresh weight was determined 5 weeks after the treatment. Values are means ± SE (n=6). Different letters indicate significant differences (P<0.05) between means within each NaCl treatment level (Duncan's multiple range test).

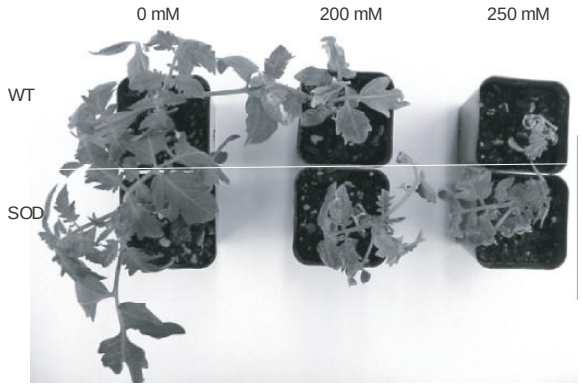

Fig. 6. Effects of salt stress on wild-type (WT) and transgenic Mn-SOD (Line S20) tomato plants. Plants were treated with 200 mM and 250 mM NaCl for 18 d in the greenhouse.

and 250 mM NaCl solution severely inhibited their shoot height growth (Fig. 6). The margins of leaflets in WT plants became necrotic following 10 days of salt treatment. Visible injury to WT was rated as 2.2. In contrast, injury to transgenic plants was significantly lower (P<0.05), showing only slight leaf area injury (scale 1). The effect of the salt treatment was still apparent but severe after 20 d. WT plants displayed visible necrotic injury (scale 3.6) after 20 d. In contrast, the transgenic seedlings showed less injury (Fig. 7A). At 250 mM NaCl, WT seedlings showed injury scale 5 after 20 d, whereas the transgenic plants exhibited less wilting injury (average scale 2.2 for S4 and 3 for S20). The differences in visible injury between transgenic and WT plants were statistically significant (P<0.05, Fig. 7B). The leaf APX activity in transgenic plants was about 4- to 5-fold higher than that in WT plants after 10 d of NaCl (200 mM) treatment (data not shown).

Discussion

Numerous studies have indicated that oxidative stress enhances SOD activity (Donahue *et al.*, 1997; El-Saht, 1998). Abiotic stresses, such as chilling, drought, and salt stress have been correlated with increase in SOD activity (Baek *et al.*, 2006; Dionisio-Sese and Tobita, 1998; Fadzilla *et al.*, 1997; Scandalios, 1993).

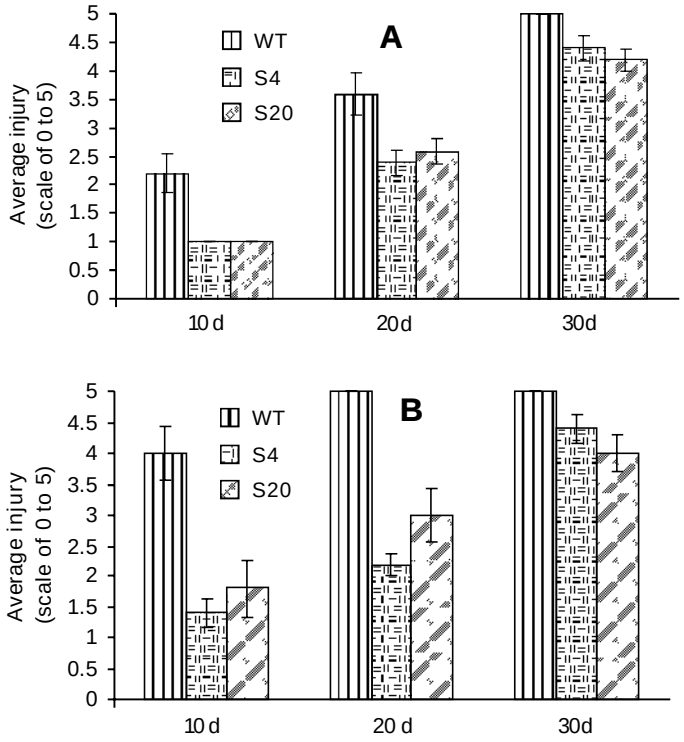

Fig. 7. Injury to wild-type (WT) and transgenic Mn-SOD (Lines S4, S20) tomato plants under stress from (A) 200 mM or (B) 250 mM NaCl treatments after 10, 20, and 30 d. No visible injury = 0; slow growth, and <20% visible injury = 1; yellowing leaves and 21-40% of leaf area with visible injury = 2; wilted plants and 41-60% visible injury = 3; seriously damaged plant unable to remain upright with 61-80% visible injury = 4; dead plant or >80% visible injury = 5. Values are means ± SE (n=5). Different letters indicate significant differences (P< 0.05) between means within each sampling time (Kruskal-Wallis test). *data for WT was also cited in Wang *et al.* (2005).

Our study indicated that transgenic tomato plants expressing the Mn-SOD gene from *Havea* displayed an enhanced tolerance to both MV and salt-induced oxidative stress. Transgenic plants had less electrolyte leakage than WT plants (Fig. 3), suggesting that overexpression of Mn-SOD in the transgenic plants reduced cellular damage caused by ROS (Bowler *et al.*, 1991). After MV treatment, APX and SOD activity was higher in transgenic plants and was related to a plant's resistance to ROS damage (Allen, 1995; Van Camp *et al.*, 1994).

An increase in ROS scavenging capacity is required to enable rapid removal of ROS produced during early seed imbibition (Gidrol *et al.*, 1994). In this study, transgenic seeds were more tolerant to NaCl than WT seeds. The transgenic plants had higher SOD and APX activity, which could prevent the accumulation of O_2^- and H_2O_2 concentration during the early stages of seed germination.

In our study, we found that shoot cuttings of transgenic tomato plants produced more root biomass than WT plants under salinity stress, indicating that overexpression of Mn-SOD enabled the transgenic plants to better deal with oxidative stress. Also, seedlings of transgenic *Mn-SOD* tomato plants showed less injury (Fig. 7) following NaCl (200 mM and 250 mM) stress than WT plants. This is consistent with the theory that increased antioxidant enzyme activity can prevent NaCl-induced oxidative stress (Fadzilla *et al.*, 1997; Tanaka *et al.*, 1999).

Our study showed that APX activity increased due to NaCl treatment and is consistent with other reports (Hernández *et al.*,

1999; Mittova *et al*., 2002; Sairam and Srivastava, 2002; Wang *et al*., 2005, 2006). This confirms earlier reports that APX plays an important role in scavenging H_2O_2 induced by NaCl stress. However, SOD activity decreased after 10 d of NaCl treatment (data not shown). The reason for this decrease in activity is not known but may be related to the long exposure to NaCl.

In this study, the overexpression of Mn-SOD in transgenic tomato plants enhanced seed germination, root development and seedling tolerance to NaCl stress. We conclude that increased antioxidant levels may play an important role in scavenging ROS when plants are exposed to salt stress.

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Some changes in postharvest physiology and activities of glutamine synthetase in broccoli head supplied with exogenous sucrose during storage

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Abstract

Sugars play indispensable roles in many metabolic processes in plants. In broccoli, the level of sugars, particularly sucrose, rapidly decline few days after harvest. This study investigated the influence of exogenous application of 10% (w/v) sucrose to broccoli heads during storage at 20°C. Hydration of the head was slowed down by sucrose treatment compared with the non-treated heads which gained weight by about 5% of the initial value at the end of the experimental period. Furthermore, sucrose application enhanced ethylene production as well as respiration rate. Glutamine synthetase (GS; EC 6.3.1.2) activity was higher in the florets of sucrose-treated heads but, like the non-treated heads, the activity continuously declined until the end of the storage period. The relatively higher GS activity during the early period of storage caused the delay of the onset of ammonia accumulation by about a day. In the branchlet portion, GS activity was higher in the sucrose-treated heads until day 2 but declined thereafter. The decline in GS activity in this portion, however, did not result to ammonia accumulation.

Key words: Ammonia, ethylene, postharvest life, respiration, broccoli

Introduction

Broccoli heads are harvested for commercial purposes when they are physiologically immature, thus making them susceptible to rapid quality deterioration when kept under ambient temperatures after harvest. During storage, major physio-biochemical changes occur in heads that lead to postharvest senescence. For instance, sucrose level rapidly declines (Pramanik *et al.*, 2004; Coupe *et al.*, 2003; Downs *et al.*, 1997) and ammonia accumulates (Baclayon *et al.*, 2004; Matsui *et al.*, 2004; King and Morris, 1994) in the florets few days after harvest. These changes have been singled out as the major contributing factors in the quality deterioration of the commodity.

Postharvest sugar application has been reported to increase the longevity of some important horticultural commodities such as roses (Ichimura *et al.*, 1999), carnation (Verlinden and Garcia, 2004) and broccoli (Nishikawa *et al.*, 2005; Irving and Joyce, 1995). It could be pointed out that the essential roles of sugars as sources of carbon skeleton for the complex biochemical metabolism in plants contribute to the postharvest life of perishable commodities. It was reported that exogenous sucrose application in broccoli can improve postharvest quality by altering ethylene metabolisms (Nishikawa *et al.*, 2005), keeping higher level of chlorophyll in the florets (Coupe *et al.*, 2003), and increasing ascorbic acid levels (Smirnoff and Pallanca, 1996). Furthermore, sucrose may affect the ammonia detoxification process by providing energy for the latter's assimilation by glutamine synthetase (GS; EC 6.3.1.2), a primary enzyme responsible for assimilating ammonia in plants. As high levels of ammonia are thought to be toxic to plant cells, it is incorporated by GS as the amide group into glutamate, thus enabling detoxification. Ratajczak *et al.* (1981) reported that GS activity in lupine embryo in media containing saccharose was induced. However, in this study, sucrose was considered since sugars are transported to the sink tissues as sucrose; other forms of sugars could be hardly transported into the cells (Nishikawa *et al.*, 2005).

As mentioned earlier, sugar application improves the shelf life of perishable produce. However, the influence of sucrose on glutamine synthetase activity and ammonia accumulation, which are believed to have important roles in the shelf life of broccoli, have not been investigated which formed the basis of this study. This report also presents the changes of some important physiological processes occurring in broccoli during storage.

Materials and methods

Plant material and treatment: Broccoli var. 'Pixcels' heads were harvested from Kagawa Agricultural Experiment Station, Miki, Kagawa, Japan. Right after harvest, the heads were trimmed and brought to the laboratory for treatment. The stem ends were immersed in a 10% (w/v) sucrose solution with 0.05% (v/v) NaClO. The same conditions were employed in the control samples except the addition of sucrose in solution. The solution was replaced with a newly prepared one every 24 h. The heads were enclosed with perforated plastic sheet and kept at 20°C. At the end of each storage period, the florets were separated from the branchlets and kept at -30°C until enzyme and ammonia extractions and analyses.

Daily weight determination: The weights of the heads were taken daily and expressed as percent weight loss or gain of the initial sample weight.

CO_2 and C_2H_4 production rate measurements: Each head was weighed and placed in a 6 L glass jar held at 20°C. Carbon dioxide and ethylene production were measured daily from an intact

head sealed in a glass jar for 1 h by taking a 10 ml (for CO_2) and 1 ml (for C_2H_4) gas sample from the glass jar and injecting the sample to the thermal conductivity detector (TCD) (GC-8 AIT, Shimadzu Co., Ltd.) and flame ionization detector (FID) (GC-14B, Shimadzu Co., Ltd.) gas chromatographs, respectively. The result was expressed in ml CO_2 $kg^{-1}h^{-1}$ for respiration rate and nl $g^{-1}h^{-1}$ for C_2H_4 production.

Extraction and assay of GS and ammonia: Approximately five grams (plus or minus % weight gain or loss of the initial fresh weight of the tissue) from each portion of the broccoli head was added with 1% polyvinyl polypyrrolidone (PVPP) proportional to the sample weight, 1 g sea sand and 5 ml buffer solution containing 50 mM tris-HCl (pH 7.6), 10 mM $MgSO_4$, 1 mM EDTA, 1 mM dithiothreitol (DDT), 12 mM 2-mercaptoethanol, 5 mM L-glutamate and 100 ml glycerol L^{-1}. The mixture was homogenized using a cooled mortar and pestle. 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 12000 x *g* at 2°C for 10 min. Enzyme activity was determined as described by Baclayon *et al.* (2006).

Ammonia content was assayed using the procedure of Kun and Kearney (1974) with few modifications. Briefly, 2 g of fresh-weight sample from each portion of the broccoli head was extracted with 20 ml 10% TCAA at 0°C (ice bath) and centrifuged at 12000 x *g* at 2°C for 10 min. The 1 ml assay mixture contained 200 µl 0.5 M tris-buffer (pH 8), 100 µl 0.1 M 2-oxoglutarate solution (pH 7.4), 30 µl 8 mM β-NADH solution, 150 µl distilled water and 500 µl of neutral extract sample. The decrease of NADH, as determined by the change of extinction at 365 nm, was used as a measure of the reaction.

Analysis of data: Data were analysed in randomized complete block design with three replications to see the significance of treatments using calculated *F*-value. Linear correlation was used to evaluate the relationships between GS activity and ammonia accumulation.

Results

Weight loss/gain: The weights of the broccoli heads treated with sucrose were constant over the first 3 days of storage, and decreased thereafter (Fig. 1). For the non-sucrose-treated heads,

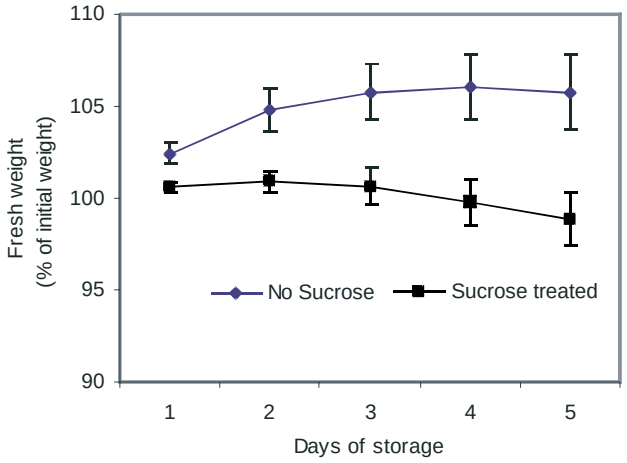

Fig. 1. Changes in fresh weight of intact broccoli heads supplied with exogenous sucrose during storage. Vertical bars indicate SE. SE bars are not shown when masked by the graph symbols.

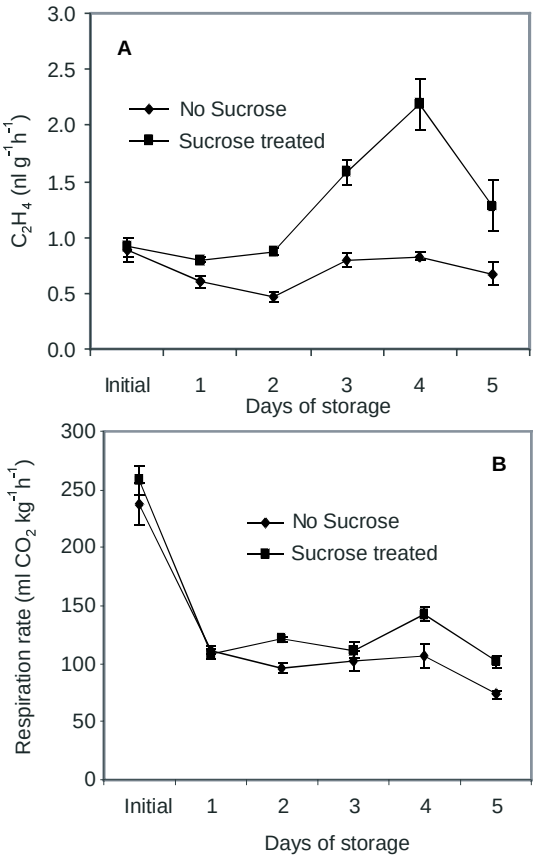

Fig. 2. Changes in ethylene production (A) and respiration rate (B) of intact broccoli heads supplied with exogenous sucrose during storage. Vertical bars indicate SE. SE bars are not shown when masked by the graph symbols.

the weights continuously increased until the last day of the storage. Weight gain was significantly higher in the heads without sucrose treatment than in the treated ones.

Production of C_2H_4 and CO_2: Ethylene production was constant in the sucrose treated heads while declined in the non-treated heads until day 2 of storage (Fig 2A). A rapid increase was observed in the sucrose-treated heads while the level in the non-treated heads was at par with the initial value until day 4. At the end of the 5-day storage period, ethylene production declined in both treatments. Although ethylene was higher in the treated heads, the pattern of decline in greenness was nearly the same as that in heads without sucrose treatment (data not shown).

Carbon dioxide production drastically declined after 24 h from harvest in both the treated and non-treated heads (Fig. 2B). However, CO_2 production in the sucrose-treated heads was consistently higher than that in the non-treated heads from day 2 until day 5.

GS activity and ammonia accumulation: The glutamine synthetase activity varied between portions of broccoli head. In the floret portion, the GS activity in both treatments increased after 24 h from harvest and continuously declined thereafter (Fig. 3A). The sucrose-treated florets had relatively higher enzyme activity than the non-treated ones throughout the entire storage duration. In the branchlet portion, sucrose treatment increased the enzyme activity until day 2 while the activity in the non-treated portion was maintained until day 3. Enzyme activity in the sucrose-treated tissue was higher than that without sucrose treatment except on day 3.

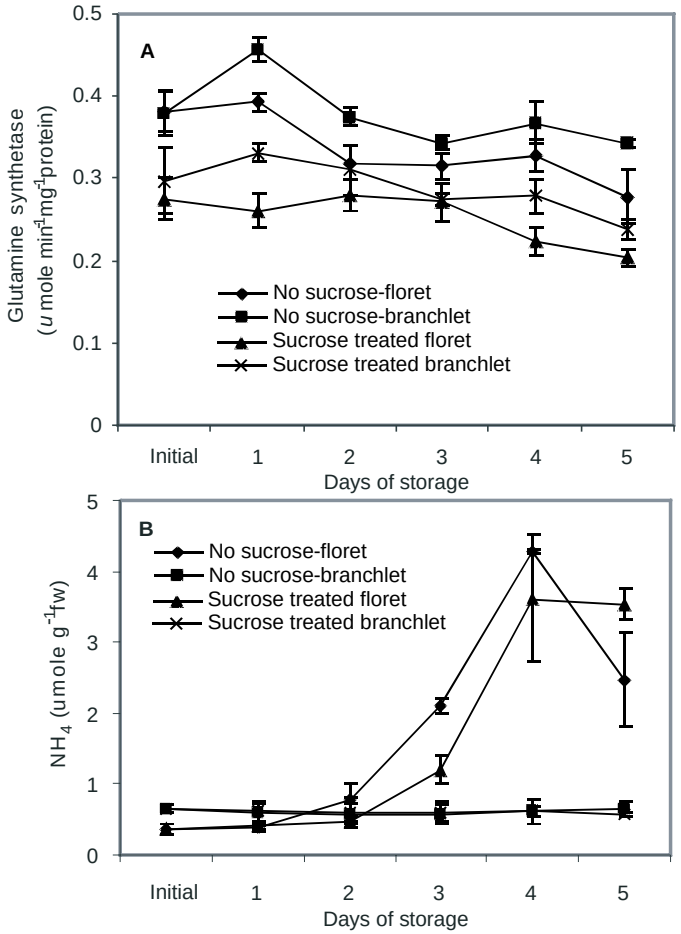

Fig. 3. Changes in glutamine synthetase activity (A) and ammonia content (B) in the branchlet and floret portions of broccoli heads supplied with exogenous sucrose during storage. Vertical bars indicate SE. SE bars are not shown when masked by the graph symbols.

Ammonia content in the florets treated with sucrose was slightly lower than that without sucrose until day 4 (Fig. 3B). Although ammonia content abruptly increased in both treatments, the onset of ammonia accumulation was delayed by about a day in the sucrose treated florets than that without sucrose. In the branchlet portion, the ammonia content was not significantly different between the non-treated and the sucrose-treated tissues, and there was no remarkable change in ammonia content throughout the storage duration.

Correlation between GS activity and ammonia accumulation: Correlation analysis revealed that there is a significant negative correlation between GS activity and ammonia accumulation in the floret portion of the sucrose–treated heads (Table 1). On the other hand, no significant correlation between GS and ammonia content was found in both portions of the non-treated heads and the branchlet portion of the treated ones.

Table 1. Correlation coefficient (*r*) values computed from linear regression analyses between glutamine synthetase activity and ammonia accumulation in the branchlet and floret portions of broccoli heads treated with exogenous sucrose during storage

Treatment	Portion	Correlation coefficient (*r*) value
Without Sucrose	Florets	-0.356
	Branchlets	-0.277
Sucrose-treated	Florets	-0.503*
	Branchlets	0.359

*significant correlation at $P<0.05$ (0.468); n=18

Discussion

Major physio-biochemical changes have been observed in broccoli head during storage (Deschene *et al.*, 1991; Pogson and Morris, 1997; Matsui *et al.*, 2004; Pramanik *et al.*, 2004). In this study, immersing the cut base of the broccoli head in a solution either with or without sucrose during storage affected the hydration rate of the head as manifested by the changes in weight during storage (Fig.1). The weights of the sucrose-treated heads remained constant until day 3, but declined thereafter. In the non-sucrose-treated heads, the weights increased continuously to about 5% of the initial content at the end of the storage period. The decline in weight after day 3 may imply that transpiration rate exceeded the hydration rate in broccoli head. The presence of solute, sucrose in this case, could have impaired the hydration process. In cut rose, treatment in a solution containing sucrose significantly retarded hydration (Durkin, 1979). Furthermore, the application of sucrose enhanced ethylene production (Fig. 2A). After day 3 of storage, ethylene rapidly increased until day 4 in the sucrose treated heads but no remarkable increase was observed in the non-treated heads. Although ethylene production was significantly higher in the sucrose-treated heads, the rate of yellowing (data not shown) was almost the same as in the non-treated ones. Nishikawa *et al.* (2005) attributed this result to the decreased sensitivity of the florets to ethylene. They suggested that sucrose may be easily transported into the sink cells, the florets, and glucose produced by sucrose hydrolysis in the cells may enhance the ethylene biosynthesis. However, it was unclear as to what regulates ethylene sensitivity, sucrose or its hydrolyzed product, glucose, in the cell. On the other hand, the respiration rate was initially high in all heads but abruptly declined after 24 h from harvest and stabilized thereafter (Fig. 2B). The high initial CO_2 production can be attributed to stress imposed during harvest and subsequent trimmings. In the sucrose-treated heads, respiration rate was consistently higher than the non-treated heads from day 2 until the end of the storage period. The ready availability or abundance of respiratory substrate could have caused the higher CO_2 production.

GS activity in the florets of both treatments reached the maximum, a day after harvest, and continuously declined thereafter (Fig. 3A). The enzyme activity was higher in the florets of the sucrose-treated heads than in the non-treated ones. The changes in enzyme activity in the floret portion of sucrose-treated heads were negatively correlated with ammonia accumulation (Table 1). In senescing wheat leaves, the accumulation of ammonia has been found to coincide with almost complete disappearance of GS (Peters and Van Laere, 1992). The result implies that due to the relatively higher GS activity in sucrose-treated head, the onset of ammonia accumulation was delayed compared to the non-treated heads. Ammonia started to rise on days 2 and 3 in the non-treated and the sucrose-treated florets, respectively (Fig. 3B). In the branchlet portion, GS activity was higher in the sucrose-treated tissue during the earlier period of storage but dropped after day 3. Although there was a decline in GS activity in this portion, there was no significant increase in ammonia content until the end of the 5-day storage period. It is likely that the level of ammonia has been efficiently assimilated by GS. The ammonia produced from protein catabolism, amino acid deamination and some biosynthetic reactions (Lam *et al.*, 1996) may not have

exceeded the required amount to repress GS activity. In addition, the higher sugar content of broccoli branchlet (Pramanik *et al.*, 2004) favoured the activity of the enzyme.

The results of this study suggest that sucrose treatment delays the onset of ammonia accumulation due to relatively higher GS activity in the earlier period of storage of broccoli heads. However, as in the non-treated heads, the GS activity continued to decline after 24 h from harvest. The relatively higher respiration rate and ethylene production in the sucrose treated heads have enhanced senescence. Thus, the benefit of delayed onset of ammonia accumulation may be diminished by the effects of other deteriorative processes. Further study to a wider range of cultivars is needed to validate these suggestions.

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Developmental influence of *in vitro* light quality and carbon dioxide on photochemical efficiency of PS II of strawberry leaves (*Fragaria x ananassa*)

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Abstract

The influence of light quality and carbon dioxide concentration on the development of photosynthetic functional structures of strawberry leaves *in vitro* was examined. We assessed the photochemical quenching parameter of chlorophyll a photochemical efficiency of photo system II (PSII) of strawberry leaves *in vitro* in a factorial set up. The main effects of light quality; averaged over CO_2 enriched, air flow and closed systems, increased the initial chlorophyll fluorescence value from 485 for yellow light developed PS II system of leaves to 1142 for white light (control) developed ones. The photochemical efficiency of PSII significantly increased from 0.64 under white light to 0.80 for yellow light developed leaves. The leaves developed under blue light were similar to that of control white light for many chlorophyll fluorescence parameters except the initial chlorophyll fluorescence level. The increase in photochemical efficiency of PSII of strawberry leaves can be attributed to lower initial fluorescence values. Under blue light the total dry weight and total chlorophyll content were increased. The possible role of high mercury peak of white light and photoinhibition during development *in vitro* is discussed.

Key words: Carbon dioxide enrichment, air flow, photo system II, photochemical efficiency of PS II (F_V/F_M), photosynthetic photon flux (PPF), photoinhibition, dry weight, chlorophyll and nitrogen.

Introduction

The leaves developed under *in vitro* conditions usually have poorly developed chloroplasts; disorganised granal structure, low chlorophyll (chl) and protein content in general (Capellades *et al.*, 1990 and Ziv and Ariel, 1995) and the leaves of *in vitro* developed strawberry plants lack photosynthetic ability (Grout, 1988). Understanding the photosynthetic potential and changes in photosynthetic efficiency of *in vitro* developed leaves, in response to *in vitro* changes in physical environmental conditions become essential to regulate the *ex vitro* adaptation conditions for quick establishment of the plants in the open. The chlorophyll fluorescence technique offers a precise and highly sensitive measure of the photochemical efficiency of PS II and the photosynthetic capacity of the leaves (Butler, 1977) by probing the internal structure of the Photosystem II (PS II) and it's functioning. Chlorophyll fluorescence measurement is rapid, extremely sensitive, and non-intrusive and can be performed on intact leaves of field plants as well as isolated chloroplast or sub chloroplast particles (Butler, 1977; Krause and Weis, 1991 and Samson *et al.*, 1999). These parameters may be good predictors of *ex vitro* performance.

The reports on the chlorophyll fluorescence parameters of *in vitro* developed leaves under conventional system are generally varied. This is mainly due to the difference in developmental conditions of the tissue culture system, like photosynthetic photon flux (PPF) (Ticha *et al.*, 1995) or photoauto/mixotrophic condition (Haisel *et al.*, 1999; Ticha *et al.*, 1998 and Pospisilova *et al.*, 1998) or the species used (Rival *et al.*, 1997 and Triques *et al.*, 1997). There are no reports on the photochemical efficiency of PS II of strawberry leaves developed in a conventional closed tissue culture system at low light intensity (< 80 µmol m^{-2} s^{-1}) or, of any plant developed under different spectral qualities and CO_2 regimes *in vitro*. We have tested the developmental influence of light spectral qualities or CO_2 concentration on the photochemical efficiency of PS II in strawberry leaves *in vitro*, while all other parameters: controllable biological, physical and chemical conditions were constant.

Materials and methods

Plant, media and growth conditions: Strawberry cultivar 'Red Joy' plantlets of uniform size, 2-2.5cm high, ≈ 0.105 g weight, with 2-3 leaves and without roots were taken from 6-8 weeks old virus free whole plant culture developed at a rate of one plantlet per 250 mL container in 30 mL of half strength MS solid media (Murashige and Skoog, 1962) supplemented with 2 µM of BAP (pH 5.6). These cultures were maintained under a PPF of 65 ± 5.5 µmol m^{-2} s^{-1} for a 16 h photoperiod from 08:00 h to 24:00 h and at a culture growth room temperature of 26 ± 1.5°C. Strawberry plantlets were grown in 250 mL glass containers with transparent polypropylene screw lids, which were fitted with two rubber septums (Shimadzu, Japan) to suit CO_2 enrichment and air systems**.**

Light spectral qualities: The emission spectra's of light spectral qualities (Phillips, Holland) used was directly measured using a computerised spectrophotometer (Ocean Optics S2000, LAS TEK, S. Australia). A beam of light, 2 cm from the light globe which was at 1/3rd distance from the distal end of the tube light was quantified (Table 1). In addition to the respective major peaks, a mercury peak between 545 – 546 nm for white or blue or yellow

or red lights with an intensity of 1100 or 1000 or 500 or 35 units (arbitrary), respectively were also observed.

Table 1. Light quality, spectral band width and major peaks of light source used

Light spectral quality	Spectral bandwidth (nm)	Major peak wavelength (nm)
White	461 - 650	438 - 440
Blue	423 - 476	435 - 436
Yellow	537 - 637	578 - 579
Red	646 - 680	658 - 662

CO_2 enrichment and air flow systems: The plantlets were enriched with CO_2 or air as per the experimental design using a gas flow system developed at the University of Queensland, Gatton campus. This system consisted of: 1) compressed CO_2 (1000 ± 5ppm) and air cylinders with respective pressure gauge, G sized 15.0 MPa, BOC Australia, 2) a cigweld gas flow meter, Comweld Groups Pty Ltd, Australia, 3) solenoid time dosing control, 4) manifolds with ten outlets for each, 5) sterile Terumo needles and Millipore filter-Millex FG 0.2 µm, and 6) transparent culture container lids with 1 or 2 rubber septums - Shimadzu Japan. These cylinders were connected with upstream (0-30000 Kpa) and down stream (0-1500 Kpa) regulators. The down stream was connected to the flow meter, which was marked from 0-15 L min^{-1} with 30 equal divisions (set for 200 Kpa). The outlet of the flow meter was connected to a solenoid time dosing control system and the flow rate was at 1 L min^{-1}. The flow rate was standardised after measuring the changes in CO_2 *in vitro* using an infra red gas analyser, prior to the experiment considering the photosynthetic rate and CO_2 accumulation due to dark respiration. The temperature and PPF inside each container measured every half an hour for a week was used as covariates of the developmental characters.

Three plants were selected from each treatment and container with the plants were covered with aluminium foil and transferred to a laminar flow for recording fluorescence parameters. From each plant two to three matured leaves were selected for measuring chlorophyll fluorescence values of *in vitro* developed leaves during the last week (fifth) of the experimentation. Adjusted mean values for number of leaves $plant^{-1}$ were used for statistical analysis. Observations on the following developmental parameters were assessed at end of five weeks. Data recorded were analysed using Generalised Linear Model of Statistical Analysis System. The whole experiment was repeated at a later date to confirm the results of the first.

Fluorescence measurements: Chlorophyll fluorescence parameters were measured by plant efficiency analyser (Hansatech Instruments Ltd., UK) with Firmware Version PO2.001, Analyser Version PO2.01 and Summary Version P2.01. The excitation light intensity was set at saturation pulse mode (3000 µmol $m^{-2}s^{-1}$) for all observations. This high light intensity was chosen to get additional information on the potential photosynthetic ability of these leaves for direct *ex vitro* planting without going through an acclimatisation procedure as in normal tissue culture plants. The intact plants with the media were deflasked and placed in a sterile Petri dish under a laminar flow. The selected intact leaf blades were dark adapted for 15 min. and the illumination was activated and parameters F_0, F_M, F_V and time were recorded, where: F_0 is the initial level of chlorophyll fluorescence, F_M is the maximum level of chlorophyll fluorescence, F_V is the variable component of fluorescence, Time is the duration for fluorescence rise in ms and F_V/F_M (calculated) is the photochemical efficiency of PS II (Butler, 1977).

Total dry weight, total chlorophyll and nitrogen content: The plants were dried in a hot air oven at 65°C for three days and the weight at ambient temperature was recorded as the total dry weight. Total chlorophyll content was determined from whole plant extracts (Porra *et al.*, 1989). Total nitrogen content of whole plant was determined by combustion analyser, LECO CNS 2000, at a temperature of 1100°C calibrated with EDTA.

The light qualities; white, blue, yellow and red, in combination with three regimes of CO_2 treatments; i) CO_2 enriched (1000 ppm) system, ii) air flow system which was similar to the CO_2 enriched system except for CO_2, compressed air was used, and iii) a closed system which was the standard tissue culture system. The details of spectral qualities of the light source are shown in Table 1. A 4 x 3 factorial set up in a completely randomised design with 5 replications for each treatment was used. The temperature and PPF measured every half an hour inside each experimental unit (container) over a period of week prior to the experiment were used as covariates of fluorescence parameters. The general factorial model to describe the dependent variable which involved terms for each factor and also terms for possible interactions between the factors was chosen.

Results

The light quality used had major impact on the chlorophyll fluorescence parameters; F_0, time for fluorescence rise and F_V/F_M of leaves of the strawberry plants developed *in vitro* (P<0.05, Figs. 1, 4 and 5). Neither CO_2 regimes nor the interaction effect of Light x CO_2 had any significant influence on development of PS II.

Fluorescence parameters- F_0, F_M, F_V and time: Blue, yellow and red lights significantly reduced the mean F_0 value, when compared to the control, white light (P<0.05, Fig. 1). The lowest F_0 recorded was under yellow light. The F_M value (Fig. 2) of leaves developed under yellow light and F_V values (Fig. 3) of white light tend to be the lowest (P>0.05). The leaves developed under white and blue light recorded less time to reach fluorescence maximum (F_M), whereas those developed under yellow and red light recorded more time (Fig. 4).

Photochemical efficiency of PS II (F_V/ F_M): Yellow and red lights increased the mean F_V/F_M ratio of leaves when compared to those leaves developed under white and blue light (P<0.05, Fig. 5). Leaves developed under the white light, (control) recorded lowest photochemical efficiency of PS II. The mean F_V/ F_M ratio of leaves developed under red light were statistically higher than that of yellow light (Fig. 5).

Total dry weight, total chlorophyll and nitrogen content: Blue lights significantly increased the total dry weight and nitrogen contents of plants developed *in vitro* compared to the rest (Figs. 6 and 8). The total chlorophyll content was highest in blue and yellow light developed plants (Fig. 7).

Relationship between wavelength and chlorophyll a fluorescence parameters: The development of PS II of strawberry leaves was favoured at longer wavelengths than

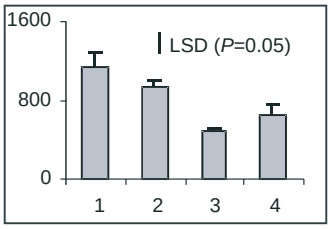

Fig. 1

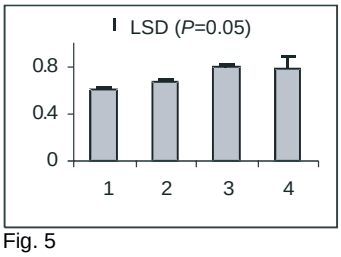

Fig. 5

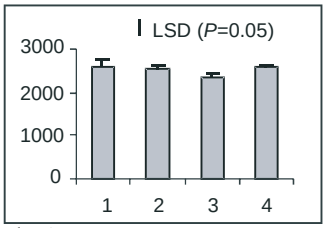

Fig. 2

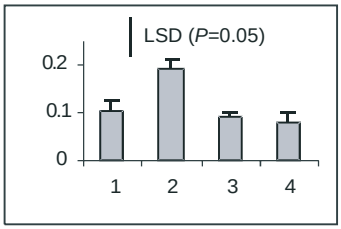

Fig. 6

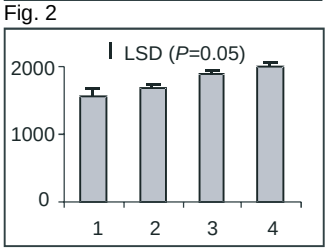

Fig. 3

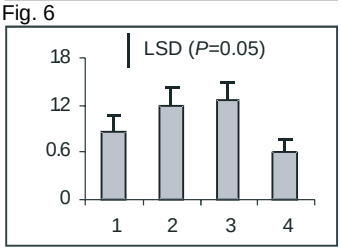

Fig. 7

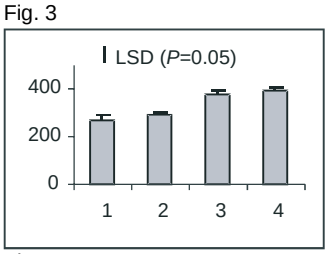

Fig. 4

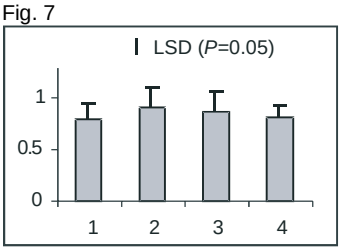

Fig. 8

Fig. 1-8. The X axis for all Figs. (1-8) represent the spectral colour of light under which the strawberry plants developed *in vitro* where; 1= white light (461-650nm), 2 = blue light (423-476nm), 3 = yellow light (537-637nm) and 4 = red light (646-680nm). In Y axes of Figs. 1-5 are chlorophyll fluorescence parameters of chl a of strawberry leaves, where Fig. 1 for mean initial chlorophyll fluorescence (F_0), Fig. 2 for mean maximum chlorophyll fluorescence (F_M), Fig. 3 for mean fluorescence variable (F_V), Fig. 4 for time for fluorescence rise from F_0 to F_M (T) and Fig. 5 for photosynthetic efficiency of PS II of Chl a (F_V/F_M), Fig. 6 for mean total dry weight (g), Fig. 7 for mean total chlorophyll content (mg g^{-1}) and Fig. 8 for mean total nitrogen content (% of total dry weight). Error bars on each mean where n=27 for each fluorescence parameter and n = 9 for all other parameters. Standard error was too small to be visible on some bars. The independent bars are least significant difference (LSD) at P =0.05.

shorter wavelengths (r = 0.875). There was a better correlation between wavelength and time for fluorescence rise (r = 0.981). There was a highly significant association between spectral peak mean wavelength and F_V (r = 0.960, $P < 0.01$).

Discussion

The light spectral qualities significantly influenced the leaf development *in vitro* whereas CO_2 regimes did not. The difference in F_0 and other parameters across lights can be due to the differences in the spectral distribution of the lights used. Though *in vitro* developed strawberry leaves lack photosynthetic ability (Grout, 1988), this study reveals that the *in vitro* developed leaves have high photochemical efficiency of PS II.

The high F_0 values and short time for fluorescence rise of chl a in leaves developed under white light can be due to the partially oxidised reaction centre or not fully active or not fully opened state instead of being fully oxidised (Figs. 5, 1 and 4). This may be a sign of photoinhibition or photodamage (Bjorkman, 1987; Krause, 1988), which occurs when the rate of degeneration of D1 protein exceeds its regeneration in the reaction centre of PS II due to unused absorbed light energy causing formation of reactive oxygen species (ROS) which fragments the D1 protein (Powels, 1984; Ohad e*t al.,* 1984; Barber, 1992; Aro *et al.,* 1993; Asada, 1999; Nikitishen e*t al.,* 2002 and Lupinkova e*t al.,* 2004). The significantly high F_0 values of white light irradiated leaves when compared to yellow or red light developed leaves may be due to the very high mercury (emission) peaks 546 – 547 nm of the spectral distribution of the light used (Fig. 1). Though the PPF was adjusted to uniformity among light qualities used, the intensity of specific peak wavelengths within the PPF was not measurable. In addition, as the CO_2 concentration reduces drastically within first four hours of light in a closed tissue culture system, as observed in this experiment and as reported previously (Falque e*t al.,* 1991; Kubota and Kozai, 1992) at CO_2 compensation point photosynthesis leads to oxygenation of ribulose 1,5- biphospate by RubisCO (Powles, 1984; Heldt, 1997) and photoinhibition or damage is possible.

The highest total dry weight, chlorophyll and nitrogen content of plants developed under blue light, irrespective of low photochemical efficiency of PS II, may be attributed to the very higher rate of photochemical (kp) reaction under blue light compared to other competing reactions in the PS II (Figs. 6-8). In the PS II photochemical reaction, $\phi P_o = k_{P\,/}\,(k_F + k_D + k_T + k_p) = (\phi F_M - \phi F_0) / \phi F_M = F_V / F_M$, where ϕP_o = Potential yield of photochemical reaction, (k_p) = the photochemical reaction, (k_F) = rate constant of pigment fluorescence, (k_D) = thermal deactivation and (k_T) = excitation energy transfer to non fluorescent pigments, are the most important competing reactions (Krause and Weis, 1991). It is known that the photosynthetic reaction demands high photon yield, and $k_P >> k_F + k_D + k_T$. When the primary quinone-type acceptor (Q_A) of PS II is oxidised (when the reaction centre is opened) the probability of fluorescence emission is low (F_0), whereas when Q_A is reduced (when the reaction centre is closed) the probability of fluorescence emission is high (FM) (Schreiber *et al.,* 1998).

In photosynthesis, the higher rate of photochemical work or the electron transfer rate or the heat released (k_D) during the transfer from second to first singlet state (Heldt, 1997) in the excitation states of chl a had a scattering effect on chlorophyll molecules than an aggregation effect during development. Thus the leaves developed under blue light with low photochemical efficiency of PS II was not a true photoinhibition and it did not have the adverse effects of chronic photoinhibition on growth, whereas leaves developed under white light had the lowest photochemical efficiency of PS II, which was due to the highest F_0 value subsequently had low dry weight and low chlorophyll and nitrogen content (Figs. 5, 1, 6 and 7). Large increase in F_0 can be due to loss of functional continuity between photon harvest and energy processing in PS II (Schreiber *et al.,* 1998) or the rate of regeneration of D1 protein was in a much slower phase than the photon harvest.

Even though photochemical efficiency of PS II was highest under

yellow and red light developed leaves, non fluorescent parameters indicated that photochemical efficiency of PS II did not have a direct effect on growth like in the blue light developed leaves. The lowest chlorophyll content under red light may be due to the root-perceived photomorphogenic inhibition of shoot greening (Tripathy and Brown, 1995) as the root system in this experiment was exposed to red light through the media (Fig. 7).

Photoinhibition under white light was caused by excess unused radiation energy from the mercury peak because the CO2 levels get depleted in a closed system within few hours of light. The highest variability of F_0 and other florescence parameters of white light developed leaves could be attributed to the influence of the mercury peak during development and warrants further investigation and due consideration may be given to an appropriate light quality to optimise in vitro plant/tissue growth.

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Characterization of environmental stress-regulated anthocyanin production and growth of cranberry callus

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Abstract

Cranberry callus was successfully induced from cranberry (*Vaccinium macrocarpon* Ait, Ericaceae) leaves by using Gamborg's B5 medium containing phytohormones at 25°C in the dark. Anthocyanin-producing cranberry callus was obtained only under conditions of continuous light exposure. Red light and UV light exposure of the callus enhanced anthocyanin content by 41.3 and 29.3%, respectively. The light-dependent anthocyanin production in the callus was regulated by temperature. Anthocyanin content in the callus decreased 81.1% at 42°C, 58.9% at 37°C, 47.0% at 30°C, and increased 10.4% at 4°C, compared to the callus maintained at 25°C after 48 hours of incubation at the given temperature. A temperature decrease of 10°C from 25 to 15°C resulted in a critical increase of the anthocyanin production in the callus, irrespective of differences in pH of culture medium. The growth of the callus cultured in medium at pH 7.0 was 6.2-fold higher than in the same medium at standard pH of 5.8.

Key words: Anthocyanin, biosynthesis, callus, cranberry, Ericaceae, growth, light, pH, temperature, *Vaccinium macrocarpon* Ait

Introduction

American cranberry (*Vaccinium macrocarpon* Ait, Ericaceae) is a non-deciduous, perennial, woody plant. It is grown as a fruit crop in bogs with acidic sandy soil primarily in temperate northern regions of the United States, such as Massachusetts and Wisconsin. The red color of cranberry fruit is due to the presence of anthocyanins, the largest subclass of flavonoids (Harborne and Grayer, 1988), and is considered to be the determining factor of the fruit's quality (Craker, 1971). Anthocyanins have a high potential as natural food colorants, and they have been found to possess important therapeutic properties, including anti-tumor (Kamei *et al.,* 1995; Koide *et al.,* 1996), anti-ulcer (Cristoni and Magistretti, 1987), antioxidant and anti-inflammatory traits (Yan *et al.,* 2002; Wang *et al.,* 1999).

Anthocyanin biosynthesis in plants is regulated by various environmental factors such as light and temperature (Chalker-Scott, 1999). Cranberry plants grown in bogs receive several stresses, which affect the growth and development of the cranberry plant and its fruit. These stresses include biotic stresses (attacks by insects, mites and fungi) and abiotic stresses. Among the abiotic stresses, both physical stresses (light, temperature change and wounding) and chemical stresses (nutrients, water supply, and secretions that are produced by fungi and other microorganisms) occur. The color content of cranberry fruit is also affected by physical (light and temperature) and chemical factors (Craker,1971; Eck, 1972; Farag *et al.,* 1992; Sapers *et al.,* 1986). Effects of decreased light interception by defoliation on carbohydrate and anthocyanin levels were also reported by Onayemi *et al.* (2006). However, very little is understood about the physiological and biochemical basis of these factors.

Part of the reason for the lack of physiological and biochemical information lies in the conditions of growth and development of the cranberry plant and fruit. These limit the adequate availability of samples for detailed analysis. Moreover, manipulation of environmental or nutritional conditions in natural bogs is difficult to control. Plant cell culture technology has been developed to overcome geographical and seasonal restriction of the plants, and it has also been applied to study factors that affect production of useful secondary metabolites. An alternative approach is to grow a cranberry callus which can be exposed to various environmental or nutritional conditions for analyzing responses to environmental stresses.

Cranberry callus has been demonstrated to produce anthocyanins (Madhavi *et al.,* 1995). Cell cultures of other species of *Vaccinium* have also been studied (Madhavi *et al.,* 1998; Smith *et al.,* 1997; Fang *et al.,* 1998; Nawa *et al.,* 1993; Meyer *et al.,* 2002). However, effects of light, temperature, and pH on anthocyanin production have been virtually ignored in previous *Vaccinium* plant cell culture studies. Herein we report studies on how these factors affect anthocyanin production.

Material and methods

Plant materials: 'Early Black' cultivar of cranberry (*Vaccinium macrocarpon* Ait, Ericaceae) was obtained from the State Bog at the University of Massachusetts Cranberry Experiment Station in East Wareham, MA.

Induction of callus: Plants were washed under tap water for one hour and surface-sterilized in 70% ethanol for 10 min then disinfected in 6% chlorine bleach for 5 min. Under sterile conditions, plants were rinsed five times in sterilized water and cut into one-centimeter sections. Each section was placed in a petridish containing Gamborg's B5 medium (Gamborg *et al.,* 1968) containing 5 µM 1-naphthaleneacetic acid (NAA), 5 µM 2,4-dichlorophenoxy acetic acid (2,4-D) and 2.5 µM kinetin. The petridishes were cultured at 25°C in the dark.

Examination of optimal culture medium: The growth of

callus was examined in basal B5, MS (Murashige and Skoog, 1962), and WP (Lloyd and McCown, 1980) media containing 0.3 mg L^{-1} NAA, 0.3 mg L^{-1} 2,4-D, 0.1 mg L^{-1} kinetin, 0.1 mg L^{-1} benzylaminopurine, 100 mg L^{-1} $FeNa_2EDTA$ (ethyenediamine-tetraacetic acid ferric-sodium salt), 100 mg L^{-1} PVP (polyvinyl-pyrrolidone), 100 mg L^{-1} myoinositol, 50 mg L^{-1} VC (ascorbic acid), and 10% coconut water, respectively. The above reagents were purchased from Sigma (St. Louis, MO). Growth was evaluated quantitatively in terms of biomass, fresh weight (FW) in g $flask^{-1}$. Callus was separated from the medium, and FW was immediately recorded.

Anthocyanin production: The light yellow-colored callus was cultured in 125 ml-flasks containing the modified WP solid medium under a continuous photosynthetic photon flux density of 25 $\mu M\ m^{-2}s^{-1}$ provided by cool white fluorescent lights (F40CW-RS, General Electric Company, Nela Park, Cleveland, OH) to induce anthocyanin production.

Physical environmental stresses

Light treatment: Anthocyanin-producing callus cultured as above were randomly divided into three groups and subjected to various light treatments (red light treatment, UV light treatment, and darkness) for continuous 48 hours. The red light, at a photon fluence rate of 12 $\mu M\ m^{-2}s^{-1}$, was produced by six 40-w fluorescent tubes (F48T12/R-660/HO, Red, General Electric Company, Nela Park, Cleveland, OH) filtered through a red plastic sheet filter (Roscolux color filter # 27, ROSCO Laboratories, Port Chester, NY). The UV light was produced by a UV-C lamp (TUV 15W/G15T8, Philips, Holland). Light measurements were made with a Model IL1400A Radiometer/Photometer (International Light, Inc., Newburyport, MA).

Temperature treatments

Temperature change treatment between 25°C and 15°C: Callus grown at 25°C under continuous cool white fluorescent light as above was randomly divided into four groups after being transferred to a fresh medium. Groups I and II were cultured at 25°C, and groups III and IV were cultured at 15°C under continuous cool white fluorescent light, for 3 weeks. Group I was then transferred to 15°C, and group III was transferred to 25°C and kept under continuous cool white fluorescent light for 1 week. Groups II and IV remained under their respective culture conditions for 1 week.

High temperature treatment: The anthocyanin-producing callus was cultured in flasks containing the modified WP solid medium at 25°C under continuous cool white fluorescent light for 3 weeks. The callus was then transferred to 15°C and kept cultured under continuous cool white fluorescent light for one additional week. The callus was then randomly divided into six groups. One group remained at 15°C under the same light condition, another group was transferred to 25°C under the same light condition. The other groups were transferred to temperatures at 42°C, 37°C, 30°C, or 25°C and kept in the dark. All were incubated for 48 hours.

Low temperature treatment: The anthocyanin-producing callus cultured in flasks containing the modified WP solid medium at 25°C under continuous cool white fluorescent lights for 4 weeks was randomly divided into two groups. One group remained at 25°C, and the other group was transferred to 4°C for the next 48 hours.

Chemical environmental stresses

pH Treatment: The anthocyanin-producing callus was cultured in flasks containing the modified WP solid medium in different pH conditions of 5.8, 6.5, 7.0, 7.5, or 8.0, at 25°C for 3 weeks then either transferred to 15°C for 1 week, or kept at 25°C for 4 weeks. The growth of callus cultured in the modified WP solid medium in different pH conditions at 25°C for 4 weeks was evaluated quantitatively in terms of biomass, FW (g $flask^{-1}$). The callus was separated from the medium, and the FW was immediately recorded. The pH of all the media was adjusted before autoclaving (at 121°C and 1.05 kg cm^{-2} pressure for 20 min).

Quantitative analysis

Sample preparation: To extract anthocyanins, the callus was removed from the medium, mixed well, and 0.250 g of callus was mixed with 1.25 ml of ethanol-1.5 N HCl (85:15) in a 1.5 ml centrifuge tube and incubated at 4°C overnight.

Anthocyanin analysis: Absorbance of anthocyanin at 535 nm was measured using a Jasco V550 UV/VIS Spectrophotometer (Jasco Corporation, Japan). The total anthocyanin content was calculated in absolute quantities using an extinction coefficient ($\varepsilon^{1\%}_{1\ cm}$) of 982 at 535 nm (Francis, 1982; Zhou and Singh, 2002; 2004).

Results

Induction of cranberry callus and anthocyanin-producing callus: Light yellow callus on the solid medium was visible after 6 weeks of incubation. The callus was separated from the mother tissue after two subculture cycles, and the cell culture was maintained by renewing modified WP medium every 4 weeks at 25°C in the dark. The anthocyanin-producing callus was induced only under the continuous cool white fluorescent light.

Optimal culture medium: Among the three culture media tested, cranberry callus grew best in the modified WP medium (Fig. 1). The growth of the callus was 19.6-fold higher in the modified WP medium than in the modified B5 medium, and 4.2-fold higher than in the modified MS medium. The modified WP medium was used in all further experiments.

Effect of light on anthocyanin production: Forty-eight-hour-treatment with red light and UV light increased the anthocyanin content of callus by 41.3 and 29.3%, respectively, as compared with the anthocyanin production of the callus kept in the dark for 48 hours (Fig. 2).

Effect of temperature on anthocyanin production: Results for callus grown at 25°C for 3 weeks then 15°C for 1 week showed that a temperature change increased anthocyanin content by 3.3-fold in comparison with callus grown at 25°C for 4 weeks without temperature change (Fig. 3). The callus initially cultured at 15°C for 3 or 4 weeks did not show any growth, even after transferring the callus to 25°C for 1 week.

In another set of experiments, the anthocyanin content in callus showed a dramatic change with different temperature treatments for 48 hours. Incubation at temperatures above 25°C decreased the anthocyanin content by 81.1% at 42°C, by 58.9% at 37°C, and by 47.0% at 30°C, in comparison with the anthocyanin content of the callus kept at 25°C (Fig. 4). On the other hand, incubation of the callus at a low temperature (4°C) increased the anthocyanin

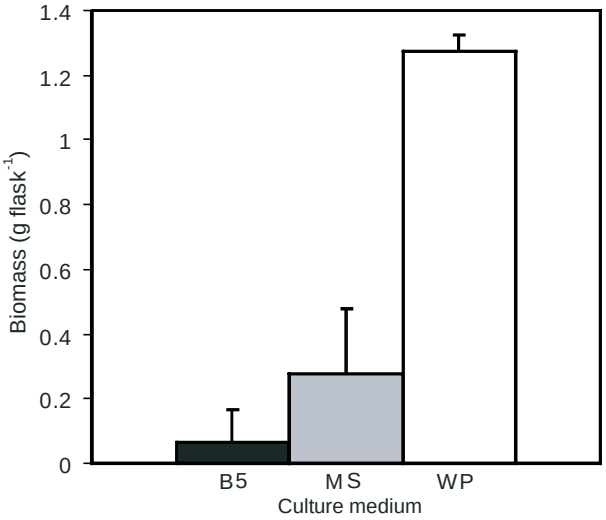

Fig. 1. Growth of cranberry callus in different culture media.The callus was cultured for 4 weeks. Values are mean from six replicates with standard error bars.

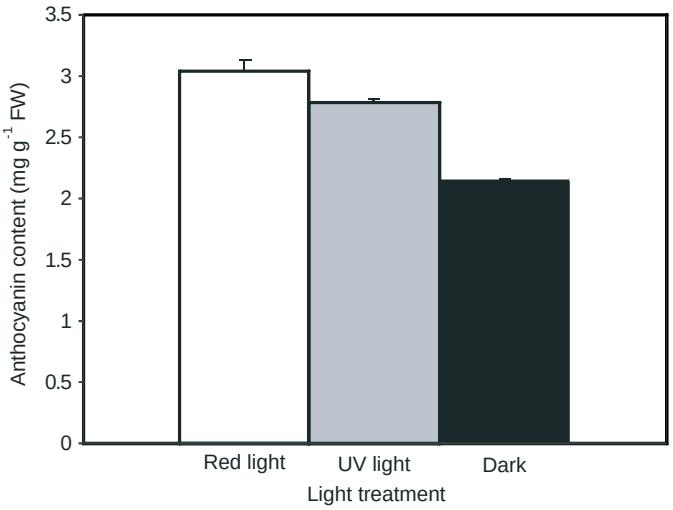

Fig. 2. Effect of light on anthocyanin production in cranberry callus. Anthocyanin-producing cranberry callus was cultured at 25°C for 4 weeks under continuous cool white fluorescent light. Anthocyanin content was analyzed after 48 hours of red light or UV light treatments and darkness. Values are mean from six replicates with standard error bars.

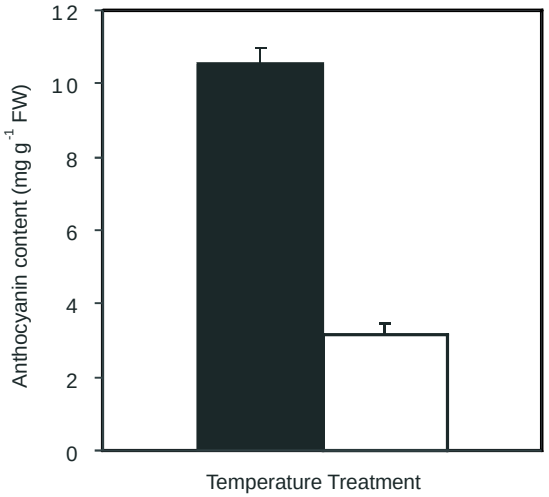

Fig. 3. Effect of temperature change from 25 to 15°C on anthocyanin production in cranberry callus. Anthocyanin-producing cranberry callus was cultured at 25°C for 3 weeks then 15°C for 1 week (black column), or cultured at 25°C for 4 weeks without temperature change (white column). Values are mean from six replicates with standard error bars.

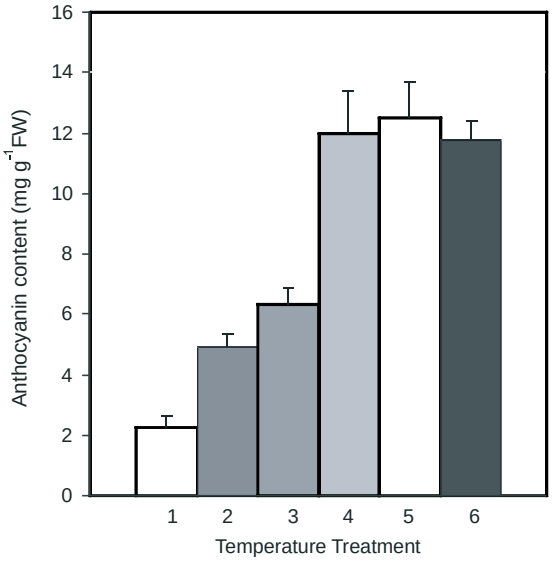

Fig. 4. Effect of high temperature on anthocyanin production in cranberry callus. Anthocyanin-producing cranberry callus was cultured at 25°C for 3 weeks, then 15°C for 1 week. Anthocyanin content was analyzed after 48 hours of incubation at different temperatures. Values are mean from six replicates with standard error bars. 1: 42°C, Dark, 2: 37°C, Dark 3: 30°C, Dark, 4: 25°C, Dark, 5: 25°C, Light, 6: 15°C, Light.

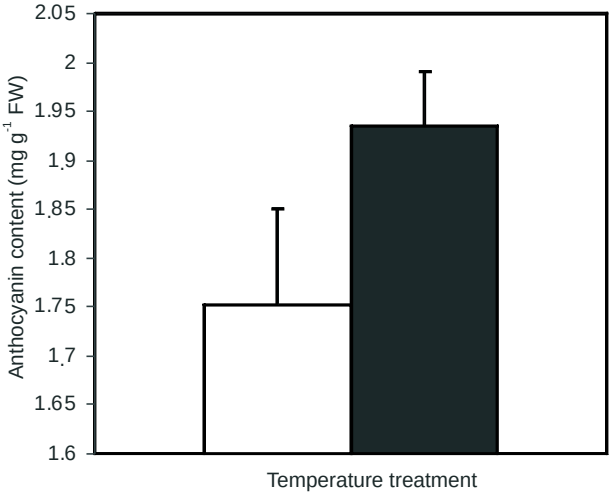

Fig. 5. Effect of low temperature on anthocyanin production in cranberry callus. Anthocyanin-producing cranberry callus was cultured at 25°C for 4 weeks, and anthocyanin content was analyzed after 48 hours of incubation at 4°C (black column), or cultured at 25°C without temperature change (white column). Values are mean from six replicates with standard error bars.

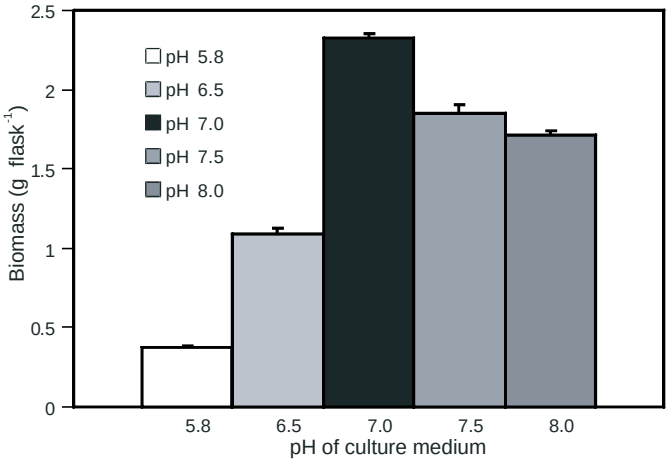

Fig. 6. Growth of the cranberry callus cultured in different pH media. Callus cultured at 25°C for 4 weeks was separated from medium and FW was immediately recorded.

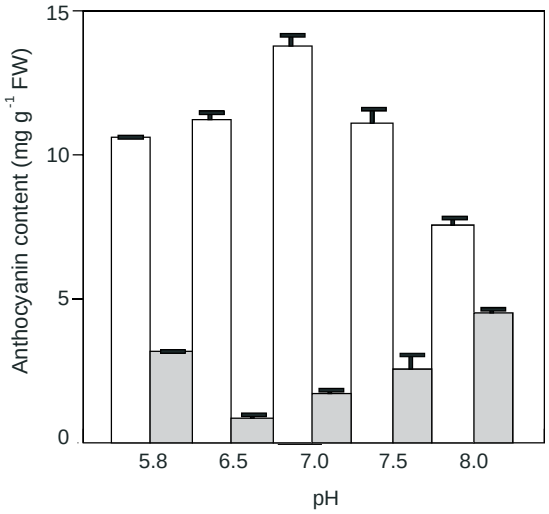

Fig. 7. Effect of culture medium pH on anthocyanin production in the cranberry callus. Anthocyanin-producing cranberry callus was cultured in the modified WP solid medium at pH conditions of 5.8, 6.5, 7.0, 7.5, and 8.0, at 25°C for 3 weeks then 15°C for 1 week (white column), or cultured at 25°C for 4 weeks without temperature change (black column). Values are mean from six replicates with standard error bars.

content by 10.4%, compared to the anthocyanin content of the callus at 25°C (Fig. 5).

Effects of culture medium pH on growth of cranberry callus and anthocyanin production: The growth of callus cultured in different pH media is shown in Fig. 6. Growth at pH 7.0 was higher (6.2-fold) than in the medium with the standard plant cell culture pH of 5.8.

Callus produced more anthocyanins after exposure to a temperature change from 25 to 15°C than with no temperature change, regardless different pH of the medium. Anthocyanin increased 3-fold when temperature changed at pH 5.8, 10-fold at pH 6.5, 6-fold at pH 7.0, 3-fold at pH 7.5, and 1-fold at pH 8.0 (Fig. 7).

Discussion

Different plants and their cell or tissue cultures require different nutrient constituents. The highest growth of the cranberry callus occurred in the modified WP medium (Fig. 1). Although the callus was induced using Gamborg's B5 medium containing phytohormones, the growth of the callus was 19.6-fold higher in the modified WP medium (Fig. 1). Therefore, the modified WP medium was used in all experiments in this study.

Anthocyanins are synthesized due to the activity of key enzymes phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) within the phenylpropanoid/flavonoid pathway; these enzymes can be induced by light (Dixon and Paiva, 1995). Our results show that anthocyanin production in the cranberry callus is light-dependent. The anthocyanin-producing callus itself is obtained only under conditions of continuous light exposure. Anthocyanin was not produced in callus kept in the dark, but red light and UV light exposure increased anthocyanin content by 41.3 and 29.3%, respectively (Fig. 2). This result is consistent with our previous study on the effect of light on cranberry fruit: anthocyanin biosynthesis in cranberry fruit is affected by light quality, and red light selectively promotes higher anthocyanin biosynthesis than UV light (Zhou and Singh, 2002).

Wang and Stretch (2001) reported that storage temperature of cranberries influences anthocyanin content, and the highest anthocyanin content occurred at 15°C storage. We examined the effect of a 15°C environment on anthocyanin production in the cranberry callus, and it was found that a temperature drop from 25 to 15°C resulted in a significant increase of the anthocyanin production in the callus (Fig. 3), which was independent of pH (Fig. 7), and chemicals (glutathione or chlorophyllin, coconut water, metal ions, data not shown) in the medium. Low temperature has been shown to induce anthocyanin synthesis in *Arabidopsis* (Leyva *et al.*, 1995) where low temperature (4°C) induced more PAL and CHS mRNAs accumulation than 20°C conditions in the light after 4 days treatment. Hall and Stark (1972) have reported that early in the fall, cranberry leaves and fruit developed more color at lower temperatures (7.2 -7.8°C in the dark, 12.8-23.9°C in the light). In this study, the cranberry callus also produced more anthocyanin at lower temperature (4°C), compared to the callus maintained at 25°C (Fig. 5).

High temperature (32°C) degrades anthocyanins (Romero and Bakker, 2000). Our high temperature treatments revealed that the higher the temperature, the lower anthocyanin content in callus (decrease of 81.1% at 42°C, 58.9% at 37°C, 47.0% at 30°C, Fig. 4), suggesting that temperature response of cranberry callus is similar to cranberry plant and fruit.

Cranberry bog soils are acidic, with a pH of 4.4 (range 3.3 to 5.5) (Chandler and DeMoranville, 1961) or 4.6 (range 3.9 to 5.9) (Davenport and DeMoranville, 1993). It was interesting to note that the pH of the culture medium not only affected the growth of callus (Fig. 6) but also affected the anthocyanin production (Fig. 7). The standard pH in plant cell culture media, such as B5, MS, WP, and NN (Nitsch and Nitsch, 1969), ranges from 5.5 to 5.8. The growth of the cranberry callus cultured in a medium at pH 7.0 was 6.2-fold higher than in the medium at pH 5.8 (Fig. 6). The anthocyanin content was 27% higher in the callus maintained at pH 7.0 than at pH 5.8 after a temperature drop from 25 to 15°C (Fig. 7). The optimum pH of cranberry culture medium was found to be 7.0.

A limited extent of color development of cranberry promoted by light has been observed (Craker, 1971). The temperature change from 25 to 15°C plays a critical role in increasing anthocyanin production in cranberry callus. This study revealed that a combination of light treatment (to induce anthocyanin production), decreasing temperature from 25 to 15°C (to increase anthocyanin content), and raising pH (to promote growth) would be useful in cranberry culture.

Acknowledgements

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Effect of forcing at different times on bud burst, flowering and fruit development of low-chill peach cultivar 'Premier'

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Abstract

Response of low-chill peach cultivar 'Premier' to early forcing culture was studied. Three-year-old trees were forced in a glasshouse from 15 November, 1 and 15 December and 1 and 15 January. Symptoms of insufficient chilling were detected when forcing was started from 15 November and 1 December. Bud break was delayed and sporadic. The flower and leaf buds forced from 15 December and 1 and 15 January rapidly burst within 15 days after the onset of the treatments. The final burst rate exceeded 70%. Generally, flowering started 10-15 days after flower bud burst. The size of the flowers from the trees forced from 15 November and 15 January was smaller than that recorded at other forcing times. Earliest harvest started under forcing from 15 December and 1 January. These results suggested that by using this low-chill cultivar, forcing could be initiated from mid-December, more than one month earlier than for high-chill cultivars, with complete dormancy release, in this region.

Key words: *Prunus persica* Batsch cv. 'Premier', low-chill peach, forcing culture, bud break, flower characteristics, fruit growth

Introduction

Peach (*Prunus persica* Batsch) is one of the most popular summer fruits in Japan. Its cultivated area spread over 10,300 ha with a production of 174,000 t in 2005 (The Ministry of Agriculture, Forestry and Fisheries of Japan, 2005). Although peaches are mainly produced under natural conditions, forcing culture in plastic greenhouses has been attempted recently. In1999, the area of plastic houses where peach is grown covered 89.3 ha and the production amounted to 1,386 t (The Ministry of Agriculture, Forestry and Fisheries of Japan, 2000). The objective of forcing culture is mainly to obtain a high market price of the products at an earlier date and to improve the fruit quality by avoiding unfavorable climatic conditions, such as frost damage during flowering and excessive seasonal rainfall before harvest as well as damage by pests and diseases (Gemma *et al.*, 1990; Yoshida, 1994).

Under forcing conditions, the growers usually start covering and heating in late January and harvest fruits from late May to early June in case of early cultivars, which is one month earlier than for the fruits grown under natural conditions. However, it is difficult to further advance harvest because Japanese cultivars require more than 900 chilling hours (CH) below 7 °C for the release of endodormancy and resumption of growth. Therefore, it would be desirable to subject low-chill peach cultivars to earlier forcing culture. The characteristics of low-chill cultivars could be valuable for early-season production because the environmental conditions of a low-chilling zone are similar to those of forcing culture. So far, however, the performance of low-chill peach cultivars under forcing conditions has not been elucidated. Moreover, it is essential to determine when the forcing should be started for this type of cultivation, in order to avoid a less bud break, abnormal bloom and delayed foliation (Gemma *et al.*, 1990).

In the present study, bud burst and flowering, flower morphology, fruit development and the characteristics of the low-chill peach cultivar 'Premier' forced at different times were observed to utilizse low-chill peach cultivars under forced culture.

Materials and methods

Plant materials: 'Premier' (150 CH) peach (*Prunus persica* Batch.) trees propagated by cuttings were used. Three-year-old trees planted in 7 L pots, filled with granite soil and bark compost (2/1 parts v/v) were grown under field conditions in the experimental field of Kagawa University prior to the forcing treatment.

Forcing dates: On 15 November, 1, 15 December, 2004 and 1, 15 January, 2005, the trees were placed in a glasshouse where the lowest temperature was kept at above 17 °C using a petroleum heater. Three trees were used per treatment.

Bud burst and flowering: The rates of flower and leaf bud burst, and flowering were observed every 5 days until 120 days after the start of heating. Flower and leaf buds were considered to have burst when the green calyx and tip of leaf appeared, respectively.

Flower morphology and pollen germinability: Five flowers per tree were collected at anthesis, and the weight of the flower, length of the pistil and ovary, length and width of the petal were measured. From these flowers, pollen grains were collected to estimate the germination rate on a medium containing 15% sucrose and 1% agar at 25 °C.

Fruit development and characteristics: After fruit-set, the fruits were thinned, leaving approximately 5-8 fruits per tree, except for the forcing from 15 November and 1 December. The fruit longitudinal and transverse diameters (cheek and suture) were

measured every week from 2-3 weeks after fruit-set to harvest. When the fruits matured, they were collected, and their weight, longitudinal and transverse diameters (suture and cheek) and total soluble solid (TSS) content in the juice were measured. The date of harvest was also recorded.

Temperature conditions: Temperature in the experimental field from October, 2004 to January, 2005 was monitored using a thermo-recorder (Model RT-11, TABAI ESPEC) placed in a ventilated case in the field. Then, chilling hour (CH) accumulation below 7.2 °C was calculated. Chilling units (CU) were also calculated according to the model suggested by Richardson *et al.* (1974). In the Richardson CU model, one unit is assigned for the range between 2.5 and 9.1 °C , 0.5 unit for the range between 1.5 and 2.4 °C and 9.2 and 12.4 °C, no unit for temperatures below 1.4 °C and for the range between 12.5 and 15.9 °C, and a 0.5 unit is subtracted for the range between 16 and 18 °C, and one unit above 18 °C.

Statistical analysis: Trees were arranged using a completely randomized design (CRD) in a controlled greenhouse. Data were analyzed statistically by analysis of variance (ANOVA) using Statistix 3.5 (NH Analytical Software, Roseville, MN, USA), and the means were separated by LSD test at $P < 0.05$.

Results

Cumulative chilling hours and units recorded at the forcing dates are shown in Table 1. Accumulation began in early November. In mid-November, the accumulation of CU and CH was small and it increased rapidly from early December.

Table 1. Comparison of calculated Richardson chilling units (CU) and cumulative recorded chilling hours (CH) < 7.2 °C in experimental field of Kagawa University

Date	Bglhkkmf t mhsr	Bglhkkmf gnt qr
15 November 2004	65	2
1 December 2004	028-4	8
15 December 2004	202-/	086
1 January, 2005	5/ 6-1	315
15 January, 2005	347-2	6/ 1

Bud burst and flowering: The pattern of flower bud burst was similar to that of leaf bud burst (Fig 1 a and c). The flower and leaf buds of 'Premier' forced from 1 and 15 January rapidly burst within 10 days after the onset of forcing. The final burst rate exceeded 80%. In the trees forced from 15 December the flower and leaf buds started to burst after 15 days, with a final rate of about 70%. In contrast, under the forcing conditions from 1 December flower buds started bursting after 30 days of forcing with a final rate of 42%. Flower buds burst slightly earlier than leaf buds. However, final leaf bud burst rate reached 48%. The duration of the period from the first bud burst to the final one under this forcing condition was much longer than that under the above three forcing conditions. Under the earliest forcing condition, flower buds started to burst 85 days after forcing, with a final burst rate of 20%. Only 1% of the leaf buds were found to burst at 120 days. Generally, flowering in all the treatments started 10-15 days after the flower bud burst (Figs. 1 a and b).

Flower morphology and pollen germinability: The morphological characteristics of the flowers at anthesis are shown

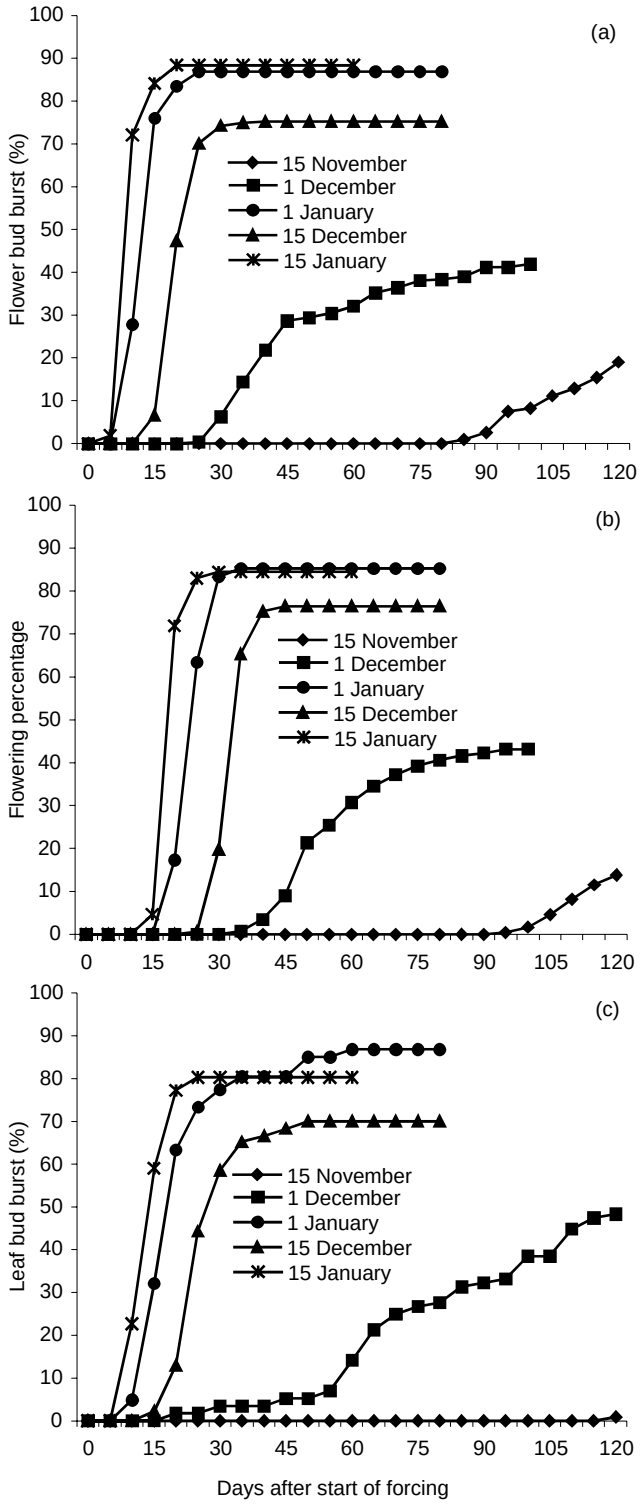

Fig 1. Effect of forcing at different times on the percentage of flower bud burst (a), flowering (b) and leaf bud burst (c) of 'Premier'.

in Table 2. Generally, the size of the flowers of the trees forced from 15 November was smaller than that of at the other forcing times, especially, the values of the weight, pistil length and petal size were lower. Moreover, the values of the flower weight and ovary length under forcing from15 January were also lower than those of other forcing times. There was no significant difference in the percentage of pollen germination.

Fruit development and characteristics: Seasonal changes in the fruit diameter and length from 2-3 weeks after fruit-set to harvest are shown in Fig. 2. In all the forcing treatments, the

Table 2. Effect of forcing at different times on morphological characteristics of the flower organs and the percentage of pollen germination of 'Premier'

Onset of forcing	Weight (g)	Ovary length (mm)	Pistil length (mm)	Petal		Pollen germination (%)
				Width (mm)	Length (mm)	
15 November, 2004	163.7±6.4b[z]	3.37±0.05ab	15.13±0.29b	10.13±0.61b	13.97±0.72b	49±10
1 December, 2004	189.3±9.8ab	3.45±0.26a	16.80±0.80a	12.33±0.67a	16.43±0.35a	63±11
15 December, 2004	190.0±7.0ab	3.73±0.17a	16.47±0.38ab	12.53±0.38a	16.73±0.44a	64±8
1 January, 2005	200.0±8.1a	3.67±0.13a	17.87±0.29a	13.20±0.16a	17.80±0.47a	72±5
15 January, 2005	171.3±14.5b	2.91±0.12b	16.51±0.49ab	12.30±0.52a	17.27±0.52a	62±3

Values are indicated as means and analyzed by ANOVA. NS denotes non-significance.
Different letters denote significant difference at $P < 0.05$ (LSD).

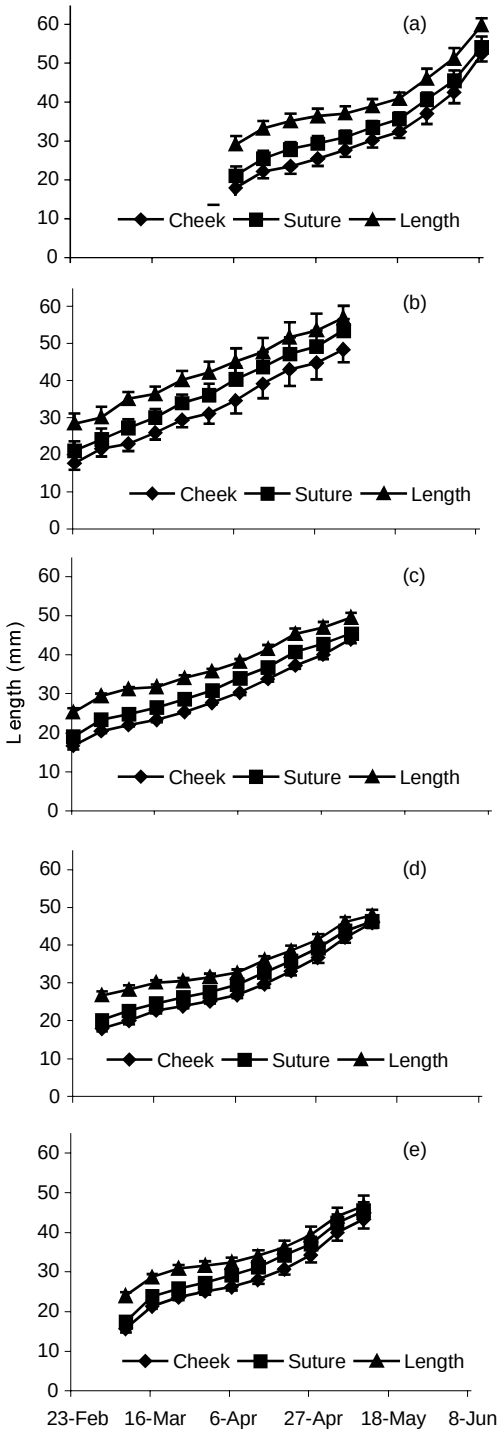

Fig 2. Effect of forcing at different times on seasonal changes in the fruit diameter (cheek and suture) and length of 'Premier' forced from 15 November (a), 1, 15 December 2004 (b and c) and 1, 15 January, 2005 (d and e).

fruits exhibited a double sigmoidal growth pattern. The fruit growth rate that had increased rapidly until 5 weeks after fruit-set (Stage 1) decreased from 5 to 8 weeks after fruit-set (Stage 2). After the short slow growth period, rapid growth resumed until harvest (Stage 3). This cultivar showed a short period of fruit development from fruit-set to maturation (about 12-13 weeks), which was even shorter in the trees forced from 15 November and 15 January.

Harvest period of the trees forced from 15 November was late in spite of the earliest forcing time. Under forcing from 1 and 15 December harvest started in early May, which was about ten days earlier than in the later forcing treatments (Table 3).

Fruit number per tree was much lower under forcing from 15 November and 1 December compared to the other treatments. Under these forcing conditions, although the fruit size was larger than that recorded under the other forcing conditions, variability was large, as indicated by the standard error. In these large fruits, the stylar tip was prominent. The percentage of total soluble solids (TSS) ranged from 11.2 to 12.7, with no significant differences among the treatments.

Discussion

Erez (2000) reported that incomplete dormancy release affected the tree behavior in three main aspects: delayed bud break, low level of bud break and lack of uniformity of leafing and bloom. In the present experiment, under forcing conditions from 15 November and 1 December similar symptoms of insufficient chilling were observed. The values of natural CH accumulation on 15 November 2004 and 1 December 2005 were 3 and 79, respectively (Table 1), which were much lower than the reported CH values for dormancy release in 'Premier' (The State of Queensland, Department of Primary Industries, 1998). On the other hand, when 'Premier' was forced from 15 December (197 CH), and 1 and 15 January an early and high level of bud break occurred. Therefore, by using this low-chill cultivar, heating could be started from mid-December, more than one month earlier than for high-chill cultivars, with complete dormancy release, in this region.

Lack of chilling also results in defective fruit-set because the flowers are poorly developed (Giesberger, 1972; Wienberger, 1950). Peach anthers fail to dehisce, pollen production is low, and the styles and stigma fail to develop (Wienberger, 1950). In the present study, although pollen germination was not significantly different when forcing was performed at different times, the values of the flower weight and pistil and petal size in the trees forced from 15 November were lower than those at other forcing times. Moreover, with 702 CH chilling, the values of the flower

Table 3. Effect of forcing at different times on the fruit characteristics of 'Premier'

Onset of forcing	Fruit number tree^{-1}	Fruit weight (g)	Fruit size (mm)			TSS	Harvest date
			Cheek	Suture	Length		
15 November, 2004	1.7	95.14±17.68^{z}a	57.25±2.61a	57.28±4.17a	60.22±2.32a	12.6±0.7	Mid-June
1 December, 2004	2.0	85.90±16.33ab	51.85±3.30ab	54.72±3.34ab	58.40±3.03a	11.4±2.1	Early May- mid-June
15 December, 2004	6.0	52.49±1.97b	47.50±0.55b	48.87±0.48b	50.50±1.60b	10.7±0.4	Early-late May
1 January, 2005	5.6	56.16±2.93b	48.03±0.74b	48.66±0.85b	51.01±1.35b	12.3±0.3	Mid-late May
15 January, 2005	4.3	55.94±4.34b	48.85±0.72b	48.87±1.03b	49.38±1.32b	11.4±0.5	Mid May- early June

Values are indicated as means and analyzed by ANOVA. NS denotes non-significance.
Different letters denote significant difference at $P< 0.05$ (LSD).

weight and ovary length were lower than in the case of the flowers subjected to 426 CH chilling. Citadin *et al.* (2001) suggested that in the low-chill cultivars, the buds might experience physiological injury, when they were exposed to excessive chilling.

Harvest was earliest when forcing took place from 1 and 15 December. However, under the former condition, the harvest period was much longer than under the latter one, probably due to sporadic bud burst. In the case of forcing from 15 January harvest was delayed by only 10 days, compared to forcing from 15 December although forcing started 1 month later, presumably due to the short periods of bud burst and fruit development. This early fruit maturation observed under forcing from 15 January might be related to temperature of the glasshouse during fruit growth, which was higher under forcing from 15 January (20.3 ^{o}C) than under forcing from 15 December (19.7 ^{o}C). Exposure to high temperature may result in early maturation, thus reducing the growth period (Dennis, 2000). This suggests that by increasing the temperature in the glasshouse during fruit growth, earlier harvest would become possible.

The fruits of the trees forced from15 November and 1 December were larger than those in the other treatments, probably due to the small number of fruits because of the low rate of flower bud burst. Fruit weight under these forcing conditions ranged from 51-129 and 42-148 g, respectively (data not shown). Maggs (1975) reported that limited chilling led to prolonged flowering, which in turn resulted in the lack of uniformity of the fruit size. Additionally, by forcing on 15 November and 1 December the stylar tip become prominent. George *et al.* (1990) attributed prominent sutures and tips in peaches grown in the subtropical region of Australia to insufficient chilling.

In conclusion, it was demonstrated that subjecting low-chill peach cultivars to forcing culture enabled to advance the flowering time, compared to Japanese high-chill cultivars. In addition, it was suggested that forcing could be started in mid-December with complete dormancy release, in this region, when this cultivar is used. However, the fruit development and characteristics of low-chill peach cultivars under forcing conditions should be evaluated under more practical growth conditions.

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2,4-D and NAA supplementation mitigates autotoxicity of strawberry in hydroponics

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Abstract

In order to mitigate the autotoxicity in growing plants in closed hydroponic systems, the effects of foliar applications of 2,4-dichlorophenoxyacetic acid (2,4-D) and 1-naphthaleneacetic acid (NAA) on the growth of strawberry were investigated. Although the growth of strawberry plantlets was not affected by the auxin treatments in the fresh nutrient solution, the auxin treatments recovered the growth in the used nutrient solution. Benzoic acid, a compound reportedly accumulating in the reused nutrient solution of strawberry hydroponics, resulted in a significant decrease in the growth of strawberry plantlets at 50 μM concentration, compared to the growth in the nutrient solution without benzoic acid. Mitigation of the growth inhibition caused by the previously used nutrient solution or addition of the high concentration of benzoic acid in the fresh solution was demonstrated by immersing strawberry leaves in the auxin solutions (0.45 and 4.5 μM 2,4-D or 5.4 and 54.0 μM NAA) for two seconds before transplanting. The number of flowers and harvested fruits, and the fruit yield of strawberry plants grown in the greenhouse for about 33 weeks were reduced by the non-renewing the nutrient solutions. These values recovered in the 5.4 μM NAA treatment and were not significantly different from the control (renewal of the nutrient solution). These results suggested that reductions in the number of flowers and the yield of strawberry in closed hydroponic systems appear to be related to the allelochemicals exuded by the plant itself. The auxin such as NAA would avoid the growth reduction of strawberry caused by autotoxicity. The 5.4 μM NAA treatment may be the most effective for alleviating autotoxicity of strawberry and increasing the yield.

Key words: Autotoxicity, 2,4-dichlorophenoxyacetic acid (2,4-D), hydroponics, 1-naphthaleneacetic acid (NAA), strawberry (*Fragaria* × *ananassa* Duch.).

Introduction

Closed hydroponics is a system used for plant cultivation in environmentally sensitive areas (Van Os, 1995) where the nutrient solution is not released into the surrounding environment, but recycled (Ruijs, 1994). For such a reason, recently, closed hydroponics has been considered for strawberry cultivation (Takeuchi, 2000; Oka, 2002; Koshikawa and Yasuda, 2003). However, in a closed hydroponic system, plants could suffer autotoxicity, due to the accumulation of toxic exudates from the roots themselves in the nutrient solution (Yu *et al.*, 1993).

We suggested that the autotoxicity of strawberry in a closed hydroponic culture was caused by root exudates such as benzoic acid (Kitazawa *et al.*, 2005). The autotoxicity of strawberry reduced the shoot and root growth, number of flowers and harvested fruit per plant, and most notably, the fruit enlargement was inhibited. The vegetative and reproductive growth inhibition caused by root exudates was mitigated by the addition of activated charcoal into the nutrient solution (Yu and Matsui, 1993; Asao *et al.*, 1998; Sato, 2004). However, the activated charcoal adsorbed Fe-EDTA in the nutrient solution and subsequently caused Fe deficiency in plants (Yu *et al.*, 1993).

Phenolic compounds disrupt the balance of endogenous hormones in plants (Rice, 1984; Asao *et al.*, 2001). Some of substituted benzoic acids or the compounds having a structure similar to benzoic acid have been considered as anti-auxin (Van *et al.*, 1951; Keitt and Baker, 1966; Karabaghli-Degron *et al.*, 1998). It is known that auxin controls several fundamental functions including hormonal regulations for the plant development (Lomax *et al.*, 1995; Hobbie, 1998; Berleth and Sachs, 2001). Callis (2005) reported that the fruit expansion and maturation of strawberry depends on auxin. Thus, benzoic acid exuded from the strawberry roots may reduce the auxin activity and inhibit the fruit enlargement. Auxin treatments could support the recovery from inhibited fruit enlargement of strawberry, which was caused by autotoxicity in a closed hydroponic culture. In this study, we investigated the effects of the 2,4-dichlorophenoxyacetic acid (2,4-D) and 1-naphthaleneacetic acid (NAA) treatments on the growth inhibition of strawberry caused by autotoxicity.

Materials and methods

Effect of auxin treatments on the growth of strawberry plantlets in the nutrient solution used for strawberry culture: Strawberry (*Fragaria* × *ananassa* Duch. cv. 'Toyonoka') plantlets, each with 4 leaves, were used for the experiment. 10 strawberry plantlets were transplanted into a formed urethane block inside a plastic container (17 × 29 × 9.5 cm) containing 3 L of the nutrient solution freshly prepared or previously used for a strawberry hydroponic culture (continuously used for 8 months). The major ion concentrations (NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+} and Fe^{3+}) in the

used nutrient solution were adjusted to be as close as possible to a half strength of 'Enshi' nutrient solution (Hori, 1966). The full-strength nutrient solution contains the following amounts of salts per 1000 L of tap water: 950 g of $Ca(NO_3)_2 . 4H_2O$; 810 g of KNO_3; 500 g of $MgSO_4 . 7H_2O$; 155 g of $NH_4H_2PO_4$; 3 g of H_3BO_3; 2 g of $ZnSO_4 . 7H_2O$; 0.05 g of $CuSO_4 . 5H_2O$; 0.02 g of $NaMoO_4 . 2H_2O$. We used a reflectometer (RQflex2; Merck, Darmstadt, Germany) for NO_3^-, the molybdenum blue molecular absorption spectrometric method (Murphy and Riley, 1962) for PO_4^{3-} and an atomic absorption spectrometer (Z-5010; Hitachi, Tokyo, Japan) for K^+, Ca^{2+}, Mg^{2+} and Fe^{3+} analyses. The solutions were not renewed during the two week experiment.

The leaves of strawberry plantlets were immersed in aqueous solutions of 2,4-D at 0 (control), 0.45 and 4.5 μM, and NAA at 0 (control), 5.4 and 54 μM with 0.4% ethanol for 2 seconds before transplanting. The containers were placed in a growth chamber at 25° with a light intensity of 74-81 μmol s^{-1} m^{-2} and a 16 h photoperiod provided by the white fluorescent tubes. The plantlets were grown for 2 weeks, and then the number of leaves per plant, the dry weights (DW) of shoots and roots, and maximum root length per plant were measured.

Effects of auxin treatments on the growth of strawberry plantlets in nutrient solution with benzoic acid: Strawberry plantlets, each with 4 leaves, were used for this experiment. A half strength 'Enshi' nutrient solution containing benzoic acid at 0 (control), 5 or 50 μM was prepared (EC=1.36 dS m^{-1}). Only the leaves of strawberry were immersed in aqueous solutions of 2,4-D at 0 (control), 0.45 and 4.5 μM, and NAA at 0 (control), 5.4 and 54 μM with 0.4% ethanol for 2 seconds before transplanting. The growth conditions were same as the previous experiment. To minimize the effect of microbial degradation of the organic acids (Sundin and Waechter-Kristensen, 1994), the solutions in the containers were renewed every 3 or 4 days. The plantlets were grown for 2 weeks, and then the number of leaves per plant, the DW of shoots and roots, and maximum root length were measured.

Effects of non-renewal of nutrient solution and auxin treatments on strawberry growth and yield in greenhouse: Strawberry plantlets, each with 5 leaves, grown in foamed urethane blocks as a support, were transplanted on 21 October, 2003, into plastic containers (54 × 34 × 20 cm) filled with 30 L of continuously aerated (3.8 L min^{-1}) half strength 'Enshi' nutrient solution in the Shimane University greenhouse. 6 ml of the aqueous solutions of 2,4-D at 0, 0.45 and 4.5 μM, and NAA at 0, 5.4 and 54 μM with 4% ethanol were sprayed on leaves every 2 weeks. These nutrient solutions were not renewed throughout the experiment period. A control treatment that the nutrient solutions were renewed at 2-week intervals was prepared. 3 plantlets were planted in each container, and 4 containers were used for each treatment as replicates. Nutrient concentrations (NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+} and Fe^{3+}) in the solution were adjusted at 2-week intervals as close as possible to be the initial concentration on the basis of chemical analyses by the same methods described previously. In all treatments, the EC and pH in the nutrient solutions ranged from 1.28 to 1.38 dS m^{-1} and 6.67 to 7.57, respectively. Pollination was aided by vibrating the plants with a soft brush at 2-day intervals. The fruits were collected when ripe. The mean air and water temperature during the experiment ranged from 7.1 to 31.2°, and from 7.4 to 28.3°, respectively. At the end of the experiment (10 June, 2004), the number of leaves per plant, the fresh weight (FW) and DW of the shoots, the DW of roots, and maximum root length per plant were measured. During cultivation, the number of flowers, flower clusters per plant, and beginning dates of harvest, mean fruit weight per plant, the number of harvested fruits, and yield per plant were recorded.

Results

Bioassays in the fresh and used nutrient solutions: In the fresh nutrient solution, the number of leaves and the DW of shoots were not significantly different among all five treatments (Table 1). In the used nutrient solutions, the number of leaves and the DW of shoots decreased to 39 and 32% of control values, respectively, and these recovered by the auxin treatments. The DW of roots was not significantly different among all treatments. The maximum root length was not significantly different among all treatments in the fresh nutrient solution. In the used nutrient solution, however, the maximum root length decreased to 45% of control value, and was recovered to the level not significantly different from that in the control, by the 5.4 μM NAA treatment.

Table 1. Effects of used nutrient solution and auxin treatment on the growth of strawberry plantlets

Nutrient solution[z]	Auxin treatment (μM)		Number of leaves plant^{-1}	DW of shoots plant^{-1} (g)	DW of roots plant^{-1} (g)	Maximum root length (cm)
Fresh	— (Control)		5.1a[y]	0.078a	0.047a	13.1a
Fresh	2,4-D	0.45	5.8a	0.067a	0.043a	12.0ab
Fresh	2,4-D	4.5	6.0a	0.070a	0.045a	10.7abcd
Fresh	NAA	5.4	5.8a	0.079a	0.049a	11.5abc
Fresh	NAA	54.0	5.8a	0.073a	0.050a	13.1a
Used	— (Control)		2.0b	0.025b	0.039a	5.9e
Used	2,4-D	0.45	5.2a	0.058a	0.042a	8.8cde
Used	2,4-D	4.5	5.2a	0.056ab	0.047a	9.5bcd
Used	NAA	5.4	6.2a	0.070a	0.048a	10.8abcd
Used	NAA	54.0	4.8a	0.048ab	0.049a	8.2de

[z]Fresh: the fresh nutrient solution. Used: nutrient solution used for strawberry culture but nutrient concentration adjusted to Fresh.
[y]Values in a column followed by a different letter differ significantly by Tukey's test (P=0.05).

Table 2. Effects of added benzoic acid in nutrient solution and auxin treatment on the growth of strawberry plantlets

Benzoic acid (μM)	Auxin treatment (μM)		Number of leaves plant^{-1}	Dry wight of shoots plant^{-1} (g)	Dry wight of roots plant^{-1} (g)	Maximum root length (cm)
0[z]	(Control)		5.5a[y]	0.098a	0.060a	11.4a
0	2,4-D	0.45	4.6a	0.112a	0.068a	12.7a
0	2,4-D	4.5	6.1a	0.080a	0.059a	10.9a
0	NAA	5.4	4.7a	0.111a	0.071a	12.7a
0	NAA	54.0	5.6a	0.118a	0.069a	12.8a
0	(Control)		5.5a	0.098a	0.060a	11.4a
5	-		5.6a	0.107a	0.056a	11.3a
5	2,4-D	0.45	6.0a	0.073b	0.054a	10.6a
5	2,4-D	4.5	7.1a	0.100a	0.060a	12.4a
5	NAA	5.4	5.3a	0.110a	0.064a	12.5a
5	NAA	54.0	5.6a	0.086a	0.054a	10.8a
0	(Control)		5.5a	0.098a	0.060ab	11.4ab
50	-		5.0a	0.075b	0.055b	10.6b
50	2,4-D	0.45	5.4a	0.090ab	0.056b	10.1b
50	2,4-D	4.5	6.0a	0.107a	0.068a	10.1b
50	NAA	5.4	5.4a	0.102a	0.059b	11.3ab
50	NAA	54.0	6.0a	0.095a	0.051b	12.6ab

[z]Benzoic acid 0 μM with non-auxin treatment was control at each concentration.
[y]Values in a column followed by a different letter differ significantly by Tukey's test (P=0.05).

Bioassay in the presence of benzoic acid: The number of leaves was not significantly affected by the foliar auxin treatments, regardless of the benzonic acid concentration (Table 2). The DW of shoots and roots, and maximum root length were significantly not affected by the auxin treatment in the nutrient solution containing 0 and 5 μM benzoic acid, except for the shoot DW in the 0.45 μM 2,4-D treatment combined with 5 μM benzoic acid in the nutrient solution. With 50 μM benzoic acid without auxin treatments, the DW of shoots decreased to 77% of the control value. All auxin treatments increased the DW of shoots to the level similar to the control. The values of DW of roots in the 4.5 μM 2,4-D treatment was greater than the control value and those in the other auxin treatments. Maximum root length was not significantly affected by the auxin treatments in the nutrient solution containing 50μM benzoic acid.

Effects of non-renewal of the nutrient solution on the growth of strawberry plants: In non-renewed nutrient solution treatment, the number of leaves, FW and DW of shoots decreased significantly to 35, 33 and 37% of control values, respectively (Table 3). These values recovered by the auxin treatments. Regardless of the auxin treatment, the DW of roots was not significantly different among the non-renewed nutrient solution treatment although the DW of roots tended to increase by the auxin treatment. The maximum root length was not significantly different among all treatments.

The number of flowers decreased significantly to 75% of the control value in the non-renewed nutrient solution treatment without auxin treatment (Table 4). It recovered by the 4.5 μM 2,4-D, 5.4 μM NAA, and 54 μM NAA treatments to 90, 99 and 91% of the control values, respectively. The number of flower clusters, the beginning dates of harvested fruit, and the mean fruit weight per plant were not significantly different among all treatments. In the non-renewed nutrient solution without auxin treatment, the number of harvested fruit and the yield decreased significantly to 56 and 58% of the control values, respectively. However, a significant recovery of fruit number and yield was found in the auxin treatments. The 5.4 μM NAA treatment had both fruit number and yield similar to those of the control.

Table 3. Effects of non-renewal of nutrient solution and auxin treatment on the growth of strawberry plants

Nutrient solution	Auxin treatment (μM)		Number of leaves plant^{-1}	Fresh weight of shoots plant^{-1}(g)	Dry weight of shoots plant^{-1}(g)	Dry weight of roots plant^{-1}(g)	Maximum root length(cm)
Renewed[z]	(Control)		31.0a[y]	108.9a	31.0a	7.8b	48.5a
Non-renewed	-		11.0b	35.6b	11.5b	8.5ab	31.5a
Non-renewed	2,4-D	0.45	38.0a	97.7a	28.9a	11.2a	51.0a
Non-renewed	2,4-D	4.50	26.5a	97.4a	27.9a	10.7a	40.0a
Non-renewed	NAA	5.40	25.5ab	121.1a	33.5a	10.3ab	46.5a
Non-renewed	NAA	54.0	32.0a	83.3ab	25.3a	10.7a	46.0a

[z]Complete renewal of the nutrient solution every other week (control).
[y]Values in a column followed by a different letter differ significantly by Tukey's test (P=0.05).

Table 4. Effects of non-renewal of nutrient solution and auxin treatment on number of flowers and flower clusters, fruit weight, harvested fruits and yield on strawberry plants

Nutrient solution	Auxin treatment (μM)		Number of flowers plant^{-1}	Number of flower clusters plant^{-1}	Beginning dates of harvested fruit (month day^{-1})	Mean fruit weight per plant (g)	Number of harvested fruits plant^{-1}	Yield per plant (g)
Renewed[z]	— (Control)		30.5a[y]	6.0a	2/24a	11.9a	49.5a	588.6a
Non-renewed	—		22.8c	4.0a	2/24a	12.5a	27.5c	340.8c
Non-renewed	2,4-D	0.45	23.5c	5.5a	2/24a	12.2a	40.0b	477.0b
Non-renewed	2,4-D	4.50	27.3b	5.5a	2/24a	12.9a	39.0b	500.3ab
Non-renewed	NAA	5.40	30.3a	5.0a	2/24a	13.0a	43.0ab	549.6ab
Non-renewed	NAA	54.00	27.8ab	5.5a	2/24a	12.9a	38.3b	492.7ab

[z]Complete renewal of the nutrient solution every other week.
[y]Values in a column followed by a different letter significantly by Tukey's test (P=0.05).

Discussion

The inhibition of vegetative growth in strawberry was reported as a result of autotoxic root exudate due to non-renewal of the nutrient solution resulted from the autotoxic root exudates (Kitazawa *et al.*, 2005). There were some reports that phytotoxic substances accumulated in the nutrient solution that was used for hydroponic culture (Yu and Matsui, 1993; Asao *et al.*, 1999). In our study, the growth of strawberry plantlets was inhibited in the nutrient solutions that were previously used for the strawberry culture (Table 1). This nutrient solution may contain potential substances inhibiting strawberry growth. Although the growth of strawberry plantlets was not affected by the auxin treatments in the fresh nutrient solution, the auxin treatments recovered the growth of strawberry plantlets in the used nutrient solution. It has been known that auxin controls several fundamental functions of the plant development such as cell expansion and division, lateral root formation, vascular differentiation, and shoot elongation (Lomax *et al.*, 1995; Hobbie, 1998; Berleth and Sachs, 2001). Thus, our results suggested that the exudates from strawberry roots inhibited vegetative growth of strawberry plantlets, which could be alleviated by the auxin treatments. The 5.4 μM NAA treatment appeared to be the most effective in ameliorating the inhibited vegetative growth of strawberry plantlet grown in the non-renewed nutrient solution.

Kitazawa *et al.* (2005) reported that benzoic acid was the strongest inhibitor of vegetative and reproductive growth in strawberry. Shann and Blum (1987) have reported that cucumber plants absorbed phenolic acid. It was suggested that phenolic compounds disrupt the balance of endogenous hormones in plants (Rice, 1984). Thus, benzoic acid exuded from the roots may be absorbed and disrupt the balance of endogenous auxin in strawberry. In our study, the effects of auxin on growth inhibition caused by benzoic acid added into the nutrient solution were investigated. The 50 μM benzoic acid resulted in a significant decrease in the growth of strawberry plantlets, compared to the strawberry growth in the nutrient solution without benzoic acid (Table 2). The inhibition of the DW of shoots caused by addition of benzoic acid could be avoided by the auxin treatments. Some of substituted benzoic acids or the compounds having a benzoic acid like structure such as *trans*-cinnamic acid (Van *et al.*, 1951), chlorobenzoic acids (Keitt and Baker, 1966), and 2,3,5-triiodobenzoic acid (Karabaghli-Degron *et al.*, 1998), are considered anti-auxin. Thus, our results suggest that benzoic acid as anti-auxin caused the reduced growth of strawberry, and the addition of auxin could ameliorate the growth inhibition.

The strawberry plantlet grown in the non-renewed nutrient solution resulted in a significant decrease as compared to the renewed nutrient solution (control) (Table 3). Vegetative growth of strawberry was probably inhibited by root exudates in the non-renewed nutrient solution. The growth inhibition could be avoided by the auxin treatments, especially in the 5.4 μM NAA treatment.

Asao *et al.* (2001) reported that the number of flowers in cucumber was unaffected and the number of harvested fruit decreased by autotoxicity. In our study, there were no significant differences in the number of flower clusters, the beginning dates of harvested fruit, and mean fruit weight in strawberry between the renewed and non-renewed nutrient solution (Table 4). The number of flowers and harvested fruit, and fruit yield were reduced by the non-renewing the nutrient solutions. These growth parameters recovered in the 5.4 μM NAA treatment and were not statistically different from the control (renewal of the nutrient solution). Callis (2005) reported that fruit expansion and maturation of strawberry depended on auxin. It was suggested that auxin affected the reproductive growth such as expression (Galum *et al.*, 1965) and formation (Kondo *et al.*, 1999; Reinhardt *et al.*, 2000) of flower. Similar to these findings, our study suggests that the number of flowers and harvested fruit of strawberry could be reduced by autotoxicity, and an auxin may have some effects to ameliorate autotoxic growth inhibition.

In conclusion, reductions in the number of flowers and the yield of strawberry in a closed hydroponic system appears to be related to the allelochemicals exuded by the strawberry plant itself. The auxin such as NAA would avoid the growth reduction of strawberry caused by autotoxicity. The 5.4 μM NAA treatment may be the most effective for alleviating autotoxicity of strawberry and increasing the yield.

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Tolerance of lilyturf (*Liriope muscari*) and four perennial ornamental grasses to preemergent herbicides

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Abstract

Tolerance of field- and container-grown lilyturf (*Liriope muscari* (Decne.)), (Liliaceae) and four species of ornamental grasses (Poaceae), perennial quaking grass (*Briza media* L.), Japanese bloodgrass (*Imperata cylindrica* (L.) Beauv. 'Red Baron'), river oats (*Chasmanthium latifolium* (Michx.) Yates) and dwarf fountain grass (*Pennisetum alopecuroide*s (L.) Spreng. 'Hameln'), to five preemergent herbicides (isoxaben, oryzalin, oxadiazon, oxyfluorfen, and prodiamine) was evaluated. Grasses were planted in the fall of 1997 and in the spring of 1998. Herbicides were applied to the fall planting in the spring of 1998. The April, 1998 plantings received herbicide applications within two or 45 days after planting. Herbicides were applied within two days of planting in May and June of 1998. All species in the field and containers were damaged most by oxyfluorfen, followed by oxadiazon; however, injury was not as severe with oxadiazon as with oxyfluorfen. The oxadiazon-treated plants recovered more quickly than oxyfluorfen-treated plants. Plants were least damaged by prodiamine, oryzalin, and isoxaben. Field-grown Japanese bloodgrass, dwarf fountain grass and lilyturf were generally less damaged when herbicide was applied in June, regardless of planting date or herbicide applied than by the April herbicide application. Prodiamine, oryzalin, or isoxaben caused few phytotoxicity symptoms in the species tested, but oxyfluorfen and oxadiazon caused unacceptable injury.

Key words: *Briza media*, *Chasmanthium latifolium*, *Imperata cylindrica*, *Liriope muscari*, *Pennisetum alopecuroides*, phytotoxicity, weed control.

Introduction

Annual and perennial weeds compete with ornamental grasses in the landscape and during production. Invasion of ornamental grass plantings by turfgrass species is likewise a problem. Herbicide application can reduce labour requirements for commercial production and landscape maintenance. Several studies have tested herbicide tolerance of ornamental grasses (Catanzaro *et al.*, 1993; Gilliam *et al.*, 1992; Green *et al.*, 1997; Hubbard and Whitwell, 1991; Neal and Senesac, 1991), but many other species and herbicides need to be explored.

Ornamental grass species vary in herbicide tolerance. In a study of 12 ornamental grasses, Hubbard and Whitwell (1991) found that 'Karl Foerster' feather reed grass (*Calamagrostis arundinaceae* (L.) Roth 'Karl Foerster') was not injured by fenoxaprop-ethyl ((±)-ethyl 2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoate) while pampas grass (*Cortaderia selloana* (Schult. & Schult.) Asch. & Grabn.), weeping love grass (*Eragrostis curvula* (Schrad.) Nees), and crimson fountain grass (*Pennisetum setaceum* (Forssk.) Chiov. 'Rubrum') were extensively damaged four weeks after treatment. The herbicides fluazifop-P-butyl ((*R*)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid), fenoxaprop-ethyl, quizalofop-P-ethyl ((*R*)-2-[4-[(6-chloro-2-quinoxalinyl)-oxy]phenoxy]propanoic acid), and sethoxydim (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) were not phytotoxic to four blue fescue (*Festuca glauca* Vill. non Lam.) cultivars, but were highly phytotoxic to ravenna grass (*Erianthus ravennae* (L.) Beauv.), switchgrass (*Panicum virgatum* (L.) 'Warrior'), and fountain grass (*Pennisetum alopecuroides* (L.)) (Catanzaro *et al.*, 1993).

The objective of this study was to determine the tolerance of field- and container-grown lilyturf and four perennial ornamental grasses to five preemergent herbicides. The herbicides tested in this study were chosen because they are labeled for weed control in turfgrass species or for weed control in field or container production of ornamentals.

Materials and methods

Liners, about 2.5 cm wide and 31 cm tall, of lilyturf and four ornamental grasses: perennial quaking grass, Japanese bloodgrass, river oats, and dwarf fountain grass, were planted in the field and in containers. The soil for the in-ground plants was a Norge loam, (fine-silty, mixed, thermic Udic Paleustols) with a pH of 7.0, and plants were spaced on 61 cm centers. The container-grown plants were potted in 11.4 L containers with a 3 pine bark : 1 peat : 1 sand medium (by volume) amended with 5.6 kg m^{-3} 17N-3P-10K slow release fertilizer (Osmocote, 17-7-12, The Scotts Co., Marysville, Ohio), 0.68 kg m^{-3} Micromax (The Scotts Co.), and 1.8 kg m^{-3} dolomite.

Four planting date and time of herbicide application combinations (planting/herbicide application dates) were used for each growing system (field and container). Field plants were planted and herbicides applied on the following dates: a) plants were planted 14 October, 1997 and herbicides applied 30 April, 1998 (197 days after planting), b) plants were planted 28 April, 1998 and herbicides applied 30 April, 1998 (2 days after planting), c) plants were planted 28 April, 1998 and herbicides applied 11 June, 1998 (44 days after planting), and d) plants were planted on 9 June, 1998 and herbicides applied 11 June, 1998 (2 days after

planting). Container plants were planted and herbicides applied on following dates: a) plants were planted on 13 October, 1997 and herbicides applied on 10 April, 1998 (179 days after planting), b) plants were planted on 8 April, 1998 and herbicides applied on 10 April, 1998 (2 days after planting), c) plants were planted on 8 April, 1998 and herbicides applied on 22 May, 1998 (45 days after planting), and d) plants were planted on 20 May, 1998 and herbicides applied on 22 May, 1998 (2 days after planting).

Japanese bloodgrass, dwarf fountain grass and perennial quaking grass were not planted in the field and perennial quaking grass was not planted in containers in October, 1997, but all of these species were tested at all other planting dates in the field and in containers. Lilyturf and river oats were tested in the field and in containers at all planting and herbicide application dates. The different planting/herbicide application dates reflect the combinations of planting/herbicide application dates that might be implemented in production or landscape maintenance. All herbicides were applied with a CO_2-pressurized sprayer with an output of 281 L ha^{-1}. Herbicides were applied at the following rates: prodiamine (2,4-dinitro-*N*^{3}-*N*^{3}-dipropyl-6-(trifluoromethyl)-1,3-benzenediamine, Barricade 65WG, Syngenta, Greensboro, NC) 1.7 kg a.i. ha^{-1}, isoxaben (N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide, Gallery 75DF, Dow AgroSciences, Indianapolis, IN) 1.5 kg a.i. ha^{-1}, oxyfluorfen (2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene, Goal 2EC, Dow AgroSciences) 1.7 kg a.i. ha^{-1}, oxadiazon (3-[2,4-dichloro-5-(1-methylethoxy)phenyl]-5-(1,1-dimthylethyl)-1,3,4-oxadiazol-2-(3H)-one, Ronstar 50WSP, Aventis Environmental Science, Montvale, NJ) 2.25 kg a.i. ha^{-1}, and oryzalin (4-(dipropylamino)-3,5-dinitrobenzenesulfonamide, Surflan A.S., Dow AgroSciences) 2.25 kg a.i. ha^{-1}. Control plants received no herbicide treatment. Plants in all treatments (including untreated control plants) were irrigated with 1.3 cm of water 48 h after herbicide application and as needed thereafter. Field plots and containers were hand weeded as necessary.

Plants were visually rated for chlorosis and necrosis on a scale of 0 to 100 (with 0 = no injury and 100 = dead plant) one and six weeks after herbicide application. Six weeks after herbicides were applied shoots of field-grown plants and shoots and roots of container-grown plants were harvested and dried at 65 °C for seven days then weighed. Root to shoot (R/S) ratio of container plants was calculated as root dry weight/shoot dry weight.

A split plot design with ten replications was used for each growing system and planting/herbicide application date. Planting and herbicide application date was the whole plot while herbicide was the subplot treatment. Visual rating data were transformed using an arcsine transformation. Data were analyzed by general linear models procedures with means separations by protected LSD or t-tests (SAS Institute, Cary, NC).

Results

Field study: Planting/herbicide application date did not interact with herbicide for damage ratings on lilyturf one or six weeks after herbicide application (data not presented). Damage ratings were highest (70) one week after application for plants planted 14 October, 1997 and sprayed 30 April, 1998. Lilyturf plants planted 28 April, 1998 and sprayed two days later were intermediate in damage (rating of 30), while those planted 28 April, 1998 or 9 June, 1998 and sprayed 11 June, 1998 were least damaged

Table 1. Damage ratings one and six weeks after herbicide treatment (WAT) of four ornamental grass species planted on various dates in the field and subsequently sprayed with selected herbicides

Planting date	Herbicide application (days after planting)	Herbicide	Damage rating[z]						
			River oats		Japanese blood grass		Dwarf fountain grass	Perennial quaking grass	
			1 WAT	6 WAT	1 WAT	6 WAT	1 WAT	1 WAT	6 WAT
28 April, 1998	2	Control	20	4	56	4	34	24	2
		Isoxaben	12^{NS}	5^{NS}	42^{NS}	13^{NS}	48^{NS}	32^{NS}	4^{NS}
		Oryzalin	15^{NS}	5^{NS}	43^{NS}	4^{NS}	36^{NS}	20^{NS}	6^{NS}
		Oxadiazon	45**	8^{NS}	66^{NS}	36*	62*	60***	16^{NS}
		Oxyfluorfen	94***	74***	95***	58**	84***	92***	54***
		Prodiamine	20^{NS}	7^{NS}	53^{NS}	32^{NS}	28^{NS}	27^{NS}	12^{NS}
28 April, 1998	44	Control	4	10	5	18	1	3	18
		Isoxaben	5^{NS}	11^{NS}	4^{NS}	26^{NS}	2^{NS}	11^{NS}	22^{NS}
		Oryzalin	7^{NS}	10^{NS}	6^{NS}	24^{NS}	2^{NS}	4^{NS}	19^{NS}
		Oxadiazon	22***	18^{NS}	8^{NS}	31^{NS}	1^{NS}	2^{NS}	26^{NS}
		Oxyfluorfen	54***	22*	14**	13^{NS}	22***	8^{NS}	38*
		Prodiamine	11^{NS}	12^{NS}	4^{NS}	20^{NS}	0^{NS}	2^{NS}	24^{NS}
9 June, 1998	2	Control	14	20	16	12	6	49	84
		Isoxaben	12^{NS}	16^{NS}	13^{NS}	8^{NS}	7^{NS}	37^{NS}	74^{NS}
		Oryzalin	10^{NS}	21^{NS}	10^{NS}	10^{NS}	6^{NS}	39^{NS}	79^{NS}
		Oxadiazon	12^{NS}	15^{NS}	12^{NS}	10^{NS}	8^{NS}	65^{NS}	87^{NS}
		Oxyfluorfen	64***	48***	32**	18^{NS}	36***	69^{NS}	92^{NS}
		Prodiamine	20^{NS}	25^{NS}	10^{NS}	6^{NS}	7^{NS}	49^{NS}	83^{NS}

[z]Rating scale was from 0 to 100 with 0 having no damage and 100 being a dead plant.
NS,*,**,*** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively, as compared to control (no herbicide applied) within each planting date by paired t test.

(ratings of 7 and 8, respectively). Damage ratings were higher on oxyfluorfen-treated lilyturf (50) than on control plants (25), but lilyturf treated with other herbicides did not differ in damage ratings from control plants (data not presented) one week after herbicide application. Six weeks after herbicide application, damage ratings did not differ among dates (data not presented). Oxyfluorfen-treated lilyturf had higher damage ratings (50) than control plants (25), but lilyturf treated with other herbicides did not differ in damage ratings from control plants (data not presented) six weeks after application. No planting/herbicide application date by herbicide interaction occurred for shoot dry weights, and shoot dry weights did not differ between plants treated with any herbicide and control plants (data not presented).

Planting/herbicide application date interacted with herbicide for damage ratings of river oats one and six weeks after herbicide application. River oats planted 14 October, 1997 and treated with herbicides the following spring (197 days after planting) had higher damage ratings one week after treatment with oxadiazon (54) or oxyfluorfen (96) than control plants(28). Similarly, river oats planted 28 April, 1998 and treated with herbicides two or 44 days later had higher damage ratings one week after treatment with oxadiazon or oxyfluorfen than control plants (Table 1). Six weeks after treatment, only oxyfluorfen-treated river oats from these same planting/herbicide application dates differed in damage ratings from control plants. Oxyfluorfen-treated river oats planted on 9 June, 1998 and sprayed two days later differed from control plants one and six weeks after herbicide application. No planting/herbicide application date by herbicide interaction occurred for shoot dry weights, and shoot dry weight did not differ between plants treated with any herbicide and untreated control plants (data not presented).

Planting/herbicide application date interacted with herbicide for damage ratings of Japanese bloodgrass one and six weeks after herbicide application (Table 1). Damage ratings of Japanese bloodgrass planted 28 April, 1998 and sprayed two or 44 days later, and those planted on 9 June, 1998 and sprayed two days later with oxyfluorfen were higher than those of control plants from the same planting and herbicide application dates one week after herbicide treatment. Six weeks after herbicide application, Japanese bloodgrass planted 28 April, 1998 and sprayed two days later with oxadiazon or oxyfluorfen had higher damage ratings than control plants, but no differences between control plants and herbicide-treated plants existed on other planting/herbicide application dates regardless of herbicide applied. No planting/herbicide application date by herbicide interaction existed for shoot dry weights of Japanese bloodgrass, nor did any herbicide affect shoot dry weights compared to control plants (data not presented).

Planting/herbicide application date interacted with herbicide for damage ratings one week after herbicides were sprayed on dwarf fountain grass (Table 1). Dwarf fountain grass planted 28 April, 1998 and sprayed two days later with oxadiazon or oxyfluorfen had higher damage ratings than control plants from the same planting and herbicide application date one week after application. Dwarf fountain grass plants planted 28 April, 1998 and sprayed 44 days later and those planted on 9 June, 1998 and sprayed two days later with oxyfluorfen had higher damage ratings one week after herbicide application than control plants. Six weeks after herbicide application no planting/herbicide application date by herbicide interaction occurred, and no differences in damage ratings existed among planting/herbicide application dates or between any herbicide-treated dwarf fountain grass plants and control plants (data not presented). Planting/herbicide application date interacted with herbicide for shoot dry weights of dwarf fountain grass. Dwarf fountain grass planted on 28 April, 1998 and sprayed 44 days later with oryzalin had smaller shoot dry weights (38.0 g) than control plants (92.7 g). Shoot dry weight was not affected by herbicide application to dwarf fountain grass two days after planting on 28 April, 1998 (data not presented). Dwarf fountain grass planted on 9 June, 1998 and sprayed two days later with oryzalin, oxadiazon or prodiamine had smaller shoot dry weights (15.4, 15.8 and 14.5g, respectively) than control plants (26.5 g).

Planting/herbicide application date interacted with herbicide for damage ratings one and six weeks after herbicide treatment to perennial quaking grass (Table 1). Perennial quaking grass planted 28 April, 1998 and treated two days later with oxadiazon or oxyfluorfen were damaged more than control plants one week after herbicide application, but six weeks after application, only oxyfluorfen-treated plants differed in damage ratings from control plants. Damage ratings of perennial quaking grass planted 28 April, 1998 and sprayed 44 days later were not affected by herbicide treatment one week after application, but oxyfluorfen-treated perennial quaking grass planted 28 April, 1998 and sprayed 44 days later had higher damage ratings six weeks after application than control plants from the same planting/herbicide application date. Damage ratings did not differ one or six weeks after herbicide application among herbicide-treated and control perennial quaking grass planted on 9 June, 1998 and sprayed two days later. Planting/herbicide application date did not interact with herbicide for shoot dry weights of perennial quaking grass, nor did shoot dry weights of herbicide-treated and control plants differ regardless of planting/herbicide application date (data not presented).

Container study: Planting/herbicide application date did not interact with herbicide for damage ratings one week after application to lilyturf. One week after application, lilyturf plants planted on 13 October, 1997 or 8 April, 1998 and sprayed 10 April, 1998 had higher damage ratings (23 and 23, respectively) than those planted on 8 April, 1998 or 20 May, 1998 and sprayed 22 May, 1998 (12 and 18, respectively). Oxyfluorfen-treated lilyturf had higher damage ratings (35) than control plants (16), but damage ratings did not differ between lilyturf treated with any other herbicide and control plants one week after herbicide application. Planting/herbicide application date did interact with herbicide for damage ratings six weeks after herbicides were applied (Table 2). Lilyturf planted on 13 October, 1997 and sprayed 179 days later with oxadiazon or oxyfluorfen had higher damage ratings than control plants. Lilyturf planted on 8 April, 1998 and sprayed with oxyfluorfen two days after planting had higher damage ratings than control plants six weeks after treatment. Damage ratings six weeks after herbicide treatment to lilyturf planted on 8 April, 1998 or 20 May, 1998 and sprayed 22 May, 1998 (45 and two days after planting, respectively) were not affected regardless of herbicide compared to control plants. No planting/herbicide application date by herbicide interaction

Table 2. Damage ratings one and six weeks after herbicide application (WAT) for four ornamental grass species and lilyturf planted on various dates in containers and subsequently sprayed with selected herbicides

Planting date	Herbicide Application (days after planting)	Herbicide	Damage rating[z]							
			Lilyturf	River oats		Japanese blood grass		Dwarf fountain grass	Perennial quaking grass	
			6 WAT	1 WAT	6 WAT	1 WAT	6 WAT	1 WAT	1 WAT	6 WAT
13 October, 1997	179	Control	10	6	10	3	1	11	---	---
		Isoxaben	23^{NS}	3^{NS}	3^{NS}	27^{*}	11^{NS}	11^{NS}	---	---
		Oryzalin	18^{NS}	16^{NS}	18^{NS}	20^{NS}	20^{NS}	4^{NS}	---	---
		Oxadiazon	37^{*}	23^{NS}	21^{NS}	40^{**}	24^{NS}	11^{NS}	---	---
		Oxyfluorfen	83^{***}	74^{***}	43^{*}	84^{***}	56^{***}	63^{***}	---	---
		Prodiamine	20^{ns}	4^{NS}	12^{NS}	19^{NS}	13^{NS}	4^{NS}	---	---
8 April, 1998	2	Control	6	40	7	22	6	15	26	10
		Isoxaben	4^{NS}	38^{NS}	4^{NS}	28^{NS}	6^{NS}	16^{NS}	30^{NS}	7^{NS}
		Oryzalin	3^{NS}	33^{NS}	10^{NS}	28^{NS}	4^{NS}	16^{NS}	24^{NS}	7^{NS}
		Oxadiazon	10^{NS}	66^{*}	17^{NS}	56^{***}	7^{NS}	24^{NS}	50^{*}	18^{NS}
		Oxyfluorfen	19^{*}	84^{***}	27^{**}	68^{***}	8^{NS}	59^{***}	71^{***}	28^{*}
		Prodiamine	3^{NS}	38^{NS}	6^{NS}	27^{NS}	8^{NS}	20^{NS}	21^{NS}	10^{NS}
8 April, 1998	45	Control	6	18	15	5	2	1	7	11
		Isoxaben	4^{NS}	10^{NS}	6^{NS}	8^{NS}	2^{NS}	2^{NS}	6^{NS}	16^{NS}
		Oryzalin	8^{NS}	22^{NS}	7^{NS}	16^{NS}	2^{NS}	17^{NS}	16^{NS}	20^{NS}
		Oxadiazon	4^{NS}	13^{NS}	8^{NS}	7^{NS}	2^{NS}	6^{NS}	16^{NS}	18^{NS}
		Oxyfluorfen	6^{NS}	28^{NS}	12^{NS}	18^{NS}	4^{NS}	23^{*}	19^{NS}	24^{*}
		Prodiamine	4^{NS}	12^{NS}	8^{NS}	9^{NS}	2^{NS}	8^{NS}	12^{NS}	18^{NS}
20 May, 1998	2	Control	12	22	9	22	6	18	37	80
		Isoxaben	6^{NS}	10^{NS}	6^{NS}	8^{NS}	5^{NS}	9^{NS}	25^{NS}	71^{NS}
		Oryzalin	20^{NS}	36^{NS}	20^{**}	23^{NS}	7^{NS}	31^{NS}	41^{NS}	74^{NS}
		Oxadiazon	8^{NS}	18^{NS}	8^{NS}	20^{NS}	5^{NS}	18^{NS}	30^{NS}	75^{NS}
		Oxyfluorfen	5^{NS}	10^{NS}	5^{NS}	10^{NS}	3^{NS}	10^{MS}	19^{NS}	56^{*}
		Prodiamine	8^{NS}	13^{NS}	7^{NS}	11^{NS}	5^{NS}	14^{NS}	30^{NS}	72^{NS}

[z]Rating scale was from 0 to 100 with 0 having no damage and 100 being a dead plant.
NS,*,**,*** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively, as compared to control (no herbicide applied) within each planting date by paired t test.

existed for shoot or root dry weight or R/S ratio, nor were shoot and root dry weight and R/S ratio affected by any herbicide compared to untreated control plants (data not presented).

Planting/herbicide application date interacted with herbicide for damage ratings of river oats one and six weeks after herbicide application (Table 2). River oats planted on 13 October, 1997 and sprayed with oxyfluorfen 179 days later had higher damage ratings than control plants one and six weeks after herbicide application. River oats planted on 8 April, 1998 and sprayed with oxadiazon or oxyfluorfen two days later had higher damage ratings than control plants one week after herbicide application, but by six weeks after application only the oxyfluorfen-treated river oats were rated higher than control plants. Damage ratings were not affected by herbicide treatment one or six weeks after the 8 April, 1998 planting date when herbicides were applied 45 days later. Similarly, damage ratings of river oats planted 20 May, 1998 and sprayed two days later were not affected by any herbicide one week after treatment, but six weeks after treatment, oryzalin-treated plants had higher damage ratings than control plants. No planting/herbicide application date by herbicide interaction occurred for shoot or root dry weight or R/S ratio (data not presented). Shoot dry weight of river oats sprayed with oxyfluorfen was smaller (5.8 g) than that of control plants (9.5 g), but shoot dry weights did not differ between river oats sprayed with any other herbicide and control plants. Root dry weight of river oats was smaller with oxyfluorfen (3.2 g) or prodiamine (4.3 g) than for control plants (5.9 g). Root to shoot ratio was not affected by any herbicide treatment compared to untreated control plants (data not presented).

Date of planting/herbicide application interacted with herbicide for damage ratings of Japanese bloodgrass one and six weeks after herbicide treatment (Table 2). Japanese bloodgrass planted on 13 October, 1997 and treated 179 days later with isoxaben, oxadiazon or oxyfluorfen had higher damage ratings one week after herbicide application than control plants. Japanese bloodgrass planted on 8 April, 1998 and sprayed two days later with oxadiazon or oxyfluorfen had higher damage ratings one week after herbicide application than control plants. Japanese bloodgrass planted on 8 April, 1998 and sprayed 45 days later or planted 20 May, 1998 and sprayed two days later did not differ in damage ratings one week after herbicide application from the respective control plants regardless of herbicide applied. Six weeks after application, only Japanese bloodgrass planted on 13 October, 1997 and sprayed 179 days later with oxyfluorfen differed from control plants in damage ratings. No planting/herbicide application date by herbicide interaction occurred for shoot or root dry weight or R/S

ratio (date not shown). No herbicide treatment decreased shoot dry weight of Japanese bloodgrass compared to control plants (data not presented). Root dry weight of Japanese bloodgrass was decreased by isoxaben (1.0 g), oryzalin (1.2 g), oxadiazon (1.0 g), oxyfluorfen (0.7 g) and prodiamine (0.9 g) compared to control plants (2.2 g). Root to shoot ratio of Japanese bloodgrass was not affected by any herbicide treatment compared to untreated control plants (data not presented).

Planting/herbicide application date interacted with herbicide for damage ratings of dwarf fountain grass one week after application (Table 2). Damage ratings were higher in oxyfluorfen-treated dwarf fountain grass one week after herbicide treatment than in control plants planted on 13 October, 1997 and sprayed 179 days later and those planted on 8 April, 1998 and sprayed two or 45 days later. No planting/herbicide application date by herbicide interaction occurred for damage ratings six weeks after application, nor did differences in damage ratings occur among planting/herbicide application dates or between any herbicide treatment and control plants (data not presented). Similarly no planting/herbicide application date by herbicide interaction occurred for shoot or root dry weight or R/S ratio, and no differences in shoot or root dry weight or R/S ratio occurred between herbicide-treated and control dwarf fountain grass plants (data not presented).

Planting/herbicide application date interacted with herbicide for damage ratings one and six weeks after herbicide application to perennial quaking grass (Table 2). Oxadiazon- and oxyfluorfen-treated perennial quaking grass that was planted on 8 April, 1998 and sprayed two days later had higher damage ratings one week after herbicide application than control plants. No differences in damage ratings one week after herbicide treatment occurred with other planting and herbicide application dates. In contrast, six weeks after treatment, oxyfluorfen-treated plants had higher damage ratings than control plants with all planting/herbicide application dates tested. Planting/herbicide application date did not interact with herbicide for shoot or root dry weight or R/S ratio of perennial quaking grass. Shoot and root dry weight and R/S ratio of perennial quaking grass were not affected by herbicide application compared to control plants (data not presented).

Discussion

In field studies, later herbicide applications in the growing season (9 June, 1998 planting with herbicide application two days later) were generally less damaging than the same herbicide applied earlier (30 April, 1998 planting date with herbicide application two days later) regardless of species tested. This difference in damage ratings might be attributed to a more favourable growing environment created by warmer air and soil temperatures later in the growing season than earlier in the season. Since the herbicides tested were for preemergent weed control, later application dates would be undesirable since many weed seeds will have germinated before the later herbicide application date.

Oxyfluorfen-treated plants generally had the highest damage ratings of field and container grown plants regardless of planting and herbicide application date. However, plants treated with oxyfluorfen generally had similar shoot dry weights to control plants in the field and in containers. This is in contrast to a study by Green *et al.* (1997) in which fresh shoot weights of container grown pampas grass treated with a 3% granular formulation of oxyfluorfen were reduced compared to those of plants in other herbicide treatments and a control. Fresh shoot weights of containerized cotoneaster (*Cotoneaster apiculatus* Rehd. & Wils.) and euonymus (*Euonymus fortunei* (Turcz.) Hand.-Mazz. 'Colorata') treated with the emulsifiable concentrate formulation of oxyfluorfen, were reduced in cotoneaster compared to the control and in euonymus compared to oxadiazon (Weller *et al.*, 1984). Our results may differ from other studies due to differences in application dates, herbicide formulations or rates, or the species to which the oxyfluorfen was applied.

Neal and Senesac (1991) found that oryzalin applied at 4.5 kg a.i. ha^{-1} injured beach grass (*Ammophila breviligulata* Fern.), pampas grass, tufted hair grass (*Deschampsia caespitosa* (L.) P. Beauv.), blue fescue (*Festuca ovina* L. 'glauca' (Lam.) W.D.J. Koch), fountain grass, and ribbon grass, and that oxadiazon at 4.5 kg a.i. ha^{-1} caused temporary damage. In our study, damage ratings from oryzalin-treated plants did not differ from those of control plants for any species or planting/herbicide application date combination except river oats planted in containers 20 May, 1998 and sprayed two days later. Neal and Senesac (1991) also reported no injury to ornamental grasses from isoxaben, prodiamine, or oxyfluorfen plus pendimethalin (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine). We saw few differences in damage ratings of plants treated with isoxaben or prodiamine compared to untreated control plants of the same species regardless of planting/herbicide application date. Our data differed from that of Neal and Senesac (1991) in the damage caused by oxyfluorfen, which we found to be highly phytotoxic. This difference in results may be attributed to their use of a granular formulation containing 2% oxyfluorfen rather than a spray application. Glaze *et al.* (1980) showed oxyfluorfen to damage pampas grass when applied as a spray.

The greater incidence of phytotoxicity with oxyfluorfen or oxadiazon than with isoxaben, oryzalin or prodiamine regardless of plant species may be attributed to different modes of action of the herbicides. Oxyfluorfen and oxadiazon are both contact herbicides that damage seedlings as they grow through the treated zone (Humburg, 1989). This contact activity likely also affected the plants that received these treatments as a direct spray in this study. In contrast, isoxaben, oryzalin and prodiamine disrupt various germination processes and affect root growth (Humburg, 1989). Therefore, they would likely not be as damaging to young plants, though they might inhibit root growth. Of the herbicides tested, isoxaben, oryzalin and prodiamine treatments exhibited least damage in the most species, though these treatments were occasionally associated with reductions in shoot or root dry weight of the species tested on various dates. Isoxaben, oryzalin, and prodiamine are better choices for preemergent weed control in the species tested since less plant damage was associated with them than with oxyfluorfen or oxadiazon.

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Effect of cultivars on storage losses in onion under hot conditions

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Abstract

In Khuzestan (South Iran), onion bulbs are usually formed and harvested in spring and consumed either in the same season or during summer while some of these are kept for seed production. In order to study the losses in onion bulbs under hot store conditions, an experiment was conducted in autumn 2002 and spring 2003. The data were collected on five cultivars being produced at Shahid Chamran University, two local cultivars and three of those under commercial cultivation. The collected bulbs were kept in 30 x 50cm boxes, 15 cm in height. The experiment was replicated three times in completely randomized design. Data on change in number, weight of the healthy bulbs and decayed bulbs were recorded every 15 days. No bulb root was produced during the storage when maximum, average and minimum temperature were 48, 33 and 25ºC, respectively with the average relative humidity (RH) of 30%. As far as storage life was concerned, there was a significant difference among the varieties. Compared to both the local and market bulbs, those produced at Shahid Chamran University showed more storage life. Another important finding of this research was that 50% of the local and university bulbs remained unspoiled after 120 days, whereas the Taxes Yellow Grano, Taxes Early Grano and G1 had the short storage life; 50% of the stock were destroyed after 60 days. In the present study, *Aspergillus niger* was found to be the most important factor responsible for onion decay in hot stores of Khuzestan.

Key words: *Allium cepa*, *Aspergillus niger*, cultivars, life storage, onion

Introduction

Onion is one of the strategic crop in Iran. Khuzestan province ranks fifth in producing onion with the average of 95514 tons per year. However, since there are no store-rooms equipped with temperature control system to keep vegetables in Khuzestan, the surplus produce is not harvested at the time of production; thus the products are kept under hot conditions.

Bulb is the edible part of the onion which naturally dormants after juvenile period under inappropriate environmental conditions (light, temperature, humidity and their interaction effects) and after some time, the bulbs start to grow. Unsuitable time for onion to grow is either hot summer or cold winter. Therefore bulbs are the reserved organs of the onion which are ready to keep the product for a long time (Brewster, 1994). Different studies have been carried out on the physiology of the onion dormancy and parameters such as changes in carbohydrate content of onion bulbs (Benkeblia and Shiomi, 2004; Grevsen and Sorensen, 2004 and Benkeblia *et al.,* 2005). The cultural management and harvest practices effectively influence the life of bulb in storage (Roos and Fouyer, 2005). Moreover, the diseases, sprouting or root production in the bulbs are the reasons of their short storage life (Lee *et al.,* 2004).

Temperature is most important parameter influenceing the storage period. The best temperature for sprouting of bulbs is between 10 to 15ºC. Under this condition, the bulbs sprout faster than the other hot or cold treatments (Abdalla and Mann,1963). After sprouting, the leaves of the bulbs, which have been kept under 15 ºC become longer and thus the leaves grow faster than those bulbs kept under 30ºC. So, the sprouting of bulbs is not similar to other physiological processes which are acceleratred with temperature increase (Brewster, 1994).

The bulbs which produce roots sprout fast. The reason lies in the fact that the roots induce the production of cytokinin (Miedema, 1994b) and both the dormancy and the sprouting are controlled by the balance between growth promoters and inhibitors within the bulb (Komochi, 1990). The use of some chemical substances stops both the production of the root and the activation of sprouts (Benkeblia, 2004). The above mentioned outcomes indicate that at the high temperature of store, the growth preventing factors cause decrease in cytokinin activity.

During the storage and dormancy of the bulbs when temperature increases up to 25ºC the sprouting is also accelerated. If, immediately after the harvest, the bulbs are kept under high temperature conditions, the rate of bulb sprouting is increased. The high temperature is usually used for curing the bulbs. Therefore it is obvious that the high temperature possibly may decrease the storage life of the bulbs (Benkeblia, 2004). In different cultivars, the resting period and their storage life are generally different. This period can be improved by curing practices. In fact, storage potential of the bulb, depends on dry matter content, pungency and the number of the thin scales (Currah and Proctor, 1990).

In humid conditions, if the bulbs are kept under 10-15ºC temperature conditions, bulbs produce roots for several days, they produce roots faster than at 30 ºC (Tanaka *et al.,* 1985). During storage period, as the storage length increases, the respiration rate increases too. At 40 ºC, respiration rate is highly accelerated (Brewster, 1994). If the outer hard skin of the onion is removed or cracked, the respiration rate doubles and the rate of the water loss also increase (Apeland, 1971). Under this condition, the bulbs will sprout faster than those with intact skins. The outer skin of the onion acts as a barrier against the intrusion of the gas (Ladeinde and Hicks, 1988). The effect of the hole or damage to the outer

skin is the same as skin removal. This causes the change in air within the bulb and thus bulb sprouts faster (Boswell, 1924).

The highly developed methods of harvesting, curing and keeping bulb have been evolved during the last 20 years. In recent past, the bulbs were kept after harvest at the depth of 3m for months (Brewster, 1994). Two methods are used to keep the bulbs in store: (1) Keeping the bulbs at a low temperature. In this method, the temperature should not be lower than 2 ^{o}C because it may cause chilling injury, (2) Keeping the bulbs at a high temperature. This method is unique in hot areas. Due to the high electricity cost, keeping the bulbs in cold storage is not often recommended. Physical phenomena such as temperature, water vapours within the store have mutual effects (Currah and Proctor,1990).

The sprouting of the bulbs causes both heat production and increase in respiration rate in CO_2 and water environment (Burton, 1982). The decay in bulb caused by diseases increases with both the water loss and the respiration rate. Increase in environmental humidity also increase respiration rate and causes temperature increase of the environment. Under hot conditions of the store, the amount of decay caused by pathogens such as *A. niger* increase (Tyson and Fullerton, 2004). The onion skin plays a basic role in physical and chemical processes during storage (Brewster, 1994). Relatively low RH in store causes cracks of the skin. Most researchers have emphasized the appropriate RH of the stores to be between 65 and 75% (Apeland, 1971; Mettananda and Edirimanna, 1999). Under this condition, the skin of the onion remains fresh and intact. Onion bulbs which are kept in controlled environment should be kept under the same condition after they are taken out of the store (Smittle, 1988).

The aim of this study was to investigate the effect of high temperature and relatively low RH on the storage life of some onion cultivars in Khuzestan.

Materials and methods

In order to study losses in onion hot stores, 10 genotypes among the most important cultivars and types existing in Khuzestan were planted and harvested in fall 2002. Five genotypes from Shahid Chamran University of Ahwaz (R1, B1, G1, Peri 79 and Peri 80), 2 of the most famous of local onions (Behbahani and Ramhormozi), three exotic cultivars existing in market (Perimavara, Texas Yellow Grano and Texas Early Grano) were used. One month after the harvest, the onion bulbs were kept in store till the curing process was over. After a month, this experiment was replicated three times in completely randomized design, each time with 50 bulbs of a diameter more than 3 cm. The bulbs were placed in boxes of 10 x 30 x 50 cm. First, they were weighed, and then the changes in the number of undamaged bulbs were recorded every two weeks. During this period, the temperature change and the RH were recorded. Finally, the collected data were analyzed using SPSS software. In order to analyze the effect of cultivar on the decrease in number the onion bulb, the linear equation $Y= a + bx$ or nonlinear $Y= a + bx + cx^2$ were used.

Results and discussion

The data available on the temperature changes in summer 2003 showed that it was lowest in April and highest in July, August and September. During the storage period, the monthly average temperature was higher than 30 ^{o}C. The highest temperature in April was 40 ^{o}C followed by 45 ^{o}C in other months. The minimum temperature (24 ^{o}C) started in April and extended to 27 ^{o}C in July and September. The relative RH was 30% from April to September (data not presented). The temperature higher than 40 ^{o}C caused increase in respiration rate in bulbs, and relative RH less than 65% caused the outer skin to separate. Accordingly, the evaporation from the surface of the bulbs increased (Tyson and Fullerton, 2004).

The RH and temperature of the store create an ideal condition for pathogens especially black mold (*A. niger*) and bacteria like *Pseudomonas allicola* to attack the bulbs. In such conditions, the losses of bulbs will be high. The most important factor responsible for destroying the bulbs in hot stores are *A. niger* and *P. allicola* (Brewster, 1994). *A. niger* acts in either of the following ways: (A) Simultaneous attack of black mold and bacteria (which mostly occurs via false stem to the bulbs). The black mold attack occured more and faster in cultivars with big bulbs and almost thick neck. Bacteria also intrude in the same way. At the early stages of the experiment, the simultaneous attack of these two pathogens to the bulbs caused the most losses to the cultivars of Texas Early Grano, G1 and Texas Yellow Grano. (B) Attack through the scales was as soon as the dry scales either died or got cracks due to dry weather and the black mold attack became severe. At the early stages of storage periods, due to the health of the scales, the attack in this way was not so remarkable. However, with advancement of time and destruction of the scales, the attack of the pathogens through this way increased. At the final stages of the experiment, the decay rate of the bulbs was more apparent in all cultivars especially the local ones. In this way, except for a black mass of mold, nothing was left in the bulbs.

After 30 days of storage period, the significant difference in the weight of the retained bulbs of the experimental cultivars was observed. In other words, the Texas Early Grano and G1 cultivars were bigger than the others, although they were not statistically different with Ramhormozi and R1 cultivars. The G1 and Texas Early Grano had superiority up to the end of the experiment. During the middle of the experiment, the average weight of Texas Early Grano decreased, but at the end it again increased. The reason could be traced back to the destruction of the big bulb cultivar which had relatively thick neck and had high disease incidence. Accordingly, the general and medium bulbs make the most of the bulb mass and the average weight decreases. At the end of the experiment, only bulbs which were physiologically mature remained in the store. The Behbahani, Ramhormozi, B1 and R1 cultivars had statistically no change. However, the bulbs of B1 and R1 cultivars were of better storage potential in the experiment (Table 1).

The maximum and minimum decrease in weight was observed in Texas Early Grano and G1, respectively. Generally, the exotic cultivars were of higher weight at the early stages of the study. The rate of the decrease in their weight was however two times as the local cultivar. As far as the damaged bulbs in the first 60 days after the growth period are concerned, the most losses refer to the Texas Early Grano, G1, Texas yellow Grano and Perimavara; but after that almost no serious difference was observed among them (Table 3). Sixty days after the storage period, 50% of Texas Early Grano, G1 and Texas yellow Grano were diseasesed and were

Table1. Effect of storage time on average bulb weight of cultivars

Genotypes	Days						
	30	45	60	75	90	105	120
R1	69.6ab*	66.2b	61.0b	58.6b	46.2b	48.5b	46.7b
B1	63.5b	60.4b	57.7b	53.8b	52.4ab	48.4b	44.8b
Peri 80	60.6b	58.5b	64.0ab	52.8b	38.7b	47.6b	46.1b
Peri 79	60.4b	58.2b	54.5b	50.7b	49.4b	47.1b	41.5b
G1	76.6a	79.6a	77.0a	74.3a	71.9a	69.7a	70.3ab
Texas Yellow Grano	65.5b	62.5b	60.0b	57.8b	55.6b	55.3b	54.0b
Texas Early Grano	78.9a	74.8ab	69.2ab	64.0b	58.0b	68.0a	80.0a
Perimavara	64.5b	60.2b	54.5b	53.5b	50.4b	48.5b	46.0b
Ramhormozi	65.4ab	62.2b	59.6b	57.0b	53.2b	49.4b	46.4b
Behbahani	62.5b	59.0b	55.6b	54.6b	50.3b	46.9b	43.8b

*Within each column, a different letter above indicates a significant difference by the Duncan's Multiple Range Test (P=0.05)

removed. It took 90 days for other cultivars such as Perimavara, Peri 79, and 105 days for Peri 80. In other words, each of these cultivars had 50% of its bulb loss at a certain time. The equation coefficients of the removed bulbs is given in Table 4. From the beginning of the study to the end, the losses of Ramhormozi, Behbahani, B1 and R1 cultivars did not exceed 50%. However the regression line slope of the equation of the damaged bulbs in both B1 and R1 was less than that of the local ones. In other words, some of the improved cultivars, at the university had higher storage life.

In the light of the above findings, it can be concluded that bulb did not develop roots during hot store conditions. There was difference among the cultivars with regard to storage life. The short storage life was in Texas Early Grano, Texas yellow Grano and Peri 79. In B1, R1, Ramhormozi and losses were the least. Behbahani in increasing order. The most important factor responsible for decay in the onion bulbs in the hot stores of Khuzestan was *Aspergillus niger*.

Table 2. Coefficients of linear and nonliner models for decrease in onion bulb weight of cultivars in hot stores (Y= a+bx or Y= a+bx+ cx^2)

Genotypes	Coefficients			
	a	-b	c	r
R1	78.4	0.29		0.95
B1	69.67	0.20		0.99
Peri 80	68.71	0.21		0.77
Peri 79	66.68	0.20		0.99
G1	81.15	0.09		0.90
Texas Yellow Grano	68.9	0.13		0.97
Texas Early Grano	107.11	1.01	0.0069	0.88
Perimavara	69.83	0.21		0.97
Ramhormozi	71.77	0.20		0.99
Behbahani	68.44	0.20		0.99

Table 4. Coefficients of regression equation fitted for decrease in bulb number of cultivars in hot stores for linear model (Y= a +b x)

Genotypes	Coefficient		
	a	b	*r*
R1	19.11	0.449	0.96
B1	-17.857	0.435	0.97
Peri 80	-23.00	0.730	0.99
Peri 79	-24.85	0.778	0.98
G1	-15.30	0.944	0.95
Texas Yellow Grano	-14.71	0.983	0.94
Texas Early Grano	-16.35	0.948	0.97
Perimavara	-20.32	0.755	0.98
Ramhormozi	-22.19	0.580	0.98
Behbahani	17.88	0.475	0.98

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Table 3. Percentage decrease in number of onion bulbs of differnt cultivars in hot store

Genotypes	Days						
	30	45	60	75	90	105	120
R1	0	0b*	5d	10c	19c	30b	38b
B1	0	1b	4d	12c	19c	30b	37b
Peri 80	0	7b	22bc	32b	44bc	55b	65ab
Peri 79	0	25a	54a	66a	76a	83a	87a
G1	0	7b	23bc	34b	55ab	59ab	63ab
Texas Yellow Grano	0	23a	56a	66a	76a	80a	86a
Texas Early Grano	0	31a	45ab	61	75a	83a	88a
Perimavara	0	14b	25bc	35b	55ab	60ab	65ab
Ramhormozi	0	1b	11cd	17bc	33bc	42b	46b
Behbahani	0	3b	8d	14c	26c	34b	40b

*Within each column, a different letter above indicates a significant difference by the Duncan's Multiple Range Test (P=0.05)

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N-NO_3 from cellular extract as an indicator of nutritional status of cantaloupe muskmelon in fertigation

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Abstract

In cantaloupe farming (*Cucumis melo* L.), the production of export quality fruit require nutritional indicators that allow an adequate management of nitrogen fertilization and irrigation water supply. This study with cantaloupe muskmelon was carried out to test four nitrogen fertilization treatments and three levels of soil moisture tension under field conditions. Nitrate content (N-NO_3) in the cellular extract was evaluated as an indicator of nutritional status. Significant correlation was found between the lowest nitrate concentrations in the petiole sap and the N fertilization doses in three different sample periods. The effect of soil moisture tension on nitrate concentration varied in both years; this was attributed to soil temperature differences. Results showed that it is feasible to establish outcome predictions of yield and quality of the fruit based on the nitrate concentration in petiole sap, concluding that this is an adequate indicator of the nutritional status of the cantaloupe plant. However, its use as a guide for managing fertilization and irrigation must include a permanent follow up of the crop that evaluates the effects of environmental factors in plant growth and uptake of the nutrients.

Key words: *Cucumis melo* L., nitrogen fertilization, petiole sap, export product, soil moisture

Introduction

Cantaloupe muskmelon (*Cucumis melo* L.) is the main produce cultivated in the state of Colima, Mexico where 75% of its production is destined to the export market. An adequate crop management is required to obtain optimum yield with excellent fruit quality, to satisfy market demand and maintain crop profitability without environmental detriment, avoiding unnecessary waste of resources such as water, fertilizers and energy.

In different crops, it has been proved that yield and production quality are greatly related to an appropriate nutrition during the plant growth cycle (Locascio *et al.*, 1997; Williams, 1996). MacKerron *et al.* (1995) found a close relationship between the crop yield, days of active plant growth and the amount of required nutrients.

Studies have shown that variations in temperature between years and crop seasons, affect the development and duration of the tomato growth cycle, consequently, the uptake rate, and the utilization of nutrients (He *et al.*, 1999). On the other hand, using adequate nutrients doses during the early stage of the crop and even before pollination, an excellent yield is obtained. In contrast, with excessive doses of nitrogen, during low nutritional requirement period, toxins can be produced in plants and increase nitrate concentrations in fruit, which has been shown in muskmelon and other crops (Jang and Nukaya, 1997; Forlani *et al.*, 1997). Some authors point out that the N-NO_3 concentration in different crops is an indicator of the relation between nitrogen fertilization doses and yield, therefore this indicator has been utilized to evaluate the necessity for applying fertilizers during the plant growth cycle (Porter and Sisson, 1991). Nitsch and Varis (1991) observed that modern instruments for N-NO_3 determination in cellular extract, facilitates the use of this type of determinations as a guide in fertilization management. However, some authors propose a critical revision of this indicator because N-NO_3 concentration is not uniform the different parts of the plant (MacKerron *et al.*, 1995).

The availability of nutrients for the plant that come from the fertilizer depends greatly on soil moisture. The effect of the level of soil moisture on the quality and yield of cantaloupe was shown in a study for improving the irrigation management, particularly at the final stage of the crop, but little research has been done on its interaction with the nutrients (Hartz, 1997).

The objective of this study was to evaluate the usefulness of the determination of N-NO_3 concentration in the cellular extract of cantaloupe petioles as a reliable indicator of the nutritional status of the crop in relation to nitrogen fertilization and in combination with soil moisture levels.

Materials and methods

Experimental site: The study was conducted in a commercial plantation, located at 17 km south east of the city of Colima, Mexico during the winter seasons of 1998 and 1999. The soil is classified as Haplic phaeozem, pH 7.9, lightly calcareous, poor in organic matter, electric conductivity of 1.08 dS m^{-1}, high contents of exchangeable bases (>95 %), and a loamy to sandy loam texture.

Soil preparation: Soil preparation was conducted in the month of January following the practices followed by the farmers. Before planting ,150 kg ha^{-1} of each P (P_2O_5) and K (K_2O) were applied to the soil as additional fertilization to all treatments. The soil moisture was maintained by controlling the irrigation application with tensiometers installed at the soil depth of 0.30 and 0.60 m.

The soil temperature was registered daily.

Experimental design: A divided blocks experimental design was implemented in the field. Three sub-plots at soil moisture tensions of 10, 20, and 45 kPa constituted each block or main plot. Each main plot was divided into four small plots (Latin arrangement) for the application of nitrogen treatments: 0, 80, 120, and 160 kg N ha^{-1}. Each treatment was replicated four times.

The variety of cantaloupe evaluated was the hybrid Ovation. The cellular sap extractions to evaluate the N-NO_3 concentrations were carried out at the following phenological stages: blossom, fruiting and ripening stages. The samples were collected according to the Warncke's methodology (Warncke,1997). In order to have a representative sample of each treatment, 8 or 10 petioles were collected in each of the 12 small plots and were stored immediately at 4ºC temperature until the laboratory analysis.

The total fruit yields were registered in each treatment. Fruit quality was evaluated using the sugar content (ºBrix) and the fruit size as indicators. In this last indicator, five groups were considered according to the market criteria. Results were analyzed using ANOVA procedures and linear regression with the statistics software STATISTIX (1998)

Results and discussion

Effect of nitrogen applied to soil: The statistical analysis showed that the effect of nitrogen fertilization treatments was not similar in the two years of study. For 1998, only in the first stage of the cycle (43 days after sowing), significant differences were registered among treatments while, in 1999, significant differences were registered at all three crop stages (28, 49 and 63 days after planting) (Table 1).

The values of correlation between the N quantities applied to the soil and the N-$N0_3$ concentrations in the cellular extract varied in the crop stages. In 1998, only at blooming stage, a high correlation coefficient was observed (r=0.88). In contrast, during 1999, at all three sampling stages, correlation coefficients were significant (r = 0.90, 0.92, and 0.95, respectively). Similar variations have been reported in other horticultural crops when these variables correlate as function of time. (Andersen *et al.*,1999; Kubota *et al.*, 1997; Rhoads *et al.*, 1996; Waterer, 1997). These results indicate that variations of correlation values depend on the age of the crop (sampling date).

The differences in correlation values found in our study between 1998 and 1999 were related to the N-$N0_3$ level in the petiole sap. During 1998, the average of N-NO_3 concentrations was 1919 mg L^{-1} and for 1999 was 1316 mg L^{-1} (Table 2). In the first year, the lowest N-$N0_3$ level occurred at the harvest stage (6 days after sowing). At this date, high correlation was observed.

Some authors have observed that increase and decrease of N-NO_3 concentration in the first and final crop stages, are characteristic of species (MacKerron *et al.*, 1995). The age intervals in which applied doses of N and N-$N0_3$ concentration in the petiole sap correlated, depends on this characteristic that explains variations in correlation values for different ages and crops. Besides this, other factors like the space variability for N-$N0_3$ concentrations among plant parts necessitate a critical revision of the use of N-$N0_3$ concentration in the petiole sap as a guide to optimize fertilization (Mackerron *et al.*, 1995; Meyer and Marcus, 1998).

Applied water effect: Even though the effect attributed to the soil moisture treatments on the N-$N0_3$ concentration proved to be significant ($P < 0.01$) for every crop stage during the first year of study, in the second year the effect was significant only in the earliest stage of the crop, which is the most important stage for N deficits correction of the plant (Pérez-Zamora e*t al.*, 2004).

The increase of N-$N0_3$ related to the applied N varied with the soil moisture tension. In 1998, this increase was observed for 45 kPa treatment (Table 2). These results were attributed to the differences in soil temperature between both crop cycles: in 1998, a low level of soil moisture tension caused high concentration of N-$N0_3$. Considering that the humidity levels in treatments were kept within the adequate humidity range for cantaloupe, the high concentration of N-$N0_3$ in the petioles of the highest humidity treatment (10kPa) is explained by the effect the soil temperature registered in 1999 over the uptake rate and because the $N0_3$ moves in the soil mainly due to mass flow.

Reports are available that low humidity levels contribute to accumulation of nitrogen in the petioles of potato plants (Middleton *et al.*, 1975), also a lack of consistency in the correlation between humidity and concentration of nitrates is reported (Kubota *et al.*,

Table1. Analysis of variance summary for the N-$N0_3$ concentrations (mg L^{-1}) in cantaloupe petioles for N doses and soil moisture tension levels.

Source of variation	Degree of freedom	1998			1999		
		DAS[1]			DAP[2]		
		43	50	63	28	49	63
Main Plots							
Rows	3	NS	NS	NS	NS	NS	NS
Columns	3	NS	NS	NS	NS	NS	NS
Nitrogen	3	**	NS	NS	**	***	***
Error a	6						
Soil moisture	2	**	***	***	**	NS	NS
Error b	6						
Nitrogen x Soil moisture	6	NS	NS	NS	*	**	NS
Error	18						
C. V. (%)		9.39	8.63	8.87	9.37	12.82	14.22

*, ** and *** are significant at P=0.05, 0.01, and 0.001, respectively; NS=not significant; [1]DAS= day after sowing; [2]DAP= days after planting

Table 2. Concentrations of $N\text{-}NO_3$ (mg L^{-1}) in the cellular extract from cantaloupe petioles under four nitrogen doses and three soil moisture tensions at three crop stages

Treatments		Crop Stages					
N (kg ha^{-1})	Soil moisture tension (k Pa)	1998			1999		
		Blooming	Fruiting	Ripening	Blooming	Fruiting	Ripening
0	10	1944	1944	1469	1763	1017	678
	25	2260	1808	1310	1831	1130	678
	45	2011	2290	1898	1492	859	746
80	10	2034	2035	1672	1893	1198	927
	25	2260	1695	1536	1863	1288	881
	45	2290	2215	2011	1559	927	859
120	10	2011	1853	1582	2237	1537	1175
	25	2280	1831	1401	1695	1220	1107
	45	2300	2214	2124	1695	1356	1041
160	10	1763	1808	1536	2057	1175	1153
	25	1831	1831	1469	1763	1424	1006
	45	2300	2011	2260	1695	1288	1107
LSD (*P*=0.05)		262	275	311	238	240	223

1997). In the case of cantaloupe muskmelon such report that the humidity effect on the $N\text{-}N0_3$ concentration in the petiole sap, does not exist.

Interaction of nitrogen fertilization and soil moisture: The effect of the nitrogen interaction with mositure was significant for samples at blooming and fructification in 1998, but it was not consistent under the different doses of fertilization in both crop cycles (Table 2). This contrasts with the results obtained in studies with potato crop, in which it is observed that an optimum combination of nitrogen and soil moisture during the plant growth cycle results in a high correlation with the $N\text{-}N0_3$ concentrations in the petioles during the whole plant growth cycle (Porter and Sisson, 1991).

Soil temperature and $N\text{-}NO_3$ concentrations: The pattern of soil temperature was different between the study years. In 1998, temperature average was 27.5ºC and showed a tendency to increase from the beginning of the crop cycle, in the month of February, until the end of the crop in the month of May. In 1999, temperature average was 25.5ºC and registered a decline during the first crop stage which was strongest during the blooming and fructification stages in the months of March and April. In the first year the $N\text{-}N0_3$ concentrations in the cellular extract of all treatments were superior to those of the second year, which showed the relation between temperature pattern and $N\text{-}NO_3$ concentration level.

Although the effect of the soil temperature on the utilization ability of N available from the cantaloupe crop has not been studied in detail. However, in other horticultural crops, the season effect has been observed on the $N\text{-}N0_3$ cellular extract levels, and the yield and quality of the fruit (Meyer and Marcus, 1998; He *et al.*, 1999).

In this study, the differences between soil temperature patterns for study years, were related with the nitrate level in the cellular extract.

Effect of $N\text{-}N0_3$ concentration on the yield and fruit quality: The effect of the $N\text{-}N0_3$ concentrations on the total production and fruit size was estimated indirectly through response functions of N applied to the soil and the production, and from applied N to the soil and concentration of $N\text{-}N0_3$. Maximum $N\text{-}NO_3$ concentration in the petiole sap, was obtained with 70 kg N ha^{-1} and 17 kPa, while the maximum yield and fruit size was also obtained with the same N and soil moisture tension indicated for the $N\text{-}NO_3$ concentration; this means that $N\text{-}NO_3$ concentration in the petiole sap is an indicator of yield and fruit size.

The results show that it is possible to establish predictions with r = 90% or higher. The production function (total yield of export fruit) for the average of two crop cycles was as following:

Yield (Mg ha^{-1}) = $60.92 - 0.126x - 1.124y + 0.00039x^2 + 0.0144y^2 + 0.00128xy$; with r = 0.90

The response surface that considers the production of fruit size 9 and 12 (export size) is shown in Fig. 1.

These results coincide with reported values in other crops by authors when yield was correlated with the $N\text{-}N0_3$ concentration in the petiole sap. They showed that the harvest stage has an effect over the correlation value, in general high "r" values (0.74 to 0.89) were reported (Andersen, 1999; Dow and Roberts, 1982; Meyer and Marcus, 1998; Rhoads *et al.*, 1996; Williams, 1996).

The fruit quality was evaluated taking the fruit size and the sugar content (ºBrix) as indicators. There were not any significant difference among treatments as far as sugar content is concerned. However, the tendency was to diminish from 10 to 9.2ºBrix when the soil moisture level rose to 10 kPa which is the moisture tension value in which maximum production (80 Mg ha^{-1}) of export fruit was obtained. The results of this research contrasted with the results of other authors who did not find moisture effect or N effect on the soluble solids concentration in watermelon (Singh and Naik, 1989; Pier and Doerge, 1995). The level of $N\text{-}N0_3$ in petiole sap was related to production and the size distribution was

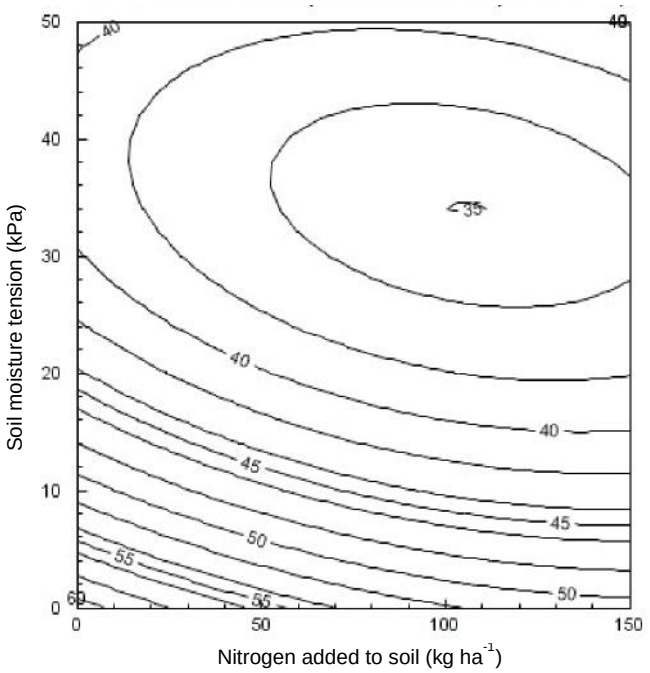

Fig.1. Response surface for cantaloupe melon, sizes 9 and 12. Lines contours are yields in Mg ha^{-1}.

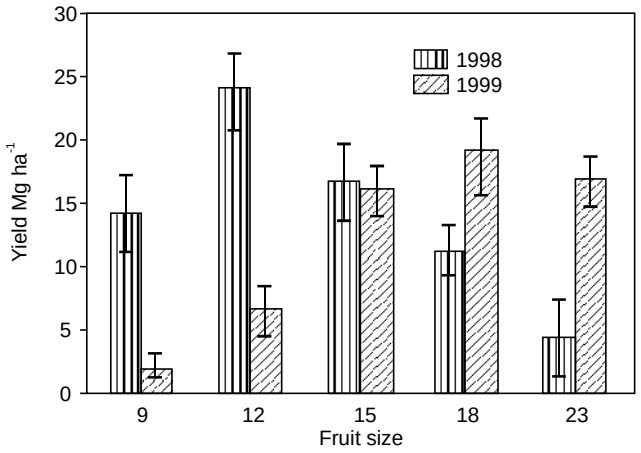

Fig. 2. Distribution by size of cantaloupe production, during two consecutive crop cycles under N fertilization and different soil moisture treatments.

strongly different for the extreme sizes, but not for intermediate size (No. 15), in both experimental cycles (Fig. 2).

The highest correlation values were obtained between the N-NO_3 concentration and the blossom stage, and the sizes 9 and 12 (r= 0.77 and r= 0.82, respectively). High correlation values between N-NO_3 concentration and the production quality (taken size as indicator) have been reported for tomato crop under field and greenhouse conditions (Anderson *et al.*, 1999; He *et al.*, 1999).

The results in this study showed that the N-NO_3 concentration in the sap petioles of the cantaloupe plant is an adequate indicator of the nutritional status of the plant and therefore constitute a useful instrument in the prediction of final yield and production quality.

The N-NO_3 analysis as a guide for management of the N fertilization and soil moisture requires a crop follow up at different stages and in different climatic conditions. It is necessary to consider the environmental conditions affecting the N absorption from soil.

The N-NO_3 concentrations reflect the effects of the interactions between N fertilization and soil moisture. An adjustment programme during the crop cycle must take in consideration both factors in order to maintain these concentrations in adequate ranges.

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Thinning response of 'Abbé Fetel' pear to lime sulphur

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Abstract

Thinning is a central management activity in the production of high quality fruit required for the domestic and export market. Early thinning of fruit trees is important since it influences fruit size and the time of application affects flower bud formation for the following season. Furthermore, finding organic blossom thinners is a major challenge as hand thinning is a costly practice. At the High Valley region of Argentina (lat. 38°56' 67°59'W), lime sulphur was evaluated as flower thinner on 'Abbé Fetel' (*Pyrus communis* L.) pear trees trained to palmette leader. Treatments were 1) control, and 2) 7 % lime sulphur applied at 30 % bloom, using an orchard sprayer. Fruit diameter (FD) was recorded two weekly (*n*=20 per date and treatment). At 144 days after full bloom (DAFB), or initial commercial harvest, fruit weight and the maturity indices were determined. Fruits were then graded into size categories. Growth equations were developed using non linear regression and mean separations were computed with Student's *t*-test. The lime sulphur sprays significantly increased mean FD, starting from 115 DAFB. Logistic model best fitted the fruit growth *vs.* time curves. Percentage of fruits with <65mm diameter was 25 % for the control and 5.26 % for lime sulphur treatment. Treatment 2 increased final fruit weight by 16.5 %, as compared to the untreated pears. At 144 DAFB, thinned trees showed firmer fruits than the controls (64.4 *vs.* 61.7 N) and there were no statistical differences among treatments in soluble solids concentration and starch index. Consequently, data indicated that lime sulphur at 7 % was an effective flower thinning agent to enhance 'Abbé Fetel' pear seasonal fruit growth and quality.

Key words: Flower thinning, fruit size, growth curves, lime sulphur, maturity, *Pyrus communis.*

Introduction

Thinning is a central management activity in the production of high quality fruit required for the domestic and export market. Flower and fruit thinning prevent the development of some fruits, allowing the remainder to become larger and more marketable (Dennis, 2002). The challenges posed by chemical thinning are among the greatest obstacles which fruit growers face in achieving profitable production. Thinning must be predictable else recommendations loose credibility and are not used. Loss of credibility is due to underthinning as much as the fear of overthinning (Jones *et al.*, 1998). It is, however, generally considered that cultural factors other than thinning are also important to achieve adequate fruit size. These include balanced fertiliser programs, dwarfing rootstocks and appropriate pruning practice (Meland, 1998b).

The chemical and its concentration, the time of application and environmental factors encountered before, during and after application, all influence the ultimate thinning response. Variation in chemical thinning efficacy between years and within years has made it difficult to accurately predict the best dose and timing for chemical application (Robinson and Lakso, 2004). The inconsistency in the results of chemical thinning practices is at least partly caused by weather factors, such as temperature and air humidity, but tree factors are also involved (Wertheim, 2000).

Early thinning of fruit trees is important since it influences fruit size in the year of application and affects flower bud formation for the following season. According to Greene (2002), efficacy of blossom thinners is less influenced by the weather than hormone type thinners, and to be effective it may not be necessary to have specific physiological conditions within the fruit. Blossom thinners are caustic; they prevent fertilization and reduce fruit-set by damaging different flower parts, including anthers, stigma, style and pollen tubes (Fallahi and Fallahi, 2004). A number of chemicals have been tried as flower thinning agents, including the foliar feeds ammonium thiosulphate (ATS) and potasium thiosulphate, lime sulphur (calcium polysulphide, CaSx), endothalic acid, pelargonic acid and sulfcarbamide, all of which are flower desiccants (Balkhoven-Baart and Wertheim, 1998; Fallahi *et al.*, 2004; Greene, 2004). Ethephon may also thin when applied at bloom (Alina, 2006); it can stimulate flower thinning by inducing flower drop and the response appears to be cultivar and temperature sensitive. Looney (1998) reported that application of MCPB ethyl (a synthetic auxin) at full-bloom significantly reduced fruit-set in 'Fuji' apple in Canada.

Under current organic production methods growers are dependent on hand thinning to reduce crop load and enhance fruit size at harvest. However, because hand thinning is not normally carried out until six to eight weeks after bloom the resulting increase in fruit size is typically less than from chemical thinning applied at or soon after bloom, and there is minimal or no enhancement of return flowering (McArtney *et al.*, 2000). The higher costs of hand thinning combined with the increased potential for biennial bearing are significant obstacles that need to be overcome in order to achieve regular annual yields under organic production systems. With the move towards the use of simple salts that act as blossom desiccants, rather than hormonal type thinning agents, there is more scope for finding suitable chemicals for organic production (Bound and Wilson, 2004).

Lime sulphur is permitted under current guidelines for organic production and impedes fruit-set by lowering the number of pollen

grains that reach the ovary at the base of the flower; this response is cultivar specific. It was found to be effective for pome and stone fruits (Meland, 1998a; Bertschinger *et al.*, 2000; Webster and Spencer, 2000; Lenahan and Whiting, 2006). Fallahi (2006) used lime sulfur and fish oil and combination of these chemicals and found them to be effective organic blossom thinners for apples and peaches.

According to Warlop (2002), CaSx is considered one of the most promising organic apple thinning agents. Chemical thinning of pears is not as generally satisfactory as with apples. Problems with inadequate fruit-set are more common and application of blossom desiccants may show different responses among cultivars, directly associated to special sensitivities presented by them to the materials. Trials conducted on the pear cultivar 'Conference' have shown that a proportion of flowers may be prevented from setting fruits using sprays of ATS applied at or around the time of full bloom (Webster, 2002).

'Abbé Fetel' is becoming a variety of interest to the pear industry because of the excellent fruit quality and the high degree of consumer demand. Fruit size is critical for marketing this cultivar. Thus, in order to set up a strategy to enhance seasonal fruit growth, a trial was carried out to evaluate lime sulphur as a flower thinning compound on 'Abbé Fetel' (*Pyrus communis* L.) pear trees.

Materials and methods

The study was conducted on 10-year-old 'Abbé Fetel' pear trees on *P. communis* L. rootstock, growing in sandy loam (Irisarri, 1987) and trained to palmette leaders at the experimental farm, Comahue National University, Argentina (38° 56'S, 67° 59'W). The trees were spaced 4.0 × 2.3 m and row orientation was north south. Surface-flood irrigation was applied in the orchard.

The experimental site was located in an arid region, with average annual rainfall of 250 mm. Relative humidity, relative sunshine duration, maximum, mean and minimum temperature were monitored in orchard with Metos, Gottfried Pessl., Weis, Austria. Meteorological data before, during and after lime sulphur application and during the growing season (2002-03) are presented in Tables 1 and 2, respectively.

Ten trees were selected for uniformity of size and fruit density. Each tree was an experimental unit and there were five replications per treatment, in a completely randomized design. Treatments were 1) control, and 2) 7 % lime sulphur applied at 30 % bloom. The applications were performed with an orchard sprayer until run off, on a cool day. Relative sunshine duration, mean temperature and relative humidity were 50.0 %, 7.9 ºC and 66.0 %, respectively (Table 1).

Fruit diameter (FD) was recorded two weekly (n=20 per date and treatment). At 144 days after full bloom (DAFB), or initial commercial harvest, fruit weight (FW) was determined with an electronic scale (model Mettler P1210, Mettler Instruments AG, Zurich, Switzerland). Fruits were then graded into size categories.

Ten-fruit samples were harvested for determination of the maturity indices. Fruit firmness was monitored with a fruit pressure tester (model FT 327, Effegi, Alfonsine, Italy) on three peeled equatorial positions. Soluble solids concentrations (SSC, %) were determined on the expressed juice with a hand-held refractometer (Brix 0-32 %, Erma, Tokio, Japan). Starch pattern index was measured by staining with an iodine-potassium iodide solution, where each fruit cut transversely in half was assessed in a scale of 1 (all tissues stained blue/black) to 6 (no staining), indicating least and maximum maturity, respectively.

Growth equations were developed using SYSTAT procedure. Model suitability was evaluated using goodness-to-fit measures. Mean separations were computed with Student's *t*-test.

Results and discussion

Growth curves: Lime sulphur sprays significantly increased (P<0.01) mean FD, starting from 115 DAFB (Fig. 1). This flower thinner reduced competition between fruits at an earlier stage in the season than was achieved using the fruitlet thinner naphthaleneacetic acid described in a previous trial (Garriz *et al.*, 2004). Under the climatic conditions of this study (Table 1), logistic models best fitted the fruit growth *vs.* time curves on treated (I) and non-thinned trees (II):

$FD=81.00/(1+e^{2.30-0.03DAFB})$, $R^2=0.98$, $P<0.001$ (I)

$FD=77.87/(1+e^{2.26-0.03DAFB})$, $R^2=0.97$, $P<0.001$ (II)

In commercial fruit-growing, knowledge of the seasonal course of fruit growth is essential for correct timing of the different cultural practices like fertilization, pruning, fruit thinning, etc. (Westwood, 1993). Different kinds of seasonal fruit growth patterns were described for the pear cultivars 'Bartlett', 'Packham's Triumph' and 'Abbé Fetel' (Garriz *et al.*, 1995, 1999, 2005).

Final fruit size: Final size grading showed that percentage of fruits with <65mm diameter was 25.0 % for the control and 5.26 % for treatment 2 (Fig. 2). Lime sulphur sprays significantly altered final fruit size in terms of FW; values were increased by 16.5 % in relation to control fruits (Table 3). Fruit size increases following blossom thinning are attributable to increased cell division as well as to cell expansion in the persisting fruits. Increased cell division in fruits leads to firmer fruits with improved texture. In 'Golden Delicious' apples, very severe thinning occurred when 3 % CaSx was applied at full bloom (Stopar, 2004).

Table 1. Relative humidity, relative sunshine duration, maximum, mean and minimum temperature in orchard from 15 September to 19 September.

Parameter	15 September	16 September	17 September	18 September	19 September
Relative humidity (%)	70	66	45	46	51
Relative sunshine duration (%)	74	50	83	54	75
Maximum temperature (ºC)	22.5	16.5	17.5	15.6	15.6
Mean temperature (ºC)	12.4	7.9	10.7	10.3	9.6
Minimum temperature (ºC)	-1.1	4	1.9	6.4	1.7

Table 2. Relative humidity, relative sunshine duration, maximum, mean and minimum temperature in orchard during the growing season

Parameters	Month						
	September	October	November	December	January	February	March
Relative humidity (%)	53	58	53	60	57	57	60
Relative sunshine duration (%)	63	57	72	63	83	81	66
Maximum temperature (ºC)	18.9	21.9	26.0	28.4	30.6	29.4	27.7
Mean temperature (ºC)	11.4	14.2	18.3	20.2	22.3	20.1	18.6

Table 3. Effects of 7 % lime sulphur (LS) on fruit diameter and weight of 'Abbé Fetel' pears at commercial harvest. Trees were treated at 30 % bloom. Means followed by different letters within columns are significantly different from one another (Student's *t*-test, $P < 0.01$)

Treatment	Fruit diameter		Fruit weight	
	(mm)	Increment in relation to control (%)	(g)	Increment in relation to control (%)
Control	68.9 a	0.00	229.8 a	0.00
LS	72.5 b	5.22	267.7 b	16.50

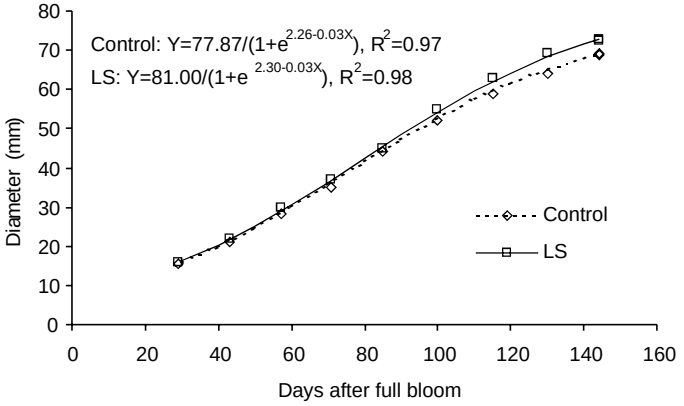

Fig. 1. Changes in 'Abbé Fetel' fruit diameter plotted on a time-from-bloom basis, as affected by lime sulphur (LS), applied at 30 % bloom. The lines are the fitted models to the data. Statistical differences ($P < 0.01$) between means at each date are indicated by the asterisk, according to Student's *t*-test.

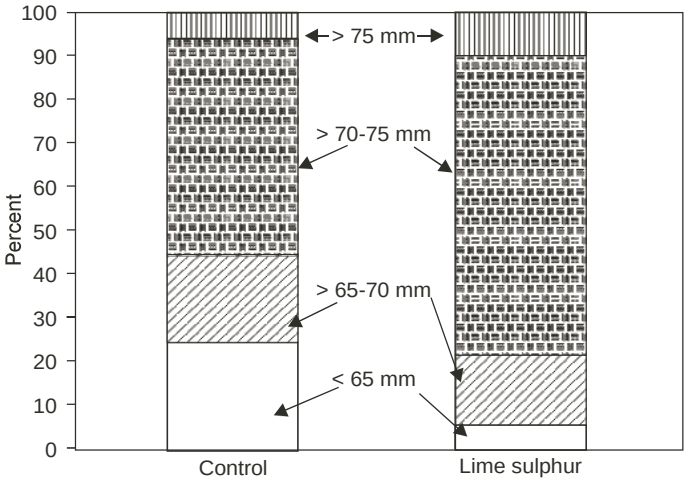

Fig. 2. Effect of thinning with 7 % lime sulphur (LS) on fruit size distribution of 'Abbé Fetel' at commercial harvest.

Fruit deformations or marks were not detected on treated pears. Kelderer *et al.* (2002) carried out thinning trials with lime sulphur and the sprays were applied during blossom to various apple varieties. In most cases, it was possible to increase the average fruit size; the influence on fruit-russeting was non-significant and the fruit deformations increased slightly. Their results showed a correlation with the amount of active ingredient, water volume and number of treatments.

Fruit maturity: Ripening changes are associated with the transition from growth to senescence and whilst these phenomena appear common to all pear cultivars, the rate of fruit development is a varietal characteristic, although there is a lesser influence of growing conditions, particularly of climate. Fruit ripening is a coordinated series of biochemical changes that renders the fruit attractive to eat; the process is under genetic regulation, but plant hormones play an essential control (Vendrell and Palomer, 1998). The maturity indices of 'Abbé Fetel' pear samples picked at 144 DAFB, or initial commercial harvest in the High Valley region are shown in Fig. 3. Blossom thinning improved fruit quality as well as size, since treated trees showed firmer fruits than the controls (64.4 *vs.* 61.7 N) and there were no statistical differences among treatments in soluble solids concentration (11.5 *vs.* 11.8) and starch index (3.6 *vs.* 3.7). Guak *et al.* (2004) treated Fuji and Gala/M9 apple trees with lime sulphur at 85 % full bloom at rates up to 4 %. Treatments caused Fuji fruits to be slightly longer than the untreated control but other fruit quality characteristics at harvest were largely unaffected. After 3 or 4 months of 1°C storage, firmness of Gala was slightly reduced by lime sulphur treatment but juice soluble solids and acidity were unaffected.

From the present study with 'Abbé Fetel' under conditions in the High Valley region of Argentina it can be concluded that Lime

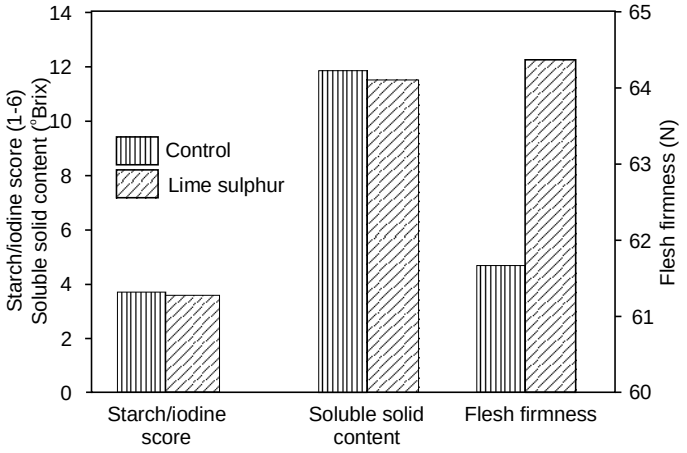

Fig. 3. Effect of 7 % lime sulphur (LS) on starch/iodine score, soluble solids concentration and flesh firmness values of 'Abbé Fetel' pears at commercial harvest.

sulphur sprays at 30 % bloom significantly altered seasonal fruit growth in terms of mean fruit diameter. Percentage of fruits with < 65 mm diameter was higher (25.0 %) in control and 5.26 % in lime sulphur spray. Final fruit weight increased by 16.5 % as compared to control fruits. More research is needed to determine how lime sulphur concentration and time of application influence the thinning response on different pear cultivars.

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Growth, fruit yield and quality of 'Golden Delicious' apple trees under fixed partial rootzone drying

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Abstract

We investigated the vegetative and productive responses of 'Golden Delicious' apple (*Malus domestica* Borkh.) trees to fixed partial rootzone drying under the dry climate of central Sicily. Soil water content (SWC), stomatal conductance, yield, fruit quality, fruit growth, and vegetative growth of conventionally irrigated trees (CI), where drip emitters on both sides of each tree were left open, were compared to that of fixed partial rootzone drying (FPRD) trees where only one side of the rootzone was irrigated for the entire season thus receiving 50% of the CI irrigation water. The irrigation season started on 31 July and ended on 13 September, 2004. Wet and dry rootzone sides showed significantly different SWC from 16 August until 14 September, whereas stomatal conductance of CI and FPRD trees differed significantly starting on 24 August. Relative growth rate of CI fruit was higher than that of FPRD fruit on 27 and 31 August, but fruit size was similar during the entire sampling period and at harvest. Trees of the two treatments had similar yields, number of fruits, crop load, fruit:leaf ratio, fruit quality, tree height, wood fresh and dry weight, canopy spread area, volume and density, shoot length and number, internode length, and leaf area. FPRD trees had higher yield efficiency, thinner shoots, lower leaf water content, higher canopy density and leaf dry weight and specific leaf weight than CI trees. Our observations suggest the extent of possible water savings without loss of yield and fruit quality using this partial rootzone drying strategy in 'Golden Delicious' apple orchards of central Sicily.

Key words: Canopy size, crop load, deficit irrigation, fruit growth, fruit quality, leaf area, shoot length, stomatal conductance, yield.

Introduction

Fruit production in semi-arid climates is subject to high evapotranspiration, increased soil salinity, and limited water availability. For these reasons, maximizing yields with minimal irrigation inputs, *i.e.*, increasing plant water use efficiency, becomes essential.

Plants growing under water deficit conditions can partly maintain cell turgor by closing stomata (Parker and Pallardy, 1985). Yet, stomatal closure for varying periods of time can impair CO_2 assimilation and may reduce the structural and energetic support for growth (Hsiao, 1973). This often leads to significant yield reductions especially in fruit crops. On the other hand, mild water deficit can induce partial stomatal closure which may result in improvements of water use efficiency due to the non-linear relationship between stomatal conductance and assimilation (Turner, 1997). Moderate water deficit may also alter resource allocation in favour of reproductive development (Yang *et al.*, 2000). Seeds and fruits are in fact, stronger growth sinks than shoot apices (Wardlaw, 1990) and under drought and limiting assimilation rates, vegetative growth is reduced more rapidly than reproductive growth (Higgs and Jones, 1991).

Regulated deficit irrigation (RDI) was developed to minimize irrigation inputs for fruit production in areas where water is a limiting resource. It consists of withholding water during certain periods to produce a moderate drought stress and to obtain beneficial consequences on fruit quality while limiting shoot growth. Results of RDI experiments have been promising in certain regions and for some fruit crops, such as peach (*Prunus persica* L.) (Chalmers *et al.*, 1981), pear (*Pyrus communis* L.) (Mitchell *et al.*, 1984; Mitchell *et al.*, 1989; Caspari *et al.*, 1994), French prune (*Prunus domestica* L.) (Lampinen *et al.*, 1995), and olive (*Olea europea* L.) (Goldhamer, 1997). In these species, vegetative and reproductive growth occur during different periods allowing for control of shoot growth without any decrease in fruit size or yields (Chalmers e*t al.*, 1981). On the other hand, apple (*Malus domestica* Borkh.) fruits and shoots grow concurrently (Forshey *et al.*, 1983) and water deficit usually reduces fruit size and yields irrespective of timing (Lötter *et al.*, 1985; Ebel *et al.*, 1993, 1995; Mpelasoka *et al.*, 2001; Caspari *et al.*, 2004b; Leib *et al.*, 2006).

Partial rootzone drying (PRD) is an irrigation technique that was recently developed in Australia for grapes (*Vitis vinifera* L.) (Dry *et al.*, 1995; Dry and Loveys, 1998). With PRD, only one half of the rootzone is irrigated whereas the other half is not. The physiological basis for PRD is that roots in drying soil produce abscisic acid (ABA) which is translocated to the shoots, indicating a developing soil-water deficit (Dry *et al.*, 1995). In leaves, ABA induces partial stomatal closure which reduces transpiration and may increase water use efficiency. At the shoot meristem, ABA may reduce shoot extension, but because the other half of the rootzone is kept well watered, the effect on plant water potential is minimal (Gowing *et al.*, 1990). Other metabolic and physiological processes associated to water stress are not affected during PRD (Dry *et al.*, 1995; Dry *et al.*, 2000). PRD relies on cyclical wetting and drying of parts of the rootzone in order to maintain root derived ABA signals (Zhang and Davies, 1987). Yet, fruit yield, stomatal conductance (g_s), and shoot growth of

raspberries (*Rubus idaeus* L.) was similar in alternated and fixed PRD where there was no switching of wet and dry sides (Grant *et al.*, 2004).

In studies conducted on 'Braeburn', 'Fuji', and 'Gala' apples (Caspari *et al.*, 2004 a, b; Einhorn and Caspari, 2004; Lombardini *et al.*, 2004), PRD should allow for a good final fruit size of apples and possibly for a reduction in shoot growth due to a lower number of nodes (rather than shorter internodes) along with a significant reduction in irrigation water. For this reason, PRD has a significant potential to become a beneficial irrigation strategy in those fruit crops where RDI has led to negative outcomes. Our objective was to examine the productive and vegetative responses of 'Golden Delicious' apple trees to fixed PRD in the semi-arid climate of central Sicily. We hypothesized that FPRD would not only save water but could also reduce vegetative growth without sacrificing 'Golden Delicious' fruit yield or quality.

Materials and methods

The study was conducted near Caltavuturo (37° 49' N and 850 m above sea level), Sicily, Italy. Trees were 42 uniform six-year-old 'Golden Delicious' apple trees grafted on MM 106 rootstock trained to a central leader. Trees were planted in single rows (north-south oriented) spaced at 4 m between rows and 1.5 m within the row and arranged in a randomized complete block design with three replicates of seven trees per irrigation treatment (described below). The soil type was a sandy clay loam with pH 7.3 and 18% active carbonates. Soil moisture content at field capacity was about 0.27 $m^3 m^{-3}$. Trees were drip irrigated using one dripper every 1.5 m and received conventional cultural care.

In July 2004, five of the seven trees (one tree at each end was left as buffer) per treatment-replicate combination (total 30 trees) were selected and labeled. For the conventional irrigation treatment (CI), all drip emitters on the line located between consecutive trees along the row were left open so that trees were receiving water on both north and south sides of the rootzone. Irrigation maintained soil water content above 80% of field capacity. For the FPRD, the drip emitter on one side of each of 15 trees was closed and the emitter on the other side was left open so that trees were receiving 50% of the CI irrigation water only on one side of the rootzone.

Wet and dry sides of the rootzone were not alternated because of the relatively short irrigation season of 44 days (typically the irrigation season in this area ranges from 60 to 75 days), the relatively constant soil water content (around 0.2 $m^3 m^{-3}$) in the dry side during the last two thirds of the irrigation season, and the significantly reduced stomatal conductance of FPRD trees for the entire second half of the irrigation season. Also, since previous trials with 33% season-long irrigation reductions using neighboring trees had led to significant fruit size and yield reductions, a treatment with 50% irrigation of CI distributed on both sides of the rootzone was not included.

Soil water content (SWC), g_s, and fruit growth were monitored twice a week from 3 August until 16 September. SWC was measured in each block on the wet and dry side of the FPRD treatment at the fixed soil depth of 40 cm by time domain reflectometry (Trase Systems-Soil Moisture Equipment Corp., Santa Barbara, CA, USA). SWC of CI treatment was assumed to be similar to the wet side of the FPRD treatment. Stomatal conductance was measured between 11:00 and 13:00 HR on two leaves, each located on one side (East and West) of the tree, with an AP4 Delta-T porometer (Delta-T Devices, Cambridge, UK). Mature, fully expanded, but non-senescent leaves on extension shoots were selected for g_s measurements. Fruit growth was monitored non-destructively on one fruit per tree. Each fruit was photographed against a white background and next to a reference tape with a digital camera, and fruit vertical cross-sectional area was determined after editing and calibration of the images. Climate data were obtained from an official weather station of the Sicilian Agro-Meteorological Information Service located nearby in the same farm. Vapor pressure deficit (VPD) was calculated from average daily temperature (T in °C) and relative humidity (RH in %).

Fruit were harvested on 22 September and total fruit weight and number per tree were determined in the field, and a sub-sample of 30 fruit per tree was taken to the laboratory for quality analysis. In the laboratory, each fruit was photographed (under identical light conditions provided by two 18-watt fluorescent lamps) and digital images were used to determine final fruit size (vertical cross-sectional area) and peel color. Peel color was determined by digital image analysis using an algorithm developed with MATLAB® software (The Mathworks Inc.) that converts images from RGB to CIE 1976 L*a*b format (by lookup tables), extracts the fruit from the image (removing the image background), and quantifies color characteristics as the weighed distance of each pixel in the image from a reference sample (best colored area interactively chosen from a well colored fruit). The output is an index ranging from 0 (green) to 1 (yellow). Subsequently, flesh firmness (with a manual pressure tester mounting a 8-mm tip, TR di Turoni & Co., Forlì, Italy), total soluble solids (with an Atago Palette PR-32 digital refractometer, Atago Co., Ltd., Tokyo, Japan), juice pH, titratable acidity (with a Crison S compact titrator, Crison Instruments, SA, Alella, Barcelona, Spain; expressed in grams of malic acid per liter), and starch pattern index (by Lugol staining) were measured on each fruit. Stained fruit sections were photographed and the same algorithm used for determination of peel color, was used to quantify staining. The output in this case is an index ranging from 0 (no staining) to 1 (fully stained).

At the beginning of October, trunk circumference was measured at about 15 cm above the graft union, trees were defoliated, all leaves of each tree were weighed, and a sub-sample of 30 leaves per tree was transported to the laboratory for determination of area, fresh and dry weight. The leaf sub-samples were photographed and their area was measured by digital image analysis; leaf area of sub-samples was used to establish a correlation with leaf weight and estimate total leaf area per tree. Trunk cross-sectional area and leaf area were used to calculate yield efficiency (kilogram of fruit per square centimeter of trunk cross-sectional area), crop load (number of fruits per square centimeter of trunk cross-sectional area), and fruit:leaf ratio (kilogram of fruit per square meter of leaf area). Subsequently, entire above-ground wood structures (trunk, limbs, and shoots) were cut at the ground level, and photographed with the digital camera against a white background from plan and side views for later acquisition of bi- and three-dimensional measurements. A measuring tape of known length was included in the picture as a reference for subsequent size

adjustments. After all images were acquired, wood structures were cut, weighed, and oven-dried at 60°C to a constant weight. Digital images were edited as described by Lo Bianco *et al.* (2003) to determine total shoot length and diameter. Briefly, the background was manually removed from original JPEG images and clean images were saved as binary TIFF files. Morphological image processing (skeletonizing algorithm) was used to separate the seasonal growth from older wood according to diameter category. ROOTEDGE software (Iowa State University Foundation Inc., Ames, IA, USA) was used to scan TIFF images and determine shoot length and diameter.

The original images were also used to calculate average internode length (dividing shoot length by number of nodes) from three shoots per tree, canopy spread area (marked as a circle or ellipse enclosing all stems in the plan views), and canopy height. Canopy shape of the young apple trees resembled a cone. Hence, canopy volume was estimated as follows:

Volume = (spread area × height)/3

Canopy density was calculated as the total length of wood portions per unit of volume.

Yield, fruit quality, and growth data were compared by analysis of variance (with irrigation treatment and replicate as factors) using SYSTAT procedures (Systat Software Inc., Richmond, CA, USA). Fruit quality data were also analyzed using crop load or yield efficiency as covariate. Repeated measures analysis of variance followed by orthogonal polynomial contrasts was used to evaluate differences in g_s, soil water content, and fruit growth between treatments and sampling dates. Pearson product moment correlation analysis was used to determine associations between g_s, SWC, and VPD.

Results

The irrigation season started on 31 July (5 days after the last relevant precipitation event and 92 days after bloom) and ended on 13 September (Fig. 1A). The total irrigation volume was 90 mm for CI and 45 mm for FPRD distributed over 20 events. Daily vapour pressure deficit varied greatly reaching peaks of over 3 kPa on particularly hot and dry days and showing significant reductions (below 0.5 kPa) on corresponding rainy days (Fig. 1B).

Wet and dry soil areas showed significantly different SWC from 16 August until 14 September, with the exception of 7 September when a problem in the irrigation system caused skipping of one programmed event (Fig. 2A). On 16 September, similar SWC in wet and dry areas was due to over 10 mm of rain early in the same day (Fig. 1A). Repeated measures analysis showed a significant effect of the irrigation treatment ($P < 0.001$), a significant change of SWC over time ($P < 0.001$), and a significant interaction between irrigation treatment and SWC over time ($P = 0.004$) indicating that SWC was changing over time in a different fashion in the wet and dry areas. In particular, SWC in the dry areas decreased exponentially according to the model

$SWC = 0.199 + 0.048\ e^{-0.205\ day}$ ($P < 0.001$, $r^2 = 0.940$).

Stomatal conductance of CI and FPRD trees differed significantly from 24 August until the end of the sampling period (Fig. 2B). The effect of the irrigation treatment was significant ($P < 0.001$), conductance changed significantly over time ($P < 0.001$), and irrigation treatment x conductance over time interacted significantly ($P = 0.002$). Initial stomatal response to changes in SWC was delayed by about eight days (Fig. 2A and B). Stomatal conductance of FPRD trees was correlated to SWC (r = 0.888, $P = 0.001$) and VPD (r = 0.709, $P = 0.010$), whereas, conductance of CI trees was correlated only with SWC (r = 0.729, $P = 0.026$).

Repeated measure analysis indicated no significant effect of irrigation treatment on fruit cross-sectional area ($P = 0.392$; Fig. 3A), but there was a significant change of cross-sectional area over time ($P < 0.001$) and a significant interaction between irrigation treatment and change of cross-sectional area over time ($P < 0.001$). Specifically, the first degree (linear) polynomial contrast explained over 98% of the variability due to changes of cross-sectional area over time. On the other hand, relative growth rate of CI fruit was significantly greater than that of FPRD fruit on 27 and 31 August (Fig. 3B). In this case, repeated measure analysis showed a significant irrigation treatment effect ($P = 0.003$), a significant change of relative growth rate over time ($P < 0.001$), but no significant interaction between irrigation treatment and change of relative growth rate over time ($P = 0.330$). The first degree (linear) polynomial contrast explained over 80% of the variability due to changes of relative growth rate over time.

Trees in the two irrigation treatments had similar yields, number of fruit, crop load, and fruit:leaf ratio, but FPRD trees were more efficient than CI trees (Table 1). Using yield efficiency or crop load as a covariate in the analysis of variance for fruit quality

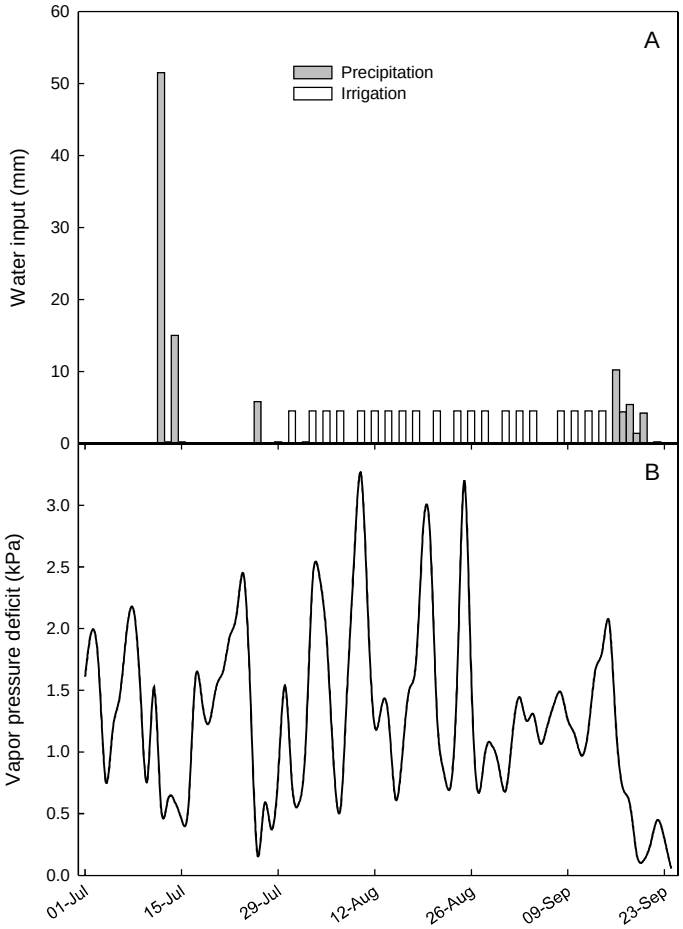

Fig. 1. Average daily water inputs (A) and vapor pressure deficit (B) during summer 2004 near Caltavuturo (37° 49' N and 850 m above sea level), Sicily.

Table 1. Yield performance of six-year-old 'Golden Delicious' apple trees under conventional irrigation (CI) and fixed partial rootzone drying (FPRD)

Yield parameters	CI	FPRD	P^z
Yield (kg tree^{-1})	12.1	14.7	0.098
Number of fruits	103	135	0.068
Yield efficiency (kg cm^{-2})	0.31	0.39	0.046
Crop load (fruits cm^{-2})	2.61	3.56	0.062
Fruit:leaf ratio (kg m^{-2})	3.70	3.65	0.933

^{z} *P* value from analysis of variance.

Table 2. Fruit quality of six-year-old 'Golden Delicious' apple trees under conventional irrigation (CI) and fixed partial rootzone drying (FPRD)

Quality paremerers	CI	FPRD	P^z
Fresh weight (g)	125	115	0.133
Cross-sectional area (cm^2)	48.4	46.2	0.436
Peel color index	0.93	0.92	0.055
Flesh firmness (kg cm^{-2})	8.52	8.88	0.295
Starch pattern index	0.94	0.94	0.545
Soluble solids (°Brix)	12.2	12.1	0.506
Acidity (g L^{-1})	4.60	4.45	0.484
pH	3.63	3.70	0.276

^{z} *P* value from analysis of variance.

Table 3. Vegetative growth of six-year-old 'Golden Delicious' apple trees under conventional irrigation (CI) and fixed partial rootzone drying (FPRD)

Growth parameters	CI	FPRD	P^z
Tree height (m)	3.11	3.06	0.437
Wood fresh weight (kg)	8.07	7.73	0.586
Wood dry weight (kg)	4.17	4.13	0.904
Canopy spread area (m^2)	4.90	4.52	0.197
Canopy volume (m^3)	5.10	4.62	0.149
Canopy density (m m^3)	12.2	13.7	0.014
Shoot length (m)	49.6	50.2	0.854
Shoot diameter (cm)	0.69	0.66	0.017
Shoot number	182	176	0.588
Internode length (cm)	3.24	3.19	0.732
Leaf area (m^2 tree^{-1})	3.76	4.06	0.377
Leaf dry weight (kg tree^{-1})	0.47	0.62	0.003
Leaf specific weight (kg m^{-2})	0.13	0.15	<0.001
Leaf water content (%)	49.9	47.0	0.004

^{z} *P* value from analysis of variance.

parameters, did not affect differences between CI and FPRD trees. Hence original means and statistics from analysis with no covariate are reported in Table 2. In particular, quality parameters of fruit of CI and FPRD trees were similar (Table 2).

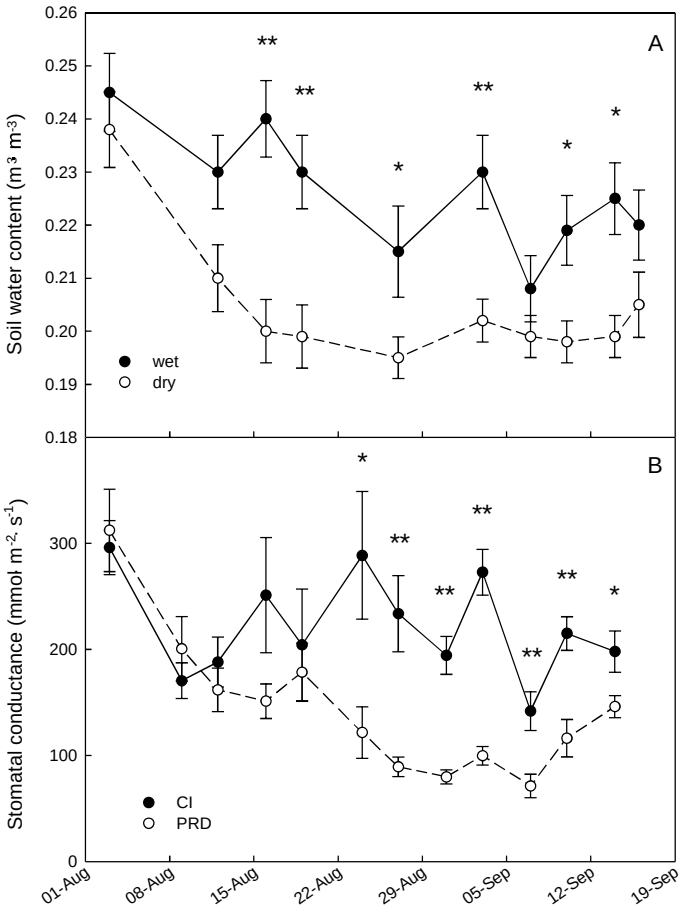

Fig. 2. Soil water content (A) and stomatal conductance (B) of six-year-old 'Golden Delicious' apple trees under conventional irrigation (CI) and fixed partial rootzone drying (FPRD). ** and * indicate significant differences between the two treatments at $P \leq 0.01$ and $P \leq 0.05$, respectively. Error bars represent standard errors of the means.

Vegetative growth showed some differences mainly due to a greater leaf water content in CI trees compared to FPRD trees. Statistically, CI and FPRD trees had similar height, wood fresh and dry weight, canopy spread area and volume, shoot length and number, internode length, and leaf area (Table 3). On the other hand, CI trees had thicker shoots and greater leaf water content, but lower canopy density, leaf dry weight and specific weight than FPRD trees (Table 3).

Discussion

This study provides further positive support in favour of PRD irrigation strategy over CI for apple cultivation in semi-arid environments in the Southern Mediterranean regions. In particular, a 50% reduction of the irrigation water applied during the entire season to only one side of the rootzone did not reduce yields compared to conventionally irrigated trees. Similarly, we did not detect any difference in fruit external or internal quality, whereas differences in water status (g_s) resulted in some reduction of vegetative growth of FPRD trees. In previous studies, there were no changes in fruit quality in response to PRD in 'Breaburn' (Van Hooijdonk *et al.*, 2004) and 'Gala' (Caspari *et al.*, 2004a) apple.

It took slightly over a week for the 'Golden Delicious' trees to reduce g_s in response to diminished soil water content. This might provide an indication of the time required for a six-year-old apple

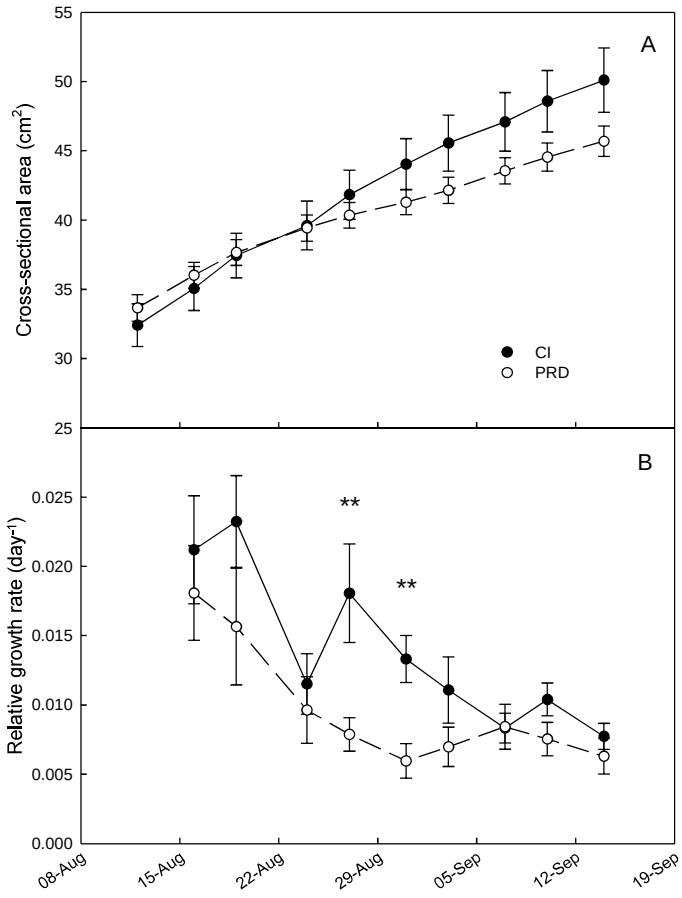

Fig. 3. Fruit cross-sectional area (A) and relative growth rate (B) of six-year-old 'Golden Delicious' apple trees under conventional irrigation (CI) and fixed partial rootzone drying (FPRD). ** and * indicate significant differences between the two treatments at $P \leq 0.01$ and $P \leq 0.05$, respectively. Error bars represent standard errors of the means.

tree to synthesize ABA in the root system and/or to translocate a sufficient amount of hormone to the leaves for the reduction in g_s to be detectable. Similar reductions in g_s have been reported in PRD apple (Gowing *et al.*, 1990), olive (Wahbi *et al.*, 2005), and grapes (De Souza *et al.*, 2003). Other studies with field-grown apple trees, however, have shown no reduction in g_s in response to PRD (Caspari *et al.*, 2004b; Einhorn and Caspari, 2004; Lombardini *et al.*, 2004; Van Hooijdonk *et al.*, 2004) so the ability of hormonal signals to reduce g_s may depend on the evaporative demand and the rate of transport of signals to the leaves (Davies *et al.*, 2002). Thus, the generally low irrigation volumes and relatively high evaporative demand during our experiment could explain differences between our results and previous studies.

In grapes, switching of the wet and dry sides every 10 to 15 days is needed to maintain the ABA signal and the consequent reduced g_s (Dry and Loveys, 1999; Loveys *et al.*, 2000). This is apparently due to the transient nature of ABA accumulation in grape roots in dry soil. In 'Fuji' apple grown in the semi-arid climate of Washington State, Leib *et al.* (2006) alternated wet and dry sides of the PRD treatment every 3-4 weeks without affecting fruit size or yield. In our study, although we did not switch wet and dry sides, g_s of FPRD trees remained significantly lower than that of CI trees for the final three weeks of the irrigation season. Probably, under our conditions a longer period of time was needed for a further decrease in SWC in the dry root zone side, and thus for the ABA signal to be canceled and for g_s to return to control levels.

In spite of the observed reductions in g_s of FPRD trees, mainly due to lower SWC, fruit growth rate was affected on only two dates and there was no difference in final fruit size, weight, yields, and fruit quality between the two irrigation treatments. Other authors have observed contrasting responses for apple fruit yield and quality depending on the season, orchard location, and climatic conditions; for example, Lombardini *et al.* (2004) observed a reduction in fruit size for apple trees under PRD. However, it is generally accepted that PRD does not affect apple fruit yield and quality (Caspari *et al.*, 2004a, b; Einhorn and Caspari, 2004; Van Hooijdonk *et al.*, 2004).

Our irrigation treatments resulted in thinner shoots and greater leaf specific weight in FPRD compared to CI trees but tree size and shape was not affected. Since wood dry weight was not influenced by irrigation, thinner shoots in FPRD trees may be the result of some reduction in shoot radial growth probably due to a decrease in the diameter of xylem elements. On the other hand, higher leaf specific weights in FPRD trees could be related to reduced cell expansion (probably due to the reduced water content), increase in cell number, and consequent increase in the deposition of cell wall structures. Growth reductions, mainly in terms of decreased shoot length, were reported in PRD raspberry (Grant *et al.*, 2004), grapes (Dry *et al.*, 2000; Santos *et al.*, 2003), olive (Wahbi *et al.*, 2005), and potted apple (Gowing *et al.*, 1990), but not in field-grown apple (Einhorn and Caspari, 2004; Lombardini *et al.*, 2004). In our case, the lack of reductions in shoot length in response to FPRD could be due to the late timing of treatment when flush of terminal shoot growth was nearly completed.

Timing of treatment imposition may have also played an important role in the behaviour of reproductive sinks. In other words, fruits may have escaped significant size reductions because most of the cell division had been already completed by 16 August when differences in SWC became significant. Fruit may have been stronger sinks for water than shoot tissues during final cell expansion. Similarly, late decreases in water potential did not reduce fruit size of 'Breaburn' apple (Kilili *et al.*, 1996).

This study suggests a potential advantage of using PRD strategies over CI in 'Golden Delicious' apple orchards for reduction of irrigation inputs in central Sicily. Continuation of field measurements in the following years with the addition of deficit irrigation treatments with reduced water amounts distributed to both sides of the rootzone and alternated PRD treatments, should allow for determination of any greater potential for PRD utilization as a common irrigation practice to save water in dry climates.

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Soil, plant and canopy resistance to water flow in bell pepper (*Capsicum annuum* L.) as affected by fertigation regimes

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Abstract

The effect of fertigation regimes on water transport properties (soil, plant and canopy resistances) through the plant to the canopy in the Soil-Plant-Atmosphere-Continuum (SPAC) was studied in bell pepper in a Mediterranean climate. The treatments consisted of fertigated drip irrigation in factorial combinations of three levels (amounts) of water application (daily, twice and once weekly) and application frequencies (2, 6 and 10 times per fertigation event). Leaf water potential and stomatal conductance were monitored while whole plant hydraulic conductance was estimated by the evaporative flux method, using the Ohm's law analogy (the slope of the water potential difference ($\Delta\psi$) versus sap fluxes). Canopy conductance (inverse of resistance) was estimated from vapour pressure deficit (vpd) and transpiration flux. Differences in the intervals between fertigation events altered the environment for root development and affected soil moisture status, stomatal conductance (gs), leaf water potential (lwp), transpiration (sap) flux, and xylem and canopy water transport capacities in bell pepper. The components of the resistance elements in the SPAC differed under the fertigation treatments. Total plant resistance (Rp) increased with transpiration flux in a linear manner in addition to a proportional decrease in stomatal (gs) and canopy conductance (gc). Canopy component constitutes the least resistance (greatest conductance) to the flow of water, estimated soil resistance was much lower than total resistance to the flow of water, and the highest within plant resistance is contained in the root system which constituted a predominant part of total plant resistance. Bell pepper has an efficient xylem sap transport system, maintains gs and plant water status under variable soil moisture regimes. Bell pepper water use is affected by soil environment, plant architectural and xylem traits. The mechanisms underlying differences in water use and plasticity of physiological functions in bell pepper under variable fertigation regimes appeared to be offered through changes in the magnitudes of component resistances of the water transport pathways in the SPAC. The implications of knowledge of the magnitudes of the resistances to water flow pathway in the SPAC to irrigation management is discussed.

Key words: Bell pepper, fertigation, hydraulics, stomata, canopy, leaf potential, water uptake

Introduction

The trend to increase crop yields has led to frequent fertigation and therefore the time intervals between successive fertigation events has diminished to hours or even less. Therefore, modern agricultural systems tend to simultaneously supply water and nutrients (fertigation) mainly by drip devices (Bar-Yosef, 1999). Frequent irrigation events enhance high water fluxes from the growing medium to the root surface. The promotion of water and nutrients availability at rates that match plant requirements and reduction in the quantities of fertilizer needed to achieve optimal production contributes to the minimisation of ecological damage to environment (Silber *et al.*, 2003). In semi-arid climates, conventional daily cycle of irrigation is 1-3h per day in comparison with 10-14h of potential photosynthesis and transpiration of plants. As a result, transpiration during the day may cause significant differences between the water content in the root zone and that in the bulk soil (Silber *et al.*, 2003). Water and nutrient uptake by plants, and the formation of a depleted zone in the immediate vicinity of the roots are the driving forces for solute movement towards the roots. During fertigation events, subsequent redistribution enables frequent supply to the root surface and its vicinity with fresh nutrients in solution. These frequent replenishments eliminate the depletion zone formed at the root surface by uptake of nutrients during period between successive irrigation events, thus decreasing the concentration gradient between the medium solution and the root surface. Frequent fertigation enhances high water fluxes from the growing medium to the root surface in contrast to non-fertigated plants where nutrients and water are supplied independently (Claassen and Steingrobe, 1999).

Soil-plant-atmosphere coupling (SPAC) explains the control exercised by the soil and atmospheric conditions (environments) on plant processes. It is reported that changes in plant water relation parameters such as leaf water potential and stomatal response can be explained in terms of changes in the hydraulic architecture of plants (Salleo *et al.*, 2000; Tyree and Zimmermann, 2002). The ease of water fluxes from the soil to the leaf (canopy) drives the architecture and physiology of plants. If a plant's hydraulic architecture is important to the maintenance of functional integrity and hence growth and productivity under soil and air drought, then, stomatal conductance and photosynthetic sensitivity to water stress may be determined by hydraulic constraints in the SPAC (Sperry *et al.*, 2002). This understanding could be a useful input in the development of prediction models for plant water requirements in regions prone to drought. The hydraulic properties of a species may influence the response of gas exchange to soil moisture deficits. Therefore, the ability of plants to maintain a favourable water status is dependent on the resistance to water

flow in the SPAC (Jones *et al.*, 1982; Stiller *et al.*, 2003). Plant hydraulic efficiency therefore regulates the diurnal and seasonal time scale of water loss and leaf water status (Stiller *et al.*, 2003; Agele *et al.*, 2005).

Under non-limiting soil water status, within plant resistance dominates soil resistance to water flow, however, soil hydraulic resistance and root densities would be the limiting resistance in dry soils (LoGullo *et al.*, 2003). Changes in soil and plant resistance in response to soil drying are important causes of changes in the overall soil to leaf hydraulic conductance (Ks-l) and water relation characteristics of plants subjected to varying cycles of drought (Sperry *et al.*, 2002). Plant resistance is not constant but according to van Honert model, it increases with decrease in transpiration (Zur *et al.*, 1982; Rieger and Motisi, 1990; Steudle, 1994). Total resistance to water flow is dependent on leaf water potential and the transpiration rate (Lascano and van Bavel, 1984), however, hydraulic resistance of plants decreased with increasing transpiration rate (Hirasawa and Ishihara, 1991; Rieger and Motisi, 1990). Baker and van Bavel (1986) opined that the conductivity of the unsaturated soil is the dominant factor controlling water flow through the soil-plant system however, Kramer and Boyer (1995) postulated the existence of higher resistance to liquid water flow in the plant than soil and the predominance of root over plant resistance. The controversial reports are due to experimental conditions which vary from differences in soil, plant and atmospheric conditions (Lafolie *et al.*, 1991; Passioura, 1988). In addition to soil water status, the need for irrigation depends also on plant water status (Hsiao, 1990). Plant water status depends on soil water status, evaporative demand of the atmosphere and other plant characters such as root distribution, and hydraulic conductance (Jones, 1990). Several physiological indicators of plant water, stem and leaf water potentials, stomatal conductance and hydraulic conductance are postulated as possible criteria for scheduling irrigation due to their sensitivity to soil water status (Jones, 1990).

Empirical relationships are commonly used to describe water flow and quantification of water uptake and plant water status in the soil-plant system. For steady state conditions, water uptake (Q) is proportional to water potential difference. The total resistance (RT) to water flow can be defined as:

$$R_T = \psi o - \psi_1/Q = \psi s - \psi l/Q \quad 1$$

where, ψo is the average water potential at sites of entry into the roots (average soil water potential) and ψ_1 is the average exit potential in the leaves (average leaf water potential). The pathway of water movement can be described using the following equations;

$$T = Q = (\psi s - \psi r)/R_S = (\psi r - \psi l)/R_p = (\psi s - \psi l)/R_T \quad 2$$

where, T is transpiration rate, Q is the root water uptake, ψs, ψr and ψl are the water potential in the soil matrix, at the root surface and in plant leaves, respectively. Rs and Rp are resistances of the soil and plant pathway, therefore,

$$R_T = Rs + Rp \quad 3$$

The diurnal time scale of plant water status and stomatal behaviour could be regulated by the hydraulic transport efficiency of sweet pepper, a herbaceous annual. It is hypothesised that in bell pepper grown under different fertigation regimes, declines in soil water availability will be accompanied by changes in within plant water uptake and transport capacities. Such regulation will contribute to the prevention of hydraulic failure by ensuring that plant water use does not exceed supply. This is important to acclimation to water stress or adaptation to soil water availability, and these attributes could partly buffer pepper growth and survival under decreased soil water availability. Experiments were conducted to evaluate the physiological behaviour of bell pepper under fertigation regime-enhanced differences in soil moisture status. The aims were to evaluate the coupling of xylem conductance and stomatal conductance and hence the regulation of water use of bell pepper by soil, xylem and canopy hydraulics.

Materials and methods

Bell pepper were grown on sandy soil in a net house and subjected to varying fertigation regimes. Treatments consisted of factorial combinations of three levels (amounts) of water application by different daily application frequencies. The treatments were replicated four times on 4 x 5m plots while pepper seedlings were planted at a spacing of 90 x 30 cm. Fertigation regimes were made up of daily, twice and once weekly water application, while the fertigation frequencies involved water (and nutrient) application (2, 6 and 10 times per fertigation event at 0600 and 1200h, 0600, 0800, 1000, 1200, 1400 and 1600h, and from 0600 and 1600h at 1-h intervals. Pressure compensated drippers supplying 2.0L h^{-1} (Netafim, Inc, Israel) were used. The daily irrigation volumes per plant were 500mL during days 1-32 after transplanting, 800mL beyond days 32 to termination of experiment, and with excess at least equal to the total evapotrasnpiration. Irrigation scheduling was automatically implemented by a computer to deliver equal amount of water at different frequencies (time of day). Plants were individually irrigated with nutrient solution via drippers located on soil surface. The N and K concentrations were 13.5mM (constant NO_3-N/NH_4-N in ratio 3:1) and 6mM K_2O, respectively. Micronutrients concentrations (mg L^{-1}) were 0.6 Zn, 0.65 Mn, 0.8 Fe, 0.04 Cu, 0.4 B and 0.03 Mo, all EDTA-based (Silber personal communication). The initial pH of the irrigating solutions was 7.1, and irrigating solution was prepared in three 2500-L tanks.

Root analysis: The development and distribution of roots (inter-row and intra-row spaces) were monitored using cubic coring tools (10 x 10 x 10 cm). The corers were drill-inserted into the soil at 5, 15 cm circumference around 10 sampled plants/plot. The excavated roots from the samples were put in 2mm sieve and were gently washed free of soil in the laboratory using moderate jets of water. Samples were taken at 10 cm interval to a depth of 60cm. This sampling procedure was advanced at least 50 cm along the row before sampling to avoid edge effects. Measurement of root geometry characteristics by image analysis was performed as described by Costa *et al.* (2000) with minor modifications using a Delta -T Scan (Delta-T Devices Ltd, UK), an interactive scanner-based image analysis of root samples. The scanner incorporates a Hewlett-Packard Scanner (Scan Jet 3c) software set to 300 dots/inch scanning resolution in a PC system. 30-g fresh weight of root samples were stained for 15 min with 0.1% (w/v) toludine blue prior to analysis. The stained roots were placed in Plexiglas trays on a 4mm layer of water.

Measurements of plant water potentials and stomatal

conductance: Simultaneous measurement of stomatal conductance and leaf water potential (lwp) were made from 0800 to early afternoon in net house grown bell pepper which received six irrigation rates. Plant water potential was measured using a pressure chamber and stomatal conductance with a steady state porometer (model LiCor 1600; LiCOR Inc. USA). Leaf water potential (ψl) was measured using pressure chamber on detached leaves from the plant and sets of measurements were taken on sunlit leaves (ψlL), shaded leaves (ψlS) and on leaves covered with aluminium foil (ψl.C). In order to allow leaf water potential to equilibrate with stem water potential, shaded leaves were taken from inside the canopy, cut and placed in plastic bag covered with aluminium foil for about one and a half hours before measurement of its water potential. The water potential of leaves covered with aluminium foil (ψlC) is therefore equivalent to stem xylem water potential. It is in equilibrium with the potential of the conducting stem vessels below the transpiring canopy (Moreshet *et al.*, 1990). Total root water potential was measured at pre dawn, midday and sunset on excised root segments.

Estimation of hydraulic resistance: During each sampling period, the measured transpiration flux and leaf water potential measured hourly were used with daily measurements of root and soil potential to calculate total resistance using Ohm's law analogy (Moreshet *et al.*, 1996; Ruggiero *et al.*, 1999; Tsuda and Tyree, 2000). The overall relationship between difference in water potential between soil and leaf and transpiration was linear, with the slope equal to average plant resistance. Transpiration rate (E) in a plant is equal to the flow of sap through the xylem, Van den Honert equation relates sap flow to leaf and soil water potential and the hydraulic conductance between the soil and the leaf (Tardieu and Simonneau, 1998). K_T was therefore obtained as the proportionality constant between sap flux (E_L) and the gradient in water potential (dψ) between the soil (ψ_{soil}) and leaf (ψ_{leaf}) needed to maintain the evaporative flux density (Tyree and Zimmermann, 2002).

The model used according to Ruggiero *et al.* (1999) was as follows:

$$T = Q = (\psi s - \psi r)/R_S = (\psi r - \psi l)/R_P = (\psi s - \psi l)/R_T \quad 4$$

$$\text{Therefore, } Q = (\psi_S - \psi r)/Rs + R_P = \Delta\psi / Rs + R_P \quad 5$$

where, Q is the water flux, Δψ is the water potential gradient between soil and root system, $R_{S\ I}$ is soil and plant resistance, respectively.

Soil water potential (weighted) was calculated as:

$$\psi s = \sum i\psi_{si} Lvi / \sum iLvi \quad 6$$

where, Lvi is the root density in soil layer i (i=0.50cm)

Plant resistance was estimated as:

$$Rp = (\psi_R - \psi_L)/Q \quad 7$$

where, ψ_R is the time-weighted average total root water potential, ψ_L is total leaf water potential and Q is transpiration flux on a root length basis. Root resistance was estimated from the difference between plant resistance, and ψ_L is the total leaf water potential and Q is transpiration flux (on a root length basis). Soil resistance was calculated as the difference between total and plant resistance for each measurement day ($R_S = R_T - R_p$).

Total resistance to water flux from the soil through the plant ($R_T = R_P + R_S$) was calculated as follows:

$$R_T = (\psi_S - \psi_L)/Q \quad 8$$

where, ψs is the average soil water potential, ψL is the total leaf water potential and Q is the transpiration flux expressed on a root length basis.

Canopy conductance (gc) was estimated using Fick's law of diffusion and is based on vapour pressure deficit and plant water use (tranpiration) (Nobel, 1983; Alacron *et al.*, 2003):

$E = gsVPD$ therefore, $gc = E.VPD^{-1}$

E (transpiration) and gc (canopy conductance) for gs (stomatal conductance), gc is the aggregate of gs throughout the canopy. Data collected were subjected to analysis of variance (ANOVA) and differences between means were tested with Fisher's Least Significant Test (LSD) (Steel *et al.*, 1997).

Results and discussion

Differences in the intervals between fertigation events (daily, twice and once weekly) altered the environment for root development and affected soil moisture status, and water relations in bell pepper. The effects of fertigation regimes were significant on root morphology evaluated in terms of root mass densities. Intervals between successive irrigation events and daily fertigation frequencies modified root mass densities (Table 1). Higher root densities were obtained for pepper irrigated twice and once weekly over those irrigated daily. This result was consistent under the different fertigation frequencies at each irrigation event. However, across intervals between successive irrigation events, fertigation frequencies at 6 times per irrigation event produced significantly higher root densities over twice and ten times. The advantages of good root development (root biomass and root length densities) may reside in greater extraction and utilisation of soil water reserves located at shallow depths and possibly in the inter-row areas. Changes in root length and root diameter of bell pepper can possibly explain the observed variability in total resistance to water flow under the fertigation regimes. The rate of root water uptake and hence sap flow within a plant constitutes an important link between stomata and the atmospheric demand. Reduction in the intervals between fertigation events increased water uptake and use. Silber *et al.* (2003) postulated that frequent irrigation eliminates depletion zone at the root-soil interface by supplying fresh nutrient solution to the root surface. Decrease in the time intervals between irrigation events enhanced transpiration flux so that water uptake of plants grown under daily fertigation was about two fold of those irrigated once a week. Decreases in frequencies of irrigation from ten to two events induced a decrease of 6 and 18 % in transpiration (water use) fluxes. High fertigation frequencies especially under well watered situation (water and nutrient application at 6 and 10 times per fertigation event), produced higher midday leaf water potentials. However, under deficit water application (weekly fertigation event), high frequency deliveries (6 and 10 times) produced significant improvement in midday leaf water potentials over less frequent daily water application (2 times per fertigation event) to the rootzone.

The results of this study indicate rapid response of the hydraulic

Table 1. Effect of fertigation frequencies on bell pepper growth characters

	Mean root diameter (mm)	Root fresh weight (g plant^{-1})	Total root length (m plant^{-1})	Root length/ unit root fresh weight (m g^{-1} FW)	Water suction (K Pa)	Transpiration / unit root length (g h^{-1} m^{-1})	Root water uptake (Q) (g h^{-1})
Means of irrigation intervals (I)							
Daily	0.889	33.7	236	7.02	-0.98	9.05	0.92
2 days interval	0.688	36.2	273	7.54	-1.07	7.89	0.69
Once weekly	0.623	38.5	297	7.71	-1.23	4.53	0.75
Means of fertigation frequencies/irrigation event (F)							
10 times	0.821	27.8	178	6.40	-0.92	8.91	0.48
6 times	0.793	24.3	211	8.68	-1.12	5.63	0.79
2 times	0.712	21.5	223	10.37	-1.19	3.06	1.04
LSD (*P*=0.05)							
Amount (I)	0.14	3.5	31.7	1.5	0.52	NS	0.51
Frequency (F)	0.01	4.7	21.4	NS	0.38	1.53	0.34
I x F	NS	*	*	NS	*	*	*

Table 2. Whole plant hydraulic resistance and water relation parameters of bell pepper as affected by fertigation regimes

Treatments	Leaf water potential midday (MPa)	Stomatal conductance (mmol s^{-1})	Hydraulic resistance (g s^{-1} MPa^{-1})			Canopy resistance RGC
			RT	RS	RP	
Means of irrigation intervals (I)						
Daily	- 0.61	191.3	0.13	0.08	0.05	0.24
2 days interval	-0.78	173.1	0.17	0.09	0.08	0.32
Once weekly	- 0.91	161.4	0.21	0.11	0.10	0.56
Means of fertigation frequencies/irrigation event (F)						
10 times	- 0.53	169.8	0.16	0.09	0.07	0.28
6 times	- 0.68	155.2	0.22	0.12	0.10	0.44
2 times	- 0.77	147.7	0.27	0.15	0.12	0.82
LSD (*P*=0.05)						
Amount (I)	0.21	25.3	0.08	0.03	0.02	NS
Frequency (F) 0.05	12.6	0.05	NS	NS		0.4
I x F	NS	*	*	NS	*	*

and stomatal apparatus in bell pepper, to rootzone water regimes. The measurements of stomatal conductance (gs) on several soil water availability treatments showed that gs is sensitive to water potentials in the soil (-0.92 to -1.23 MPa, Table 1) and leaf (-0.53 to -0.91 MPa, Table 2). Over deficit irrigation, superior within plant xylem transport and canopy resistances and plant water potentials were recorded in daily irrigated bell pepper (Table 2). Regardless of decreases in transpiration fluxes, hydraulic conductance in the vascular system (R_p) was maintained. Thus it appears that plant water stress did not attain level of cavitation threshold and disruption of water conducting system. Although, reduced ability of the soil and within plant water transport capacity to supply water to the shoot system could have induced stomatal closure in water stressed plants, little changes in leaf water potential supported the concept of homeostasis of the hydraulic architecture under variable soil moisture conditions. The changes in transpiration fluxes indicated stomatal adjustment of transpiration under variable soil moisture status (irrigation regimes). The estimated value of R_T confirmed increased hydraulic resistance under deficit water application (Ruggierro *et al.*, 1999). Under water stress condition, the increases in resistance to water movement through plant to the canopy could have caused declined stomatal and canopy conductances. Soil drought increased the resistances (soil and plant) in the pathway of water flow in the SPAC via decreases in root water uptake and transpiration fluxes. Therefore, as water depletes in the rootzone, water uptake and transport within the xylem system adjust as necessary. The increase in plant resistance under low soil moisture status (deficits) observed may be due to loosening of root-soil contact and an altered hydraulic properties in this interfacial region. Associated with increasing soil resistance for water uptake is decreased hydraulic conductance from soil to canopy (Ks-l). However, root surface and its vicinity are frequently supplied with fresh water (nutrients) in solution by subsequent redistribution following fertigated drip irrigation events. Transpiration during the day may cause significant differences between the water content in the root zone and that in the bulk soil. Silber *et al.* (2003) reported that frequent replenishments eliminate the depletion zone formed at the root surface by uptake of water during period between successive irrigation events and decreases the concentration gradient between the medium solution and the root surface.

The components of the resistance elements in the SPAC changed as a function of the status of water in the root zone (RZ). When the total plant resistance was separated into its components, the trend observed was a greater water consumption in well irrigated pepper plants (Table 2). Soil resistance to water flow averaged about 30 % of the total resistance, and constituted a predominant part of the total resistance in the SPAC. In general, the magnitudes of plant resistance were smaller than those of soil system. The estimated value of soil and plant resistances confirmed greater soil and plant hydraulic resistance under deficit water application

resulting from remarkably larger root and canopy resistances. The resistance elements were normalised to the leaf area, stem cross section and root length in order to obtain resistances in relation to plant attributes (Table 3). The relative effect of treatments was similar when resistances were expressed per unit leaf and sapwood area (specific resistance). Plant attribute specific resistances (hydraulic resistance expressed on root length, leaf area and stem cross section) are in the range of those reported for herbaceous species on the field (Steudle and Peterson, 1998; Ruggiero *et al.*, 1999). The observed trends following the scaling of the hydraulic resistance elements with root length, sapwood and leaf areas possibly explains the sufficiency of the hydraulic system in the maintenance of favourable plant water status and canopy water use. The trade-off between within plant water transport and canopy water use is an ecophysiological attribute with strong impact on the performance of a species under variable environmental conditions (Agele *et al.*, 2005). Low hydraulic resistance was responsible for greater water uptake per unit leaf and sapwood area under well watered situation. The homeostatic balance between the areas of leaf and sapwood/stem cross section (Huber value) could serve to maintain similar water potential gradients and hence water demands between the stem and canopy despite differences in soil moisture availability.

Soil drying (deficit irrigation condition) brought about declined leaf Ψ and increased soil and plant resistances. In circumstances of low soil water status, soil conductivity decreases sufficiently, leaf Ψ declines more than soil Ψ to overcome the increase in resistance to water uptake. Hence, stomata may be induced to close sufficiently by low soil Ψ or high evaporative demand causing a reduction in transpiration and hence a smaller difference in potentials between soil and leaf. Therefore, plants can suffer water stress on days of high evaporative demand even when the soil is well supplied with water (atmosphere induced water stress). This behaviour complicates the use of sole plant indicators for scheduling irrigation since these indicators would call for irrigation on days of high evaporative demand despite that soil is wet. The need to use plant indicators in addition to soil indicators cannot be over emphasized. It is therefore necessary to evaluate plant indicators in the context of evaporative demand and transpiration. There is a minimum level of soil water allowable before irrigation is intended to keep leaf Ψ at or above a given limit. For example, under high frequency fertigation, frequent replenishment of depleted water in the root zone means that the limit of soil Ψ would be extended because of smaller difference in potential needed between the soil and leaf to drive water uptake. Under deficit irrigation (once weekly), plant can be allowed to deplete soil water to drier status under high daily frequent fertigation. Larger allowable depletion would imply fewer frequencies of irrigation and more water application per irrigation.

Table 4. Important relationships among water relation parameters in bell pepper

Parameters	Regression equations	r^2	P
swp and lwp	y=-1.05+1.90	0.95	*P*<0.05
swp and gs*	y=-1.23x+304.1	0.94	*P*<0.05
swp and Tn	y=-16.29+21.64	0.87	
swp and Q	y=-1.42x+2.36	0.91	*P*>0.05
swp and gc	y=-1.63x+2.3	0.90	
swp and R_T	y=-0.57x+0.84	0.93	*P*<0.05
Swp and Rp	$y=0.13x^{-3.57}$	0.79	
Lwp and Q	y=1.37x–0.22	0.97	*P*>0.05
Lwp and R_p	y=0.29–0.12	0.97	*P*<0.05
Lwp and gc	y=1.55x–0.61	0.98	
gs and gc	y=2.15Ln(x)10.48	0.97	*P*<0.05

*gs is stomatal conductance, swp is soil water potential, lwp is leaf water potential, R_T and R_p are total and plant hydraulic resistance, gc is canopy conductance , Q is root water uptake, Tn is transpiration flux

The relation between water potential difference and transpiration flux and hence plant resistance to water flow is influenced by soil moisture status. Our results were consistent with those reported by Steudle (2000). Hydraulic characteristics optimizes water uptake from the soil and moderate canopy water use. The stomata regulates leaf water potential and leaf area is adjusted as necessary to maximize water uptake and avoid loss of hydraulic contact with the soil. Fertigation regimes and hence root zone water status influenced the responses of stomata and canopy conductance (gc), water uptake and within xylem transport capacity (Kh) in bell pepper. Changes in hydraulic properties and stomatal behaviour enable pepper plants to sense root zone water status and to adjust canopy water loss (transpiration) adequately. Therefore, within xylem transport capacity (Kh) could serve as a signal controlling stomatal closure under soil moisture deficit.

Significant relationships were established among physiological attributes of bell pepper and fertigation regime enhanced differences in soil water potential (Table 4). The measured physiological parameters differed in their sensitivity to irrigation regimes. The strong association among measured physiological parameters (r^2 ranging from 0.70 and 0.90) appeared to indicate adjustment of crop water use under variable soil moisture conditions.

Table 3. Whole plant hydraulic conductance normalized with plant attributes (root length, leaf area and stem cross section) of pepper

Treatments	Leaf area	Stem cross section	Huber value	Specific resistance					
				Root length		Leaf area		Stem section	
				R_T	R_p	R_T	R_p	R_T	R_p
Means of irrigation intervals (I)									
Daily	0.22	0.00011	0.00050	0.00055	0.00021	0.59	0.23	1545.5	454.6
2 days interval	0.20	0.00010	0.00050	0.00062	0.00029	0.85	0.40	1700.0	800.0
Once weekly	0.19	0.000097	0.00051	0.00071	0.00034	1.11	0.53	2164.9	1030.9
Means of fertigation frequencies/irrigation event (F)									
10 times	0.23	0.00013	0.00057	0.00090	0.00039	0.30	0.31	1230.8	438.5
6 times	0.22	0.00012	0.00055	0.0010	0.00047	0.46	0.43	1833.3	833.3
2 times	0.20	0.00010	0.00050	0.0012	0.00054	0.60	0.57	2700.0	1200.0
SE	0.02	0.00004	0.00003	0.0004	0.00005	0.12	0.21	673.7	97.5

The knowledge of water flow within the SPAC and its control of plant's physiological processes is important to the choice of plant based indicators of water status which is directly a basic component of irrigation management strategies. The model used in this study was able to explain the mechanisms involved in the changes in resistance to water flow in the SPAC as influenced by fertigation regimes in bell pepper. Soil drought increased the resistances (soil and plant) in the pathway of water flow in the SPAC via decreases in root water uptake and transpiration fluxes. Therefore, as water is depleted in the rootzone, water uptake and transport within the xylem system adjust as necessary. Under drought, leaf water potential and transpirational water loss adjusted to xylem hydraulic sufficiency and soil water status. A coupling between the canopy and the root system may mean that both systems were tightly synchronized in response to soil moisture status. Homeostatic balance was established between the areas of leaf and sapwood (stem cross sectional), and could serve to maintain similar water potential gradients and hence water demands between the stem and canopy despite differences in soil moisture availability. Sensitive physiological indicators of plant water status which integrate all plant characters are relevant to the understanding of the mechanisms by which plants sense soil water status and can be useful in irrigation scheduling. Plant and weather based tools can be integrated for the development of crop models for estimation of crop water use and productivity, irrigation and water resources management. The observed trends of transpiration fluxes, leaf water potential, soil, plant and total resistance to flow of water and canopy conductance are therefore useful in modeling pepper crop water use.

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Chlorine disinfection: effects on hydroponics lettuce

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Abstract

Disinfection by chlorination was applied to the solution of a soilless closed system of *Lactuca sativa* varieties, Gallega and Mantecosa. The aim was to study the effect of the addition of different doses of chlorine on the production (fresh weight and dry matter), quality (nitrates, vitamin C and nitrogen contents), and phytotoxicity to lettuce (chlorosis) with regard to the chemical properties of the solution (pH, electrical conductivity EC and chlorides). Four treatments were applied: 0.55, 5.5 and 11 mg L^{-1} (ppm) of chlorine and a control without addition of chlorine. The 11 mg L^{-1} treatment produced a decrease in production of Gallega, presenting a 40 % lower fresh weight than the control. Both varieties presented high contents of nitrates. Gallega presented the maximum values (2920- 8158 mg kg^{-1}) and showed values under the permissible limit with the 0.55 and 5.5 mg L^{-1} treatments. Mantecosa showed lower maximum values (3787- 5291 mg kg^{-1}), although with all the values above the limits of permission. The contents of nitrogen for both varieties exceeded the levels of sufficiency in all the treatments. This fact was related to the high nitrogen supply provided by the fertirrigation that contributed to the high nitrate contents. Gallega presented larger contents of vitamin C (19.3-28 mg.$100g^{-1}$) than Mantecosa (15.3-19.98 mg $100g^{-1}$). Chlorination did not affect the chemical properties of the solution (pH and EC remained between the appropriate range for the species). Chloride contents in the nutrient solution were larger at the 11ppm doses; however the values remained under the toxicity levels for the species. For both the varieties, 0.55 mg L^{-1} treatment produced the higest fresh weight and vitamin C contents and the lowest nitrate contents and toxicity symptoms (chlorosis), while 11 mg L^{-1} treatment resulted more chlorosis and necrosis of leaves, diminishing the commercial quality of the plants.

Key words: *Lactuca sativa,* hydroponic, chloride, nitrate, vitamin C, nitrogen, phytotoxicity, pH, electrical conductivity

Introduction

There is a strong environmental legislative pressure worldwide that forces the producer to apply measures that contribute to sustainable and competitive agriculture. The soilless closed culture systems are considered a strategy for alternative production that makes possible a better use of water (20- 30 %) and nutrients (25 and 45%) associated with a less environmental pollution caused by the leaching of fertilizers (www.infoagro.com/abonos/9917asp). Some of the disadvantages of the closed soilless systems are the risk of the fast dissemination of root-infecting pathogens due to the recirculation of the nutrient solution. Different methods (cultural, physical, biological and chemical) are applied to reduce or remove the pathogenic microorganisms or potential pathogens of the nutrient solution (Van Os, 2000; Van Os and Postma, 2000**;** Van Os *et al.,* 2001). Chlorination constitutes a chemical method of low cost and easy application. The oxidation power of the hypochloric acid helps in inhibiting the development of pathogenic organisms, but the addition of high doses can cause damages in the culture. Therefore, it is necessary to establish the effective doses for each plant species and the time of application to control different pathogenic microorganisms without causing damage to the plants or producing undesirable effects on the production and the quality. Tests were performed to select the doses of chlorine, analysing the effects on production, quality and toxicity in plants of *Lactuca sativa*, as a prior step to the effectiveness tests with the presence of *Pythium* sp in lettuce. Two varieties, Gallega and Mantecosa, with different seasonal sensitivity were compared for their response.

Materials and methods

Lettuce was greenhouse grown on wooden benches with galvanized canals, covered with black polyethylene. Pots were placed in each canal and perlite-growing medium was used. A plant and its corresponding drip emitter were placed in each pot. The irrigation took place with Enshi solution (CETTEFFHO, pH: 6.58; EC: 0.016 dS m^{-1} and Cl^-: 0. 56 mM L^{-1}), prepared with water previously subjected to reverse osmosis. The canals presented a slope of 5% at their ends to collect the leaching and lead them towards the storage tank of the nutrient solution. Two leafy varieties Mantecosa and Gallega were used in the study. The chlorine stock solution was prepared with 100 ml of commercial sodium hypochloride diluted in 10 L of water. The corresponding doses for each treatment were: 0.55 ppm (0.015mM L^{-1}) with 1 ml stock solution L^{-1}; 5.5 ppm (0.15mM L^{-1}) with 10 ml L^{-1}, and 11ppm (0.30mM L^{-1}) with 20 ml L^{-1}. No sodium hypochloride was added to the control treatment.

According to their seasonal characteristics of growth and production, Gallega variety was studied during July and August (winter), and Mantecosa in October (spring). In both the cases the steps previously described were followed. The plants were placed in the same greenhouse at INTA-CETEFFO-CASTELAR. The phytotoxicity effects for each plant were quantified every four days and measured as number of affected leaves / number of total leaves. Throughout the tests, the presence of disease symptoms by natural infections was assessed. The production as aerial fresh weight (performed with a digital scale ACCULAB,

GS200) and production of dry matter (dried for 48 h at 70°C) were evaluated. In addition, the effects in relation to quality were quantified: vitamin C contents (AOAC, 1980); nitrates (Cataldo, 1975), nitrogen (Kjeldahl). Weekly controls of conductivity (conductimetry), pH (potentiometric) and chlorides (volumetric determination with silver nitrate) were performed in the recirculated nutrient solution recovering the consumed volume of nutrient solution and the corresponding doses of chlorine and fertilizer.

The experimental design was completely randomized with four treatments (including control) and 20 replicates for each treatment.

The results were statistically analyzed by ANOVA and means were compared by LSD values ($P \leq 0.05$) using SPSS software (Field, 2000).

Results and discussion

Phytotoxicity: The phytotoxic effects caused by the chlorination were quantified by the percentage of affected leaves based on the total leaves per plant. Mantecosa recorded 15.75 - 42.68 % and Gallega 22.61 - 41.1% of damaged leaves. The phytotoxicity (damage in leaves) was positively correlated to the presence of the largest doses of chlorine for both varieties. Disease symptoms caused by natural infections were not observed during the experiment in any of the varieties. All the observed symptoms of necrosis were caused by phytotoxicity of chlorine, at the beginning, starting at the top of leaves and then moving towards the edges, and finally affecting the whole leaf. Fig. 1 shows the values of percentage of affected leaves corresponding to the last day of evaluation.

Production and quality: Time required to reach the commercial maturity was 45 days for Gallega and 46 days for Mantecosa. There were differences for the fresh weight of both varieties with regard to weight and the response to the chlorination treatments. Gallega presented lower fresh weights (13-33 %) than Mantecosa. The 11 mg L^{-1} dose of chlorine affected the production in Gallega, presenting a negative correlation of the fresh weight and the chlorine addition, while the addition of chlorine did not affect the fresh weight of Mantecosa (Fig. 2).

No significant difference was found for percent dry matter production among treatments. Gallega showed higher values (6.11-7.85%) than Mantecosa (5.52-6.27 %), due to varietal characteristics.

Both varieties presented high contents of nitrates (Fig. 3). Gallega showed the highest contents of nitrates, presenting only the treatments with 0.55 and 5.5 mg L^{-1} of chlorine contents under the 3500 mg kg^{-1} limit values permitted in European legislation (Gazzetta Ufficialle, 1995). The lower content of nitrates of these treatments could be related to a larger fresh weight and an effect of dilution and to larger chloride content compared to control, due to the existence of an antagonism between chloride and nitrates (Behr and Wiebe, 1992). The largest nitrate contents of Gallega could also be related to the environmental conditions, particularly to light or radiation (Blom- Zandstra, 1990), as this variety was harvested 30 days before Mantecosa. Mantecosa over passed the allowed values for nitrates by the European commission (Gazzetta Ufficialle, 1995), for all treatments. The high levels of nitrates of both varieties could be related to a high doses in the nitrogen fertilization (Rincón Sanchez *et al.*, 2002), as well as to the NO_3/NH_4 relation in the nutrient solution (Van Der Boon *et al.*, 1990), resulted by the replacements of nutrients in the recirculating solution. The content of nitrates presented a negative correlation with the fresh weight of plants in both varieties.

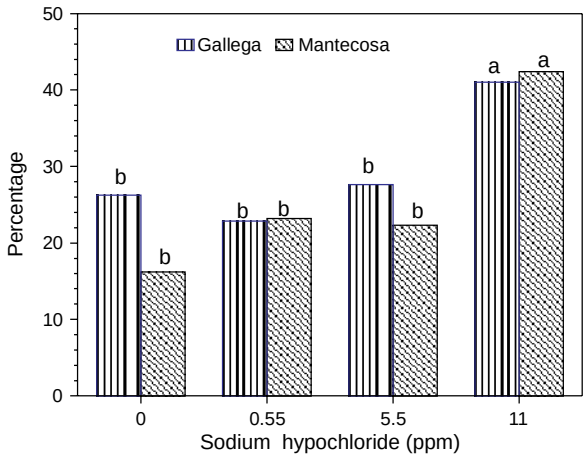

Fig. 1. Effect of the addition of sodium hypochloride in hydroponics on *Lactuca sativa* leaves damage (%) in varieties Gallega and Mantecosa. Different letters represent significant differences ($P \leq 0.05$)

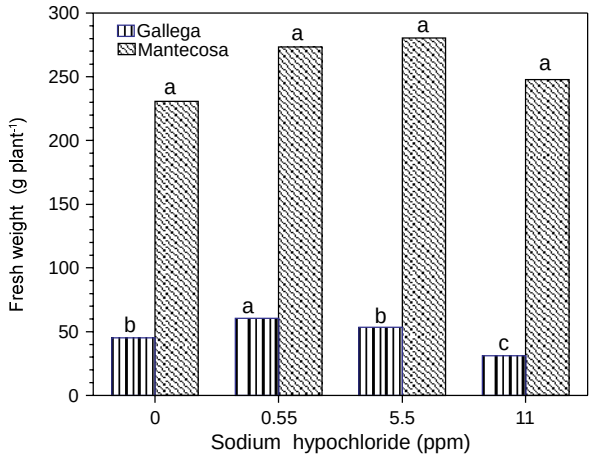

Fig. 2. Effect of the addition of sodium hypochloride in hydroponics on *Lactuca sativa* fresh weight of plants in varieties Gallega and Mantecosa. Different letters represent significant differences ($P \leq 0.05$)

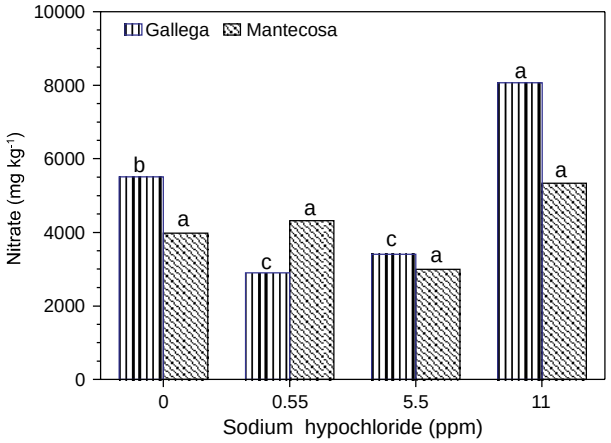

Fig. 3. Effect of the addition of sodium hypochloride in hydroponics on *Lactuca sativa* nitrate content in varieties Gallega and Mantecosa. Different letters represent significant differences ($P \leq 0.05$)

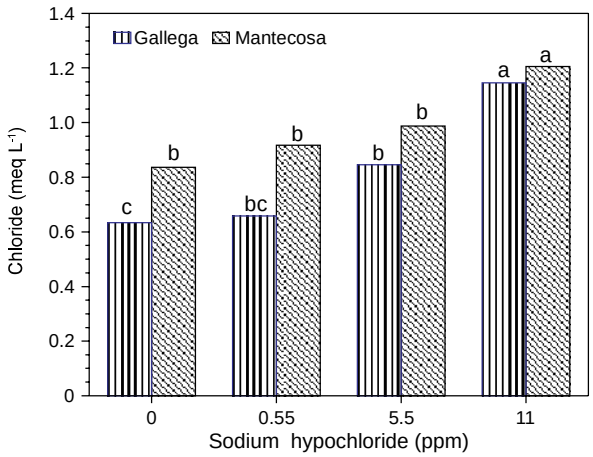

Fig. 4. Effect of the addition of sodium hypochloride in hydroponics on *Lactuca sativa* chloride content of recirculated solutions. Different letters represent significant differences ($P \leq 0.05$).

No differences for the contents of vitamin C among treatments were observed in Mantecosa (15.3-19.98 mg $100g^{-1}$). Gallega (19.3-28 mg $100g^{-1}$) presented differences among treatments (Table 1) and showed a negative correlation between the nitrate (in leaves) and the chloride concentrations (in the nutrition solution). The larger levels of vitamin C of Gallega could be related to a concentration effect due to the smaller size of the plants and to the larger dry weight of this variety. The vitamin C contents of both varieties presented negative correlation with the fresh weight (Drews *et al.*, 1997) and a positive correlation with the dry matter. These correlations were related to the presence of light and consequently with a higher photosynthesis rate and a larger presence of carbohydrates; and therefore a larger production of dry matter. The synthesis of vitamin C is improved in the presence of light (Blom- Zandstra *et al.*, 1985). A higher photosynthesis is associated with a higher production of biomass and a larger fresh weight; which, in turn, is associated with a larger number of leaves (in leaf type lettuce) and a higher shading effect, so the synthesis of the vitamin C is affected.

Table 1. Effect of the addition of sodium hypochloride to *Lactuca sativa* var. Gallega and Mantecosa on vitamin C (mg $100g^{-1}$) content

Treatments of chlorine (mg L^{-1})	Variety	
	Gallega	Mantecosa
0 (Control)	28 ± 5ab	18 ± 6a
0.55	24 ± 5b	20 ± 7a
5.50	31 ±6a	15 ± 5a
11.00	19 ± 2c	15 ± 4a

The values represent the average of 20 replications and different letters indicate significant differences among treatment means (LSD, $P \leq 0.05$)

The nitrogen contents in both varieties (3.83-4.6%) were similar. In Mantecosa, in spite of showing no differences among treatments, the lowest content of nitrogen was with 11 mg L^{-1} of chlorine. The nitrogen contents were over the values of sufficiency for all the cases (Hochmuth, 1994). This fact could be related to the high nitrogen contribution supplied with the fertilization.

For both the varieties, an increase in pH of the recirculated solution appeared before the addition of increasing doses of chlorine, although only significant in the Mantecosa variety with the 11 ppm treatment compared to the control and 0.55 ppm treatment. The addition of chlorine did not produce modifications in pH outside the range recommended for the species (Table 2) in any variety.

Table 2. Effect of the addition of sodium hypochloride on the pH of recirculating nutrient solution

Treatments (mg L^{-1})	Variety	
	Gallega	Mantecosa
0 (Control)	6.06 ± 0.49a	5.36 ± 0.70b
0.55	6.24 ± 0.28a	5.72 ± 0.81b
5.50	6.32 ± 0.20a	5.91 ± 0.74ab
11.00	6.37 ± 0.21a	6.14 ± 0.52a

The values represent the means and standard deviation of 20 replications. Different letters indicate significant differences ($P \leq 0.05$)

The recirculated solution of variety Mantecosa (Table 3) presented a larger electrical conductivity with the values negatively correlated to the values of pH.

Table 3. Effect of sodium hypochloride addition on electrical conductivity of the recirculated nutrient solution

Treatments (mg L^{-1})	Variety	
	Gallega	Mantecosa
0 (Control)	2.13 ± 0.06a	2.62 ± 0.23ab
0.55	1.94 ± 0.13a	2.62 ± 0.44ab
5.50	1.95 ± 0.21a	2.67 ± 0.45a
11.00	2.07 ± 0.09a	2.41 ± 0.48b

The values represent the means and standard deviation of 20 replications. Different letters indicate significant differences ($P \leq 0.05$)

The contents of chloride in the recirculated nutrient solution for both varieties (Fig. 4) were larger with the dose of 11 ppm, although none of the values surpassed the toxicity levels of the species (Reed, 1999)

For both lettuce varieties, the addition of 0.55 mg L^{-1} chlorine produced the largest fresh weight and vitamin C contents and the lowest nitrate contents and toxicity symptoms (chlorosis), suggesting this dose as the most suitable (among the tested ones) for this species. Doses between 0.55 and 5.5 mg L^{-1} may be selected for a future evaluation of the effectiveness for the control of *Pythium* sp in hydroponics culture of lettuce.

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Tulip cultivar response to Flurprimidol preplant bulb soaks

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Abstract

Flurprimidol preplant bulb soaks (10 to 40 mg L^{-1}) were applied to tulip (*Tulipa* sp. L.) bulbs for growth control. Three tulip cultivars ('Page Polka', 'Prominence' and 'Red Present') were used to determine if the efficacy of flurprimidol varied by cultivar. Flurprimidol was compared to paclobutrazol (50 and 100 mg L^{-1}) and uniconazole (10 and 20 mg L^{-1}). Flurprimidol preplant bulb soaks significantly ($P \leq 0.05$) controlled tulip plant height during the greenhouse forcing when applied to 'Page Polka' and 'Prominence' at concentrations $\geq$ 15 mg L^{-1} and $\geq$ 10 mg L^{-1}, respectively. A concentration of 40 mg L^{-1} was needed to control plant height during the postharvest evaluation for 'Page Polka' while concentrations $\geq$ 15 mg L^{-1} controlled postharvest plant height for 'Prominence'. No control during forcing or postharvest was provided by any concentration tested on 'Red Present'. The differences observed indicate that the efficacy of flurprimidol as a preplant bulb soak varied with cultivars. In order to determine optimal cultivar doses, growers will need to conduct their own tulip cultivar trials, with flurprimidol concentrations ranging between 10 and 40 mg L^{-1}.

Key words: Paclobutrazol, piccolo, plant growth regulators, uniconazole

Introduction

During greenhouse production and postharvest blooming, plant growth regulators (PGRs) are often needed to control excessive stem stretch of tulips. A number of PGRs have been recommended for use on tulips. Ancymidol (α-cyclopropyl-α-(*p*-hmethoxyphenyl)-5-pyrimidinemethanol) (A-Rest, SePRO Corp., Carmel, IN) has been recommended by Dole and Wilkins (2005) as a substrate drench of 0.125 to 0.5 mg a.i. per pot, while Barrett (2002) recommended ancymidol substrate drenches of 1 to 5 mg L^{-1} per pot within the first two days of greenhouse forcing. Paclobutrazol [(±) -(R*,R*)-β-[(4-Chlorophenyl)methyl]-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol] (Bonzi, Syngenta, Greensboro, NC) drenches of 0.31 to 2.5 mg a.i. per pot or 1-h preplant bulb soaks in a 2 to 5 mg L^{-1} solution are recommended on the label. Tulips are listed on the uniconazole [(E)-(+)-(S)-1(4-chlorophenyl)4,4-dimethyl-2(1,2,4-triazol-1-yl)pent-1-ene-3-ol] (Sumagic, Valent USA, Marysville, OH) label, but no recommended concentrations are provided. In initial trials, flurprimidol preplant bulb soaks at a concentration of 25 mg L^{-1} for 10 min or substrate drenches of 0.5 mg a.i. per pot were recommended for the cultivar 'Prominence' (Krug *et al.*, 2004).

In previous experiments, only a single tulip cultivar was evaluated. Cultivar variations may occur as reported with sunflowers (*Helianthus annuus* L.) (Whipker and McCall, 2000) and hyacinth (*Hyacinthus orientalis* L.) (Krug *et al.*, 2006) or cultivar response may be similar as in geraniums (*Pelargonium ×hortorum* L.H. Bailey) (Whipker *et al.*, 2000). In order to provide guidelines for the use of flurprimidol preplant bulb soaks, a cultivar comparison trial was conducted with three cultivars of tulip.

Materials and methods

Noncooled tulip ('Polka', 'Prominence', and 'Red Present') bulbs were planted in 10.2 cm diameter plastic pots (575 ml) on 24 October, 2003. The root substrate was Berger BM 6 (Berger Peat Moss, St. Modeste, Quebec, Canada), which contained 75% to 80% Canadian sphagnum peat and 20 to 25% perlite. From date of potting to 5 January, 2004, bulbs were held at 5.0 ºC. On 5 January, 2004 the cooler temperature was lowered to 1.1 ºC. The bulbs were removed from the cooler on 4 February, 2004 at sunset and allowed to acclimatize overnight. Greenhouse forcing began on 5 February, 2004 with day/night set points of 20.0/18.3 ºC. Plants were forced under natural day length. The experiment was a completely randomized design with six single-plant replications of each of the 12 treatments for each of the three cultivars.

Plant growth regulators: On 24 October, 2003, the following treatments were applied as a preplant bulb soak for 10 min (in mg L^{-1}): flurprimidol (0.38%) at 10, 15, 20, 25, 30, 35, or 40; paclobutrazol (Piccolo, Fine Americas, Inc., Walnut Creek, CA) at 50 or 100; uniconazole at 10 or 20; and nontreated controls. Anthesis date (all petals fully colored and beginning to separate), and total plant height at anthesis (measured from the soil line to the uppermost part of the inflorescence) were recorded.

Postharvest study: Four plants, randomly selected from each treatment, were placed in a growth chamber with a temperature at 20.0 ºC after anthesis. Fluorescent bulbs provided light at 24 to 75 $\mu mol \cdot m^{-2} s^{-1}$ for a 12-h photoperiod. Plant height was recorded 10 d after anthesis.

Data analysis: Data were tested by analysis of variance (ANOVA) using general linear model (SAS Institute, Cary, NC) and means separation by least significant differences (LSD). Forcing and postharvest plant height values were regressed using PROC REG procedure to determine the best-fit, linear or quadratic, model. Terms of the model were evaluated for significance based on a comparison of F values at $\alpha = 0.05$. Models were compared to determine the best fit based on r^2 values.

Results and discussion

'Page Polka': Flurprimidol concentrations ≥ 15 mg L^{-1} controlled plant height, resulting in plants ≤ 14.0 cm tall, which was ≥ 21% shorter than the nontreated control (Fig. 1A). Paclobutrazol resulted in plants ≥ 19% shorter than the nontreated control at the concentrations tested. Plants were ≥ 22% shorter than the nontreated control when uniconazole was used at concentrations of 10 and 20 mg L^{-1}. Anthesis was not delayed by any of the treatments trialed (data not presented). During the postharvest evaluation plant height was controlled by flurprimidol at a concentration of 40 mg L^{-1} resulting in plants 29.0 cm tall, which were 20% shorter than the nontreated control. Paclobutrazol and uniconazole did not significantly ($P \geq 0.05$) control height during the postharvest evaluation at any concentration used. A concentration of 40 mg L^{-1} flurprimidol was needed to control postharvest stretch for 'Page Polka'.

'Prominence': Flurprimidol concentrations ≥ 10 mg L^{-1} controlled plant height, resulting in plants ≤ 16 cm tall, which was ≥ 20% shorter than the nontreated control (Fig. 1B). Paclobutrazol resulted in plants ≥ 28% shorter than the nontreated control at the concentrations tried. Plants were ≥ 23% shorter than the nontreated control with the concentrations of uniconazole used. Anthesis was not delayed by any treatment used (data not presented). During the postharvest evaluation plant height was controlled by flurprimidol at concentrations ≥ 15 mg L^{-1} resulting in plants ≥ 10% shorter than the nontreated control. Paclobutrazol at 100 mg L^{-1} controlled plant height during the postharvest evaluation resulting in plants 25.7 cm tall, which were 27% shorter than the nontreated control. Neither concentration of uniconazole (10 and 20 mg L^{-1}) controlled plant height during the postharvest evaluation. Based on regression analysis (Fig. 1B) a flurprimidol concentration of 38.3 mg L^{-1} would be required to obtain similar control as 100 mg L^{-1} paclobutrazol during the postharvest evaluation.

Previous trials by Krug *et al.* (2004) indicated that flurprimidol preplant bulb soaks at a concentration of 25 mg L^{-1} applied to 'Prominence' resulted in plants 32.8 cm tall during the postharvest evaluation. In this year's experiment, plants at postharvest evaluation were similar in height (31.3 cm) when treated with 15 mg L^{-1} flurprimidol preplant bulbs soaks. The amount of stem stretch which occurred in the cooler was greater in the initial experiment (Krug *et al.*, 2004) and may explain why 25 mg L^{-1} flurprimidol was needed then to obtain similar heights as the 15 mg L^{-1} recommended in this study.

'**Red Present**': None of the treatments of flurprimidol, paclobutrazol, or uniconazole controlled plant height during greenhouse forcing or the postharvest evaluation. Anthesis was delayed by 2.5 d ($P \leq 0.05$) when flurprimidol was used at a concentration of 40 mg L^{-1} (data not presented); however, this would not be considered commercially important. 'Red Present' is a short cultivar and PGRs would not be required to control postharvest stem stretch.

During greenhouse forcing flurprimidol preplant bulb soaks did not control height of the tulip cultivar 'Red Present'. Flurprimidol controlled greenhouse forcing height of tulip cultivars 'Page Polka' and 'Prominence' at concentrations of 15 and 10 mg L^{-1} respectively. When 'Prominence' tulip bulbs were treated with

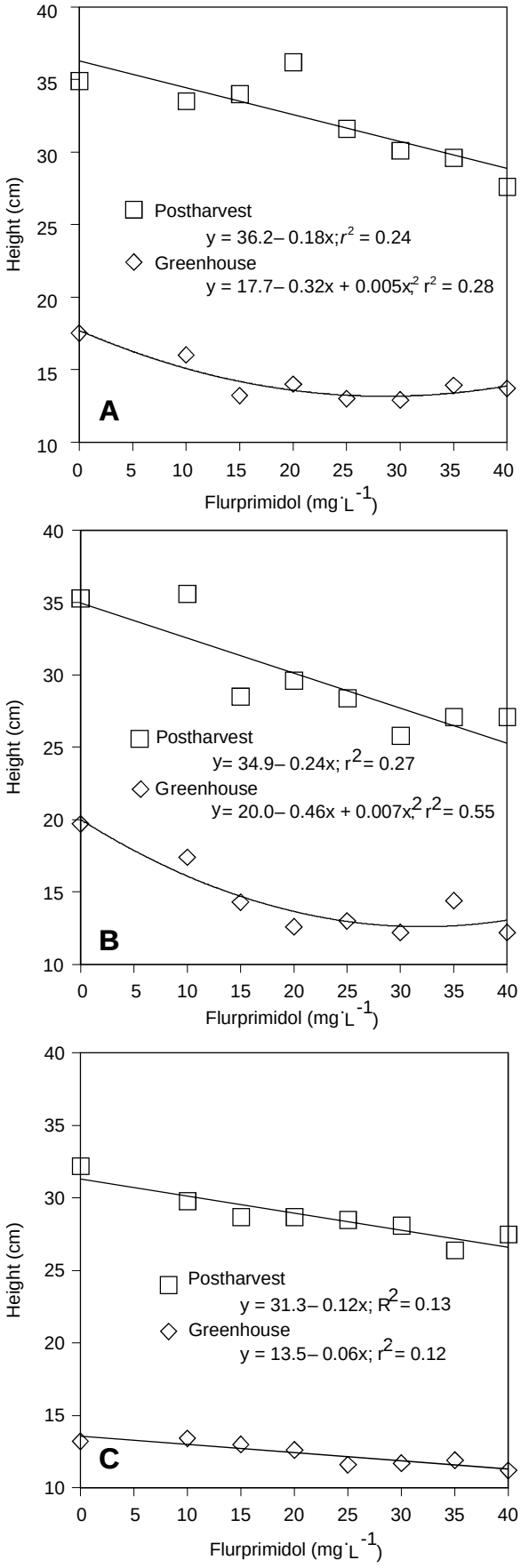

Fig. 1. The effects of flurprimidol preplant bulb soaks on tulip cultivars 'Page Polka' (A), 'Prominence' (B), and 'Red Present' (C) plant height at anthesis and during postharvest evaluation.

a 25 mg L^{-1} preplant bulb soak, plants were 32.8 cm tall, which were 28% shorter than the untreated control (Krug *et al.*, 2004). Flurprimidol preplant bulb soaks at 25 mg L^{-1} applied to 'Page Polka' and 'Prominence' resulted in plants 12% and 17%, shorter plants, respectively than the nontreated controls during the postharvest evaluation.

Differences in PGR efficacy among cultivars have been found with other bulb crops and PGRs. Optimal paclobutrazol preplant bulb soak recommendations for potted freesia range from 50 to 300 mg L^{-1} depending on the cultivar (DeHertogh, 1996). When paclobutrazol and uniconazole preplant bulb soaks were applied to Oriental and LA-hybrid lilies (*Lilium* L.), the response varied significantly among cultivars. Paclobutrazol preplant bulb soaks of 50 mg L^{-1} to 'Star Gazer' Oriental lily bulbs resulted in plants 9% shorter than the nontreated control, while the same concentration applied to 'Tom Pouce' lily resulted in plants 15% shorter than the nontreated control (Ranwala *et al.*, 2002).

Analysis of our results indicates that among tulips cultivar differences exist against flurprimidol efficacy as a preplant bulb soak during greenhouse forcing and the postharvest evaluation. However, the difference in efficacy during greenhouse forcing is usually not a concern for commercial producers as the plants are shipped to consumers before stem elongation occurs. Flurprimidol preplant bulb soaks at concentrations from 15 to 40 mg L^{-1} for tulip cultivars should be used for commercial production to control postharvest stem stretch. Growers will need to conduct on-site trials to determine the optimal concentration for individual cultivars of tulips.

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Effect of Ni on yield, quality and N assimilation of cucumber (*Cucumis sativus* L.) grown with urea or nitrate

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Abstract

The effects of Ni concentrations in the nutrient solution on the yield, quality and N assimilation of cucumber plants were evaluated in plants grown either with urea or nitrate as the sole N source. The cucumber plants (*Cucumis sativus* cv RS189 and Vikima) were treated with two N sources, urea and nitrate as $NaNO_3$ at 200 mg L^{-1}, and three concentration of Ni as $NiSO_4.6H_2O$ (0, 0.5, and 1 mg L^{-1}). Treatments were arranged in a randomized block design with six replicates. The highest concentration of Ni in the leaves (1.2 mg kg^{-1} DW) was observed in the urea-fed plants at 1 mg L^{-1} Ni concentration. Addition of Ni up to 0.5 mg L^{-1} had no effect on the fruit Ni concentration in the both urea and nitrate-fed plants. Ni supplement (0.5 mg L^{-1}) increased the yield significantly (10 and 15% in RS189 and Vikima, respectively), in urea-fed plants but decreased when 1 mg L^{-1} Ni applied to the solutions. Nitrate-fed plants had higher percentage of total soluble solids compared to urea-fed plants. Nitrate concentration of the fruits in urea-fed plants in both cultivars was approximately 50% less than those nitrate-fed plants. The reduction of nitrate concentration in the fruits became more pronounced as the Ni concentration increased in the solution. The rate of photosynthesis (Pn) in urea-fed plants continuously increased with the increase of the Ni concentration in the solution. Both N concentration and NR (Nitrate Reductase) activity of young leaves were higher in urea-fed plants at 0.5 mg L^{-1} Ni concentration. Ni supplements enhanced the growth and yield of urea-fed plants by the increase of Pn, N concentration and NR activity. It can be concluded that Ni supplements (0.5 mg L^{-1}) improves yield, quality and NR activity in urea-fed cucumber plants.

Key words: Ni, cucumber, N, yield, quality, urea, nitrate

Introduction

Nickel (Ni) is an essential nutrient for higher plants because of its role as a component of urease enzyme (Dixon *et al.*, 1975 and Brown *et al.*, 1987). This element is required for N metabolism where N is applied in the form of urea (Eskew *et al.*, 1984). The deficiency of Ni depresses urease enzyme activity (Eskew *et al.*, 1983) and other enzymes responsible for the nitrate reduction (Brown *et al.*, 1990), consequently reducing synthesis of protein and N compounds (Brown *et al.*, 1990). This lead to the accumulation of urea, nitrate, and certain amino acids resulting in the incidence of leaf chlorosis and meristem necrosis of cucumber plants (Watanabe and Shimada, 1990). Therefore, low level of Ni is crucially important for the achievement of optimal yield of commercial crops (Eskew *et al.*, 1983, 1984). However, it appears that there is a difference among cucumber cultivars in response to Ni supplements.

Terrestrial plants are able to uptake different forms of N including nitrate, ammonium and urea. Urea is an important source of N fertilizer which is applied in soil and soilless culture systems. In hydroponic cultures urea can replace those nitrate N fertilizers provided that the adverse effects of urea accumulation on plant growth can be overcome (Zhu *et al.*, 1997). The main problem which is associated with urea use for higher plant nutrition is the unavailability of urea for the plant metabolism unless hydrolyzed to ammonium and carbon dioxide by urease (Marschner, 1995; Watanabe and Shimada, 1990).

Many studies showed that Ni is a part of the active centre of urease (Dixon *et al.*, 1975) and that activation decrease depends upon Ni in the plants (Marschner, 1995). A critical Ni concentration in the leaf of the plants could depend upon employing the N source; therefore different Ni concentrations might be required for the achievement of optimal plant growth. In this study, the effects of Ni concentration in the solution on the N assimilation, yield and fruit quality of cucumber plants grown in hydroponic supplied either with urea or nitrate was investigated.

Materials and methods

Plant growth conditions and treatments: Seeds of cucumber (*Cucumis sativus* cv. RS189 and Vikima) were sown in the propagation cubes. When two leaves fully expanded, four plants were planted in the bags (each bag was 100×20×10cm) filled with mix of perlite and vermiculite (1:1). Six bags were laid out on the floor in six rows with 1.2 m between rows. Each bag was considered as a plot and each row as block (3.3 plants per m^2). The first and last slabs on the rows were considered as guard rows. The treatments were randomized within rows to give a randomized complete block design with six replicates.

The greenhouse was under natural sunlight during spring and summer and the temperature was set 28±3 and 20±3 ^oC in day and night, respectively. The basic nutrient solution was prepared based on Xue *et al.* (2000) containing K_2SO_4: 2, $CaCl_2.2H_2O$: 1.5, $MgSO_4.7H_2O$: 1 and $NaH_2PO_4.2H_2O$: 2.3 mM and all micronutrients in half strength of Hoagland's solution. The concentration of N in all nutrient solutions was kept at 200 mg L^{-1} by adding either urea or NO_3 as $NaNO_3$. Three concentrations of Ni as $NiSO_4.6H_2O$ (0, 0.5 and 1 mg L^{-1}) were applied. The solution pH was adjusted to 6.5 by adding H_2SO_4. The bags were

equipped with drippers (4 L h^{-1}) which enabled to supply nutrient solutions with different solutions to each bag. The drippers were placed at the base of each plant and a timer was used to ensure that all plants receive an equal volume of solutions; an excess of 20% solution was applied to minimize EC and pH changes inside the bags.

Data collection and chemical analysis: The fruits (50-60 g) were harvested twice per week from the beginning of June until the end of September for 16 weeks and the fresh weight of the fruits was recorded. At the end of experiment, all plants from each treatment were taken to measure leaf area and weight. The leaf area was measured using leaf areameter (Li-Cor, model Li -1300, USA). After weighing the leaves they were dried at 80°C in an air forced oven for 48 h.

Fruit quality was measured in a representative sample collected at the same position from plants in each treatment. Total soluble solids (TSS) were measured in undiluted juice with a hand-held refractometer. A thin layer of the middle of fruit skin (0.5 mm), was removed by a sharp razor and fruit color or the extent of greening was measured using a chlorophyllmeter (SPAD-502, Minolta, Japan). The concentrations of N and Ni in the youngest fully expanded leaves were determined by Kjeldal method and atomic absorption spectrophotomtry, respectively (Perkin Elmer, Model 110, USA).

Photosynthetic rates of the mid-lamina portion of the youngest fully expanded leaves of two plants from each treatment were measured using a portable photosynthesis meter (Walz, Model Da1010, Germany). The flow rate and PAR were set to 800 min and 1500 µmol $m^{-2}s^{-1}$, respectively. Single measurement was carried out between 9:00 and 14:00 O'clock.

Nitrate reductase (NR) was measured in the young leaves (third or forth from top) according to Klepper *et al.* (1971). The leaf tissue (0.2 g fresh weight) was placed in reaction mixture containing 0.1 M potassium phosphate buffer (pH 7.5), 0.02 M KNO_3, 50% isopropanol, 0.05 chloramphenicol at 30°C for 1 h in the dark. The indicative Grease reagent containing 0.001 g 1-naphtyl-ethylene diamine, 0.01 g suphanilic acid, and 0.9 g tartaric acid was added to each sample. The concentration of nitrite formed during the reaction was measured spectrophotometrically.

Nitrate concentration in the dried fruits and leaves was determined according to Cataldo *et al.* (1975). Approximately 0.2 g of dried tissue powder was placed in 125 ml container and 25 ml hot water was added. The samples were shaken for 30 min on a Wristaction shaker and filtered through Whatman No 42 filter paper. Nitrate in the filtered solution was determined by adding a 0.2 ml sample aliquot to 0.8 ml of 5% (w/v) salicylic acid (dissolved in H_2SO_4) and 19 ml 2 N NaOH. Samples were allowed to cool at room temperature for 1 h, and developing color was measured at 410 nm by spectrophotometer (Motic, CL-45240-00, China).

A statistical analysis was done using analysis of variance in the SAS 8.2 software and the means were separated by LSD test (*P*=0.05). The graphs were drawn in Excel software.

Results

Ni concentration of leaves and fruit in both urea and nitrate-fed plants increased significantly with the increase of the Ni concentration in the solutions (Table 1). In the leaves, the higher concentration of Ni was observed in the 1 mg L^{-1} Ni concentration in both cultivars. Like leaves, addition of Ni to the solution increased the Ni concentration in the fruits. However, no significant difference was found between 0.5 and 1 mg L^{-1} Ni concentration. In this experiment, the N source had no significant effect on the Ni concentration in the leaves. No visual symptom of urea toxicity was observed in the cucumber plants at various rates of Ni.

Table 1. Influence of N source and Ni concentraion on the concentration of Ni in the leaves and fruits

Treatments		Ni concentration in leaves (mg kg^{-1} DW)		Ni concentration in fruits (mg kg^{-1} DW)	
		RS189	Vikima	RS189	Vikima
Ni_0	Nitrate	0.07b	0.06b	0.04b	0.03b
$Ni_{0.5}$		0.37a	0.39a	0.06b	0.04b
Ni_1		1.19a	1.35a	0.18a	0.13a
Ni_0	Urea	0.06b	0.07b	0.02b	0.03b
$Ni_{0.5}$		0.23a	0.25a	0.03b	0.04b
Ni_1		1.20a	1.30a	0.08a	0.10a
F values					
Ni×N		1.30*	1.70*	0.0 7 ns	0.08 ns
N source		2.70**	2.90**	1.06 *	1.35 *
Ni		1.80 ns	0.82	0.05 ns	0.02 ns

*, ** significant at *P*=0.05, *P*=0.01, respectively; ns- non significant

Either N source or Ni concentration had no significant effect on the fresh weight of leaves, however dry weight of RS 189 in urea-fed plants was higher than that with nitrate-fed plants (Table 2). In general, leaf area was not affected by N form supplements however, in both cultivars the highest leaf area was found in urea-fed with Ni at 0.5 mg L^{-1} concentration (Table 2). The yield of cucumber significantly increased in urea-fed plants (Table 2). The highest yield in urea-fed plants, 2.1 and 1.8 kg $plant^{-1}$ was obtained from RS189 and Vikima, respectively. Ni supplement (0.5 mg L^{-1}) increased the yield significantly (10 and 15% in RS189 and Vikima, respectively), but decreased when 1 mg L^{-1}Ni applied to the nutrient solution in urea-fed plants.

The effect of Ni concentration and N source on fruit quality is given in Table 3. Dry matter and colour of fruit were not affected by the treatments. TSS in the plants with nitrate nutrition was higher than that with urea nutrition. Nitrate concentration of the fruits in urea-fed plants in both cultivars was approximately 50% less than those nitrate-fed plants (Table 3). The reduction of nitrate concentration in the fruits became more pronounced as the Ni concentration increased in the solution so that the lower nitrate concentration was observed with urea-fed plant at 1 mg L^{-1} Ni concentration. Both N concentration and NR activity of young leaves were higher in urea-fed plants at 0.5 mg L^{-1} Ni concentration (Table 4). The lowest NR activity was observed in urea-fed plants without supplying Ni. Nitrate concentration of the leaves was not significantly affected by the treatments.

Although leaf chlorophyll index were low in both without Ni supplements and at 1 mg L^{-1} Ni concentration, the concentration of Ni in nitrate or urea-fed plant had no significant effect on chlorophyll index (Fig. 1).

Table 2. Effect of N source and Ni concentration on the vegetative characteristics and yield of cucumber plants

Treatments		Leaf FW (g plant^{-1})		Leaf DW (g plant^{-1})		Leaf area (cm^2)		Yield (kg plant^{-1})	
		RS189	Vikima	RS189	Vikima	RS189	Vikima	RS189	Vikima
Ni_0	Nitrate	331.0	330.1	23.0	20.8	6566.2b	6416.8b	1.9b	1.4b
$Ni_{0.5}$		359.6	330.7	24.1	22.4	6817.1b	6408.7b	1.9b	1.6a
Ni_1		353.3	321.8	23.1	22.2	6746.0b	6476.3b	1.6b	1.4b
Ni_0	Urea	374.3	332.7	26.4	23.4	7000.9b	6395.8b	1.9b	1.5b
$Ni_{0.5}$		365.0	327.3	26.8	22.5	7781.6a	6668.0a	2.1a	1.8a
Ni_1		363.6	315.0	24.9	21.7	7327.5b	6253.1b	1.4b	1.5b
F value									
N source		3.16ns	0.06 ns	2.6 *	0.68 ns	4.5*	0.31 ns	0.04 ns	0.46 ns
Ni		0.26 ns	0.64 ns	0.47 ns	0.12 ns	9.9**	8.30*	2.23**	2.80*
Ni×N		1.16 ns	0.08 ns	0.15 ns	1.20 ns	0.33 ns	1.34 ns	0.06 ns	0.43 ns

*, ** significant at P=0.05, P=0.01, respectively; ns- non significant

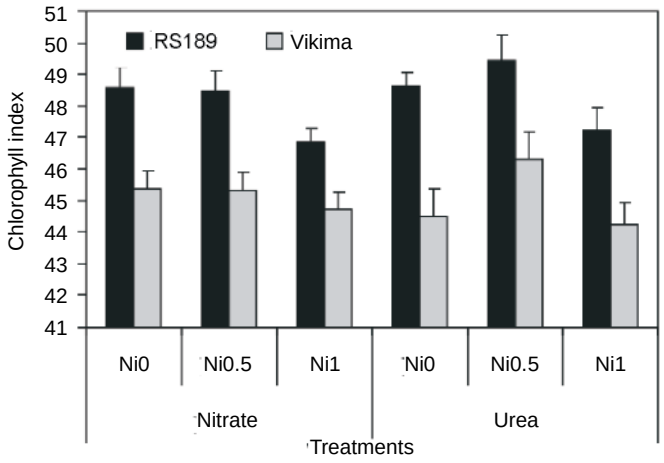

Fig. 1. Influence of N source and Ni concentraion on the chlorophyll index

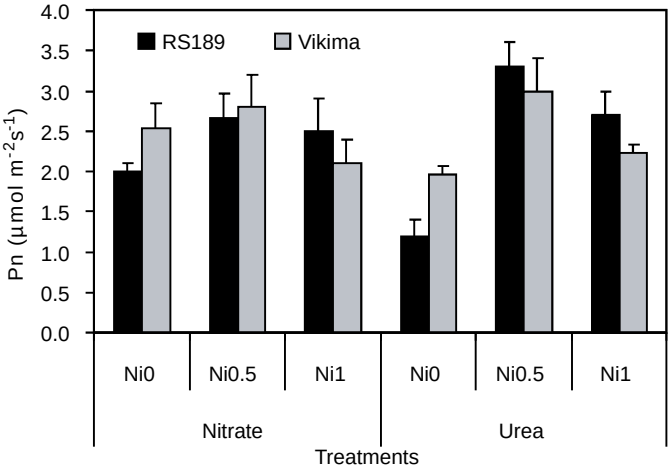

Fig. 2. Influence of N source and Ni concentraion on the Pn

The rate of Pn increased with the increase of the Ni concentration in the solution in urea-fed plants (Fig. 2). The rate of Pn impaired in the both without and high concentration of Ni in urea-fed plants. No significant difference in Pn was found between N sources. Both high and low concentration of Ni reduced the rate of Pn in the urea fed plants.

Discussion

Many studies on Ni have been focused on N metabolism and its related enzymes in higher plants in both monocots and dicots. Only very limited papers have been published concerning Ni application in relation to the yield and fruit quality. The results of this experiment indicated that Ni supplements enhanced the growth and yield of urea-fed plants by the increase of Pn and N concentration. Both high and low level of Ni impaired the growth of cucumber plants in terms of leaf area, fresh weight of leaves and yield. Therefore, supplying of Ni (at least 0.5 mg L^{-1}) to the nutrient solution for the urea-fed cucumber plant grown in hydroponics is required. In this work under Ni_0, the presence of Ni was observed in the leaves of plants implies that the plants were not strictly Ni limited. The presence of Ni in the fruits indicated that Ni is able to accumulate in the fruits however; the concentration of Ni in the fruits reduces as a result of rapid growth (dilution effect). The possible Ni contamination may be from chemical compounds (fertilizers) used for making solutions. Ni is one of the toxic compounds for human and environment hence, further attention should be paid in order to reduce the accumulation of Ni in the edible parts of the plant.

The quality and quantity of cucumber plants were improved with the supplements of Ni to the solution. The stimulating effect of a moderate Ni supply for urea-fed plant is well demonstrated (Shimada and Matsuo, 1985; Krogmeier *et al.*, 1991 and Xue *et al.*, 2000). The growth of cucumber plants without supplying Ni was not restored to the same level as for the nitrate-fed plant which agrees with earlier finding of Gerendas and Sattelmacher, (1977). Although Ni has been considered as an ultra-trace nutrient (Brown *et al.*, 1987), it is required at least 0.5 mg kg^{-1} DW for the normal growth of cucumber plant where urea is used as N source in the nutrient solution. This finding is in contrast with the report of Gerendas and Sattelmacher (1999) who observed non significant reduction in growth without Ni supply in *Brassica napus*. It seems that there may be differences among various plants in response to Ni supplements. Although Ni concentration in the leaves of the cucumber plants was higher where plant supplied with 1 mg L^{-1} Ni, no visible symptoms of toxicity was observed. It implies that cucumber plants are able to tolerate high concentration of Ni. Yang *et al.* (1997) found that the tolerance of some species to Ni concentration depends on the interaction of Ni with organic acids. The concentration of Ni in the cowpeas plants up to 3000 mg kg^{-1} DW is reported by Walker *et al.* (1985). Cucumber needs more N for the production of optimum yield, therefore any reduction in N content may reduce yield or plant

Table 3. Influence of N source and Ni concentraion on the fruit quality

Treatments		Fruit color Index		TSS (%)		Dry matter (%)		NO_3 (mg kg^{-1} DW)	
		RS189	Vikima	RS189	Vikima	RS189	Vikima	RS189	Vikima
Ni_0	Nitrate	41.5	35.7	5.0	5.5	5.2	5.3	69.7a	92.3a
$Ni_{0.5}$		44.1	31.8	5.1	5.1	5.3	5.2	43.6a	97.7a
Ni_1		43.7	33.7	5.3	5.0	5.6	5.1	40.0a	90.0a
Ni_0	Urea	44.8	33.0	4.8	4.3	5.6	5.2	38.0a	62.6a
$Ni_{0.5}$		44.3	38.0	5.0	5.0	5.3	5.1	32.0b	55.0b
Ni_1		45.8	32.5	4.6	5.0	5.6	5.0	30.6b	31.0b
F values									
N source		1.00 ns	0.56 ns	2.20*	8.5*	0.65 ns	0.22 ns	5.6*	6.71*
Ni		0.24 ns	1.10 ns	0.03 ns	0.2 ns	0.80 ns	1.04 ns	7.11**	3.28*
Ni×N		0.24 ns	1.70 ns	0.42 ns	1.7 ns	0.42 ns	0.03 ns	2.00 ns	0.85 ns

*, ** significant at *P*=0.05, *P*=0.01, respectively; ns- non significant

Table 4. Influence of N source and Ni concentraion on the concentration of N, NO_3 and NR activity in the leaves

Treatments		N (mg g^{-1}DW)		NO_3 (mg kg^{-1} DW)		NR (µmol h^{-1} g^{-1} FW)	
		RS189	Vikima	RS189	Vikima	RS189	Vikima
Ni_0	Nitrate	40.0 a	30.6 b	193.9	346.8	1.0 a	0.7 b
$Ni_{0.5}$		30.8 b	30.8 b	433.3	492.8	0.9 b	0.6 b
Ni_1		40.2 a	30.5 b	541.4	532.0	0.9 b	0.6 b
Ni_0	Urea	30.0 b	30.1 b	499.7	673.5	0.7 b	0.7b
$Ni_{0.5}$		40.4 a	40.6 a	221.7	320.0	1.2 a	1.0 a
Ni_1		30.6 b	30.0 b	280.0	492.8	0.8 b	0.6 b
F values							
N source		0.30 ns	2.70 ns	0.57 ns	1.80 ns	0.37 ns	0.02 ns
Ni		7.70*	6.90*	1.56 ns	1.35 ns	1.01*	1.40**
Ni×N		1.80 ns	0.82	1.50 ns	0.82 ns	0.09 ns	0.99 ns

*, ** significant at *P*=0.05, *P*=0.01, respectively; ns- non significant

growth. Xue *et al.* (2000) found that Ni supplements at 0.01 mg L^{-1} to the urea-fed tomato plants improved growth and N metabolism. The plants with urea without Ni supplements were smaller in size as reported by Gerendas and Sattelmacher, 1997, 1999; Mordy and Atta, 1999). There is no clear information about the critical toxicity concentration of Ni for cucumber; however the concentration of 1.2 mg kg^{-1} DW, with respect to cultivars, is most likely to reduce cucumber plant growth and yield. The reduction in growth of urea-fed plants without supplying Ni was reported by many researchers. They have demonstrated that urease activity as bio-indicator which is impaired in the plants grown with urea, consequently urea accumulate in large amounts in all parts of plants. The urea-fed plants without Ni supplements have substantially low amino acid N content in all parts of plants (Gerendas and Sattelmacher, 1997, 1999). These plants were not able to use the urea N provided due to reduced urease activity. In this work urease activity was not measured.

Dry matter percentage and colour of fruits were not affected by the treatments, however reduction in TSS was observed in the plants supplied with urea without Ni supplements. The reduced TSS in this treatment may be due to the reduced Pn which led to the reduction in sugar accumulation. The significant reduction of nitrate in the fruit of urea-fed plants, particularly with the increase of Ni concentration, is an important crucial issue. The reason of the reduction in nitrate concentration of fruit may be due to the increased activation of NR converting nitrate to amino acids (Marschner, 1995). A supplement of Ni to the urea-fed plants significantly reduced the nitrate concentration in spinach (Khan and Watanabe, 1999). In nitrate-fed plants the activity of NR was reduced when Ni concentration increased suggesting that the critical concentration of Ni in nitrate fed plant is much less than that of urea-fed plants. The reduction in nitrate in nitrate-fed plants by Ni supplements has not been reported. Changes in the content of organic acids and other solutes like nitrate might result from secondary events of disturbance in nitrogen metabolism in Ni-deficient plant (Marschner, 1995).

In this study, Ni concentration had no effect on chlorophyll index which is in contrast to Xue *et al.* (2000) findings in tomato. They reported that chlorophyll concentration increased in urea-fed plants because of urea assimilation. In this experiment, a presence of small amount of Ni in the leaves of without Ni supplemented plants could be the reason of the no reduced chlorophyll content. Furthermore, high concentration of N in all treatments may promote the chlorophyll content in the leaves.

The concentration of total N in urea-fed plants with Ni supplement at 0.5 mg L^{-1} was higher than those without Ni and high concentration of Ni, indicating that the absorption of urea is increased by Ni supplement up to 0.5 mg L^{-1} and then decreased at 1 mg L^{-1} Ni concentration. The activity of NR seems to play a key role in N metabolism in urea-fed plants (Table 3). The activity

of NR was strongly affected by Ni concentration in urea-fed plants suggesting the beneficial effect of Ni on the NR activity. Both high and low concentration of Ni in the plants reduced the activity of NR. NR is an enzyme that is regulated by several factors namely, enzyme synthesis, reversible inactivation, and concentration of substrate and effectors (Solomonson and Barber, 1990). Accumulation of urea without Ni supplements (Mordy and Atta-Aly, 1999; Watanabe and Shimada, 1990) and toxic effect of high concentration of Ni (1 mg L^{-1}) appeared to reduce Pn consequently reducing plant growth and yield.

Deprivation of Ni in barley led to lower content of amino acids and nitrate accumulation (Brown *et al.*, 1990). Changes in organic acids content and other solutes might result from secondary events of disturbance in nitrogen metabolism in Ni-deficient plant. It has not been known to what extent these various effects of Ni deficiency are directly related to the function of Ni in the urease. It has been demonstrated that urea is produced in a normal metabolite regardless supplements of N form. The ornithine cycle or degradation of protein for urea biosynthesis is likely to be general importance (Walker *et al.*, 1985). Therefore, Ni supplements might be playing an important role in all plant in the secondary events in nitrogen metabolism regardless N form nutrition. However, Gerendas and Sattelmacher (1999) suggested that Ni may not be strictly essential for *Brassica napus* plants receiving mineral N, or that the critical level is well below 25 µg kg^{-1} DW. The essentiality of Ni in nitrate assimilation in nitrate-fed plants seems to be unclear.

These results have important implications for the cucumber growers and physiologists. Use of Ni in the nutrient solutions containing urea has an important role to promote cucumber plants growth and increase the yield in commercials production. Furthermore, the reduction of nitrate concentration of the fruits in urea-fed plant with Ni supply at 0.5 mg L^{-1} improves the fruit quality. However, further attention should be paid to prevent the accumulation of Ni in the edible parts. Finally, impairing the NR enzyme in both high and low concentration of Ni and the effect of Ni on the nitrate assimilation need more investigations.

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Effect of different fertilizer sources on the qulaity of head cabbage

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Abstract

The influence of different fertilizer sources on head cabbage (*Brassica oleracea* var. *capitata*, cv. 'Pructor') yield and quality was studied. The field experiment was carried out on alluvial - meadow soil (*fluvisol –FAO*) pH 6.5. The trial included mineral fertilizer, farmyard manure and foliar fertilizer. The highest yield values were obtained with mineral fertilization. The best quality parameters in the cabbage leaves – dry weight, total soluble sugars, cellulose, vitamin C and nitrates content were obtained in the treatments with foliar fertilization followed by the treatments with organic fertilization. The observed decrease of N and K residuals after the harvest of head cabbage crop in comparison with the initial soil reserves indicated complete absorption of fertilizers supplied and this is a very important result from ecological point of view.

Key words: Head cabbage, quality parameters, foliar fertilization, fertilizer application

Introduction

Forms and amounts of applied fertilizers influence the yield and quality of vegetable plants to a great extent (Sidiras *et al.*, 1999). Vegetable plants response positively to organic and mineral fertilization. According to some reports (Kovacheva *et al.*, 1999; Panayotov, 2001) these crops also respond positively to foliar feeding. The head cabbage is considered among the most valuable vegetables becuase of its chemical composition, taste and nutritional value (Alipieva, 1986). Cabbage is a good source for carbohydrates (mainly sugars), vitamins, minerals, amino acids and other biologically active substances (Huxsoll *et al.*, 1989). Vitamin C content is very high in cabbage leaves and especially varieties appropriate for storage are rich in vitamin C (Volodina, 1987). The head cabbage is a crop with high nutrient requirements to the nitrogen as well as potassium and phosphorus because it accumulates large vegetative biomass in a relatively short period. The right proportion between the nitrogen and other nutrients leads to the ballanced mineral nutrition. Unbalanced nitrogen application resulted in quality deterioration - reduction of carbohydrates and accumulation of nitrates were observed, cabbage turned sleazy and disposed to bursting (Soyergin *et al.*,1999; Rozek *et al.*, 2001).

The aim of the present study was to investigate the effect of different fertilizer sources (mineral, organic and foliar fertilizers) on the yield and quality parameters of head cabbage.

Material and methods

White head cabbage (*Brassica oleracea* var. *capitata* cv. 'Pructor') plants were grown in a field experiment on alluvial – meadow soil (*fluvisol – FAO*) with pH 6.5 and low content of total nitrogen and humus – 0.052 and 0.70%, respectively. The alluvial – meadow soil is distinguished with relative density 2.53- 2.71 and volume density within the range 1.54-1.66g cm^{-3}.

Soil samples were collected both before the planting and after the harvest of the cabbage. The arable horizon (0-30 cm) had the following agrochemical characteristics: NH_4^+ - N = 12.35 mg kg^{-1} soil, N-NO_3^- =18.53 mg kg^{-1} soil. The content of movable P and K forms were P_2O_5 = 63 mg kg^{-1} and K_2O = 265 mg kg^{-1} soil. Soil samples have been collected for determination of soil mineral nitrogen (spectrophotometrically after Kjeldal digestion), phosphorus and potassium after acetate - lactate method (Ivanov, 1984).

A randomized block design with 4 replicates was used at plant density 33 x 10^3 plants per hectare. Each experimental plot was 40 m^2 in area and consisted of 12 rows (11 plants in row). Plants were grown at optimal fertilizer rates previously determined in model pot experiments (Mitova *et al.*, 2005). The following treatments were tested:

B_1- Control, without fertilization.

B_{2-} Mineral nitrogen, applied as NH_4NO_3, 150 kg ha^{-1} active substance, P as one time application as a triple super phosphate and K applied as a K_2SO_4 (100 kg ha^{-1} P_2O_5 and 100kg ha^{-1} K_2O, respectively). For the nitrogen and potassium fertilizers split application was performed: for NH_4NO_3 2/3 as a base fertilization +1/3 as a dressing and for K_2SO_4 1/2+1/2, , respectively.

B_3- Farmyard manure –24t ha^{-1}. (The composition of the farmyard manure was: total N – 0.64%, total P_2O_5 – 1.84% and K_2O - 0.51%.). The farmyard manure fertilized rate was calculated to be relevant to the NPK content in the applied mineral fertilizers.

B_4- Foliar fertilizer Agroleaf (Agroleaf total) from Scotts company, Ohio, USA and distributed by VLADI Company in Bulgaria. Agroleaf distinguishes with high purity, N:P:K –20:20:20 + all important microelements. Agroleaf chemical characteristics have been described in details in our previous study (Stancheva *et al.*, 2004).

Agroleaf was applied with high pressure spray, 5 times during the vegetation period at 10 day intervals at rates 5 kg ha^{-1} or 0.5% solution starting 3 weeks after planting. After young seedling formation, each plot was covered by a pellucid plastic film to avoid penetration into the soil of the flier foliar fertilizer.

At the cabbage harvest the following parameters were measured: yield of fresh biomass, dry weight, vitamin C content and cellulose (Ermakov *et al.*, 1952), total soluble sugars (Dubois, 1956). The content of nitrates was determined by Nitrachek from Hawk Creek Laboratory Inc. USA.

Data are expressed as means ± standard error. Comparison of means was performed by the Fisher LSD test (P = 0.05) after performing multifactor ANOVA analysis. The STASTICA (version 6.0) package was used for statistical analysis.

Results and discussion

The highest cabbage yield (121 t ha^{-1}) was obtained from the treatments with mineral fertilization followed by organic fertilization (Fig.1). The yields obtained from the foliar fertilization were higher than the control but differences were not significant.

Cabbage quality parameters at crop harvest are shown in Table 1. Changes in dry weight, vitamin C, sugars and cellulose dependence on fertilizer source are more indicative for the white head cabbage quality. Dry matter is one of the main parameters of quality and it is genetically determined (Alipieva, 1986). Dry weight showed maximal values in the organic fertilized cabbage closely followed by mineral fertilization. As reported earlier by Stoicheva *et al.* (2002) the productivity of the vegetable crops grown in a crop rotation was determined mainly by the N- rate rather than the N - way of application and N - source.

Cabbage is a good source for fiber and vitamins, these characteristics are considered as quality parameters. The highest value of sugars and vitamin C were observed in cabbage leaves with foliar fertilization. King and Bolin (1989) suggested that increased protein content and reduced carbohydrates deteriorate quality and storage of the vegetable produce. The vitamin C values in the cabbage leaves are much higher in comparison with other leafy vegetables and negative correlation was found between

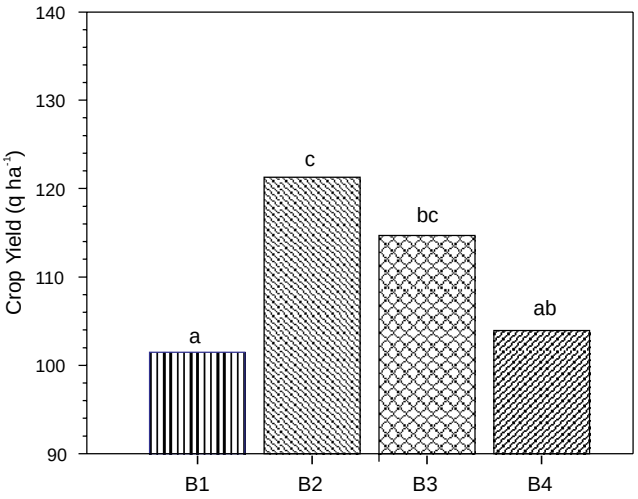

Fig. 1. Head cabbage crop yield under different treatments. Different letters indicate significant differences assessed by Fisher LSD test (P≤0.05) after performing ANOVA.

nitrate nitrogen and vitamin C in the cabbage leaves sap (Volodina, 1987). As it was shown in our previous study (Atanasova and Stancheva, 2004) vitamin C accumulation was found to be higher in the varieties with prolonged period of vegetative phase.

Among the carbohydrates with high molecular weight, pectin and cellulose occupy an important place. The cellulose content in the foliar fed plants was low while the highest cellulose values were observed under mineral fertilization.

High nitrates accumulation in the leaves of some leafy vegetables is closely related to the nitrogen nutrition (Gonnella *et al.*, 2000) and duration of the vegetative phase (Reinken, 1992). Head cabbage is among the vegetable crops which can accumulate considerable nitrate dependending upon the climate, soil composition and properties, fertilizer sources and rates (Rankov, 1990; Amelin, 1996). Nitrate content was maximum in the leaves of mineral fertilized plants, while in the foliar fed plants the lowest values were found. The content of nitrates in the leaves of the plants remained much lower than the acceptable limit concentration – 500 mg kg^{-1} fresh weight.

The soil content of accessible forms of the main macronutrients N, P, K after harvest of the cabbage indicated (Table 2) availability

Table 1. Effect of fertilization on cabbage quality parameters

Treatments	Dry weight (%)	Vitamin C (mg 100g^{-1} fresh weight)	Soluble sugars (mg100g^{-1} dry weight)	Cellulose (% dry matter)	NO_3^- (mg kg^{-1} fresh weight)
B_1 (Control)	6.24±0.29*a	42.60±2.2a	1.02±0.04b	9.36±0.47b	68±3.8c
B_2 (Mineral fertilization)	7.08±0.35b	63.85±3.3c	0.82±0.03a	10.53±0.53c	316±14.1d
B_3 (Organic fertilization)	7.22±0.44b	52.80±2.6b	1.07±0.06bc	8.82±0.44ab	32±2.1b
B_4 (Foliar fertilization)	6.69±0.23ab	74.80±4.4d	1.16±0.07c	8.42±0.42a	16±0.9a

*Values are means ± S.E., n=4. Different letters indicate significant differences assessed by Fisher LSD test (P≤0.05) after performing ANOVA

Table 2. Changes in chemical parameters of soil after harvest of cabbage crop

Treatments	Mineral nitrogen (mg kg^{-1} soil)			Mobile P and K forms (mg kg^{-1} soil)		pH	
	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	Total	P_2O_5	K_2O	(H_2O)	(KCl)
Initial level	12.35	18.53	30.88	63	265	6.5	6.1
B_1 (Control)	3.35	5.3	8.65	63	92	6.7	6.1
B_2 (Mineral fertilization)	5.7	3.68	9.38	84	62	6.6	5.9
B_3 (Organic fertilization)	6.37	5.70	12.07	110	62	6.9	6.2
B_4 (Foliar fertilization)	6.03	4.36	10.39	72	62	6.7	5.9

of residual quantities of macronutrients, compared to the initial levels in the soil. The content of NH_4^+- N , NO_3^- - N and K_2O after the crop harvest was lower than the initial levels in all fertilized treatments, therefore no residuals of nitrogen and potassium accessible forms were found. An increase of phosphorus level was observed in the all fertilized treatments especially in the treatments with organic fertilization, which could be due to the high phosphorus content in the farm yard manure. Changes in soil pH in the treatments with applied mineral, organic and foliar fertilizers are negligible (Table 2). Soil agrochemical analyses after head cabbage crop harvest indicated that the nitrogen and potassium from the applied fertilizers were mostly absorbed by the plants.

Significantly higher yields of cabbage cv. 'Pructor' grown on the alluvial – meadow soil (*fluvisol- FAO*) with different fertilizer sources was obtained after application of mineral fertilizers followed by the treatments with organic fertilizers. Fresh cabbage produce with best quality parameters (maximal levels of total soluble sugars and vitamin C and minimal content of cellulose and nitrates) were obtained as a result of foliar fertilization. Supplied optimal fertilizer rates were assimilated by the plants because no N and K residuals in the arable soil horizon were found and no nitrate accumulation above acceptable limit concentration was measured.

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Effect of sucrose concentration on somatic embryogenesis in carnation (*Dianthus caryophyllus* L.)

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Abstract

The effect of sucrose concentration was investigated on callus induction and differentiation of embryogenic callus derived from petal explants of carnation cultivar 'Nelson'. Embryogenic calli were produced on MS culture medium containing 9 µM 2,4-dichlorophenoxy acetic acid (2,4-D), 0.8 µM 6-benzyladenine (BA) and different concentrations of sucrose alone or in combination with sorbitol. In constant osmotic potential medium, number of explants containing embryogenic calli was significantly enhanced by increasing the sucrose concentration. Somatic embryos were induced on a hormone-free media containing various concentrations of sucrose alone or in combination with sorbitol. Different sucrose concentrations from 50 to 150 mM significantly increased somatic embryos. No callus and embryo formed when sorbitol was the sole carbon source. In the presence of a constant sucrose concentration, increasing the osmotic potential with sorbitol led to increase in the frequency of somatic embryos. In medium containing low concentration of sucrose (50 and 100 mM), reduced development of embryos was recorded. 90% of somatic embryos were regenerated to form the entire plantlets when they were transferred onto the half-strength MS culture medium containing 3% sucrose. Plantlets also continued to grow under greenhouse condition.

Key words: Carnation, embryogenic callus, somatic embryos, sucrose.

Introduction

Carnation (*Dianthus caryopyllus* L.) is an important floricultural crop with high commercial interest worldwide (Burich *et al.*, 1996). Somatic embryogenesis is a desirable mode of plant regeneration (Williams and Maheswaran, 1986). Besides being the fastest method of plant micropropagation, somatic embryos may also be encapsulated in various gels to form synthetic seed that can be easily stored and transported to long distances (Ghosh and Sen, 1994). Due to the presence of well-developed root and shoot primordia, somatic embryos germinate easily to produce plantlets without additional step of rooting (Laux and Jugens, 1997).

Carbohydrates play various essential roles in plants. They are substrate for respiration, play a role in the synthetic pathways of many compounds and are building blocks of macromolecules. In addition, carbohydrates may control developmental processes (Gibson, 2000; Smeekens, 2000).

Plant cell, tissue or organ culture normally requires the incorporation of a carbon source to the culture medium (Karhu, 1997). The nutritional importance of an adequate carbon source in a culture medium is widely known. However, the addition of medium components, especially macronutrients and carbon sources, represent considerable decrease in the medium osmotic potential (George, 1993). The substitution of the culture medium carbon source by osmotically active solutes has shown that sugars act as a carbon source and as osmotic regulators. In this case, the most frequently used solutes are the two alcohol sugars sorbitol and mannitol (George, 1993).

It has been observed that sugar concentration affects the formation of somatic embryos in culture medium (Kamada *et al.*, 1989; Gray *et al.*, 1993; Lou and Kako, 1995; Lou *et al.*, 1996; Nakagawa *et al.*, 2001). Among the many available carbon sources, sucrose has been the most tested carbon source and osmoticum for somatic embryogenesis in plant species (Fuentes *et al.*, 2000). Sucrose concentration of 3% was used as a carbon source for the induction of somatic embryos in carnation (Frey *et al.*, 1992; Nakano *et al.*, 1993; Sankhla *et al.*, 1995; Yantcheva *et al.*, 1998). The effect of different sucrose concentrations in somatic embryogenesis of carnation has yet to be studied. In the present study, the effect of different sucrose concentrations has been investigated on somatic embryogenesis of carnation.

Materials and methods

Plant materials: Immature flower buds of the carnation (*D. caryophyllus*) cultivar 'Nelson' were harvested from greenhouse-grown plants and stored at 4°C for 3-4 weeks. They were then surface-sterilized with 70% ethanol for 30 sec, 2% commercial bleach for 20 min, followed by three times rinses with sterilized distilled water. Sepals and receptacles were removed from the buds and the pieces of approximately 4 mm in length of basal petals part were excised as explant and placed on the solid culture medium. For producing callus, explants were placed on Murashige and Skoog (1962) medium supplemented with 9 µM 2,4-dichlorophenoxy acetic acid (2,4-D) and 0.8 µM of 6-benzyladenine (BA) in combination with various concentrations of sucrose (50, 100, 150, 200, 250 and 300 mM) and sorbitol (145 and 335 mM) alone or with both in the following combinations: 50 mM sucrose + 280mM sorbitol, 100 mM sucrose + 232mM sorbitol, 150 mM sucrose + 149 mM sorbitol, 200 mM sucrose + 109 mM sorbitol and 250 mM sucrose + 54 mM sorbitol. Number of explants producing callus were recorded after 9 weeks of culture. Calli were transferred to growth regulator free MS medium containing various concentrations of sucrose and sorbitol alone or combinations of both to produce somatic embryo. Numbers

of embryos were recorded after 5 weeks time. Somatic embryos were then transferred to the half-strength MS medium containing 3% sucrose for plant regeneration. All cultures were incubated in growth room at 24°C and 16/8 hours photoperiod with 30µmol m^{-2} s^{-1} illumination provided by cool white fluorescent lamps. The pH of the culture media were adjusted to 5.8 using1N NaOH before adding 7 g L^{-1} of gelling agent (Agar-Agar, Merck) to the media and were autoclaved at 121°C for 15 min.The osmolarity of each medium was measured on complete liquid medium using a cryoscopic osmometer (Hermann Roebling, Berlin, Germany) according to the manufacturer's instructions.

Transferring to soil: Agar-based medium was removed from roots of plantlets (approximately 25 mm length) under running water and then transferred to plastic pots containing mixture of sand and compost in the ratio 2:1. Pots were maintained in the growth room (18 ± 2°C, 16-h photoperiod and 25 µmol m^{-2} s^{-1}). After four weeks, well established plants were transferred to pots containing garden soil and acclimatized in greenhouse for two weeks prior to field plantation.

Histological examination: For histological investigation, somatic embryos were fixed in FAA (formalin- acetic acid- ethanol, 2:1:17 v/v) for 24 hours, dehydrated in serial grades of alcohol and then embedded in paraffin. Serial sections (7 µm) thick were cut and stained with heamatoxylin.

Statistical analysis: Almost 140-150 explants or 200mg callus were considered for each treatment conducted. The data were presented as mean ± S.E. The ANOVA was performed for analysis of the data obtained for each experiment and the means were tested by LSD test ($P < 0.05$).

Results and discussion

Callus induction: Two types of callus were recognized according to color, texture, and time of callus initiation. Type I calli were soft and yellowish green (Fig. 1A), and on all media, their initiation started at the cut edges of the explants within 2-3 weeks of inoculation. A high-frequency of growth rate (95-100%) was observed on this type of calli. Type I callus were apparently non-embryogenic. Type II calli were creamy-white, compact in structure and appeared nodular (Fig. 1B), and grew slowly. The callus initiation was observed on the edges of the petal explants which grew rather slowly and known as embryogenic.

The high concentrations of sucrose have been found to promote somatic embryo formation in chrysanthemum (May and Trigiano, 1991), cucumber (Lou *et al.*, 1996), iris (Jehan *et al.*, 1994), Spindle tree (Biahoua and Bonneau, 1999) and melon (Nakagawa *et al.*, 2001). In this study, number of explant containing embryogenic calli (type II calli) was significantly enhanced by increasing the sucrose concentration (Table 1) and also in presence of the same osmotic potential provided by sorbitol, the number of explants producing embryogenic calli was significantly ($P<0.05$) enhanced by increasing the sucrose concentration (Table 1). Explants cultured on media devoid of sucrose never induced callus. In the presence of a constant sucrose concentration, increasing the osmotic potential with sorbitol did not increase the frequency of embryogenic calli (data not shown).This suggests that the role of sucrose in embryogenic calli and high frequency may be related to the source of carbon supply to plant cells.

Table 1. Effect of different sucrose concentrations on embryogenic calli induced from petal of Nelson cultivar of carnation after 9 weeks on MS meduim containing 9 µM 2,4-D and 0.8 µM BA*.

Carbon source	Calculated osmotic potential (MPa) at 25 ºC**	Number of explants with embryogenic calli ***
Sucrose 50 mM	-0.330	3.66±0.57d
Sucrose 100 mM	-0.512	7.33±0.57c
Sucrose 150 mM	-0.750	15.66±1.15a
Sucrose 200 mM	-0.910	17.66±1.52a
Sucrose 250 mM	-1.025	11.66±1.00b
Sucrose 300 mM	-1.220	6.66±0.57c
Sorbitol 335 mM	-1.220	0.00
Sorbitol 280 mM + Sucrose 50 mM	-1.223	3.00±0.33d
Sorbitol 232 mM + Sucrose 100 mM	-1.225	8.00±1.00c
Sorbitol 149 mM + Sucrose 150 mM	-1.215	16.66±1.00a
Sorbitol 109 mM + Sucrose 200 mM	-1.218	16.00±1.52a
Sorbitol 54 mM + Sucrose 250 mM	-1.217	10.00±0.52b
Sorbitol 149 mM	-0.754	0.00

*****30 explants/ replicate, six replicate/ treatment.
****** The osmolarity of each medium was measured using a cryoscopic osmometer.
******* Mean ± S.E, means having the same letter in column were not significantly different by LSD test ($P < 0.05$).

Table 2. Effect of different sucrose concentrations on somatic embryo induction from type II calli from petal Nelson cultivar of carnation after 5 weeks on hormone-free MS medium*****

Carbon source	Calculated osmotic potential (MPa) at 25 ºC**	Number of embryos induced from embryogenic calli***
Sucrose 50 mM	-0.325	40.33 ± 5.33 e
Sucrose 100 mM	-0.510	78.33 ± 7.33 d
Sucrose 150 mM	-0.746	122.33 ± 9.33 c
Sucrose 200 mM	-0.911	186.66 ± 8.00 a
Sucrose 250 mM	-1.010	112.66 ± 11. 66 c
Sucrose 300 mM	-1.218	72. 66 ± 5.66 d
Sorbitol 335 mM	-1.214	0.00
Sorbitol 280 mM + Sucrose 50 mM	-1.217	141.66 ± 9.8 b
Sorbitol 232 mM + Sucrose 100 mM	-1.220	193.66 ± 11. 33 a
Sorbitol 149 mM + Sucrose 150 mM	-1.219	190.66 ± 8.66 a
Sorbitol 109 mM + Sucrose 200 mM	-1.218	184.33 ± 10 .00 a
Sorbitol 54 mM + Sucrose 250 mM	-1.215	123.66 ± 7.66 c
Sorbitol 149 mM	-0.750	0.00

*****200 mg embryogenic calli / replicate, six replicate/ treatment
******The osmolarity of each medium was measured using a cryoscopic osmometer
*******Mean ± S.E, means having the same letter in column were not significantly different by LSD test ($P < 0.05$).

Somatic embryo induction and development: Somatic embryos were induced within 1-2 week from embryogenic calli when transferred to hormone-free medium containing different concentrations of sucrose. Histological studies showed that globular and cotyledonary somatic embryos had no vascular connection to the callus (Fig. 1C and F). After one week, globular embryos were further developed into heart and torpedo-shaped embryos. Cotyledonary embryos were observed on most of the calli (90-95%) 4 weeks after being subcultured (Fig. 1E). No embryo induction occurred on non-embryogenic calli (type I

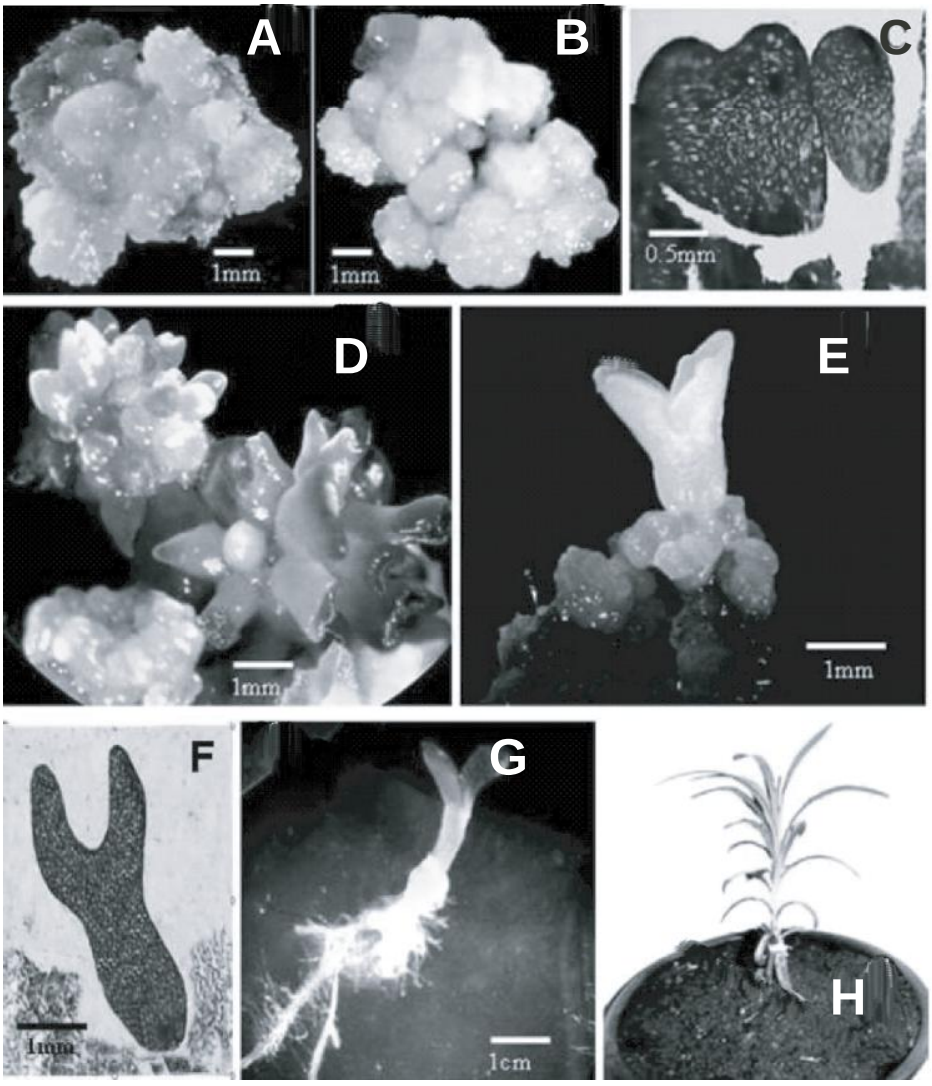

Fig 1. Somatic embryogenesis and plant regeneration in carnation (*Dianthus caryopyllus*). **A**: Type I callus on MS medium containing 9 µM 2,4-D and 0.8 µM mg L^{-1} BA, 4 weeks after culture. **B**: Type II callus on MS medium containing 9 µM mg L^{-1}2,4-D and 0.8 µM mg L^{-1} BA, 9 weeks after culture. **C**: Histological section of somatic embryos in globular and heart stage. **D**: Somatic embryogenesis at different developmental stages on medium containing 150 mM sucrose, 4 weeks after culture. **E:** Cotyledonary embryo on medium containing 200 mM sucrose. **F**: Histological section of somatic embryo in cotyledonary stage. **G**: Plantlet regenerated from a somatic embryo cultured on half-strength MS medium after 2 weeks. **H:** A potted plant in greenhouse.

calli) on medium similar to that used for embryogenic calli.

Table 2 shows the number of embryos on embryogenic calli at different sucrose concentrations. Different sucrose concentrations from 50 to 150 mM significantly increased somatic embryogenesis ($P < 0.05$). With constant osmotic potential medium by sorbitol, number of embryos on embryogenic calli was not enhanced significantly by increasing the sucrose concentration (Table 2). In the presence of a constant sucrose concentration, increasing the osmotic potential with sorbitol led to increase in the frequency of somatic embryos (Table 3). No embryo formed when sorbitol was the sole carbon source. It suggest that the role of sucrose in somatic embryos frequency may be as a result of osmotic potential and carbon supply or their combined action. Our results were consistent with the findings of Litz (1986), May and Trigiano (1991) and Biahoua and Bonneau (1999) who indicated that osmotic potential resulted from higher concentration of sucrose was related to somatic embryogenesis.

The high concentrations of sucrose (250 and 300 mM) reduced embryogenic calli and somatic embryo formation (Table 1 and 2). This may be due to toxic effects of this sugar and not due to the increase in osmotic potential.

Lower concentrations of sucrose alone (50 and 100 mM) promoted precocious germination and reduced development of normal embryos, but higher concentrations of sucrose alone or in combination with sorbitol promoted the development of normal embryos and prevented precocious germination. Higher concentrations of sucrose were shown to improve maturation of somatic embryos in other plant species (Lu and Thorpe, 1987; Themblay and Themblay, 1991; Ricci *et al.*, 2002). Increasing osmotic activity of the medium due to elevated concentrations of carbohydrate may create the osmotic stress, thus improving the somatic embryogenesis. Therefore, it could be suggested that osmotic effect of the sucrose and sorbitol (in this study) may cause normal development of somatic embryos. The positive effect of high osmolarity may mimic changes in osmolarity that occur in the environment surrounding the embryo within the seed (Merkle *et al.*, 1995).

Table 3. Effect of sorbitol concentrations on the induction of somatic embryos from embryogenic calli in cv. Nelson, 5 weeks after culture on hormone-free MS medium containing 100 mM sucrose

Sorbitol (mM)	Calculated osmotic potential (MPa) at 25 ºC**	Number of embryos induced from embryogenic calli***
0	-0.510	40.33 ± 5.33 e
50	-0.800	78.33 ± 7.33 d
100	-0.990	122.33 ± 9.33 c
150	-1.200	186.66 ± 8.00 a
200	-1.350	112.66 ± 11. 66 c
250	-1.400	72. 66 ± 5.66 d
300	-1.690	0.00 ± 0.00
350	-1.805	141.66 ± 9.8 b

*****200 mg embryogenic calli / replicate, six replicate/ treatment
******The osmolarity of each medium was measured using a cryoscopic osmometer
*******Mean ± S.E, means having the same letter in column were not significantly different by LSD test ($P \leq 0.05$).

Plant regeneration: After 4 weeks, induced somatic embryos were transferred into half-strength hormone free MS medium where they developed into perfect plantlets within 2 weeks (Fig. 1G). Average germination rate of somatic embryos was about 90 to 95%. As the somatic embryogenesis was asynchronous, plantlets obtained from the same culture media were at different developmental stages. Roots failed to develop, when somatic embryos were not separated from calli cultured on half-strength hormone free MS medium (medium suitable for rooting). A high percentage of rooted plantlets (approximately 95%) were successfully transferred to soil (Fig. 1H) and were developed to normal plants in the greenhouse with an average of 95% survival. All acclimatized plants were transferred to field plots and grew normally in the natural environmental condition.

In conclusion, the results of this study showed that the induction of embryogenic callus on petal explants and production of somatic embryos on embryogenic calli could be obtained by changing sucrose concentration, whether supported by an osmotic potential provided high sucrose concentrations and carbon supply or a combined action of the two. This study also showed that high percentages of somatic embryos could successfully be regenerated into entire normal plants. Establishment of conditions required for the high frequency of regeneration via somatic embryogenesis would facilitate genetic transformation and artificial seed production in carnation.

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Response of tomato to transplant drench, foliar organic-complex Ca, B, K and yield enhancement amendments

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Abstract

Field studies were established in 2004 and 2005 to determine the effects of transplant drench and foliar applications of organic-complex Ca, B, K amendments and a yield enhancement product, 'Perc Plus™', on the flowering, fruiting, fruit yield and market value of Italian-cv. 'Classica' and large-fruited cv. 'Amelia' tomato (*Lycopersicon esculentum* Mill.). Treatments were an organic yield enhancement applied as a transplant drench and then foliar 7 days later (TD); once a week foliar amendments of organic-complex Ca, B, and K nutrients beginning at first bloom for 4 weeks (FA); a combination of the drench and foliar treatments (TD+FA); and a control (CON). Fruit-set of 'Classica' was significantly higher for the FA and TD+FA than the other treatments in 2004, however there was no effect on yield and quality of harvested fruit. Flowering and fruit-set of 'Amelia' were not affected by drench and/or foliar amendments in either year. Total fruit yield and quality of the treated plots were not significantly different than the CON for either cultivar or year.

Key words: Tomato, foliar nutrient amendments, B, K, Ca, yield enhancement, fruit-set, biostimulants, *Lycopersicon esculentum*

Introduction

Commercial vine-ripened tomato producers are constantly seeking innovative and inexpensive management practices to enhance yield and quality. Various companies market supplemental nutrient and bio-stimulant products to vegetable growers that are reported to increase yields and profits. Investigations of soil applied, transplant drench and foliar applications of nutrients and various organic biostimulant compounds as a way to enhance fruit-set, growth, size, fruit quality and overall yields of tomato have produced inconsistent results. Foliar applications of Ca + B nutrients were shown to significantly decrease incidences of shoulder check defects (Huang and Snapp, 2004) and a biostimulant increased early fruit size and yields on a sandy soil when injected through the drip lines at 14 and 21 days after transplant (Cszinszky, 2002). However, foliar applications of biostimulants and nutrients have not produced consistent increases in yield or quality (Castro *et al.*, 1988; Csizinszky, 1996). Foliar applied K did not increase fruit yield or quality when compared to drip-line K injection (Hartz *et al.*, 2005). In apples, foliar B amendments after bloom increased fruit-set and yield (Wojcik *et al.*, 1999).

The literature reflects a complicated and inconsistent response of fruit-set and growth to biostimulant application and foliar fertilization. Environmental parameters such as temperature (Peet and Bartholemew, 1996; Sato *et al.*, 2000; Adams *et al.*, 2001), assimilate supply and demand (McAvoy and Janes, 1989; Bertin and Gary, 1992; Bertin, 1995), sucrose synthase enzyme (D'Aoust *et al.*, 1999), genotype (Abdul-Baki and Stommel, 1995) and even management practices such as planting depth of transplants (Vavrina *et al.*, 1996) are other factors which have been shown to influence tomato fruit-set and growth and could possibly influence the effects of applied amendments for some cultivars. The type of organic compound could also affect plant response. Our objectives were to investigate the effects of a fulvic acid derived biostimulant and foliar applied fulvic acid complexes of B, Ca, and K on the flower, fruit-set and fruit yields of two contrasting tomato - a small-fruited Roma type, and a large-fruited commercial cultivar.

Materials and methods

Field studies were conducted at the University of Arkansas at Monticello, Monticello, Arkansas in the 2004 and 2005 growing seasons. Transplants of 'Classica', an Italian type fruited cultivar, and 'Amelia', a popular large-fruited type grown by commercial vine-ripened tomato producers in the region were planted on 6 April 2004 and 18 April 2005 in a raised bed with black-plastic mulch, micro-irrigation culture. Tensiometers at 0.15 m depth were used to schedule irrigation events when readings reached -0.25 bars. The soil was a Sacul loam and preliminary soil test data (Melich-3 extract, Mehlich, 1984) revealed high P and K nutrient levels and a favorable pH (Table 1).

Table 1. Soil pH and nutrient levels

Depth (cm)	pH	Nutrient (kg ha^{-1})			
		P_2O_5	K_2O	Ca	Mg
0-15	6.9	196	434	7665	548

The experimental design was a randomized complete block of four treatments and five replications. Treatments were a biostimulant soil transplant drench with early foliar application (TD), foliar nutrient application programme (FA), a combination of the drench and foliar programmes (TD+FA) and a control (CON). The biostimulant 'PercPlus™' (a fulvic acid based organic complex according to company literature) was applied as a soil drench at 0.1 L plant^{-1} of a solution containing 0.001135 L L^{-1}, followed by a foliar application of 'PercPlus™' at 0.454 kg ha^{-1} (mulched). The foliar programme consisted of an application of Ca+B at first cluster set followed by four, weekly applications

of Ca+B+K beginning at second cluster set. The Ca, B and K sources were fulvic acid based organic complex solutions of calcium nitrate, boric acid, and potassium carbonate foliar-applied at 0.023-, 0.0055- and 0.25 kg ha^{-1} Ca, B, and K, respectively at an application rate of 187 L ha^{-1}. Within-row transplant spacing was 0.61 m and the raised bed row spacing was 1.5 m. Sub-plot size was four plants with fruit yield data harvested from the inner two plants. Number of flower clusters, flowers and set fruit of 1 cm diameter or larger were evaluated in mid-May of each year (Table 2). Fruit was hand harvested two to three times per week for four weeks and graded using current United States Department of Agriculture classification for grades of fresh tomatoes (USDA, 2006). Statistical analyses of the data were performed using analysis of variance procedures.

Table 2. Calendar of field activities

Activity	2004	2005
Transplants set	6 April	18 April
First foliar treatment	27 April	11 May
Cluster evaluations	24 May	19 May
First fruit harvest	14 June	16 June

Results and discussion

The nutrient and biostimulant treatments had little effect on the early flowering and fruiting of the 'Amelia' cultivar in either year of evaluation (Table 3). There was a significant treatment effect on clusters set in 2004 but it was not consistent with the treatments and numbers of flowers. The measured reproductive parameters in 2005 were less than 2004 because of a 12 day delay in transplanting. Treatment effect on the number of fruit-set for the 'Classica' cultivar was significant in 2004 but not in 2005 (Table 4). A one-degree of freedom contrast of the 'treatments' vs. 'CON' in a general linear model (analysis not shown) was significant at P=0.01.

Table 3. Mid-May evaluation of reproductive progress for 'Amelia' (plant^{-1})

Treatment	Clusters	Flowers	Fruit-set	Clusters	Flowers	Fruit-set
	2004			2005		
TD	6.1	19.1	5.0	6.2	22.5	1.4
FA	6.9	20.9	5.6	5.9	24.4	1.6
TD+FA	5.0	17.5	4.4	6.1	25.3	1.5
CON	6.6	17.4	4.5	6.0	24.4	1.8
	**^{z}	NS	NS	NS	NS	NS

^{z}Not significant (NS) and significant treatment effect at P=0.05 (*) and P=0.01 (**)

Fruit yields of both types of tomatoes were not effected by treatment in either year of study (Tables 5 and 6). A general trend of higher premium grade fruit was noticed for both cultivars in 2005. With regard to the production efficiency of marketable fruit (defined here as percent of total yield meeting USDA Grades 1 and 2), 'Amelia' was not effected by the treatments (Table 7). There were significant effects on the 'Classica', but it cannot be attributed to improvements in efficiency due to the applied amendments since the untreated plots had high efficiency. The FA treatment reduced the percentage of harvestable fruit in 2005. The rainfall was much higher in 2004 than in 2005 (Table 8) which seemed to affect the percentage of harvested fruit more for the Italian fruited cultivar 'Classica' rather than the large-fruited 'Amelia'. Rainfall for the month of June in 2004 was 32.8 cm compared to only 3.81 cm in 2005. This increased partial lodging

Table 4. Mid-May evaluation of reproductive progress for 'Classica' (plant^{-1})

Treatment	Clusters	Flowers	Fruit-set	Clusters	Flowers	Fruit-set
	2004			2005		
TD	13.3	35.0	22.0	7.7	32.7	1.6
FA	12.1	37.9	20.0	7.5	29.8	2.4
TD+FA	11.1	32.4	18.6	7.2	30.1	2.0
CON	12.9	35.1	16.6	8.2	32.3	1.0
	NS	NS	**	NS	NS	NS

^{z}Not significant (NS) and significant treatment effect at the 0.05 (*) and 0.01 (**) level of probability.

Table 5. Harvest yields of 'Amelia' (kg plant^{-1}) under different treatments

Treatment	XL-1	L-1	No. 2	XL-1	L-1	No. 2
	2004			2005		
TD	0.64	0.12	1.64	1.64	0.33	2.90
FA	1.28	0.20	1.74	1.49	0.01	2.97
TD+FA	1.22	0.20	1.95	1.72	0.11	2.43
CON	1.16	0.22	2.28	2.12	0.07	2.74
	NS^{z}	NS	NS	NS	NS	NS

^{z}Not significant (NS) and significant treatment effect at P=0.05 (*) and P=0.01 (**)

Table 6. Harvest yields of 'Classica' (kg plant^{-1}) under different treatments

Treatment	Marketable	Total	Marketable	Total
	2004		2005	
TD	1.56	4.75	2.22	3.69
FA	2.02	5.46	1.92	4.13
TD+FA	1.83	4.55	1.79	3.19
CON	2.40	5.06	2.05	3.66
	NS^{z}	NS	NS	NS

^{z}Not significant (NS) and significant treatment effect at P=0.05 (*) and P=0.01 (**)

Table 7. Market efficiency (%) of 'Amelia' and 'Classica' cultivars

Treatment	'Amelia'		'Classica'	
	2004	2005	2004	2005
TD	64	68	33	60
FA	76	68	37	46
TD+FA	79	67	40	56
CON	80	74	46	56
	NS^{z}	NS	*	*

^{z}Not significant (NS) and significant treatment effect at P=0.05 (*) and P=0.01 (**)

Table 8. Water balance during 2004 and 2005

Year	Irrigation	Water received (cm)		
		Irrigation	Rainfall	Total
2004	12	11.2	61.5	72.7
2005	19	15.2	22.4	37.6

of the 'Classica' plants more than the 'Amelias' during a time of rapid fruit development and resulted in a higher percentage of sun scalding and thus, lower market quality.

Our results did not show any consistent benefits to fruit yield and quality of a large- or Italian-fruit type tomato fruit from supplemental amendments of biostimulants and fulvic acid complex Ca, B and K. The native fertility of the soil in this study was very high and plots were well-watered. Future studies should investigate the effects of these amendments on soils that are less fertile at the beginning of the growing season. Given the extra time and expense needed to apply supplemental nutrients and

biostimulants, tomato growers may be more likely to be benefitted by improving soil fertility, irrigation efficiency, pest control and general management practices.

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Variation in growth, dry matter production, nitrogen and potassium uptake by six *Musa* genotypes in a soilless culture

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Abstract

Six genotypes comprising a landrace and a hybrid from each of the three *Musa* major genomic groups were evaluated in a soilless potting mix. Effect of genotype on most of the growth parameters was non-significant. But the uptake (total quantity accumulated, distribution pattern and tissue concentration) of N and K was significantly ($P < 0.05$) influenced by genotype (G), age of plant at sampling (AP) and G x AP interaction. Dessert bananas had higher N uptake while 'PITA 22' (a plantain hybrid) demonstrated an exceptional propensity for K uptake. Nitrogen and potassium concentration varied with tissue, genotype and age of plant at sampling. Nitrogen concentration in roots and leaves decreased with plant age while it increased in the corm. Potassium concentration in roots, corm and leaves increased progressively with plant age in all the genotypes. Significant differences in the quantity of N and K accumulated per plant, even though all the genotypes were planted in the same potting mix, suggested differential nutrient mining capacity of the genotypes. Implying that nutrient uptake and consequently nutrient demand varies with genotype, supplemental application would vary accordingly. The study suggested that genotype that had higher nutrient uptake will impoverish the soil faster, and thus require more external nutrient inputs to maintain/restore soil productivity.

Key words: Bananas and plantains, genotypic differences, nutrient uptake.

Introduction

Bananas and plantains (*Musa* species L.) are important staples in West and Central Africa sub-region where they contribute to the socio-economic wellbeing of the people. Per capita consumption of plantain in some traditional production areas could be as high as 150 kg (Vuylsteke *et al.*, 1997).

The introduction of improved cultivar is one of the most effective and cost-efficient means of enhancing crop productivity and farmers' incomes (Kueneman, 2002). However, ensuring sustainability of production of improved cultivar in farmers' field requires agronomic packages based on sound scientific principles. Soil fertility management on small farms in the tropics has become a major crop production issues, as a result of continued land degradation and rapid population growth (FAO, 1981) and external nutrient inputs are essential to improve and sustain crop production on these soils (Hossner and Juo, 1999).

Earlier fertilizer recommendations in Nigeria were based on landrace genotypes (Ndubizu, 1978; Obiefuna *et al.*, 1981; Obiefuna, 1984; Baiyeri, 2002). But recent *Musa* germplasm evaluation studies in Nigeria had identified high yielding and disease resistant hybrids that are adapted to some specific agro-ecological niches (Baiyeri *et al*, 2004; 2005). But before final release of a variety for large scale production, standardization of the basic agronomic package will ensure sustainable production in farmers' field. Most important is the nutritional demand for optimum production. In assessing nutrient requirements, quantitative information should be obtained between yield and nutrient uptake and between nutrient supply and crop demand (Keerthisinghe *et al.*, 2003).

Thus, an increased knowledge of the growth pattern and nutrient uptake of the new hybrids can lead to a better understanding of their responses under commercial conditions or in farmers' field. Nitrogen and potassium are the key nutrient elements for optimum growth and yield in *Musa* species (Twyford and Walmsley, 1974; Lahav and Turner 1989; Lahav, 1995). Therefore, the general objective of this study was to evaluate genotypic differences in growth, biomass yield and uptake of key nutrient elements in *Musa* germplasm. More specifically, genotypic differences in nitrogen and potassium mining capacity were determined to establish differential nutrient demand by landrace and hybrid genotypes.

Materials and methods

The experiment was conducted in the Department of Crop Science, University of Nigeria, Nsukka, Nigeria, between May 2004 and June 2005. Suckers were generated following the method described by Baiyeri and Aba (2005). The method ensured that uniform sucker plantlets at a similar physiological age were used. Besides, since the study was to compare growth and nitrogen and potassium uptake by different *Musa* genotypes, the plantlets were cut-back to allow a re-growth under the experimental conditions provided. Six *Musa* genotypes belonging to the three major *Musa* genomic groups were evaluated (Table 1). Each genome group consisted of a landrace and a hybrid genotype.

The potting medium was ricehusk mixed with poultry manure at 3:2 (v/v) and composted for about five months before use. The medium was analyzed for elemental composition (Table 2). Potting bag measured 60 x 60 cm in dimension and spaced 1 x 1 m after planting. The experimental layout was completely randomized design; each genotype was replicated nine times. Watering frequency followed the recommendation of Baiyeri (1996).

Table 1. List of genotypes evaluated

Genome group	Name/Code	Breeding status	Source
Plantains	Agbagba [AGB]	Landrace	Southern Nigeria
	PITA 22 [P22]	Hybrid	International Institute of Tropical Agriculture (IITA), Nigeria.
Dessert bananas	Nsukka Local [NSK]	Landrace	Southeastern Nigeria
	FHIA 17 [F17]	Hybrid	Fundacion Hondurena de InvestigacionAgricola, Honduras.
Cooking bananas	Fougamou [FOU]	Landrace	Southeast Asia
	BITA 7 [BT7]	Hybrid	IITA

Table 2. Nutrient composition of the soilless culture (ricehusk plus poultry manure in 3:2 v/v)

Nutrients	Quantity
Nitrogen (%)	1.86
Phosphorus (mg $100g^{-1}$)	18.34
Potassium (mg $100g^{-1}$)	8.15
Calcium (mg $100g^{-1}$)	1900.00
Magnesium (mg $100g^{-1}$)	346.67
Sulphur (mg $100g^{-1}$)	11.22

Data were collected monthly from the fourth month after planting (MAP) to the sixth MAP. This growth period is physiologically significant in *Musa* because, it signals the transformation of the plant from vegetative to reproductive growth stage (Ndubizu *et al.*, 1981; Stover and Simmond, 1987). Plant height, girth, number of leaves and leaf area were measured. Leaf area was determined following Obiefuna and Ndubizu (1979) method. Fresh weight of plant components was determined and samples were dried at 70°C until constant weight was obtained, thereafter dry weight was measured. Nitrogen and potassium content were determined using dried samples milled to pass through 0.2 mm sieve using Thomas Wiley Hammer Mill. Leaf number three from the top was used for N and K determination following standard procedures as recommended by AOAC (1981). Data collected were analyzed as factorial completely randomized design to compare the main effect of genotype, age of plants at sampling and the interaction of these two factors. Significance test of variance components was by least significant difference.

Results

The elemental composition of the potting medium after five months of composting is shown in Table 2. The relative abundance of potassium was low while others were high. Growth parameters of the six genotypes were in most cases similar irrespective of age at sampling. However, significant variations were obtained in leaf area at four and five months after planting (MAP), and in plant girth and height at four and six MAP, respectively (Table 3). Total dry matter production at the four and five MAP was similar in each genotype; however, there was a tremendous increase in dry matter yield at six MAP (Fig 1a). Total dry matter yield was generally higher in the landrace genotypes. Partitioning of dry matter to above and below ground components varied with age and genotypes (Fig. 1b). Partitioning pattern in 'Nsukka Local' was nearly equal between the above and the below ground components. Exceptionally high proportion of the total dry matter was reported in above ground part in 'BITA 7' and 'PITA 22' at six MAP. The proportions of the total photo assimilate in different plant parts are shown in Fig. 2. Generally, higher proportion of the total dry matter was contributed by the leaf and corm irrespective of genotype and age at sampling. The contribution of root to the total dry matter decreased with plant age in most genotypes while the contribution of leaf remained fairly stable over time in most genotypes. There was inconsistent proportion of contribution of the corm to the total dry matter yield.

The concentration of N and K in specific plant tissues was significantly ($P < 0.05$) influenced by genotypes (Table 4). The N concentration in root was highest in landrace dessert banana 'Nsukka Local' and lowest in cooking banana landrace 'Fougamou'. The quantity of N in the corm was highest in landrace plantain 'Agbagba' and was closely followed by 'Nsukka Local'. 'BITA 7', a cooking banana hybrid had the highest percent N in the pseudostem and leaf, followed by 'Nsukka Local'. Generally,

Table 3.The effects of age after planting on growth parameters of six *Musa* genotypes in a soilless culture

Genotype	Plant girth (cm)			Plant height (cm)			Number of leaves			Leaf area (cm^2)		
	M4	M5	M6	M4	M5	M6	M4	M5	M6	M4	M5	M6
Agbagba	13.7	15.3	20.5	34.6	44.0	65.7	7.1	7.4	9.8	624.2	689.4	1114.7
PITA 22	13.2	14.5	20.5	38.0	39.5	61.5	7.7	8.3	10.5	554.9	578.0	965.0
Nsukka Local	11.6	15.6	24.3	30.8	39.3	58.5	7.8	8.2	9.8	381.3	624.1	958.3
FHIA 17	14.6	17.0	21.3	36.0	41.6	60.8	9.1	8.3	9.9	658.6	836.3	1005.5
Fougamou	15.7	15.5	21.3	32.4	40.8	60.8	7.9	8.2	9.9	568.9	681.6	1005.5
BITA 7	12.9	15.2	19.7	31.9	39.5	57.4	8.2	8.7	9.3	513.3	680.7	983.4
LSD (*P*=0.05)	2.5	NS	NS	NS	NS	6.2	NS	NS	NS	174.3	153.1	NS

M4, M5, M6: Plants at four, five and six months after transplanting, respectively. NS: Non-significant.

Table 4. Variation in nitrogen and potassium concentration in different plant components as influenced by genotypes

Genotype	Nitrogen (%)				Potassium (mg $100g^{-1}$)			
	Root	Corm	Pseudostem	Leaves	Root	Corm	Pseudostem	Leaves
Agbagba	1.34	1.60	1.10	1.81	70.0	74.0	19.0	49.0
PITA 22	1.43	1.11	1.35	1.69	197.0	250.0	219.0	189.0
Nsukka Local	3.08	1.50	2.02	2.20	89.0	75.0	21.0	39.0
FHIA 17	2.50	1.45	1.72	1.76	85.0	61.0	84.0	54.0
Fougamou	0.77	0.80	0.68	1.47	103.0	89.0	21.0	33.0
BITA 7	1.82	1.14	2.43	2.22	136.0	80.0	93.0	58.0
LSD (*P*=0.05)	0.10	0.01	0.04	0.04	2.7	4.5	3.1	2.7

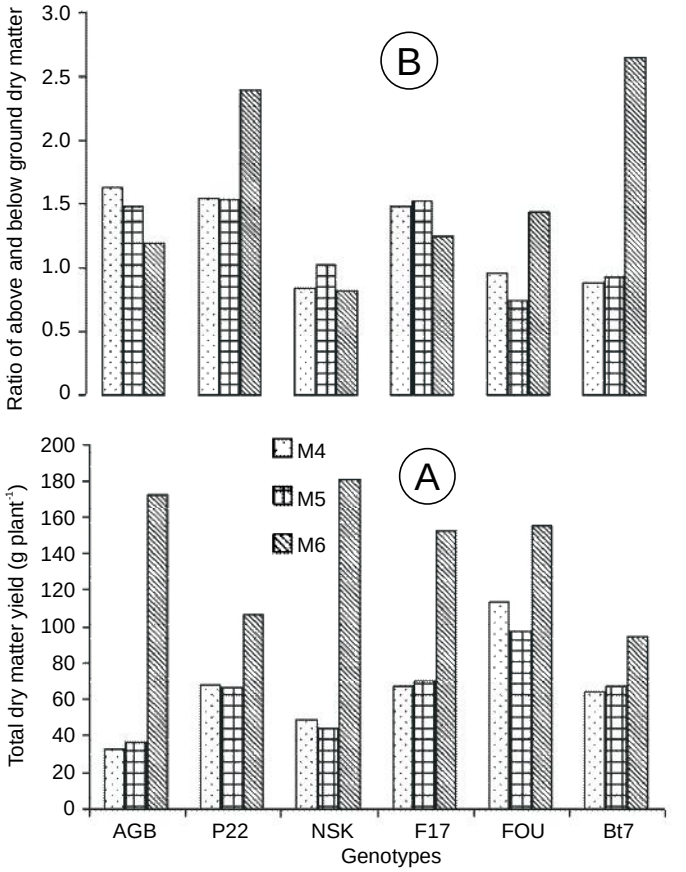

Fig. 1. The effects of genotype and age at sampling on [A] total dry matter yield and [B] ratio of dry matter above ground to the dry matter below ground.

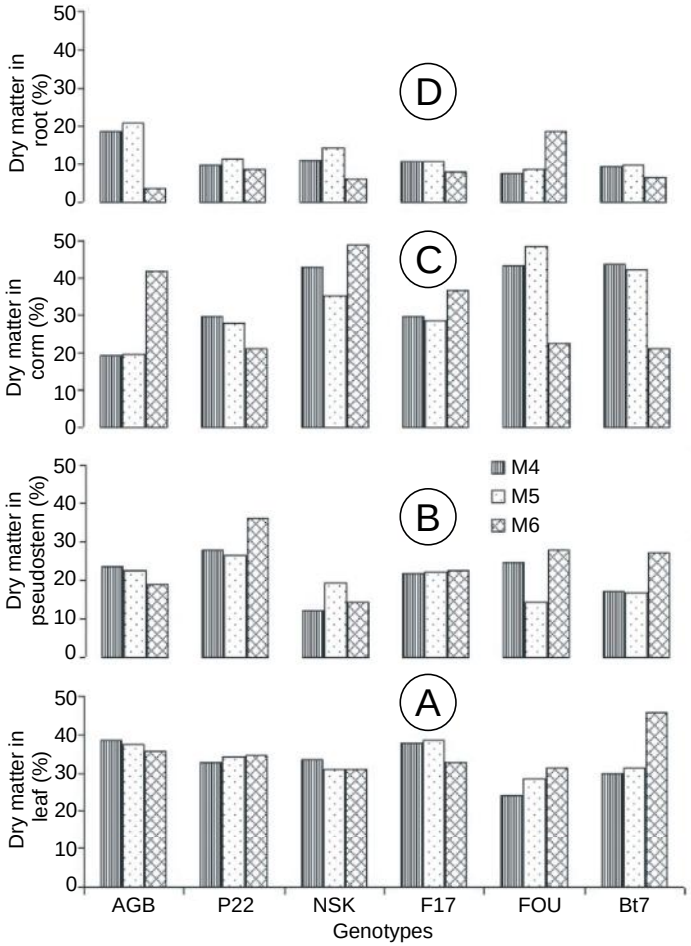

Fig. 2. Dry matter distribution pattern in six *Musa* genotypes as influenced by age at sampling and plant components (A: leaf; B: pseudostem; C: corm and D: root).

the dessert bananas had higher tissue N while 'Fougamou', a landrace cooking banana, had the lowest value of N in all the plant components considered. 'PITA 22', a plantain hybrid, contained the highest concentration of K in all the plant parts sampled. Also, 'BITA 7' had relatively high concentration of K in the tissues than the remaining four genotypes. Earlier field observation by the first author revealed that these two genotypes had impressive growth under limited water conditions when nine other genotypes went in to growth dormancy.

Table 5. Variation in nitrogen and potassium concentration in different plant components as influenced by age at sampling and genotypes

Genotype		Nitrogen (%)				Potassium (mg 100g^{-1})			
	Age	Root	Corm	Pseudostem	Leaves	Root	Corm	Pseudostem	Leaves
Agbagba	M4	1.64	1.79	1.23	2.05	35.0	73.0	1.2	46.0
	M5	1.65	1.85	1.42	2.01	51.0	76.0	2.0	48.0
	M6	1.37	2.26	1.44	1.95	137.0	87.0	3.0	53.0
PITA 22	M4	1.91	1.23	1.64	2.05	111.0	13.0	100.8	70.0
	M5	1.81	1.25	1.70	2.00	117.0	20.0	126.7	80.0
	M6	1.64	1.67	1.78	1.63	490.0	907.0	576.7	547.0
Nsukka Local	M4	4.37	1.64	2.19	2.46	84.0	75.0	1.1	25.0
	M5	3.85	1.68	2.41	2.44	83.0	76.0	2.0	30.0
	M6	3.03	2.01	2.40	2.28	132.0	90.0	2.0	40.0
FHIA 17	M4	3.28	1.78	2.19	2.32	81.0	46.0	84.1	50.0
	M5	3.31	1.75	2.15	2.27	90.0	48.0	92.0	59.0
	M6	2.73	1.89	2.05	1.71	101.0	92.0	80.0	50.0
Fougamou	M4	0.82	0.70	0.55	1.37	73.0	81.0	1.1	23.0
	M5	0.84	0.70	0.62	1.39	76.0	88.0	3.0	28.0
	M6	0.72	1.37	0.66	1.37	185.0	137.0	3.0	31.0
BITA 7	M4	2.19	1.10	3.01	2.87	148.0	64.0	86.2	56.0
	M5	2.15	1.16	3.13	2.81	156.0	69.0	92.7	57.0
	M6	1.58	1.50	3.03	1.96	177.0	138.0	117.0	69.0
LSD (*P*=0.05)		0.23	0.06	0.08	0.09	6.2	10.4	6.8	6.3

M4, M5 and M6 are plants at four, five and six months after transplanting, respectively.

Table 6. Variability in total nitrogen, total potassium and percent N and K distribution in plant components as influenced by genotype

Genotype	Total N (g plant^{-1})	Nitrogen distribution (%)				Total K (mg plant^{-1})	Potassium distribution (%)			
		Root	Corm	Pseudostem	Leaf		Root	Corm	Pseudostem	Leaf
Agbagba	1.49	13.8	30.8	17.3	38.0	44.3	17.2	44.6	1.0	37.2
PITA 22	1.30	10.9	21.9	31.4	35.7	248.3	13.3	15.1	40.3	31.2
Nsukka Local	1.98	19.7	35.8	17.2	27.3	53.5	20.0	64.8	0.6	14.6
FHIA 17	1.94	14.8	28.3	22.8	34.1	66.9	13.7	29.6	29.1	27.6
Fougamou	1.12	9.8	35.9	15.0	39.4	76.1	23.9	62.3	0.9	12.9
BITA 7	1.50	8.4	21.7	30.7	39.2	64.3	16.6	35.7	24.2	23.5
LSD (*P*=0.05)	NS	5.3	11.4	5.3	8.2	69.3	6.6	11.2	3.2	7.4

NS: non-significant

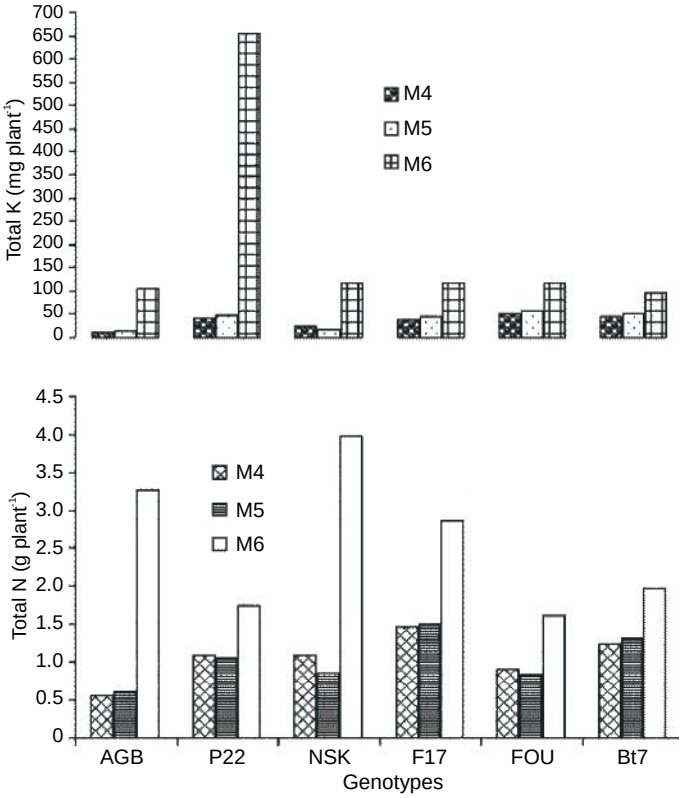

Fig. 3. The effect of genotype and age at sampling on total nitrogen (g plant^{-1}) and total potassium (mg plant^{-1}) per plant.

There was significant ($P < 0.05$) genotype by sampling age interaction on N and K concentration in the entire plant tissues sampled (Table 5). Generally, root N decreased with age while N concentration in the corm increased with age in all genotypes. N in pseudostem was relatively stable over time for each genotype, however, leaf N slightly declined with age of sampling in all genotypes. There was progressive increase in K concentration in the roots and corm with age of plant in all genotypes. Similarly, leaf K increased with plant age in all genotypes. K concentration was exceptionally low in the pseudostem of all the landrace genotypes. The quantity of K in 'PITA 22', a plantain hybrid, was very high in all the tissues sampled at the sixth MAP.

The total N accumulation (g plant^{-1}), K (mg plant^{-1}) and the percent contribution to these totals by the different plant parts is shown in Table 6. The total N was statistically similar for all the genotypes evaluated; however, higher quantity was accumulated by the dessert bananas ('Nsukka Local' and 'FHIA 17'). More than 60% of total N accumulated by the landraces was contributed by the corm and the leaves. But for the hybrid (except 'FHIA 17') total N was largely due to the contribution by the pseudostem and the leaves. The highest proportion of the total plant N was contributed by the leaves except in 'Nsukka Local'. The quantity of K accumulated varied significantly ($P < 0.05$) with genotypes. Total K accumulated was highest in 'PITA 22'(a plantain hybrid) whereas 'Agbagba' (a plantain landrace) contained the lowest amount of K. Data in Table 6 revealed that the quantity of K in the corm contributed most to the total K in landraces, whereas in the hybrid it was due to the leaves and pseudostem.

The quantity of N and K as influenced by age at sampling and genotype is shown in Fig. 3. Accumulation of N and K was highest at six MAP for all genotypes. The quantity of N was generally highest in the dessert bananas while the highest amount of K was found in 'PITA 22' at six MAP.

Discussion

Variability in N and K with age of plant as recorded in this study has been reported by other authors also (Twyford and Walmsley, 1974; Samuels *et al.* 1978; Lahav, 1995). This suggested the need for periodic external nutrient inputs. Periodic input through judicious fertilizer management will ensure adequate tissue concentration of these important elements. Adequate concentration will support optimum plant growth and development.

The significant differences in quantity of N and K accumulated by the genotypes depicted differential nutrient mining capacity, and so, fertilizer recommendation for optimum plant growth and development will vary. There was an evident difference in N and K uptake between landrace and hybrid within a genomic group, an indication that nutritional requirement varied, and fertilizer recommendation will therefore, also vary.

Non-significant differences in dry matter yield was probably due to differences in nutrient use efficiency (NUE). The implication is that detailed fertilizer study to elucidate NUE in *Musa* germplasm is urgently needed to assure profitable fertilizer input to bunch yield output ratio. However, Samuels *et al.* (1978) reported that dry matter yield of plantain is similar within the first five months of growth. They reported a surge in growth from the sixth month of planting similar to the results obtained in this experiment.

Pattern of N and K partitioning was influenced by the combined effects of plant age and genotype. Meaning that nutrient uptake from the rhizosphere and distribution pattern within the plant tissues was dependent upon plant type and age. Nutritional status of *Musa* is usually monitored via leaf analysis; the lamina of the third youngest leaf is recommended for sampling (Turner and Lahav, 1983; Lahav, 1995). Leaf N and K in this study was significantly influenced by genotype and age of plant. This

result further supports the view that nutritional management for optimum productivity should be genotype specific. While K increased with plant age, significant N decline at the 6 MAP was probably because available nitrogen in the potting mix had decreased through leaching and plant uptake. However, this result might suggest a more regular N supplementation via fertilizer application.

Evidences from the study suggested that higher N supplementation was needed for dessert bananas, whereas K uptake by 'PITA 22" was exceptionally high, suggesting the need for high K supplementation to sustain productivity in a ratooning cropping system. High K uptake by 'PITA 22' explained its high water economy under water stressed savanna environment (unpublished data of the first author). Potassium improves water use efficiency via osmotic regulation of plant stomata by modulating transpiration of water and the penetration of atmospheric carbon dioxide into the leaf (Fischer and Hsiao, 1968; Sawhney and Zelitch, 1969; IPI, 2006). Therefore, breeding *Musa* genotypes for water limited environment will be efficient by selecting genotypes that possessed high K mining capacity. Furthermore, results from this study revealed that in each genome group, hybrids accumulated higher quantity of K. Given the fact that both hybrids and landraces were planted in the same potting mix, it suggested that hybrids had higher K mining capacity, thus selection for high K uptake is possible within *Musa* germplasm. Higher K uptake by the hybrids probably explained higher productivity of hybrids than the landraces as commonly reported in relevant literatures.

Further studies to elucidate the physiological roles of K in *Musa*, especially with regards to sustainable production under water limited environments should urgently be carried out. This should enhance breeding *Musa* germplasm for water stress prone areas.

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