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ISSR, anthocyanin content and antioxidant activity analyses to characterize strawberry genotypes

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Abstract

Data on molecular markers, anthocyanin contents and antioxidant activities are increasingly used in breeding programs of many horticultural crops. Inter simple sequence repeat (ISSR) analysis, anthocyanin contents and antioxidant activities were used to characterize 10 strawberry (*Fragaria* x *ananassa* Duch.) cultivars and nine breeding lines. Fifteen primers generated 240 polymorphic ISSR-PCR bands. Cluster analysis by the unweighted pair-group method with arithmetic averages (UPGMA) revealed a substantial degree of genetic similarity among the genotypes ranging from 45% to 73% that were in agreement with the principal coordinate (PCO) analysis. Wide genetic diversity was observed among the strawberry genotypes for anthocyanin contents and antioxidant activities. The ISSR analysis together with data for antioxidant activities and anthocyanin contents in strawberries could be used for germplasm management and more efficient choices of parents in current strawberry breeding programs.

Key words: Anthocyanin content, antioxidant activity, *Fragaria* x *ananassa* Duch., ISSR markers, strawberry

Introduction

The cultivated strawberry (*Fragaria* x *ananassa* Duch.), an octoploid (2n = 56) hybrid between the Scarlet or Virginia strawberry (*F. virginiana* Duch.) and the pistillate South American *F. chiloensis* (L.) Duch., is a dicotyledonous, perennial low-growing herb grown in most arable regions of the world and is enjoyed by millions of people in all kinds of climates including temperate, Mediterranean, subtropical and taiga zones (Hancock *et al.,* 1991). Strawberries are good sources of natural antioxidants including carotenoids, vitamins, phenols, flavonoids, dietary glutathionine and endogenous metabolites and exhibit a high level of antioxidant capacity against free radical species: superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen (Wang and Jiao, 2000). The benefits of these high antioxidant activity fruit include reduction of carcinogens in humans (Chung *et al.,* 2002), protection against tumor development (Kresty *et al.,* 2001) and reversal of age-related effects on memory (Bickford *et al.,* 2000). Anthocyanins are typically present at high levels in strawberries and are thought to significantly contribute to the total antioxidative activity of this fruit (Wang *et al.,* 1997). Total antioxidant activity, as well as total anthocyanin content, can vary among cultivars, and these may affect overall protective benefits of human health and are worth further investigation.

There is a pressing need to develop reliable methods for identifying strawberry cultivars and for assessing genetic diversity/relatedness in strawberry genotypes for practical breeding purposes and proprietary-rights protection. Molecular markers are increasingly used in breeding programs of many horticultural crops. The introduction of molecular biology techniques, such as DNA-based markers, allows direct comparison of different genetic material independent of environmental influences (Weising *et al.,* 1995). The degree of similarity between the banding patterns can provide information about genetic similarity, and relationships between the samples studied.

Strawberries have been extensively analyzed for clone identification, mapping and diversity studies using randomly amplified polymorphic DNA (RAPD) markers (Graham *et al.,* 1996; Degani *et al.,* 2001; García *et al.,* 2002; Kuras *et al.,* 2004), amplified fragment-length polymorphisms (AFLPs) (Degani *et al.,* 2001; Tyrka *et al.,* 2002) and the simple sequence repeats (SSRs) (Cipriani *et al.,* 2006; Sargent *et al.,* 2006). Gil-Ariza *et al.* (2006) described expressed sequence tags (ESTs)-derived microsatellites from cultivated strawberry and their potential use for varietal identification and diversity study. Inter simple sequence repeat markers (ISSRs) (Zietkiewicz *et al.,* 1994) have been used successfully in a number of horticultural crops including blueberry (Debnath, 2009), lingonberry (Debnath, 2007) and strawberry (Arnau *et al.,* 2002; Debnath *et al.,* 2008). The ISSR primers target microsatellites that are abundant throughout the plant genome (Wang *et al.,* 1994). These markers have proven to be more reproducible than RAPD markers and generally reveal higher levels of polymorphism because of the higher annealing temperature and longer primer sequences (Qian *et al.,* 2001). They cost less and are easier to use than AFLPs and do not require prior knowledge of flanking sequences like SSRs (Reddy *et al.,* 2002).

The purpose of this study was to examine the level of genetic variation among 10 strawberry cultivars and nine advanced selections for antioxidant activity and anthocyanin content, to study genetic diversity using ISSR- PCR fingerprints and to compare the genotypic classification based on molecular marker with those of chemical marker. Although the scientific literature does include reports of ISSR analysis for strawberry (Arnau *et al.,* 2002; Kuras *et al.,* 2004; Debnath *et al.,* 2008), anthocyanin

content and antioxidant activity in relation to molecular analysis have not been documented in a given set of strawberries. The goal of the study was to combine data on anthocyanin contents and antioxidant activities with DNA analysis to better identify genotypes used in breeding programs.

Materials and methods

Plant material: For this study, the strawberry cultivars and advances selections (Table 1) developed by Agriculture and Agri-Food Canada Research Centres in St-Jean-sur-Richelieu, Quebec (QC); Kentville, Nova Scotia (NS); and in Agassiz, British Columbia (BC) in Canada were grown in 10.5 cm^2 plastic pots containing ProMix BX (Premier Horticulture Limited, Rivière-du-Loup, QC, Canada) potting medium, in the greenhouse under natural light conditions at a maximum photosynthetic photon flux of 90 µmol m^{-2} s^{-1} at 20 ± 2°C, 85% RH. Irrigation and artificial fertilization were applied when necessary.

DNA extraction: Leaf tissues used for DNA extraction were collected from actively grown shoots, immediately placed on ice and then frozen at -80°C until extraction. DNA was extracted by the GenElute Plant Genomic DNA Miniprep extraction kit (Sigma Chemical Co., Oakville, ON, Canada) following the manufacturer's instructions. The concentration of DNA was estimated spectrophotometrically (Ultrospec 2000, Pharmacia Biotech, Cambridge, UK) at 260 nm. DNA purity was measured by the ratio of the absorbance at 260 nm over 280 nm. Template DNA with an A_{260}/A_{280} ratio of 1.8: 2.1 in a dilution of 10 ng μL^{-1} was used for PCR.

ISSR primers, PCR amplification and electrophoresis: A set of 100 primers (UBC set 9) was procured from the Biotechnology Laboratory, University of British Columbia, Vancouver, Canada. Out of these, 15 (Table 2) that gave clear banding patterns were used in the final study. Different concentrations of template DNA and *Taq* polymerase were tested for optimal amplification. The optimized amplification reaction mixture (25 µL) contained 10 ng of DNA template, PCR buffer [50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$ and 0.001% (w/v) gelatin], 30 pmol primer, 200 µM of each dNTP, 1.25 U of *Taq* DNA polymerase (Sigma) and PCR grade dH_2O (Sigma). DNA Reaction mixtures were amplified in a PTC-100 Programmable Thermal Controller (MJ Research Inc., Watertown, MA, USA) using an initial hot start of 94°C for 10 min, followed by 45 cycles of 1 min at 94°C, 1 min at 45°C and 2 min at 72°C. The reaction was terminated with a final extension at 72°C for 10 min before holding the sample at 4°C for analysis. Amplified fragments, along with a 1kb DNA ladder (Invitrogen, Burlington, ON, Canada) used as a molecular weight standard, were resolved by 1.6% agarose gel electrophoresis in tris-borate-EDTA (TBE). After ethidium bromide staining for 35 min (0.5 µg L^{-1} of TBE) and a distilled water wash of 20 min, DNA was visualized using a GeneGenius gel documentation system (Syngene**,** Beacon House, Cambridge, UK). DNA amplification with each primer was repeated at least twice and congruence between replicates was verified. Gels were scored for polymorphic and monomorphic bands. Non-replicated bands were eliminated from analysis. Bands of similar molecular weight and migration distance across individuals were assumed to be homologous (Adams and Rieseberg, 1998).

Fruit extraction for chemical analysis: Mature ripe fruit, with a well-developed red colour, were harvested from three replicates per genotype and frozen immediately at -20°C until analysis. Four g of berries from each genotype were homogenised overnight at 4°C in 2.5 mL ethanol : 1.5 M HCl (85:15 v/v) to extract anthocyanins. The extracts were filtered through 0.2 µM syringe filters before analysis.

Total anthocyanin content: The total anthocyanin content was measured in triplicate by the pH differential method as described

Table 1. Parentage, anthocyanin contents and antioxidant activities of strawberry genotypes used in inter simple sequence repeat (ISSR) analysis

Genotype	Code	Parentage	Origin in Canada[a]	Anthocyanins (mg $100g^{-1}$ fr wt)	DPPH (ED_{50})[b]
Cultivar					
Bounty	BO	(Jerseybelle × Senga Sengana)	Kentville, NS	27.3 ± 5.5	27.5 ± 7.4
Cavendish	CA	(Glooscap × Annapolis)	Kentville, NS	31.3 ± 4.3	9.4 ± 0.42
Clé des champs	CL	(SJ89244-6E × SJ8518-11)	Saint-Jean-sur-Richelieu, QC	16.1 ± 4.3	23.1 ± 4.0
Kent	KE	[(Redgauntlet × Tioga) × Raritan]	Kentville, NS	25.4 ± 3.1	23.2 ± 7.3
Micmac	MC	(Tioga × Guardsman S1)	Kentville, NS	27.0 ± 4.7	10.7 ± 0.13
Mira	MR	(Scott × Honeoye)	Kentville, NS	21.0 ± 14.7	39.5 ± 1.6
Rosalyne	RO	[Fern × (SJ9616-1 × Pink Panda)]	Saint-Jean-sur-Richelieu, QC	23.6 ± 2.9	19.0 ± 11.0
Saint-Pierre	SP	(Chandler × Jewel)	Saint-Jean-sur-Richelieu, QC	14.1 ± 2.3	11.6 ± 0.10
Stolo	SL	(Puget Reliance × Whonnock)	Agassiz, BC	16.3 ± 2.5	16.7 ± 1.3
Wendy	WE	[{Sable x (Cavendish x Selkirk)} H Evangeline]	Kentville, NS	35.1 ± 6.1	20.3 ± 4.0
Advanced selections					
APF9313-126	AP	Unreleased breeding line	Saint-Jean-sur-Richelieu, QC	22.6 ± 1.0	19.0 ± 0.32
BC92-20-85	B1	(Cavendish × Nanaimo)	Agassiz, BC	43.7 ± 5.3	20.2 ± 2.7
BC96-1-7	B2	(Marmolada × Nanaimo)	Agassiz, BC	15.4 ± 2.5	27.6 ± 7.5
FIN005-7	F1	Unreleased breeding line	Saint-Jean-sur-Richelieu, QC	23.7 ± 5.0	20.63 ± 6.5
FIN005-55	F2	Unreleased breeding line	Saint-Jean-sur-Richelieu, QC	22.2 ± 5.0	21.0 ± 1.9
FIN0016-115	F3	Unreleased breeding line	Saint-Jean-sur-Richelieu, QC	32.5 ± 1.3	23.4 ± 4.7
KRS-10	KR	(K94-15 × K95-24)	Kentville, NS	10.5 ± 4.4	18.6 ± 1.5
SJO001-99	S1	Unreleased breeding line	Saint-Jean-sur-Richelieu, QC	6.3 ± 1.2	19.4 ± 3.2
SJO9611-23	S2	Unreleased breeding line	Saint-Jean-sur-Richelieu, QC	41.7 ± 4.8	18.7 ± 4.4

[a]BC = British Columbia, NS = Nova Scotia, QC = Quebec. [b]ED50 value is used to express the concentration of an antioxidant required to quench 50% of the initial 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Each value is expressed as mean ± standard deviation (n = 3).

by Foley and Debnath (2007). Absorbance was measured in a spectrophotometer (Ultrospec 2000; Pharmacia Biotech, Cambridge, UK) at 510 nm and 700 nm in buffers at pH 1.0 and pH 4.5. Absorbance readings were converted to total milligrams of cyanidin 3-glucoside per 100 g fresh weight of strawberry using the molar extinction coefficient of 26 900 and absorbance of $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$ (Meyers *et al.,* 2003).

Anti-oxidant activity analysis: The anti-oxidant activity in each fruit extract was measured, in triplicate, using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH, from Sigma-Aldrich Co., St. Louis, MO, USA) method as previously described (Anttonen *et al.,* 2006; Foley and Debnath, 2007). Using a calibration curve with different amounts of DPPH, the ED_{50} was calculated. The ED_{50} is the concentration of an antioxidant that is required to quench 50% of the initial DPPH radicals under the given experimental conditions.

Data analysis: The ability of the most informative ISSR primers to differentiate between genotypes was assessed by calculating their resolving power (Rp) (Gilbert *et al.,* 1999):

$Rp = \sum Ib$, where, $Ib = 1 - (2 \times |0.5 - p|)$,
where, p is the proportion of the 19 genotypes containing the band. The value of Ib was calculated for 15 informative ISSR primers.

Presence or absence of each ISSR fragment was coded as 1 and 0, where 1 = the presence of a specific allele, and 0 = its absence. Since ISSR markers are typically dominant, it was assumed that each band represented the phenotype at a single bi-allelic locus. The presence of a band was interpreted as either a heterozygote or a dominant homozygote, and the absence of a band as a recessive homozygote. The basic data structure finally consisted of a binomial (0/1) matrix, representing the scored ISSR markers. The similarities were calculated using the following coefficients, described by Sneath and Sokal (1973): *Simple Matching coefficient*: $S_{ij} = (a+d)/(a+b+c+d)$; *Dice's coefficient*: $S_{ij} = 2a/(2a+b+c)$, equivalent to the coefficient of Nei and Li (1979); and *Jaccard's coefficient*: $S_{ij} = a/(a+b+c)$, where S_{ij} is the similarity between two individuals, i and j; a = number of bands shared by both individuals, b = number of bands presented by i, but not by j; c = number of bands presented by individual j but not by i; and d = number of bands absent in both. Jaccard's and Nei and Li's (1979) coefficients calculate similarities based on the shared presence, but not the absence, of DNA bands. The similarity matrix was used as input data for cluster analysis by applying unweighted pair-group method with arithmetic averages (UPGMA), and to compute a principal coordinate (PCO) analysis (Gower, 1966) using NTSYS-pc (Version 2.1) software (Rohlf, 1998). The SAHN option was used to cluster the data according to the UPGMA method. Co-phenetic matrices were created from the dendrogram and compared with the similarity matrix using the Mantel matrix comparison function in NTSYS to test whether clusters in the dendrogram agreed with information from the similarity matrix. The cophenetic correlation coefficient (CCC) value obtained gives a goodness of fit for the clusters (Rohlf ,1998).

Data for anthocyanin contents and antioxidant activities were subjected to analysis of variance (ANOVA) using the SAS statistical software package (Release 8.2; SAS Institute, Inc., Cary, NC, USA). Statistical F-tests were evaluated at $P \leq 0.05$. Differences among treatments were further analysed using Duncan's multiple range test. For cluster analysis, variables were standardized using STAND module, similarity matrices (in SIMINT) were generated using Euclidean distance, and the dendrogram was constructed using the UPGMA with NTSYS-pc (Version 2.1) software (Rohlf, 1998).

Results

ISSR amplification: Of the 15 selected primers, 12 (seven 3′ mono-anchored and five 3′ di-anchored) were designed to anneal to di-nucleotide repeats, two to tetra-nucleotide repeats and one to mixed nucleotide repeats. The primers revealed the presence of polymorphisms in the amplified DNA fragments in a range from 300 to 4100 bp. The number of polymorphic bands produced ranged from 13 for the 811 and 879 primers to 20 for the 876 primer, with an average of 16 bands per primer (Table 2). The ability of the 15 most-informative primers to differentiate strawberry genotypes was assessed on the basis of Rp. The Rp values varied from 5.5 for primers 811 and 879 to 11.5 for 876. Primers with higher Rp values were generally able to distinguish more genotypes and showed higher polymorphic bands (Table 2).

Genetic relationships among strawberry genotypes: The Dice, Jaccard and the Simple Matching coefficients were used to generate similarity matrices for ISSR cluster analysis. The CCC relates the level of distoration between the similarity matrix and cluster analysis, a higher CCC indicates a better fit. The CCCs of the Dice, Jaccard and Simple Matching-based cophenetic matrices were 0.83, 0.83 and 0.81, respectively. Despite the similar discriminating power of both Dice-and Jaccard-based co-phenetic matrices, genetic similarity values were lower for Jaccard than for Dice (data not shown). This result is expected because the Dice index differs from the Jaccard index by the higher weight that it gives to coincidences of the presence of a band in relation to non-coincidences. The UPGMA clustering algorithm based

Table 2. Inter simple sequence repeat (ISSR) primers: their sequence, anchored end, repeat motif and the data on DNA profile and polymorphism generated in 10 strawberry cultivars and nine advanced selections using 15 ISSR primers

Primer Code[a]	Sequence[b]	Polymorphic bands		Resolving power
		Number	Size range (bp)	
807	$(AG)_8$ T	15	400-2200	7.4
808	$(AG)_8$ C	18	500-2400	8.4
810	$(GA)_8$ T	18	500-3100	9.3
811	$(GA)_8$ C	13	500-2700	5.5
817	$(CA)_8$ A	18	400-3200	10.4
823	$(TC)_8$ C	14	500-3300	6.8
827	$(AC)_8$ G	18	400-3700	7.9
834	$(AG)_8$ YT	17	300-2300	7.5
835	$(AG)_8$ YC	15	400-2400	6.8
836	$(AG)_8$ YA	16	500-2100	10.4
840	$(GA)_8$ YT	15	400-2500	8.8
841	$(GA)_8$ YC	14	400-2200	6.8
876	$(GATA)_2$ $(GACA)_2$	20	400-4100	11.5
879	$(CTTCA)_3$	13	700-3300	5.5
895	AGA GTT GGT AGC TCT TGA TC	16	500-2800	7.3

[a] Primer numbers follow those in UBC set 9 (no. 801 - 900).
[b] Y: C,T.

on Dice's and Jaccard's similarity matrix gave similar results (data not shown). Results on Dice-based matrix clustering are described hereafter. UPGMA clustering identified three clusters at a similarity index of approximately 0.54 leaving APF9313-126 as an outlier at 0.45 similarity index. Within Cluster I, two sub-clusters were resolved at approximately 57% similarity value: the first one with seven cultivars and three breeding lines, and the second sub-clusters with two cultivars and two breeding lines. In the first sub-cluster of Cluster I, Bounty, FIN005-7 and FIN0016-115 were grouped together with about 60% similarity and were separated from six cultivars and one advanced line that formed a sub-sub-cluster, at a similarity index of approximately 0.59. Cavendish and Kent had the maximum similarity (73%), and both were separated from BC96-1-7 at a similarity value of about 0.64. Mira, Saint-Pierre, Wendy and Stolo formed a group in the first sub-cluster of Cluster I at approximately 0.65 similarity index. In the second sub-cluster of Cluster I, Micmac was separated from KRS-10, Rosalyne and FIN005-55 at a similarity value of approximately 0.57. The later two were grouped together with 63% similarity and both were separated from KRS-10 at about 0.60 similarity index. Clé des champs and BC92-20-85 formed Cluster II with 56% similarity, and SJO001-99 and SJO9611-23 formed Cluster III with approximately 58% similarity (Fig. 1).

PCO analysis on the frequencies of occurrence of the polymorphic ISSR markers among the genotypes showed that the plotting of the first three components, representing 11.0, 9.2 and 8.2% of the total variation for the first, second and the third component, respectively, indicating existence of significant variation among the genotypes. Most of the genotypes are separated by the first or second or third PCO (Fig. 2) which demonstrates distinct groups of genotypes corresponding to cluster analysis (Fig. 1). The genotypes Kent, Stolo, Wendy, Saint-Pierre, Mira and BC96-1-7 were grouped together by the third axis (Fig. 2) as was also observed by cluster analysis where they were grouped together at a similarity value of 0.63 within the first sub-cluster of Cluster I (Fig. 1). Similarly, Rosalyne, FIN005-55 and KRS-10 were in a group corresponding to cluster analysis where they were grouped together within second sub-cluster of Cluster I at a similarity value of about 0.60. However, FIN005-55 and FIN0016-115 were tightly clustered in PCO analysis although they were well separated in cluster analysis (Fig. 1). The third axis revealed another level of separation for BC92-20-85 and Clé des champs (Fig. 2) and these were not apparent from the dendrogram.

Antioxidant activities and anthocyanin contents: Significant differences ($P \leq 0.05$) were observed among genotypes tested for anthocyanin contents and for antioxidant activities (data not shown). Among the 19 genotypes tested for anthocyanin contents, BC92-20-85 fruits had the highest total anthocyanin content and it was followed by SJO9611-23. The unreleased advanced line SJO001-99 had the lowest anthocyanin content (Table 1).

The strawberry genotypes differed in their activity to react and quench DPPH radicals (Table 1). The ED_{50} value is used to express the concentration of an antioxidant required to quench 50% of the initial DPPH radicals under the experimental conditions given. A smaller ED_{50} value corresponds to a greater DPPH radical scavenging activity. While Cavendish was the best for antioxidant activity, Mira was the worst (Table 1). The correlation between total anthocyanin content and antioxidant activity was positive but insignificant (r = 0.06).

The relationships between the 19 genotypes revealed by UPGMA

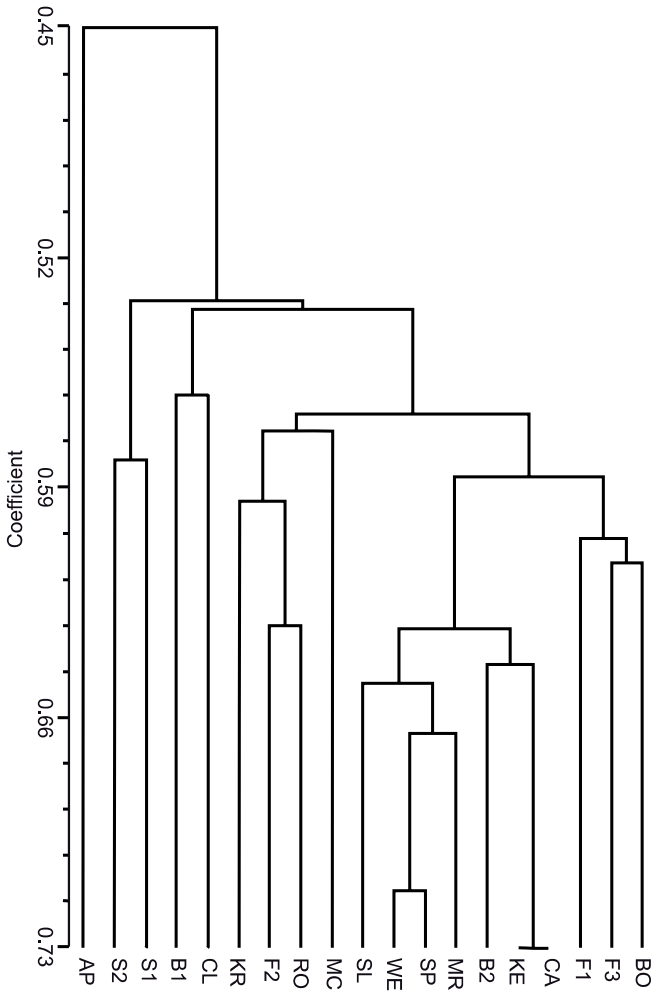

Fig. 1. Unweighted pair-group method with arithmetic averages (UPGMA) dendrogram estimating the genetic distance among 19 strawberry genotypes designated by codes given in Table 1, based on inter simple sequence repeat (ISSR) coefficient- derived Dice matrix.

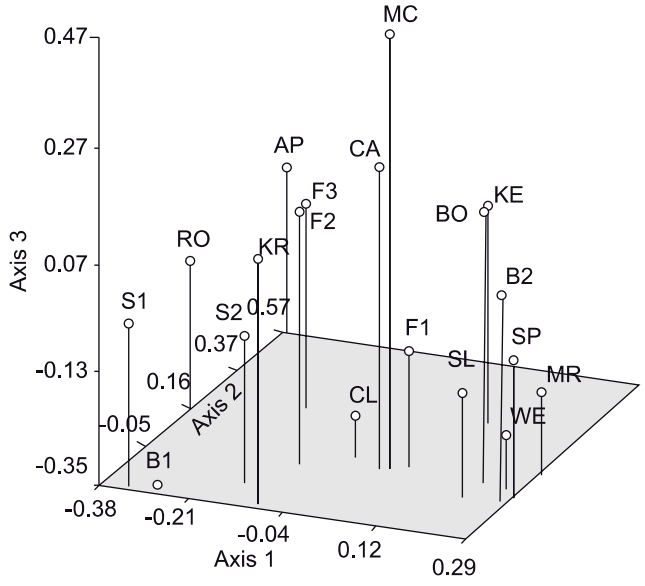

Fig. 2. Three-dimensional plot of the principal coordinate (PCO) analysis of distance among 19 strawberry genotypes designated by codes given in Table 1, on inter simple sequence repeat (ISSR) markers.

cluster analysis based on anthocyanin content and antioxidant activity data and Euclidean distance are presented in Fig. 3. Two major clusters were resolved leaving FIN005-7 as an outlier. While all cultivars and six advanced selections formed Cluster I, two advanced lines BC92-20-85 and SJO9611-23 were grouped in Cluster II. As was observed in the ISSR analysis (Figs. 1 and 2), the cultivars were intermixed with selections in both anthocyanin content and antioxidant activity-based dendrogram. Within Cluster I, two sub-clusters were evident. Within the first sub-cluster of Cluster I, Bounty, Micmac and Kent formed a group and were separated from the other group consisting of Mira, Rosalyne, APF9313-126 and FIN005-55 at a coefficient value of approximately 0.42. These two groups were again separated from a sub-sub-cluster of Cluster I consisting of Cavendish, FIN0016-115 and Wendy, at approximately 0.86 coefficient value. Within the second sub-cluster of Cluster I, Clé des champs and Stolo possessed the minimum distance (0.02 coefficient value) with each other and both cultivars grouped with BC96-1-7 and Saint-Pierre at a coefficient value of approximately 0.19 forming a sub-sub-cluster within the second sub-cluster of Cluster I. Two advanced selections, KRS-10 and SJO001-99 formed another sub-sub-cluster within the second sub-cluster of Cluster I (Fig. 3).

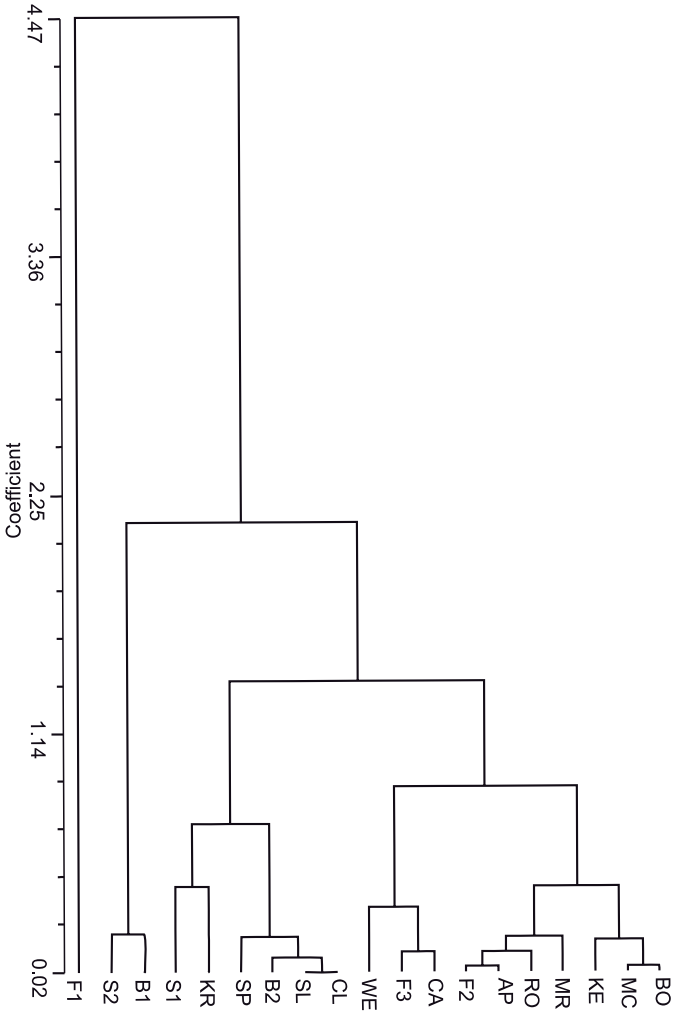

Fig. 3. Unweighted pair-group method with arithmetic averages (UPGMA) dendrogram estimating the genetic distance among 19 strawberry genotypes designated by codes given in Table 1, based on anthocyanin content and antioxidant activity data.

Discussion

The ISSR and chemical markers were used in this study to evaluate the levels of genetic relatedness among 10 strawberry cultivars and nine advanced selections. Fifteen primers detected enough genetic variation and relatedness among the genotypes to allow for complete differentiation as was revealed from PCR-derived gel analysis. The ISSR primers varied in their ability to distinguish strawberry genotypes, and primers with high Rp values were generally more effective in distinguishing between genotypes (data not shown). This is in agreement with the findings in lupin (Gilbert *et al.,* 1999) and lingonberry (Debnath, 2007). The results clearly demonstrate that ISSR markers can be used in a genetic diversity study as well as in genotypic identification of strawberries, as noted by Kuras *et al.* (2004) and Arnau *et al.* (2002) or in helping breeders to select diverse parents with similar phenotypes for breeding.

Two different methods of multivariate molecular analysis, PCO and cluster analysis, were used to group the individuals in this study, as each method is designed to sort the data on a different basis and therefore can be used comparatively to elucidate relationships. The UPGMA clustering algorithm assumes a constant evolutionary rate among accessions or clones. The results of the present study showed a significant level of genetic relatedness among strawberry cultivars and lines. The strawberry cultivars and advanced selections showed 45 to 73% genetic similarity. Graham *et al.* (1996) found a level of similarity for strawberry cultivars ranging from 62 to 89% indicating their closely related nature even though they resulted from four independent breeding programs (Scotland, England, USA and The Netherlands). Similar results have also been reported by Tyrka *et al.* (2002) confirming small gene pool variation in strawberry cultivars. In the present study, out of 19 strawberry cultivars and lines, 14 were grouped in one cluster with about 54% similarity. The five cultivars originating in NS: Bounty, Cavendish, Kent, Mira and Wendy, have 59% similarity. Degani *et al.* (2001) reported 73 and 86% similarity by RAPD and AFLP analysis respectively, between two NS cultivars, Annapolis and Kent. Although BC92-20-85 did not cluster with its parent Cavendish, they had a relatively high similarity value of 54%. Cavendish and Kent, although developed from two different crosses, were grouped together with 73% similarity (Fig. 1). This is unexpected as these cultivars have very different genetic backgrounds. Similar results were also reported by Degani *et al.* (2001) where a UPGMA-derived dendrogram based on genetic similarities from banding data did not reflect known pedigree information. These might be due to incomplete coverage of the genome (Degani *et al.,* 2001).

Strawberries are a good source of dietary antioxidants. The present data provide evidence of a genetic similarity and divergence for anthocyanin contents and for antioxidant activities among the tested genotypes. Previous studies reported variation in anthocyanin content and antioxidant activity in strawberry genotypes (Meyers *et al.,* 2003; Anttonen *et al.,* 2006). The observed poor correlation between anthocyanin content and antioxidant activity indicated that this fruit antioxidant did not play a major role in increasing antioxidant activity in the present material. Similar results have also been reported in strawberry (Hansawasdi *et al.,* 2006).

Out of 19 strawberry genotypes, 14 were grouped together in one cluster by ISSR and 16 by chemical UPGMA analysis (Figs. 1 and 3). However, clustering based on ISSR data was different from that based on the chemical data. ISSR markers are distributed throughout the genome and in the majority of cases most regions of the genome are not expressed at the phenotypic level. The non-coding regions (un-expressed) of genome are not accessible to phenotypic expression and might have resulted in disagreement between the chemical and molecular diversity. The weak correspondence between genetic distances from chemical and ISSR data most probably implies that these markers differ in their degree of genomic coverage. García *et al.* (2002) failed to correlate morphological and RAPD characterization in strawberry.

In conclusion, this study should facilitate the use of chemical analysis of fruits and of ISSR fingerprints for marker-assisted applications in strawberry breeding. The study identified promising genotypes with anthocyanin contents and high antioxidant activities. Fifteen ISSR primers that generated substantial polymorphism among the strawberry genotypes have also been identified. As ISSRs have higher reproducibility and reveal higher levels of polymorphism than RAPDs (Qian *et al.,* 2001), cost less and are easier to use than AFLPs, and as they do not require prior knowledge of flanking sequences like SSRs (Reddy *et al.,* 2002), similar genetic analysis might be applicable to other strawberry genotypes. Given the resource limitations on identifying genotypes at the molecular level, this study demonstrated the use of ISSR markers combined with chemical markers as potential quality-assurance tools to identify and maintain diverse genotypes and helps assess the genetic diversity of strawberry genotypes. The resultant molecular markers can also be used for pre-selection in seedling populations, to discard unfavourable genotypes at an early stage and to identify successful crosses between different strawberry genotypes.

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The effect of high daytime temperatures on inhibition of flowering in 'Koroneiki' olives (*Olea europaea* L.) under chilling and non-chilling nighttime temperatures

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Abstract

Regulation of flowering in 'Koroneiki' olives by various regimes of daytime and nighttime temperatures was investigated. The trees flowered profusely under chilling (2.5°C; 569 inflorescences tree^{-1}) and non-chilling nighttime temperatures (8.3°C; 729 inflorescences tree^{-1}) when daytime temperatures were kept optimal (18.3°C). Chilling nighttime temperatures (2.5°C) did not produce any greater number of inflorescence than non-chilling temperatures of 8.3°C. High daytime temperatures (26.6°C) strongly inhibited flowering at both chilling and non-chilling nighttime temperatures (*i.e.*, 0.5 and 0.0 inflorescences tree^{-1} under chilling and non-chilling temperatures, respectively). Mildly high daytime temperatures (23.9°C) also inhibited flowering but there were significantly more inflorescences per tree at 23.9°C (220 and 127 inflorescences tree^{-1} under chilling and non-chilling nighttime temperatures, respectively) than at 26.6°C. There was no significant difference in the number of inflorescences tree^{-1} between chilling and non-chilling nighttime temperatures at both inhibitory daytime temperatures; *i.e.* 23.9°C and 26.6°C. The trees that were kept vegetative by high daytime temperatures (26.6°C), but given flower inducing nighttime temperature for three months, when returned to optimal flower inducing conditions did not flower before the normal induction period (70-80 days), indicating that inhibitory daytime temperatures canceled any effects of nighttime flower inducing temperatures. Surprisingly, trees kept vegetative in growth chamber at a high daytime temperature (26.6°C) produced fewer inflorescences compared to trees kept vegetative in the greenhouse where temperatures were less controlled but generally, with a few exceptions, remained between 15-20ºC in the night and 25-30ºC during day**.**

Key words: flowering, inflorescence, *Olea europaea* L., olive, temperature effects.

Introduction

Temperature induced regulation of flowering in olives is extensively studied by Hartmann and associates (Hackett and Hartmann, 1964, 1967; Hartmann, 1953; Hartmann and Opitz, 1980; Hartmann and Porlingis, 1957; Hartmann and Whisler, 1975). Denney and McEachern (1983), reviewed Hartmann and associates work and defined ≤7.2°C as the "daily minimum temperature" for olive flowering while minimum temperatures ranged between 1.7-4.4°C and maximum between 15.6-18.3°C were considered optimal temperatures. Similarly, Lavee (1985) reviewed olive literature and suggested that maximal flower induction in olives required temperature changes "from low (2-4°C) to high (15-19°C)". It takes 70-80 days of exposure to optimal temperature conditions to obtain optimum inflorescence in olive trees (Hartmann and Whisler, 1975).

While possibilities of flower initiation in olive without significant chilling were proposed by Pinney and Polito (1990), we experimentally demonstrated that flowering in 'Arbequina' cultivar can be achieved without any significant chilling hours (Malik and Bradford, 2005b); none of the researchers listed above included 'Arbequina' (an important oil producing cultivar) in their studies. From this finding, it was hypothesized that inhibition of flowering in areas like Weslaco, Texas, is perhaps due to the inhibitory effects of high daytime temperatures rather than lack of sufficient chilling hours. This hypothesis was supported by the results where flowering in 'Arbequina' in the field at Weslaco was achieved through shading and evaporative cooling to protect from inhibitory effects of high daytime temperatures (Malik and Bradford, 2005c). Further confirmation of this concept was provided by growth room experiments where flowering was strongly inhibited by a few degrees higher daytime temperature while the nighttime temperatures were maintained just above chilling temperatures (Malik and Bradford, 2006).

In these studies we further investigate the interactions of day and nighttime temperatures in regulating flowering in olives. First, another early maturing (*i.e.* the trees start flowering within two years) cultivar 'Koroneiki' was tested for flowering under non-chilling conditions and the effectiveness of these temperatures to induce flowering was compared with the extent of flowering in trees that were given optimal chilling temperatures. Similarly, the effect of high daytime temperatures was also studied at non-chilling (low temperature of 8.3°C) and at chilling temperatures (2.5°C). In addition, plants in which flowering had been inhibited during winter induction period due to high daytime temperatures (even though they were given flower-inducing temperatures in the night) were placed back in a growth room with optimal flower inducing conditions to see if flowering in these trees would occur in a shorter period of time. Results of these studies are reported here.

Materials and methods

Three-year-old olive trees of cultivars 'Koroneiki' (also labeled I-38 which is a clonal selection of 'Koroneiki'; commonly used in Texas) were purchased from Texas Olive Ranch in Carrizo Springs, Texas (N 28.5 latitude; W 99.9 longitude and 184 m

elevation). The trees were repotted in 16 L pots filled with peat, perlite, and sand mixture and supplied with nutrients as described previously (Malik and Bradford, 2006). The trees were grown at the USDA-ARS facility (in open field under 30% shade) in Weslaco, Texas for 8 months before testing in growth chamber experiments. During this 8 months period (March 15-November 15, 2006), temperatures in field varied considerably. The average monthly, maximum/minimum temperatures (°C), from March to November were; 27/15, 29/18, 31/24, 32/25, 31/26, 31/25, 30/26, 30/17, and 27/15. Four walk-in type growth chambers that were locally constructed based on our previously published design were used for testing different day and night temperature regimes, as described below for each experiment, on flowering in 'Koroneiki' olives (Malik and Bradford, 2005a). Temperatures in each growth chamber and the greenhouse were monitored at 30 min intervals and the data transferred to Excel spread sheet for processing as described previously (Malik and Bradford, 2006). Time of appearance of floral buds in stage 1, as described earlier (Malik and Bradford, 2007), was noted in each treatment, and total number of inflorescence and number of flowers per inflorescence were measured at the conclusion of the experiment. In addition, height of the trees (m) and the diameter of the trunk (mm), 8 cm above the soil, were measured. The following experimental conditions were used for each of the experiments.

Experiment 1: Fifteen replicate trees were tested under each of the following treatments in the four walk-in growth chambers.

Treatment 1. Nighttime temperature 2.5 ± 0.5°C, daytime temperature 18.3 ± 0.5°C.

Treatment 2. Nighttime temperature 2.5 ± 0.5°C, daytime temperature 26.6 ± 0.5°C.

Treatment 3. Nighttime temperature 8.3 ± 0.5°C, daytime temperature 18.3 ± 0.5°C.

Treatment 4. Nighttime temperature 8.3 ± 0.5°C, daytime temperature 26.6 ± 0.5°C.

Nighttime and daytime temperature regimes in growth chambers were maintained for 8 hrs each period. At the end of each period the temperature was gradually increased or decreased as reported previously (Malik and Bradford, 2006). Details of daily temperature changes in each treatment are given in Table 1. The growth chambers utilize natural light (Malik and Bradford, 2005a) and therefore light intensities changed with time in the day and on cloudiness; on a bright day, light intensity measured in the growth chambers was 1303 µmole m^{-2} s^{-1}. The above temperature regimes were strictly maintained during the three month winter induction period (November 15 to February 14). By that time, flowering buds became visible under non-chilling conditions such as Treatment 3, but for chilling treatments nighttime temperatures were gradually increased (2.5°C→5.5°C→8.3°C→11.1°C) to release flower buds from dormancy. Denney and McEachern (1983) defined chilling temperatures (after thoroughly reviewing previous research) for olives as "daily minimum temperature ≤ 7.2°C". They also defined optimal temperatures when maximum temperatures are within 15.6-18.3°C and minimum temperatures are within 1.7-4.4°C. We have followed this terminology here, and therefore, for the purpose of differentiating we have referred to 8.3°C as non-chilling temperature here. Temperatures in the greenhouse were less controlled and fluctuated considerably as daytime temperatures varied between 25-30°C and night temperatures between 15-20°C except a few days when the daytime temperatures dropped below 25°C and for only a few hours nighttime temperatures dropped below 15°C in November and then again in January.

Experiment 1A: Trees from Treatments 2 and 4 of the above Experiment 1 where flowering was strongly inhibited due to high daytime temperatures, as described above, were placed in optimal flowering conditions in a chamber maintained at Treatment 3 temperature (see Table 1 for details on temperature regimes). Temperature conditions of Treatment 3 have consistently given extensive flowering in the past and in this study (Malik and Bradford, 2005b, 2005c, 2006). In addition to 15 replicate trees for each of Treatments 2 and 4 of Experiment 1, four trees kept in the greenhouse were also placed in the same chamber under optimal flowering conditions of original Treatment 3. Thus, Treatment 1 of Experiment 1A was previously Treatment 2 of Experiment 1. Treatment 2 of Experiment 1A was previously Treament-4 of Experiment 1. Treatment 3 of Experiment 1A was previously trees kept in the greenhouse. After placing all the trees of treatments 1-3 in one growth chamber, the temperature in the growth chamber was maintained at nighttime temperature of 8.3 ± 0.5°C, and daytime temperature of 18.3 ± 0.5°C for ninety days. Under these conditions, inflorescences begin to appear in 70 days.

Table 1. Daily temperature regimes for each treatment in each experiment

Time			Experiment 1 , Treatments				Experiment 1A, Treatments			Experiment 2 , Treatments		
From	Until	Hours	1	2	3	4	1[b]	2[c]	3[d]	1	2	3
0:00	5:00	**5:00**	**2.5**[a]	**2.5**	**8.3**	**8.3**	**8.3**	**8.3**	**8.3**	**2.5**	**8.3**	**8.3**
5:00	8:00	3:00	7.2	10.0	12.8	12.8	12.8	12.8	12.8	10.0	12.8	12.8
8:00	10:00	2:00	12.8	23.3	15.6	23.3	15.6	15.6	15.6	18.3	20.0	15.6
10:00	18:00	**8:00**	**18.3**	**26.6**	**18.3**	**26.6**	**18.3**	**18.3**	**18.3**	**23.9**	**23.9**	**18.3**
18:00	19:00	1:00	15.6	23.3	15.6	23.3	15.6	15.6	15.6	18.3	20.0	15.6
19:00	20:00	1:00	12.8	18.3	12.8	18.3	12.8	12.8	12.8	12.8	18.3	12.8
20:00	21:00	1:00	7.2	10.0	10.0	10.0	10.0	10.0	10.0	7.2	10.0	10.0
21:00	24:00	**3:00**	**2.5**	**2.5**	**8.3**	**8.3**	**8.3**	**8.3**	**8.3**	**2.5**	**8.3**	**8.3**

[a]Temperature in °C ± 0.5.

[b]After the conclusion of Experiment 1, its Treatment 2 was placed under this inductive temperature regime.

[c]After the conclusion of Experiment 1, its Treatment 4 was placed under this inductive temperature regime.

[d]Trees from Greenhouse under none inductive conditions throughout (since the start of Experiment 1) were placed under inductive conditions.

The temperature of the growth chamber was raised after 90 days and total number of inflorescence were counted after 2 weeks.

Experiment 2: A second experiment was conducted to study differences, if any, between chilling and non-chilling nighttime temperatures on the inhibitory effects of high day time temperatures but approximately 3 degrees less than the temperatures used in Experiment 1 (*i.e.* 23.9°C instead of 26.6°C). Four replicate trees from the greenhouse were placed in each of the following temperature regimes (Table 1).

Treatment 1. Nighttime temperature 2.5 ± 0.5°C, daytime temperature 23.9 ± 0.5°C.

Treatment 2. Nighttime temperature 8.3 ± 0.5°C, daytime temperature 23.9 ± 0.5°C.

Treatment 3. Nighttime temperature 8.3 ± 0.5°C, daytime temperature 18.3 ± 0.5°C.

These temperature regimes were also maintained for three months.

Statistical analysis: Results of different replicate of each treatment in the experiment were subjected to one way analysis of variance using SAS software package (version 9.1). The L.S.D. test of significance between means was determined at $P < 0.05$.

Results and discussion

It is noteworthy that nighttime chilling temperatures of 2.5°C under optimal daytime temperatures (18.3°C) did not produce any greater number of inflorescence compared to 8.3°C nighttime temperatures (Table 2) even though earlier researcher reported a temperature of 2.5°C as optimal chilling temperature for inducing maximal flowering in olives (Badr and Hartmann, 1971; Lavee, 1985). On the contrary, there appeared nearly 28% increase in inflorescences per tree under non-chilling nighttime temperatures (8.3°C) over the trees that were kept under optimal chilling conditions (2.5°C) (Table 2). There was also no difference in number of flowers per inflorescence in trees kept under optimal nighttime chilling conditions versus the ones that were placed in a non-chilling environment (Table 2). While these results are consistent with our previous findings that non-chilling nighttime temperatures (8.3°C) are quite effective for inducing flowering in another cultivar ('Arbequina') of olive (Malik and Bradford, 2005b, 2005c), the present finding go one step further by demonstrating that equally effective flowering, compared to trees kept under optimal chilling conditions (2.5°C), can be achieved in 'Koroneiki' olives when they were never exposed to temperatures below 7.2°C; *i.e.* chilling temperatures. Rallo and Martins (1991) have shown the release of dormant floral buds to produce inflorescence at elevated temperatures (10/21 °C night/day), but the optimal inflorescence only occurred in their explants that were taken from trees that had already experienced hundreds of chilling hours, thus their results are very different from our results described here. It would, however, be pertinent to mention here that 'Arbequina' or Koroneiki' cultivars (important oil producing cultivars) that we used in our experiments were not included in the tests of researchers mentioned above.

High daytime temperatures (26.6°C) strongly inhibited flowering at both chilling (2.5°C) and non-chilling (8.3°C) nighttime temperatures; the non-chilling nighttime temperatures under optimal daytime temperatures do produce extensive flowering (Table 2). Inhibition of flowering by high daytime temperatures (26.6°C) and optimal nighttime temperatures (8.3°C; *i.e.*, non-chilling temperature) is consistent with our previous finding with 'Arbequina' cultivar (Malik and Bradford, 2006). These studies, however, demonstrate for the first time that such inhibitory daytime temperatures (26.6°C) were equally effective in inhibiting flowering even when plants were given optimal nighttime chilling temperatures (2.5°C) (Table 2).

Since daytime temperatures of 26.6°C drastically inhibited flowering in the 'Koroneiki' cultivar (*i.e.*, 0.5±0.4 inflorescence per tree under chilling conditions and 0±0 per tree under non-chilling conditions) we decided to test the effect of nighttime chilling versus non-chilling at relatively lower daytime temperatures (23.9°C) to see if quantitative difference between the two nighttime conditions could be measured. At a daytime temperature of 23.9°C the trees kept at both chilling and non-chilling nighttime temperatures, produced significantly more inflorescences (220.0 ± 17.7 versus 0.5 ± 0.4, and 126.8 ± 30.6 versus 0 ± 0 under chilling and non-chilling nighttime temperatures, respectively) than the trees kept at 26.6°C (Table 2 and 3). Still, even at 23.9°C daytime temperature, flowering was strongly inhibited; *i.e.* 15-25 % of the optimal daytime temperatures, and there was no significant difference between chilling and non-chilling nighttime temperatures (Table 3). Further experiments involving minimal daytime inhibitory temperatures may shed more light between interactions of chilling and non-chilling nighttime temperatures in the presence of mild daytime inhibitory temperatures.

The possibility exists that the flower inhibitory effect of high daytime temperatures may simply suppress the flowering induced by optimal nighttime temperatures thus the flowering would quickly occur if optimal daytime temperatures are given to the trees that were previously kept under high daytime temperatures but inductive nighttime temperatures. Alternatively, the flower inhibitory effect of high daytime temperatures could cancel the flower inducing effects of optimal nighttime temperatures, in

Table 2. The effect of high daytime temperatures under chilling and non-chilling nighttime conditions on flowering in 'Koroneiki' olives

Treatment	Temperature (°C) minimum/maximum	Inflorescences per tree	Flowers per inflorescence	Diameter (mm)	Height (m)
Tr. 1	2.5/18.3	568.7±45.5^{a}	21.6±0.9^{d}	13.2±1.1^{f}	1.4± 0.1^{g}
Tr. 2	2.5/26.6	0.5±0.4^{c}	3.8±2.7^{e}	12.3±0.4^{f}	1.5± 0.1^{g}
Tr. 3	8.3/18.3	728.7±43.8^{b}	23.9±0.7^{d}	12.5±0.7^{f}	1.4±0.1^{g}
Tr. 4	8.3/26.6	0.0±0.0^{c}	0.0±0.0^{e}	13.6±0.7^{f}	1.4± 0.1^{g}

Details of temperature regimes in each treatment are given in the Table 1 under Experiment 1. Mean number of inflorescence per tree, and mean number of flowers per inflorescence, in treatments 2 and 3 are significantly lower ($P < 0.05$) than treatments 1 and 4 respectively. Numbers with similar superscript letters in each column are not significantly different but numbers with different letters are significantly different at $P < 0.05$.

Table 3. The effect of mildly high daytime temperatures under chilling and non-chilling nighttime conditions on flowering in 'Koroneiki' olives

Treatment	Inflorescences per tree	Flowers per inflorescence	Diameter (mm)	Height(m)
Tr. 1 (2.5/23.9)	220 ± 17.7[a]	14.9 ± 0.7[d]	13.3 ± 0.4[f]	1.4 ± 0.0[g]
Tr. 2 (8.3/23.9)	126.8 ± 30.6[a]	13.8 ± 0.8[d]	13.3 ± 0.5[f]	1.3 ± 0.1[g]
Tr. 3 (8.3/18.3)	873.8 ± 50.5[b]	20.9 ± 1.3[e]	13.7 ± 0.3[f]	1.3 ± 0.1[g]

Details of temperature regimes in each treatment are given in the Table 1 under Experiment 2. Mean number of inflorescence per tree in treatment 1 and 2 are significantly lower ($P < 0.05$) than treatment 3. Numbers with similar superscript letters in each column are not significantly different but numbers with different letters are significantly different at $P < 0.05$.

Table 4. Flowering in trees whose flowering was initially inhibited in Experiment 1 after placing them under optimal flowering conditions.

Treatment (Temperature °C, from minimum/ maximum to minimum/maximum)	Inflorescences per tree	Flowers per inflorescence	Diameter (mm)	Height (m)
Tr. 1 (2.5/26.6 to 8.3/18.3)[1]	504.6 ± 65.2[a]	19.52 ± 1.5[c]	12.1 ± 0.3[d]	1.5 ± 0.1[e]
Tr. 2 (8.3/26.6 to 8.3/18.3)[2]	625.7 ± 41.7[ab]	21.4 ± 0.8[c]	12.9 ± 0.5[d]	1.4 ± 0.1[e]
Tr. 3 (Greenhouse to 8.3/18.3)[3]	873.8 ± 50.5[b]	20.9 ± 1.3[c]	13.7 ± 0.3[d]	1.3 ± 0.1[e]

[1]After the conclusion of Experiment 1, its Treatment 2 was placed under this inductive temperature regime

[2]After the conclusion of Experiment 1, its Treatment 4 was placed under this inductive temperature regime

[3]Trees from Greenhouse under none inductive conditions throughout (since the start of Experiment 1) were placed under inductive conditions

Details of temperature regimes in each treatment are given in the Table 1 under Experiment 1 and 1A. Numbers with similar superscript letters in each column are not significantly different but number with different letters are significantly different ($P < 0.05$).

which case, putting them back under optimal conditions would take a normal time to flower. To test these possibilities, trees of Treatments 2 and 4 of Experiment 1 were placed back in a growth chamber maintained at optimal daytime temperature (same as in Treatment 3 of Experiment 1). In addition, four replicate trees (there were 15 replicate trees for Treatment 2, and 4, of Experiment 1) kept in the greenhouse were placed in the same growth chamber at optimal flowering conditions (Treatment 3 of Experiment 1). Interestingly, there was no hastening of flowering in trees that were given optimal flower inducing nighttime conditions (at both chilling and non-chilling), but inhibitory daytime temperatures, compared to the trees that were transferred from the greenhouse kept under non-inducing daytime and nighttime temperatures (15-20°C). For example, it took about 75 days for the trees (50% of trees) in Treatment 3 of Experiment 1 to produce inflorescence [inflorescences begin to appear under these conditions without changes in temperatures, and therefore, it was straightforward to note the time of appearance of inflorescence. Trees kept under chilling conditions (*e.g.*, 2.5°C) must be brought to non-chilling condition to produce inflorescence], whereas it took 70 days to produce inflorescences in trees of Experiment 1A. These results indicate that inhibitory daytime temperatures cancel any flower inducing effect of optimal nighttime temperatures. Lavee (1985) described reversal of inhibitory effect of high temperatures that may interrupt winter inductive period in field grown trees, during early induction period, but this reversal does not occur after February. Thus, based on our findings under controlled condition and those of Lavee's observations on field grown plants further studies are needed, to determine the length and intensity of high temperature interruption, at various times during induction period, that are reversible and when they become irreversible.

It is puzzling to note that the trees that were first exposed to chilling nighttime temperatures and inhibitory daytime temperatures (Treatment 1 of Experiment 1A) produced significantly fewer inflorescences compared to the tree that were kept in greenhouse (Table 4). Considering that temperatures in growth chambers were stringently controlled (Malik and Bradford, 2006), while temperatures were not that tightly controlled in the greenhouse, we compared the number of hours in different temperature groups between the two conditions. We found that while trees in the greenhouse and growth chamber were subjected to a similar number of hours of flower inhibitory temperatures (>25°C), the trees in the greenhouse received 4-5 times more hours of temperatures (18-22°C optimal; or 18-23°C) that promote flowering (Fig. 1). The flower promoting temperatures in the greenhouse are different from the nighttime chilling and non-chilling temperatures in growth chambers discussed above [tree under greenhouse conditions never flower in greenhouse and take 70 -75 days of inductive period to flower]. However, it is difficult to assert at this time that the difference in temperature categories between the two conditions (greenhouse versus growth chamber) was responsible for fewer inflorescences in trees initially kept under flower inhibitory conditions in the growth chambers versus in greenhouse. It is, however, `interesting information that deserves further studies; and we intend to conduct detailed studies on interactions between various lengths of flower inhibitory temperatures on various lengths of flower promoting temperatures on the extent of flowering in olives.

In conclusion, "Koroneiki' olives flower profusely at both chilling (2.5°C) and non-chilling temperatures (8.3°C), when daytime

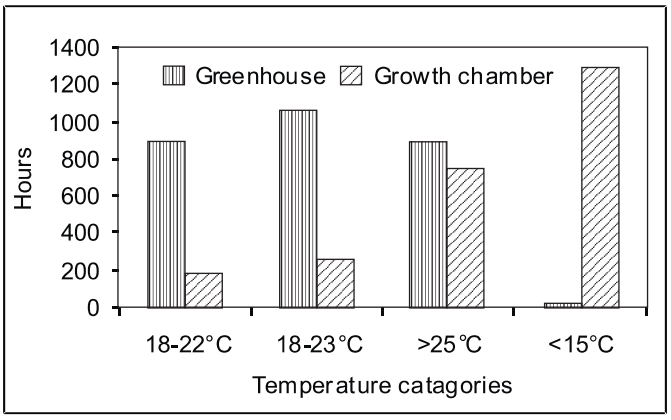

Fig. 1. Numbers of hours 'Koroneiki' trees were subjected to different temperature categories in the growth chamber (conditions of treatment 4 of experiment 1) and greenhouse parallel to the three month induction period in growth chambers.

temperatures are maintained at 18.3°C, and that equal flowering intensity in this cultivar can be achieved under non-chilling conditions. These results are similar to previously reported results with cultivar, 'Arbequina', except that they provide further evidence that chilling temperatures (2.5°C) do not produce higher number of inflorescence than non-chilling temperatures (8.3°C). High daytime temperatures (26.6 and 23.9°C) strongly inhibited flowering at both chilling and non-chilling nighttime temperatures. Inhibitory effects of high daytime temperatures canceled any flower promoting effects of optimal nighttime temperatures because trees kept at a 26.6°C daytime temperature, and optimal nighttime temperatures, when returned to optimal flowering inducing conditions did not flower earlier, but required the 70 days period before inflorescence emergence.

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Leaf N and P in different growth habits of peach: Effects of root system morphology and transpiration

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Abstract

Adequate mineral nutrition is critical for high fruit quality and sustained yield of fruit trees. In this experiment, peach [*Prunus persica* L. (Batch)] trees with different shoot and root growth habits were evaluated for leaf nitrogen (N) and phosphorus (P) concentrations after fertilizer applications in the greenhouse and field. In the field during 2008, Compact trees had higher root length density than Pillar and Standard trees (6.2, 3.8, and 3.7 mm cm^{-3}, respectively). Compact trees also had higher foliar P (0.21%) but the same N (1.3%) as Standard and Pillar trees (P concentrations of 0.14 and 0.11%, respectively) when fertilizer was applied once in the greenhouse. Following multiple applications of fertilizer, Compact tree leaves had the same P (approximately 0.21 and 0.29% in the greenhouse and field, respectively) as the other growth habits. After multiple fertilizer applications, Pillar trees had the greatest increase in foliar N and P, which was associated with high transpiration rates. Pillar, Compact, and Standard had transpiration rates of 3.0, 2.1, and 2.3 mmol H_2O m^{-2} s^{-1}, respectively. The data indicate that peach trees with fibrous roots systems may have an advantage to absorb nutrients such as P that move primarily by diffusion, when the nutrient is present in low concentrations in the soil. However, under conditions of high soil fertility, fibrous root systems did not improve nutrient uptake and trees with greater transpiration rates absorbed greater levels of nutrients. Different growth habits of peach have diverse root systems and transpiration rates that affect nutrient uptake and, consequently, the selection of tree growth habit should be considered in orchard soil management plans. Growth habits with more fibrous root systems may require reduced inputs of nutrients with low diffusion coefficients.

Key words: Orchard management, tree root system, tree nutrient uptake, *Prunus persica*

Introduction

Root systems anchor plants and absorb water and nutrient elements that are vital for growth. As with all crops, successful production of tree fruit crops will depend on the capacity of the tree to obtain soil nutrients that may be available in limited quantities. In the eastern U.S., peach trees are often planted in vegetation-free rows with grass alleys between tree rows so that the ground cover helps prevent soil degradation (Tworkoski and Glenn, 2008). Under such mixed planting schemes, the tree root system morphology and fine root distribution can affect competition among trees within the row and between trees and grass (George *et al.,* 1996). Competition for nutrients such as nitrogen (N) and phosphorus (P), which are often present in low quantities, can limit plant growth. Fine roots and root hairs are highly important for soil exploitation to acquire less mobile nutrients such as P (Lopez-Bucio *et al.,* 2003). Root proliferation in response to soil nutrient availability appears to be under genetic control and mediated, at least in part, by endogenous hormones (Lopez-Bucio *et al.,* 2003). Selecting and breeding crops that have roots with strong capacity for P foraging can contribute to food production, particularly in regions with low fertility soils and limited availability of fertilizers (Lynch and Brown, 2001).

Trees with different shoot growth habits have been found to also have distinguishing root system characteristics. In greenhouse experiments, peach trees with highly branched shoots (Compact growth habit) had three-times more lateral roots within 10 cm of the root collar than Pillar, which had less branched and more vertical shoots, or Standard growth habits (Tworkoski and Scorza, 2001). In addition, Compact trees had more and longer higher order lateral (*i.e.* fibrous) roots. If these root traits are expressed under field conditions, the fibrous root system of Compact trees could be beneficial in acquiring nutrient elements with low diffusion coefficients, such as P (Fitter and Hay, 1987). Conversely, a fibrous root system might have less impact on uptake of nutrients with high diffusion coefficients, such as nitrate, that move to roots readily by mass flow.

Fruit trees with improved root systems (own-rooted or as rootstocks) may be beneficial in infertile soils and for efficient use of fertilizer. Experiments were conducted in the greenhouse and field with the objectives to: (1) verify the fibrous root system morphology of Compact peach trees in the field and (2) determine if growth habits with fibrous roots affect leaf concentrations of N and P.

Materials and methods

Greenhouse: Peach trees with different growth habits were grown from seed in a greenhouse in 20 L pots (Hagerstown silt loam) in beginning of February 1998 as described by Tworkoski and Scorza (2001). Trees were put in cold storage in October 1998, and placed back in the greenhouse in May 1999. Fertilizer was applied once to all trees in July 1999. Half the trees received no additional fertilizer while the rest received fertilizer (1.75 g of 20N- 8.8P-16.6K applied per tree) every four days for six weeks. In August 1999, ten leaves per tree were collected from the middle of shoots located throughout the canopy, dried, and total N was determined with a Nitrogen Determinator (LECO

Corp., St. Joseph, MI). Total P was measured colorimetrically (Murphy and Riley, 1962) after digestion with $HClO_4$ (Adler and Wilcox, 1985). Also in August, transpiration and photosynthesis (CIRAS-1, PP Systems, Haverhill, MA) were measured on three leaves of each replication at approximately midday on each of five days. The experimental design included three replications of each level of two main effects (three growth habits and two levels of fertilizer) that were tested by ANOVA with mean separation by the LS Means procedure (SAS, 2001) at the 0.05 level of significance.

Field: Peach trees with different growth habits that were germinated and grown in the greenhouse in 1998 were planted in the field in the same soil as was used in the greenhouse experiment at Kearneysville, WV on 4 August 1998 (4 x 4 m spacing). Trees were not pruned but received fertilizer (10N-4.4P- 8.3K, 44 kg ha^{-1}) each spring from 1999 through 2006. Diuron (4.4 kg ha^{-1}) and solicam (3.3 kg ha^{-1}) were applied each spring to maintain a 2-m weed-free tree row. In 2007, trees were fertilized and pruned to remove dead wood. In April 2007 and 2008, urea (4.49 g N) and granulated triple super phosphate (5.6 g P) was applied to each of four 1 m^2 ground areas adjacent to the tree trunk and located north, east, south, & west of each tree. Twenty leaves per tree were harvested and pooled in July 2007 and 2008 and leaf nutrient concentrations were measured by the Agricultural Analytical Services Laboratory of the Pennsylvania State University, University Park, PA. Roots were measured in soil samples that were collected in September. 1999 and 2008 with two 5 cm-diameter soil cores per tree per year at 0 to 25 cm and at 25-to-50 cm depths. Cores were collected within 50 cm from the tree trunk. After separating roots from the soil, root lengths were measured with a root imaging device (CID, Inc., Vancouver, WA) and root length density (RLD) was calculated as the total root length divided by the total volume of the soil cores. The experimental design included three replications of each level of two main effects (three growth habits and two levels of fertilizer) that were tested by ANOVA with mean separation by the LS Means procedure in SAS at $P = 0.05$.

Results and discussion

In the greenhouse, a significant and meaningful relationship was found between leaf N concentration and transpiration, likely due to N moving in the soil by transpiration-driven mass flow from soil to root (leaf N = 0.7692 x (transpiration) + 0.2263; $r^2 = 0.74$; Fig. 1A). The relationship between leaf P concentration and transpiration was not significant (leaf P = 0.0281 x (transpiration) + 0.1255; $r^2 = 0.16$). Since mass flow contributes more strongly to plant acquisition of N than P, it is logical that transpiration had less impact on P uptake (Cramer *et al.,* 2008). Leaf N concentration significantly affected photosynthesis (photosynthesis = 9.0145 x (leaf N) - 1.5651; $r^2 = 0.82$; Fig. 1B). Peach tree productivity, as represented by photosynthetic capacity, was regulated by leaf N concentration that, in turn, depended on capacity to absorb nutrients.

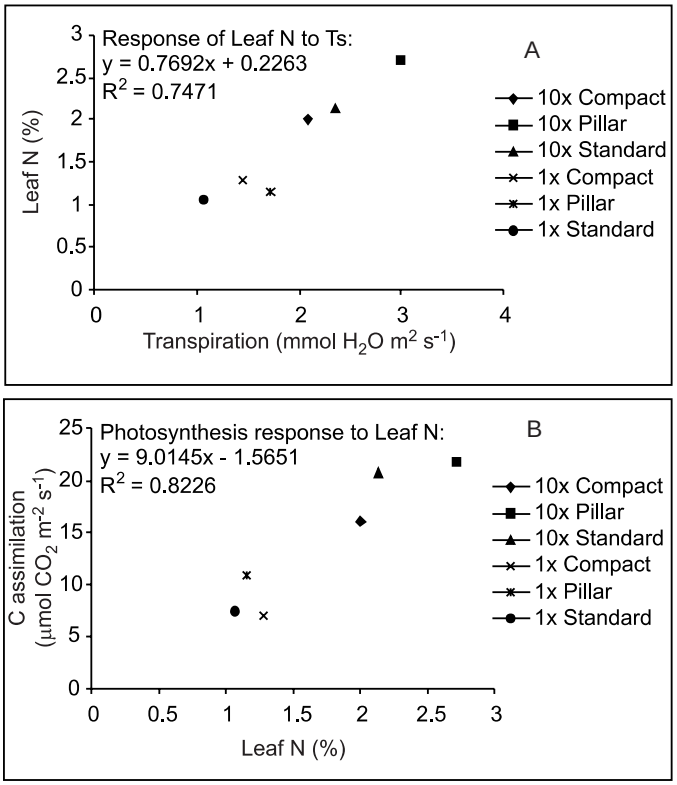

Fig. 1. Relationship of leaf nitrogen with translocation (A) and photosynthesis (B) of three growth habits of peach trees that were grown in the greenhouse and fertilized once (1x) or ten times (10x) during the 1999 growing season. Regressions were based on individual replications and mean values are shown.

In the greenhouse after one fertilizer application, Compact trees had higher leaf P than the other growth habits (Table 1).

Table 1. Leaf N and P concentrations in three growth habits of peach that were grown in the greenhouse and fertilized once or ten times during the 1999 growing season

Growth habit	One application		Ten applications		Increase between one and ten applications	
	N (%)	P (%)	N (%)	P (%)	N (%)	P (%)
Compact	1.3 a	0.21 a	2.0 b	0.21 a	0.7 c	0 c
Pillar	1.2 a	0.11 b	2.7 a	0.21 a	1.5 a	0.10 a
Standard	1.1 a	0.14 b	2.1 b	0.21 a	1.0 b	0.06 b

Within each column, means followed by the same letter do not differ at $P = 0.05$.

Table 2. Leaf photosynthesis (Pn) and transpiration (Ts) in three growth habits of peach that were grown in the greenhouse and fertilized once or ten times during the 1999 growing season

Growth habit	One application		Ten applications	
	Pn (µmol CO_2 m^{-2} s^{-1})	Ts (mmol H_2O m^{-2} s^{-1})	Pn (µmol CO_2 m^{-2} s^{-1})	Ts (mmol H_2O m^{-2} s^{-1})
Compact	7.1 a	1.4 a	16.1 b	2.1 b
Pillar	10.9 a	1.7 a	21.8 a	3.0 a
Standard	7.4 a	1.1 a	20.8 ab	2.3 b

Within each column, means followed by the same letter do not differ at $P = 0.05$.

Physiological sufficiency range of P is from 0.15 to 0.3% leaf dry weight; therefore, Compact trees had adequate P after one or ten fertilizer applications. Pillar and Standard trees achieved P sufficiency only after ten fertilizer applications in the greenhouse (Table 1). This supports the hypothesis that the fibrous root system of Compact trees measured in greenhouse conditions (Tworkoski and Scorza, 2001) provided greater access to nutrients that move more by diffusion than by mass flow from soil to root surfaces.

After ten fertilizer applications, Pillar trees had the greatest increase in leaf N and P (Table 1). This result was unexpected since previous results indicated that Pillar trees had a smaller root system and a larger shoot-to-root dry weight ratio than Compact trees (Tworkoski and Scorza, 2001). However, Pillar trees had greater transpiration rates than other growth habits which may affect mass flow of nutrients in water moving from soil to roots (Table 2). After ten fertilizer applications, leaf transpiration in the greenhouse-grown trees was greatest in Pillar and less in Standard and Compact trees (Table 2). Pillar trees achieved N sufficiency (2.5 to 3.4% leaf N) after ten fertilizer applications in the greenhouse, but Compact and Standard trees did not (Table 1).

Field root studies supported greenhouse experimental findings that Compact trees have more fibrous root systems than Pillar or Standard trees (Tworkoski and Scorza, 2001; Fig. 2). In both 1999 and 2008, Compact trees had greater lengths and weights of roots less than 3 mm diameter than Pillar trees at soil depths less than 25 cm (Fig. 2). The abundance of roots in shallow soil may have contributed to the higher leaf N and P levels in Compact than Pillar trees in the field following one fertilizer application (Table 3). Standard tree root weight density was intermediate between Compact and Pillar trees in 2008. Root abundance in shallow soil can be significant to fruit tree nutrition. Most P uptake in apple (*Malus domestica* Borkh.) was associated with root activity at soil depths less than 30 cm (Atkinson, 1974). In all growth habits in the current field experiment, over 90% of the peach roots were in the 0 to 25 cm soil depth and approximately 98% of the root length was less than 3 mm in diameter (data not shown).

Foliar N and P results from the field and greenhouse generally agree. In the field, Compact trees had greater P leaf concentration than Pillar or Standard trees after one but not two fertilizer applications (Table 3). Pillar trees had less foliar P concentration than Compact trees after one fertilizer application but foliar P concentrations increased most in Pillar trees after two fertilizer applications (Table 3). In the field, all growth habits achieved N and P sufficiency, probably due to fertilizer applications made in the orchard over previous years (Table 3).

In this experiment, differences in root system morphology and transpiration were found among the peach growth habits that may have affected the tree nutritional status and its response to fertilization. Compact trees had greater root length density (Fig. 2).

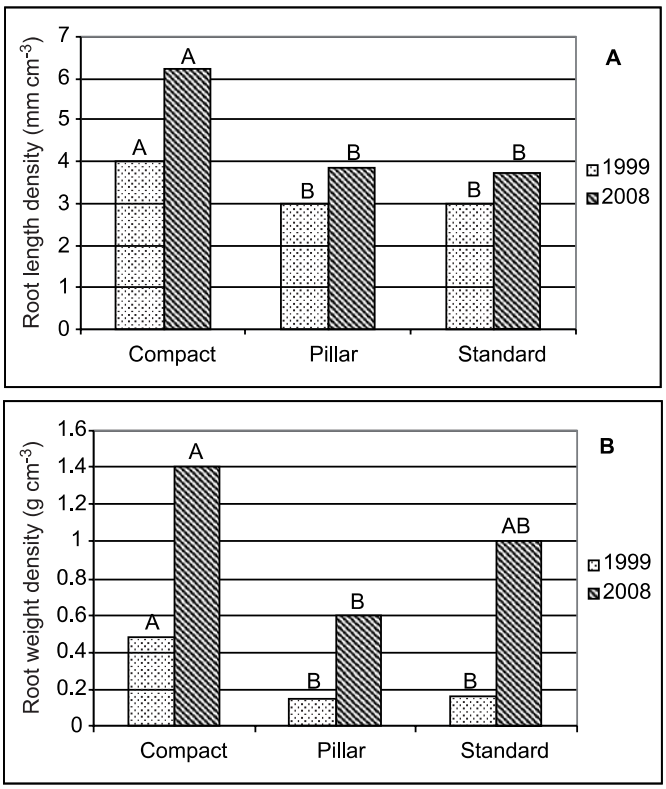

Fig. 2. Root length density (A) and root weight density (B) of roots less than 3 mm in diameter in the upper 25 cm of soil within 50 cm of the trunk of three growth habits of peach trees planted in 1998 in the field and sampled in 1999 and 2008. Within the same year, bars with the same letter above them do not differ $P = 0.05$.

Previously, Compact trees were found to have a larger number of root tips compared with Pillar trees (Tworkoski and Scorza, 2001). The large number of fine roots and root tips of the Compact growth habit may facilitate exploration of new soil as peach trees forage for nutrients. In contrast, Pillar trees may not as effectively explore the soil but leaf N and P of Pillar trees increased the most in response to fertilization application. Pillar trees have shorter root axes (links) between lateral roots (Tworkoski and Scorza, 2001) that may facilitate transpiration when soil water is abundant and also may enable Pillar trees to benefit more from a fertilizer drench than the other growth habits. Sorgonà *et al*. (2005) found that genetically-controlled root morphology of citrus affected nutrient acquisition and that nitrate adsorption was greater in trees with large numbers of root tips.

The results of this experiment have implications for orchard productivity and fertilizer inputs. Growth habits with more fibrous root systems may require reduced inputs of some nutrients such as P. Growth habits that acquire N efficiently can provide higher leaf N, increased photosynthesis, and may improve tree productivity (Fig. 1). Future research can compare nutrient use efficiency of peach cultivars on peach root systems with different

Table 3. Leaf N and P concentrations in three growth habits of nine-year-old peach trees that were grown in the field and fertilized once (2007) or twice (2007 and 2008)

Growth habit	One application		Two applications		Increase between one and two applications	
	N (%)	P (%)	N (%)	P (%)	N (%)	P (%)
Compact	2.7 a	0.25 a	3.1 a	0.29 a	0.3 b	0.03 b
Pillar	2.4 b	0.21 b	3.3 a	0.29 a	0.8 a	0.08 a
Standard	2.5 ab	0.22 b	3.2 a	0.27 a	0.6 ab	0.05 ab

Within each column, means followed by the same letter do not differ at $P = 0.05$.

morphology and transpiration rates. The current research findings suggest that nutrient uptake capacity varies within the peach variteies. Consequently, fertilization could be modified for a particular growth habit to efficiently use nutrients and to reduce unnecessary nutrient load into the environment.

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Influence of fungicides and *Phytophthora capsici*-resistant/ tolerant cultivars on bell pepper yield and farm-gate revenues

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Abstract

Phytophthora blight, caused by *Phytophthora capsici* Leonian, is a widespread and destructive disease of bell pepper (*Capsicum annuum* L.). Bell pepper yield, farm-gate revenues and Phytophthora blight incidence were determined during 2005 and 2006 in a *P. capsici*-infested field near Shawneetown, Illinois. The study evaluated 12 bell pepper cultivars (one resistant, three tolerant, and eight susceptible to *P. capsici*) with or without a recommended fungicide treatment (mefenoxam at transplant and dimethomorph + copper alternated with manganese ethylenebisdithiocarbamate + copper at 10 day intervals). Bell pepper plants receiving fungicide applications showed less Phytophthora blight incidence throughout the growing season and produced greater yield and farm-gate revenues compared to untreated plants. Additionally, *P. capsici*-resistant 'Paladin' and *P. capsici*-tolerant 'Alliance', 'Aristotle X3R', and 'Revolution' produced greater yields (≥ 17,800 and 33,800 kg ha^{-1} for 2005 and 2006, respectively) and farm-gate revenues [≥ $12,700 and $27,000 (USA) ha^{-1} for 2005 and 2006, respectively] compared to the susceptible cultivars. Therefore, in fields with a high incidence history of Phytophthora blight, 'Paladin' could be a reliable choice for commercial bell pepper production. However, 'Alliance', 'Aristotle X3R', and 'Revolution' may be preferred by growers due to the added benefits of bacterial spot [*Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye] resistance and better fruit quality compared to 'Paladin'. Furthermore, this research indicates that plant resistance and/or tolerance should not be relied upon as the only method of *P. capsici* control and growers should also incorporate fungicides into their management program to provide additional protection.

Key words: *Capsicum annuum*, chemical control, economics, *Phytophthora capsici*, Phytophthora blight, disease management, disease resistance/tolerance

Introduction

Phytophthora capsici causes Phytophthora blight, which is a devastating disease affecting many vegetable crops including bell peppers. Recently, the incidence of this rapidly spreading disease has dramatically increased in Illinois and has caused yield losses of up to 100% in pepper fields (Babadoost, 2001; Islam and Babadoost, 2002).

P. capsici is a soil-borne oomycete that thrives under warm (25 to 30°C) and excessively wet conditions; furthermore, Phytophthora blight is often observed on plants in low-lying or poorly drained areas of the field (Hausbeck and Lamour, 2004). Crown rot is usually the first symptom of Phytophthora blight observed on bell pepper plants in the field and generally results in rapid collapse and death of plants (Babadoost, 2001). During the aerial phase of the disease, sporangia are produced on the surface of the lesion, spread to healthy plants by splashing rain, and release zoospores causing the development of stem lesions in branch axils (Babadoost, 2001; Kline and Johnston, 2005). These girdling lesions lead to plant death above the affected area. Other disease symptoms on bell pepper include root rot, fruit rot, seedling damping-off, and to a lesser extent, leaf spot (Babadoost, 2001).

Although there is no single method to provide adequate control of *P. capsici* in bell pepper production, practices such as planting on raised beds, removal of symptomatic plants, managing field moisture levels, mulching plants, planting resistant or tolerant cultivars, and timely fungicide applications may reduce the incidence of this disease (Islam and Babadoost, 2002). Plant resistance and/or tolerance are often the basis of disease management programs and can be used as part of disease management program to provide *P. capsici* control in bell pepper production (Hausbeck and Lamour, 2004). Bell pepper cultivars tolerant to *P. capsici* include Alliance, Aristotle X3R, and Revolution, while Paladin is considered resistant (Anonymous, 2006b).

Although plant resistance is an important part of managing *P. capsici*, it is often combined with fungicide applications to achieve effective control of this pathogen. Recommended fungicide treatments for *P. capsici* control include soil applications of mefenoxam (Ridomil Gold EC, Syngenta Crop Protection, Inc., Greensboro, N.C.) at 1.17 L ha^{-1} during transplanting. This is followed by the application of dimethomorph (Acrobat 50WP, BASF, Research Triangle Park, N.C.) at 0.45 kg ha^{-1} or famoxadone plus cymoanil (Tanos 50WP, DuPont, Wilmington, Del.) at 0.56 to 0.70 kg ha^{-1} alternated with manganese ethylenebisdithiocarbamate (Maneb 80, Cerexagri, Inc., King of Prussia, Penn. or Manex, DuPont, Wilmington, Del.) at 1.68 to 3.36 kg ha^{-1} or 1.14 to 2.27 L ha^{-1}, respectively, during production (Egel *et al.,* 2005). Therefore, the objectives of this study were to determine the efficacy of plant resistance and recommended fungicide applications on bell pepper yield, farm-gate revenues, and Phytophthora blight disease incidence in a *P. capsici*-infested field.

Materials and methods

The experiment was conducted during 2005 and 2006 near Shawneetown, Illinois in a field infested with *P. capsici*. The field soil type was an Alvin-Roby-Ruark association (with Alvin a coarse-loamy, mixed, mesic, Typic Hapludalf; Roby a coarse-loamy, mixed, mesic, Aquic Hapludalf; and the Ruark a fine-loamy, mixed, mesic, Typic Ochraqualf) (Wallace and Fehrenbacher, 1969). A split-plot design was used with three replications. The main plots were: 1) fungicide treatment, and 2) no fungicide treatment. The fungicide treatment consisted of: 1) mefenoxam (Ridomil Gold EC) applied at 1.17 L ha^{-1} during transplanting, and 2) a spray application at 10 day intervals of dimethomorph (Acrobat 50 WP) at 0.45 kg ha^{-1} + copper (Tenn-Cop, Griffin LLC, Valdosta, GA) at 3.6 L ha^{-1} alternated with manganese ethylenebisdithiocarbamate (Maneb 80) at 2.8 kg ha^{-1} + copper (Tenn-Cop) at 3.6 L ha^{-1}. The subplots were twelve bell pepper cultivars: Aladdin X3R, Alliance, Aristotle X3R, Brigadier, California Wonder, Camelot X3R, Commandment, King Arthur, Legionnaire, Paladin, Red Knight X3R, and Revolution. All seeds were obtained from Seedway, Inc., Elizabethtown, PA.

Bell pepper seeds were planted into 72-cell plastic trays (TLC Polyform Inc., Plymouth, MN) containing Pro-Mix (Premiere Horticulture Inc., Quakertown, PA), a soilless medium, which consists of 2:1:1 peat moss, vermiculite, and perlite, respectively, in a greenhouse. Seedlings remained in the greenhouse for approximately six weeks and were then hardened-off in an outdoor cold frame for five days prior to transplanting.

Raised beds were formed on 1.8 m centers and covered with 0.03175 mm black plastic mulch with drip irrigation. Bell pepper seedlings were transplanted on double rows spaced 0.5 m apart, with 0.5 m in-row spacings. Plots were 2.5 m in length with 10 plants in each plot. Trickle fertigation was used to supply the water and nutrients necessary for bell pepper plant growth and development (Sanders *et al.,* 1995), with approximately 135 kg ha^{-1} N applied over the growing season. No insecticides or additional fungicides were applied to plants. Weeds between plastic were controlled by paraquat dichloride (Gramoxone Max, Syngenta Crop Protection, Inc., Greensboro, N.C.) at 2.3 L ha^{-1}.

Disease incidence (number of plants per plot with visible Phytophthora blight symptoms) was rated at four week intervals [4, 8, 12, and 16 weeks after transplanting (WAT)] during the growing season. Visible symptoms included plant wilting, crown rot, stem lesions, and fruit rot. Farm-gate revenues were calculated using a $10 (USA)/12.6 kg box conversion factor which was based upon the average St. Louis, Mo. produce terminal market prices for 2005 and 2006. Pepper fruit were harvested every 10 to 14 days with a total of 6 harvests. Fruit harvests began on 14 or 21 July and ended on 21 or 14 September for 2005 and 2006, respectively. Fruit were weighed and graded into marketable (Fancy, No. 1 and No. 2) and cull (misshapen or decaying fruit) based on USDA standards (Anonymous, 2005).

Bell pepper plants with Phytophthora blight symptoms were collected from the experimental site each year. *P. capsici* was isolated using the method described by Islam *et al.* (2004). 'California Wonder' seedlings were inoculated in a greenhouse with the isolated *P. capsici*; and, in all instances, Phytophthora blight disease symptoms developed. *P. capsici* was then successfully re-isolated from the infected seedlings.

Data were subject to analysis of variance procedures appropriate for a split plot design using the PROC GLM procedure of SAS (SAS Inst., Cary, N.C.). Fisher's least significant difference (LSD) tests were used to separate differences among fungicide treatment and bell pepper cultivar means at $P \leq 0.05$.

Results

The data were combined over the 2005 and 2006 growing seasons and analyzed. Interactions of year with cultivar and fungicide treatment ($P \leq 0.05$) were observed for Phytophthora blight incidence, yields, and farm-gate revenues indicating that cultivar and fungicide treatment performance for these variables depended on year. Therefore, results for these variables are presented by year. However, cultivar by fungicide treatment interactions ($P > 0.05$) were not observed during either year, indicating that cultivar performance was consistent across fungicide treatments.

Phytophthora blight incidence: The fungicide treatment used reduced Phytophthora blight incidence on bell peppers compared to those not receiving fungicide applications ($P \leq 0.0001$) for both 2005 and 2006 growing seasons (Tables 1 and 2). Although Phytophthora blight incidence increased as the season progressed, the fungicide treatment significantly reduced the number of plants with visible symptoms.

Table 1. Effect of fungicide treatment and cultivar on Phytophthora blight incidence in bell peppers during 2005 in a *P. capsici*-infested field near Shawneetown, Illinois

Treatment	Phytophthora blight incidence(%), weeks after transplanting			
	4	8	12	16
Fungicide[z]				
Non-treated	27	39	80	97
Treated	2	7	26	76
Significance	***	***	***	***
Cultivar[y]				
Paladin[x]	3a	5a	15a	42a
Revolution[w]	8a	20ab	43a	73b
Alliance[w]	13a	15ab	42ab	82bc
Aristotle X3R[w]	10a	10ab	42ab	85bc
King Arthur[v]	23a	32ab	68b	90bc
Brigadier[v]	22a	32ab	62b	92bc
Camelot X3R[v]	18a	30ab	53b	92bc
Commandment[v]	15a	27ab	53b	92bc
Legionnaire[v]	12a	23ab	67b	93bc
Cal. Wonder[v]	12a	22ab	65b	98bc
Aladdin X3R[v]	17a	20ab	53b	100c
Red Knight X3R[v]	18a	40ab	72b	100c

[z]Fungicide treatment: non-treated = no fungicide treatment and treated = fungicide treatment of: 1) mefenoxam (Ridomil Gold EC, 1.17 L ha^{-1}) at transplant and 2) a spray application of dimethomorph (Acrobat 50WP, 0.45 kg ha^{-1}) + copper (Tenn-Cop, 3.6 L ha^{-1}) alternated with manganese ethylenebisdithiocarbamate (Maneb, 2.8 kg ha^{-1}) + copper (Tenn-Cop, 3.6 L ha^{-1}) at 10 day intervals.

[y]Cultivars are ranked according to least amount of Phytophthora blight incidence at 16 WAT. Visible symptoms included wilting of plants, crown rot and stem lesions, and fruit rot. Means followed by the same letter in each column are not significantly different at $P \leq 0.05$.

[x]*Phytophthora capsici*-resistant

[w]*Phytophthora capsici*-tolerant.

[v]*Phytophthora capsici*-susceptible.

Table 2. Influence of fungicide treatment and cultivar on Phytophthora blight incidence in bell peppers during 2006 in a *Phytophthora capsici*-infested field near Shawneetown, Illinois

Treatment	Phytophthora blight incidence (%) weeks after transplanting			
	4	8	12	16
Fungicide[z]				
Non-treated	4	7	14	42
Treated	2	3	7	18
Significance	NS	NS	*	***
Cultivar[y]				
Paladin[x]	2a	2a	3a	3a
Aristotle X3R[w]	0a	0a	2a	7ab
Revolution[w]	0a	0a	0a	8a-c
Alliance[w]	3a	3a	7a	25a-e
Legionnaire[v]	2a	7a	8a	30a-e
Camelot X3R[v]	8a	10a	13a	37a-e
Red Knight X3R[v]	2a	2a	8a	37a-e
Commandment[v]	5a	8a	20a	42b-e
Brigadier[v]	3a	7a	18a	47de
Cal. Wonder[v]	8a	8a	15a	47de
Aladdin X3R[v]	0a	3a	12a	52de
King Arthur[v]	5a	7a	20a	58e

[z]Fungicide treatment: non-treated = no fungicide treatment and treated = fungicide treatment of: 1) mefenoxam (Ridomil Gold EC, 1.17 L ha^{-1}) at transplant and 2) a spray application of dimethomorph (Acrobat 50WP, 0.45 kg ha^{-1}) + copper (Tenn-Cop, 3.6 L ha^{-1}) alternated with manganese ethylenebisdithiocarbamate (Maneb, 2.8 kg ha^{-1}) + copper (Tenn-Cop, 3.6 L ha^{-1}) at 10 day intervals.

[y]Cultivars are ranked according to least amount of Phytophthora blight incidence at 16 WAT. Visible symptoms included wilting of plants, crown rot and stem lesions, and fruit rot. Means followed by the same letter in each column are not significantly different at $P \leq 0.05$.

[x]*Phytophthora capsici*-resistant

[w]*Phytophthora capsici*-tolerant.

[v]*Phytophthora capsici*-susceptible.

[NS, *, ***]Non-significant and significant at $P \leq 0.05$ and $P \leq 0.0001$, respectively.

In 2005, *P. capsici*-resistant 'Paladin' had the lowest disease incidence rating at 42% at 16 WAT which differed ($P \leq 0.05$) from all other bell pepper cultivars (Table 1). The tolerant cultivars, Alliance, Aristotle X3R, and Revolution, tended to express less Phytophthora blight incidence of 82, 85 and 73%, respectively, compared to all susceptible cultivars that had ≥ 90% incidence at 16 WAT. For 2006, *P. capsici*-resistant 'Paladin' had the lowest Phytophthora blight disease rating (3%) at 16 WAT; furthermore, two of the *P. capsici*-tolerant cultivars, Aristotle X3R and Revolution, had disease incidence ratings of 7 and 8%, respectively (Table 2). All susceptible cultivars expressed ≥ 30% Phytophthora blight disease incidence at 16 WAT.

Fungicide treatment: For both 2005 and 2006, bell pepper plants receiving fungicide applications produced greater marketable weights and farm-gate revenues compared to non-treated plants (Table 3). Under high *P. capsici* disease pressures in 2005, fungicide-treated plants generated almost double the marketable yields and farm-gate revenues compared to non-treated plants.

Bell pepper cultivar – 2005: *P. capsici*-resistant 'Paladin' produced the highest yields and farm-gate revenues compared to any other cultivar (Table 3). Farm-gate revenue produced by 'Paladin' ($20,800 ha^{-1}) was more than double of those from most susceptible cultivars. High marketable yields (≥ 19,600 kg ha^{-1}) and farm-gate revenues (≥ $15,600 ha^{-1}) were also produced by 'Alliance' and 'Aristotle X3R'. Although 'Paladin' had the lowest Phytophthora blight incidence of 42% at 16 WAT compared to all other cultivars (Table 1), it did not differ ($P > 0.05$) from *P. capsici*-tolerant Alliance, Aristotle X3R, or Revolution and susceptible King Arthur for marketable weights and farm-gate revenues (Table 3). Conversely, 'California Wonder' produced the lowest marketable yields (7,900 kg ha^{-1}) and farm-gate revenues ($6,300 ha^{-1}), and had 98% Phytophthora blight incidence at 16 WAT.

Bell pepper cultivar – 2006: Lower Phytophthora blight incidence during the 2006 growing season directly resulted in higher total yields and farm-gate revenues for all bell pepper cultivars compared to 2005 (Table 3). *P. capsici*-tolerant

Table 3. Impact of fungicide application and cultivar resistance/tolerance on bell pepper marketable weights and farm-gate revenues in a *Phytophthora capsici*-infested field near Shawneetown, Illinois during 2005 and 2006

Treatment	2005 (x 1,000)[z]		2006 (x 1,000)[z]	
	MW (kg ha^{-1})	FGR ($ ha^{-1})	MW (kg ha^{-1})	FGR ($ ha^{-1})
Fungicide[y]				
Non-treated	10.3	8.2	29.1	23.2
Treated	19.3	15.4	34.6	27.6
Significance	**	**	**	**
Cultivar[x]				
Paladin[w]	26.0a	20.8a	33.8b-f	27.0b-f
Aristotle X3R[v]	19.9ab	15.9ab	34.8b-d	37.7b-d
Alliance[v]	19.6a-c	15.6a-c	40.1ab	32.0ab
Revolution[v]	17.8a-d	12.7a-d	42.6a	34.0a
King Arthur[u]	16.6a-d	13.2a-d	33.9b-e	27.0b-e
Commandment[u]	13.3b-d	10.6b-d	25.2h-j	20.1h-j
Red Knight X3R[u]	12.9b-d	10.3b-d	38.6a-c	29.2a-c
Legionnaire[u]	12.1b-d	9.6b-d	33.5b-g	26.7b-g
Aladdin X3R[u]	11.3b-d	9.0b-d	24.7h-j	19.7h-j
Brigadier[u]	10.9b-d	8.7b-d	31.0c-h	24.7c-h
Camelot X3R[u]	9.3b-d	7.4b-d	27.3e-i	21.7e-i
Cal. Wonder[u]	7.9d	6.3d	18.7j	14.9j

[z]MW = marketable weights and FGR = farm-gate revenues. FGR was calculated using a $10 (USA)/12.6 kg box conversion factor which was based upon the average St. Louis, Mo. produce terminal market prices for 2005 and 2006. Means followed by the same letter in each column are not significantly different at $P \leq 0.05$.

[y]Fungicide treatment: non-treated = no fungicide treatment and treated = fungicide treatment of:

1) mefenoxam (Ridomil Gold EC, 1.17 L ha^{-1}) at transplant and 2) a spray application of dimethomorph (Acrobat 50WP, 0.45 kg ha^{-1}) + copper (Tenn-Cop, 3.6 L ha^{-1}) alternated with manganese ethylenebisdithiocarbamate (Maneb, 2.8 kg ha^{-1}) + copper (Tenn-Cop, 3.6 L ha^{-1}) at 10 day intervals.

[**]Significant at $P \leq 0.01$.

[x]Cultivars are ranked according to total marketable yields for 2005. Visible symptoms included wilting of plants, crown rot and stem lesions, and fruit rot.

[w]*Phytophthora capsici*-resistant

[v]*Phytophthora capsici*-tolerant.

[u]*Phytophthora capsici*-susceptible.

'Revolution' produced the highest yields and farm-gate revenues throughout the growing season, which differed from most other cultivars, including Paladin. Similar to 2005, 'California Wonder' produced the lowest marketable yields (18,700 kg ha^{-1}) and farm-gate revenue ($14,900 ha^{-1}) but did not differ ($P > 0.05$) from 'Aladdin' or 'Commandment'. Furthermore, these cultivars had high Phytophthora blight incidence ratings (≥ 42%) at 16 WAT (Table 2). *P. capsici*-resistant 'Paladin' and *P. capsici*-tolerant 'Alliance', 'Aristotle X3R', and 'Revolution' were in the top half of cultivars with respect to yields and farm-gate revenues, as well as having the lowest Phytophthora blight incidence after 16 weeks at 3, 25, 7 and 8%, respectively (Table 2).

Discussion

Although the two years provided distinctly different growing conditions, Phytophthora blight was prevalent in bell peppers during both years of the study. Overall Phytophthora blight incidence ratings on bell peppers after 16 weeks in 2005 and 2006 were 76 and 18%, respectively, for those receiving fungicide applications and 97 and 42%, respectively, for those without fungicide treatment. Furthermore, marketable yields and farm-gate revenues were lower in 2005 compared to 2006 (Table 3). These results indicate that overall Phytophthora blight incidence in bell peppers was not as high in 2006 compared to 2005 which most likely resulted from: 1) differences in rainfall patterns between the two years, with excessive amounts during July and August 2005 (Anonymous, 2006a) that led to flooding of the field and water often standing between rows of black plastic; and, 2) planting the experiment in a higher, better-drained area of the field in 2006. This further shows that *P. capsici* thrives in wet conditions, particularly in low-lying or poorly-drained areas of the field where water accumulates (Hausbeck and Lamour, 2004).

This research indicated that 'Paladin' generally produces high yield and farm-gate revenues under high Phytophthora blight disease pressures, and this bell pepper cultivar could be a reliable choice for growers in Illinois who have problems with this disease (Table 3). The *P. capsici*-tolerant cultivars, Alliance, Aristotle X3R, and Revolution tended to have a lower incidence of Phytophthora blight and increased yield and farm-gate revenues compared to the susceptible cultivars evaluated.

Although 'Paladin' offers *P. capsici* resistance, other factors such as poor horticultural characteristics and susceptibility to other diseases [*e.g.*, bacterial spot (*X. campestris* pv. *vesicatoria*)] must also be taken into account when choosing bell pepper cultivars for commercial production (Rowell *et al.,* 2006). Kline and Johnston (2005) indicated that 'Aristotle X3R' and 'Revolution' have better fruit quality characteristics than 'Paladin'. In addition, 'Alliance', 'Aristotle X3R', and 'Revolution' have bacterial spot resistance along with *P. capsici*-tolerance (Egel *et al.,* 2005, Rowell *et al.,* 2006, Anonymous, 2006b). Therefore, if growers require bell pepper cultivars having resistance to several diseases or better horticultural characteristics, Alliance, Aristotle X3R, and Revolution may provide a better choice. The multiple disease resistance in these three bell pepper cultivars is important in areas such as the southern United States where bacterial spot disease incidence is high and Phytophthora blight is also a problem (Rowell *et al.,* 2006).

Although plant resistance and/or tolerance are often the basis of disease management programs, these methods should not be relied upon alone to achieve effective control of *P. capsici* (Hausbeck and Lamour, 2004). Furthermore, this research shows that regardless of the level of resistance to *P. capsici,* fungicide applications provided greater bell pepper yields and farm-gate revenues compared to those that were not treated. Although yield and farm-gate revenues were high for the resistant/tolerant cultivars in most cases, fungicide applications provided extra protection by reducing Phytophthora blight incidence compared to non-treated plants. Furthermore, the utilization of *P. capsici*-resistant/tolerant cultivars coupled with fungicides and cultural practices, such as planting into raised beds and managing field moisture levels, may provide growers with further protection from Phytophthora blight-induced bell pepper losses.

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Evaluation of the FAO CROPWAT model for deficit-irrigation scheduling for onion crop in a semiarid region of Ethiopia

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Abstract

Deficit irrigation conserves water and minimizes adverse effects of excess irrigation. In this study, the applicability of the CROPWAT model in management of deficit irrigation was evaluated at Sekota Agricultural Research Center, Ethiopia. Water was applied using low head drippers. There were eight treatments with three replications: stress at 1st, 2nd, 3rd, and 4th growth stages and partial stresses of 50% ETc, 75% ETc with two controls of 25% ETc and 100% ETc of the water requirement throughout the growing season. The input data for CROPWAT program were climatic, rainfall, crop and soil data. Yield reductions simulated by CROPWAT program were comparable with yield reduction measured under field condition. Model efficiency and correlation coefficients of 98% were obtained. Based on the above comparative analysis, CROPWAT program could adequately simulate yield reduction resulting from water stress.

Key words: Growth stages, deficit irrigation, Ethiopia, CROPWAT model, onion.

Introduction

Regulated deficit irrigation provides a means of reducing water consumption while minimizing adverse effects on yield. CROPWAT model could play a useful role in developing practical recommendations for optimizing crop production under conditions of scarce water supply and instrument limitations for soil moisture monitoring (Smith, 1993). CROPWAT is a computer program for irrigation planning and management developed by FAO (Doorenbos and Pruitt, 1977). Based on daily water balance computations, the CROPWAT model can evaluate alternative water supply conditions and associated reductions in crop yield. However, it has been tested only in limited areas and only for few crops (Smith *et al.,* 2002). Results reported by the limited earlier evaluations of CROPWAT were not conclusive.

In north eastern parts of Ethiopia (Sekaota), onion is grown under irrigation for domestic use as well as for commercial purposes. The water application method is generally a low head or gravity drip irrigation. Because of acute water shortage during the dry season, much of the cultivable land in close proximity to the irrigation canals is left as fallow. In addition, water related dispute among farmers are common in the area. Therefore, optimal allocation of locally available water resources is a key to increase crop production. In this study, the FAO CROPWAT model was evaluated by comparing its predicted yield reductions against results of field experiment using onion as a test crop.

Materials and methods

Description of the study area: The field experiment was conducted at *Woleh* irrigation scheme, a research site of *Sekota* Dryland Agricultural Research Center in north eastern part of Ethiopia. The test crop, onion, is one of the major irrigable and marketable crops in the area. The mean annual rainfall in the area is 635 mm. The mean annual temperature ranges from 8°C to 21°C. The soil is medium textured.

Treatment setting: Bombay Red variety of onion was planted with a spacing between crops of 10 cm and spacing between rows of 20 cm in a 3 x 4 m plots. The experimental design consisted of completely randomized blocks with three replications. Within each block, eight irrigation regimes were randomly distributed (Table 1).

Installation of drip system: Low head drip irrigation was used for applying water. Each irrigation treatment consisted of 15 drip lines of length 4 m and each line serves 40 onion plants with a total of 600 plants in each plot. A total of 24 barrels were put on an elevation of one meter above ground to supply water to each of the treatments. The barrels height was calibrated and marked at

Table 1. Treatment setting for deficit irrigated onion field experiment at *Sekota* (Ethiopia)

Treatment*	Growth stage/period				Description
	P1	P2	P3	P4	
	Control				
1111 (T1)	1	1	1	1	All normal watering
0000 (T2)	0	0	0	0	All stress
	One period stress				
0111 (T3)	0	1	1	1	Stress during P1
1011 (T4)	1	0	1	1	Stress during P2
1101 (T5)	1	1	0	1	Stress during P3
1110 (T6)	1	1	1	0	Stress during P4
	Partial stress (%)				
50% Deficit (T7)	50	50	50	50	Throughout the growing season
25% Deficit (T8)	25	25	25	25	Throughout the growing season

*1- indicates normal watering - watering 100% of ETc.
0- (75% deficit) indicates stressed watering - watering 25% of ETc.
25%- Deficit was watering 75% of ETc.
50%- Deficit was watering 50% of ETc.

Table 2. Onion crop parameters for input into FAO CROPWAT deficit irrigation program in *Sekota* (Ethiopia)

Parameter	Growth stage (planted on 11th November 2005)				
	Initial stage	Development	Mid-season	Late season	Total/ Seasonal
Length of growing season (days)	20	30	30	15	95
CROPWAT standard Kc (FAO, 1998)	0.70	>>>+	1.05	0.95	
Crop height (m)	0.12	0.30	0.40	0.40	
Rooting depth (m)	0.10	0.20	0.30	0.30	
Depletion level (fraction)	0.30	>>>	0.45	0.50	
Yield response factor (Ky) (FAO, 1998)	0.80	0.40	1.20	1.00	1.00

+ Intermediate values

one litre intervals. The amount of water that is needed to reach the plants was controlled by throttle valves on the sub-main lines.

CROPWAT input data: Calculations of water and irrigation requirements utilize inputs of climatic, crop and soil data, as well as irrigation and rain data. The climatic input data required are reference evapotranspiration and rainfall. Reference evapotranspiration was calculated using Eq. (1). The crop parameters used for estimation of crop evapotranspiration, water balance calculations, and calculation of yield reductions due to water stress include crop coefficient Kc, length of the growing season, critical depletion level, p and yield response factor Ky. Literature values of these parameters were adopted (Table 2) (Allen *et al.,* 1998).

The soil data include information on total available soil water content, initial soil water content at the start of the season, and maximum infiltration rate by double ring infiltrometer for runoff estimates.

Equations of FAO CROPWAT deficit irrigation model: The FAO Penman-Monteith method (Allen *et al.,* 1998) was used to calculate ET_o in CROPWAT Program as:

$$ET_o = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T+273} U_2(e_a - e_d)}{\left[\Delta + \gamma(1 + 0.34U_2)\right]} \quad (1)$$

Where, ETo = reference crop evapotranspiration (mm/day),
R_n = net radiation at crop surface (MJ/m^2.day),
G = soil heat flux (MJ/m^2.day),
T = average temperature (^{o}C),
U_2 = wind speed measured at 2m height (m/s),
e_a-e_d = vapour pressure deficit (kpa),
Δ = Slope vapor pressure curve (kpa/^{o}C),
γ = Psychometric constant (kpa/^{o}C),
900 = conversion factor.

Crop water requirements (ET_c) over the growing season were determined from ET_o according to the following equation using crop coefficient K_c as:

$$ET_c = K_c \times ET_o \quad (2)$$

Where, ET_c = Crop water requirement, K_c = Crop coefficient, ET_o = reference evapotranspiration.

Crop coefficient K_c values as presented by (Allen *et al.,* 1998) were used. Crop irrigation requirements were calculated assuming optimal water supply and effective rainfall. Given water input into and output from the irrigation system, soil water retention and infiltration characteristics along with estimates of rooting depth; the model performs a daily soil water balance calculation to predict root zone soil water content.

Stress conditions in the root zone were in terms of critical soil water content, expressed as the fraction of total available soil water (soil water held between field capacity and wilting point), The critical soil water content is defined here as the soil moisture level below which crop transpiration is limited by soil water content. It varies for different crops and different crop growth stages and is determined by the root density of the crop, evaporation rate, and to some extent by the soil type. Allen *et al.* (1998) updated the estimates of critical soil moisture, representing onset of stress, previously reported by Doorenbos and Pruitt (1977) and Doorenboss and Kassam (1979). The effect of water stress on yield was quantified by relating the relative yield decrease to the relative evapotranspiration deficit with an empirical yield response function (Eq. 3):

$$\frac{Y}{Y_m - 1} = K_y\left(1 - \frac{ET_a}{ET_m}\right) \quad (3)$$

Where, Y and Y_m are expected and maximum crop yields, corresponding to ETa and ETm = ETc, actual and maximum evapotranspiration, respectively; K_y is a crop yield response factor that varies depending on species, variety, irrigation method and management, and growth stage when deficit evapotranspiration is imposed.

Treatment setting for the CROPWAT program: The selected crop, onion was subjected to different watering regimes using the FAO-CROPWAT program in deficit irrigation mode. Four treatments in relation to stress during a specific growth stage, additional two treatments in relation to putting the crop (onion) under stress with a certain amount of irrigation water throughout the whole growth stages, and two controls, so a total of eight treatments were implemented.

Evaluation of the CROPWAT model as a deficit irrigation scheduling tool: Data from field experiment was used to verify the utility of the CROPWAT model in simulating deficit irrigation scheduling. The field experiment applied various irrigation water levels to onion crop, thus inducing water stress at various growth stages and throughout the growing season. Climatic, soil, and crop data collected through field experiments were used as inputs to the CROPWAT model. The yield of onion crop was used to validate the yield reduction extracted from the CROPWAT model.

Model efficiency (ME) developed by Nash and Sutcliffe (1970) is commonly used and seems appropriate to evaluate performance of models. The model efficiency was similar to the coefficient of determination (r^2). However, the residual variation is calculated using the means of actual observations rather than values from the best regression line between observed and predicted values (Risse *et al.,* 1994; De Roo, 1993). Model efficiency was used to

determine goodness of fit between model prediction and measured values. It is defined as:

$$ME = \frac{\sum_{t=1}^{n}(Y_o - Y_p)^2}{\sum_{t=1}^{n}(Y_o - Y_m)^2} \quad (4)$$

Where, ME if model efficiency, Y_o and Y_p are measured and predicted values for event t and Y_m is the mean value of measured values for all events considered in the simulation study, and n = total number of events .

Results and discussion

Irrigation depths of water applied to each treatment is presented in Table 3. The depth of application presented is with variable depth and variable intervals to refill the soil moisture deficit. Irrigation was applied daily.

Table 3. Irrigation depths of deficit irrigated tomato experiment at *Sekota* (Ethiopia)

Date	Interval	Treatments							
	(d)	T1	T2	T3	T4	T5	T6	T7	T8
		Irrigation (mm)							
15 Nov	4	11.7	2.9	2.9	11.7	11.7	11.7	5.8	8.8
21 Nov	6	14.1	3.5	3.5	14.1	14.1	14.1	7.0	10.6
27 Nov	6	14.0	3.5	7.0	7.0	14.0	14.0	7.0	10.5
3 Dec	6	14.2	3.6	14.2	3.6	14.2	14.2	7.2	10.7
9 Dec	6	15.5	3.9	15.5	3.9	15.5	15.5	7.8	11.6
15 Dec	6	17.0	4.3	17.0	4.3	17.0	17.0	8.6	12.8
21 Dec	6	18.4	4.6	18.4	4.6	18.4	18.4	9.2	13.8
27 Dec	6	19.8	5.0	19.8	5.0	19.8	19.8	10.0	14.9
2 Jan	6	21.2	5.3	21.2	21.2	5.3	21.2	10.6	15.9
8 Jan	6	21.8	5.5	21.8	21.8	5.5	21.8	11.0	16.4
14 Jan	6	22.3	5.6	22.3	22.3	5.6	22.3	11.2	16.7
20 Jan	6	22.7	5.7	22.7	22.7	5.7	22.7	11.4	17.0
26 Jan	6	22.6	5.7	22.6	22.6	5.7	22.6	11.4	17.0
31 Jan	5	19.6	4.9	19.6	19.6	19.6	4.9	9.8	14.7
6 Feb	5	23.2	5.8	23.2	23.2	23.2	5.8	11.6	17.4
12 Feb	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	95	278.1	69.8	251.7	207.6	195.3	246.0	139.6	208.8

Yield and water use efficiency of onion: Treatment T2 (0000) that received only one-fourth of the optimal irrigation water throughout the growing season produced 5.5 t ha^{-1}. As presented in Table 4, water deficit at first and fourth growth stages, resulted in non-significantly different yields from the optimum application T1 (1111). However, in no case the yields were higher than that in T1 (1111). When the water deficit is at the second and third growth stages (treatments T4 (1011) and T5 (1101), or during all stages T2 (0000), 50%, and 75%, the yields were significantly different from treatment T1 (1111).

Simulation of yield reduction of onion with CROPWAT model: Climatic, soil, and calibrated onion crop parameters presented in Table 2 were entered into FAO CROPWAT program for simulation of yield reduction. The next step was to analyze the treatments by entering the net irrigation requirement to refill the soil moisture deficit in the CROPWAT program to achieve the yield reduction to water stress imposed during the various crop growth stages and throughout the growing season. The simulated onion crop depletion levels in the experimental area were 0.30, 0.38, 0.45, and 0.50 at the initial, development, mid season, and late season stages respectively (Table 2). This implies that onion can not tolerate depletion levels of more than 30, 38, 45 and 50% at the initial, development, mid, and late stages, respectively. There will not be yield reduction until the respective depletion levels are reached at the respective stages. Yield reduction starts whenever the depletion is beyond the above indicated limits at the respective growth stages.

Table 4. Mean yield (t ha^{-1}) comparison using Duncan's Multiple Range Test (DMRT)

Treatment	Yield*
T1 (1111)	25.00a
T2 (0000)	5.50e
T3 (0111)	24.00a
T4 (1011)	20.76b
T5 (1101)	17.50c
T6 (1110)	23.86a
T7 (50%)	13.80d
T8 (75%)	21.26b
Mean	18.96
Coefficient of variation	5.80

*Values followed by similar letters are not significantly different,

The simulated yield response factor values (K_y) in the respective growth stages indicated that whenever the value of K_y is less than unity, the relative yield reduction is less than relative evapotranspiration deficit. Stressing during those stages was advantageous to increase the overall water use efficiency. This means stressing at first and second stage was advantageous than stressing at the third stage. Table 5 presents the simulated yield reductions under different deficit irrigation levels using FAO CROPWAT program.

Table 5. Simulated yield reductions of onion under different water stress levels

Treatment	Irrigation (mm)	Yield reduction (%)	Rank
T1 (1111)	278.1	0.0	8
T2 (0000)	69.8	63.8	1
T3 (0111)	251.7	8.0	6
T4 (1011)	207.6	22.1	4
T5 (1101)	195.3	24.8	3
T6 (1110)	246.0	6.6	7
T7 (50%ETc)	139.6	41.6	2
T8 (75%ETc)	208.8	19.7	5

Comparison of yield reduction under field conditions and CROPWAT model simulation: Table 6 presents comparisons of measured yield reduction for each treatment at field conditions with the yield reductions simulated by the CROPWAT model. The yield reductions were expressed as percentages of the yield obtained under optimal irrigation T1 (1111).

From Table 6 it can be observed that the measured yield reductions are comparable to the simulated ones. Both model efficiency and correlation coefficient were 98%. From the student's t-test, the value of t was calculated to be 17.15. Further, in the two tailed test, the value of r was calculated to be r>0.707 and r<-0.707. Even if the value of r^2 was 0.98, the model was efficient at 5% significance level for the r values greater than 0.707 and less than -0.707 on the two tailed graphs.

Furthermore, in agreement to the field data, the simulated results

Table 6. Comparison of measured and CROPWAT simulated yield reductions for onion

Irrigation treatment	Measured		CROPWAT
	Yield (kg ha^{-1})	Yield reduction (%)	Yield reduction (%)
T1 (1111)	25000	0.0	0.0
T2 (0000)	5500	78.0	63.8
T3 (0111)	24000	4.0	8.0
T4 (1011)	20750	17.0	22.1
T5 (1101)	17500	30.0	24.8
T6 (1110)	23860	4.5	6.6
T7 (50%ETc)	13750	45.0	41.6
T8 (75%ETc)	21250	15.0	19.7

show that the impact of water stress on onion crop yield depends on growth stages. For example, water stresses at the third growth stage leads to a larger yield reduction than stress at second stage. Smith *et al.* (2002), in the validation of CROPWAT for deficit irrigation, had stated that yield reduction at third stage was more severe than yield reductions at first, second and fourth stages.

Data from field study were used to verify the applicability of the CROPWAT model in simulating deficit irrigation scheduling in the area. The yield reductions simulated by CROPWAT were comparable with the measured yield reduction at field condition with model efficiency and coefficient of correlation values of 98%. Furthermore, the simulated results reflected the impact of stress at different growth stages on yield reduction: stress at third stage leads to a higher yield reduction than stress at first, second and fourth stagees. Based on the above comparative analysis, it can be concluded that the CROPWAT model could adequately simulate yield reduction resulting from water stresses in the study area.

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Evaluation of grafting effect on tomato crop yield and *Fusarium* crown and root rot disease

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Abstract

Tomato, *Lycospersicon esculentum*, is an important vegetable crop in Tunisia and many other Mediterranean countries. *Fusarium* crown and root rot of tomato are new diseases in the area, first reported during 2000-2001 crop season, threatening tomato production. Being a soil-borne pathogen, effective disease control methods of *Fusarium* crown and root rot are limited thus requiring the alternative measures for disease management. In this study the efficacy of grafting commercial Tomato cultivars Bochra and Amal, used as scions, onto a new rootstock Beaufort and Kemerit RZ was examined in controlled and natural conditions. Grafting was found, in this study, to be an effective method to attenuate the impact of *Fusarium* wilt, *Fusarium* crown and root rot. Moreover, grafting increased tomato growth parameters, yield and improved fruit quality.

Key words: Tomato, *Lycospersicon esculentum,* graft, *Fusarium* crown and root rot, grafting, Beaufort x Bochra, Beaufort x Amal, Kemerit x Bochra and Kemerit x Amal, rootstock

Introduction

Tomato, *Lycospersicon esculentum* Mill. (Solanaceae), is one of the world's most important vegetable crops with a worldwide fresh weight production of 80 million tons from a cropped area of about 3 millions hectares (FAO, 2005). The fruit improves the supply of vitamins and minerals in human nutrition (Sabongari and Aliero, 2004). *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (*FOL*), is one of the most devastating diseases of tomato (Walker, 1971). *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*FORL*) which induced *Fusarium* crown and root rot of tomato is also an important and widespread vascular wilt pathogen of tomato (Jarvis, 1989; Jarvis and Shoemaker, 1978). Its epidemiology and geographical distribution have been studied extensively (Jarvis, 1977). In Tunisia, it was first reported during 2000-2001 crop season in some tomato geothermal greenhouses (Hajlaoui *et al.,* 2001). Although Fusarium wilt-resistant tomato cultivars have been developed, none of them have been widely used in disease control (Thibodeau and Simard, 1978).

For many years, control of Fusarium crown and root rot of tomato has been limited to the use of conventional soil sterilizing procedures together with the application of fungicides (Rowe and Farley, 1981). However, complete eradication of *FORL* from soil by steam-sterilization and fumigation with chemicals could not be achieved (Jarvis and Thorpe, 1980). Therefore, there is an urgent need to find alternatives that may protect plants from soilborne fungal pathogens, especially in covered crops. Tomato grafting is now widely used in various areas of the Mediterranean countries. In Tunisia, grafting is common for watermelon and muskmelon, but grafting tomatoes onto resistant rootstocks is very limited. The aim of the present study was to evaluate the resistance of tomato plants to *Fusarium* spp., so that they could be used as rootstocks for solanaceous plants.

Materials and methods

Plant material: Two tomato rootstocks, Beaufort and Kemerit RZ, as well as two tomato cultivars, Bochra and Amal, used as scions, were considered for this study (Table 1). The grafted plants tested were Beaufort x Bochra, Beaufort x Amal, Kemerit x Bochra and Kemerit x Amal.

Table 1. Characteristics of tomato cultivars (scions) and rootstocks used in this study

Cultivars or rootstocks	Resistance	Fruit form	Growth
Bochra	TMV, V, F_2	Oblong	Indeterminate
Amal	ASC, F_1, F_2, N, ST, V	Elongate	Vigorous
Beaufort (De Ruiter)	TMV, K, N, V, F_2, Fr	-	-
Kemerit RZ	TMV, V, F_2	-	-

TMV: Tomato mosaic virus, ASC: Alternaria stem canker, ST: Stemphilium, V: *Verticillium* spp., F_1, F_2: *Fusarium oxysporum* f. sp. *lycopersici* races 1 and 2. Fr: *Fusarium oxysporum* f. sp. *radicis-lycopersici*, K: *Pyrenochaeta lycopersici* (Corky root), N: Nematodes (most common species).

Fungal isolates: Two isolates of *Fusarium* were used to evaluate the reaction of tested tomato rootstocks and cultivars to *Fusarium* crown and root rot disease. Isolate of *F. oxysporum* f. sp. *lycopersici* named *FOL* and an isolate of *F. oxysporum* f. sp. *radicis-lycopersici* abbreviated as *FORL*.

Root-dip inoculation: Root-dip inoculation was performed with seedlings showing 3 to 5 expanded leaves. Regular surveys were carried out to detect the eventual presence of fungal diseases.

Tomato rootstocks and scions resistance to *Fusarium* wilt: Resistance of rootstocks and scion cultivars to *Fusarium* spp. affecting tomato, were evaluated upon artificial inoculation in growth chamber on peat substrate.

Evaluation of agronomic parameters in controlled conditions: To evaluate the effect of grafting on plant development, some parameters were measured to compare the behaviour of grafted and non-grafted plants: i) Number of leaves: this parameter was recorded on 10 seedlings in each kind of grafted plants and non-grafted ones. The counting of number of leaves was carried out after 15 days of grafting then this operation was repeated each week during one month. ii) Stem height: it was measured from the collar to until the apical bud of the seedlings. iii) Diameter of the stem: this parameter enabled to detect the effect of the grafting on the strength of the seedlings.

Evaluation of agronomic parameters in natural conditions: The grafted seedlings were planted in greenhouse in October in twinned lines, with mulching and on each line there were grafted seedlings on 1 arm, others on 2 arms and non grafted seedlings considered as controls. Agronomic parameters were evaluated to compare grafted and non grafted tomato plants as listed below: i) Tomato yield was recorded on selected plants in each line. Yield of 24 plants was evaluated for grafted plants with 2 arms and 24 plants for grafted plants with 1 arm and on the same number for control. The yield was estimated by weighing the fruits for each harvest. ii) The distance between the ground and the 1st flowers bunch, which is a very important parameter and this distance differs between grafted plant and non grafted ones. iii) The number of bouquets per seedling. iv) Diameter of the stems, this parameter indicates the effect of the grafting on growth of plants. Using a slide caliper the stems diameter of the grafted and non grafted plants was measured. v) Gauge of the fruits, the fruits were classified according to the gauges small, medium and large and vi) The gauges, they can be either homogeneous, or heterogeneous

Statistical analysis:. Variance analysis of the treatment effect was made using SPSS software. Means were compared by Duncan multiple test at 5% level.

Results

Evaluation of agronomic parameters in controlled conditions

Leaves number: Results showed significant differences between the number of leaves on grafted and non grafted plants. The mean number was more than 7 leaves per seedling for Kemerit RZ x Bochra plants, whereas it was around 6 for Beaufort x Bochra and Beaufort x Amal. For Kemerit RZ x Amal, this number was similar to non grafted plants (5 leaves plant^{-1}). This result shows the difference of affinity between scion and rootstock (Table 2).

Table 2. Evaluation of agronomic parameters of tomato cultivars (scions) and rootstocks used in this study in controlled conditions

Grafted tomato plant or cultivar	Leaves number seedling^{-1}	Stem height (cm)	Stem diameter (cm)
Beaufort x Bochra	6.2^{c}*	25^{b}	5^{c}
Beaufort x Amal	6.0^{c}	25^{b}	4.5^{b}
Kemerit RZ x Bochra	7.8^{d}	28^{c}	5.2^{d}
Kemerit RZ x Amal	5.2^{b}	20^{a}	4.5^{b}
Bochra	5.0^{a}	22^{a}	3.8^{a}
Amal	5.0^{a}	22^{a}	3.8^{a}

* Values with the same letter(s) are not significantly different at α=5% accor ding to Student-Newman-Keuls test

Stems height: These results revealed that grafted plants Kemerit RZ x Bochra showed the highest value of stems height (28 cm). However, for Kemerit RZ x Amal the stem height was of 20 cm (Table 2).

Stem diameter: Results showed a significant difference between grafted and non grafted plants according to the scion and the rootstock used. These results revealed that grafted plants Kemerit RZ x Bochra had the highest value of stems height (28 cm). However, for Kemerit RZ x Amal the stem height was of 20 cm (Table 2).

It seems that the grafting had a positive effect on stem diameter. Affinity and compatibility between the scion and the rootstock could influence the root system development and the rootstock strength and thus improves the vegetative growth. The two rootstocks used in this study (Kemerit RZ and Beaufort) showed the highest values of these three measured parameters (leaves number, stem height and diameter) when the scion was Bochra.

Resistance of grafted plants against *FOL* and *FORL* in controlled conditions: Results analysis of grafted plants inoculated by *FOL* and *FORL*, showed a highly significant difference according to the rootstocks used. The grafting of Amal cultivar on rootstock Beaufort seemed to improve its resistance to *Fusarium* spp. Moreover, this variety grafted on Kemerit RZ seemed to be highly resistant to *FOL* and *FORL* (0%). When using Beaufort as rootstock, seedlings appeared also to be very resistant to *FOL* and *FORL* (0%). The re-isolation of the pathogen from inoculated plants showing typical symptoms of the diseases confirmed that damages observed were due to the inoculation by the two *Fusarium* species used in this study, thus fulfilling the Koch postulate's (data not shown).

Evaluation of agronomic parameters in natural conditions

The distance between the ground and the 1st flower bunch: Statistical analysis showed a significant difference of the distance between the ground and the 1st flowers bunch during time. It was of 38 cm at the beginning of the growth cycle then decreased to 35 cm (Table 3). The variation of the distance between the ground and the 1st bunch was highly significant for the control compared to the grafted plants. However, this variation was not significant for grafted plants with 1 arm and 2 arms. This distance was of 30 cm for the grafted plants and of 47 cm for the control (Table 3).

Distance between 1st bunch and 2nd bunch: Statistical analysis revealed that the variation of the distance between 1st bunch and 2nd bunch during time was significant. This distance was of 22.5 cm in February and March then it decreased to 20.5 cm since the beginning of April (Table 3). Distance between 1st bunch and 2nd bunch was not significantly different between the different modes of culture. This distance was of 21.5 cm for the control plants and the grafted plants with 2 arms and it is of 22.5 cm for the grafted plants with 1 arm (Table 3).

Height of the plants: The variation of the height according to time and the mode of control of the plants was non significant. The height was 1.84 m for the control, 2.16 m for grafted plants with 1 arm and 2.02 m for the grafted plants with 2 arms (Table 3).

Diameter of the stems: Statistical analysis indicated that the variation in stem diameter over the period was non significant (data not shown). The variation of the diameter according to the mode of control of the seedlings was highly significant for the grafted plants with one arm. This diameter difference between the grafted plants with one arm (1.55 cm) and 1.15 cm for the control was significant (Table 3).

Table 3. Evaluation of agronomic parameters of tomato plants in natural conditions

Agronomic parameters	Time			Mode of control		
	February	March	April	Non grafted	Grafted (1 arm)	Grafted (2 arms)
Ground to 1st flower bunch (cm)	38[b]*	35[a]	35.44[a]	47.44[b]	30.22[a]	30.22[a]
1st to 2nd flower bunch (cm)	22.55[b]	22.44[b]	20.56[a]	21.67[a]	22.44[b]	21.44[a]
Height of plants (cm)	-	-	-	1.84[a]	2.16[b]	2.02[b]
Diameter of stems (cm)	-	-	-	1.15[a]	1.55[b]	1.24[a]
Number of bunches	10.77[a]	11.33[b]	10.44[a]	7.44[a]	9.67[a]	15.44[b]
Yield (kg m^{-2})	-	-	-	6.49[a]	8.29[a]	9.67[b]

* Values with the same letter(s) are not significantly different at α=5% according to Student-Newman-Keuls test

Number of bunches: The variation of the number of bunches was non significant. The number of bunches increased in March (11 bunches) and decreased gradually (Table 3). The variation of the number of bunches according to the mode of control of the plants was, however, highly significant. We noted that this parameter did not differ between the control (8-10 bunches) and the grafted plants with 1 arm (8-10 bunches) but the difference was clear between grafted plants with 2 arms (15 bunches) and that of 1 arm (Table 3).

Yield: The variation of the yield was highly significant. Results showed that the total weight did not differ for the grafted plants with 1 arm (10.54kg) and the control plants (9.18kg). However, the difference was clear between the grafted plants with 2 arms (12.46kg) and those grafted with 1 arm (Table 3).

Fruit size and bunch homogeneity: We noticed that the fruits resulting from the grafted plants had a gauge better than those from the control plants. It is clear that the majority of bunches were homogeneous, for the grafted plants, from the point of view of gauges and stage of maturity (Fig. 1A, B, C).

Discussion

In Tunisia, *Fusarium* crown and root rot of tomato is a new disease identified since 2000-2001 crop season, causing serious damages reaching 90% in certain greenhouses (Hajlaoui *et al.,* 2001). *Fusarium oxysporum* f. sp. *radicis-lycopersici* is responsible for this problem. Similarly, Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* is also a serious disease affecting tomato cropped in greenhouse. While exhibited heavy losses on tomato production, no or some effective disease control methods are available and there is no approved fungicide to control. In the past, grafting tomato plants was considered too expensive but at present it is used at a commercial level in Tunisia and elsewhere. Resistant rootstocks provide excellent control of many tomato soilborne pathogens, particularly *F. oxysporum* f. sp. *radicis-lycopersici*, *F. oxysporum* f. sp. *lycopersici, Pyrenochaeta lycopersici* and *Meloidogyne* spp. Furthermore, tomato grafting gave other advantages such as plant growth promotion, yield increase, extension of yield period and improvement of fruit quality (Rivero *et al.,* 2003).

In our growth chamber experiments, it was found that the two rootstocks Beaufort and Kemerit RZ were resistant to *FORL* and *FOL*. So, they could be used as rootstocks for grafting tomatoes. Hibar *et al.* (2006) reported that Beaufort was effective against *FORL*. Data obtained from this study revealed that grafting can control *Fusarium* crown and root rot and *Fusarium* wilt. Similar results were shown by Trionfetti *et al.* (2002) and Miguel *et al.* (2004) on controlling *Fusarium* wilt by grafting two muskmelon cultivars and triploid watermelon onto commercial rootstocks, respectively. On Tomato, our results show that grafting of susceptible tomato cvs Bochra F_1 and Amal F_1 onto the rootstocks Beaufort and Kemeit RZ increased tomato growth parameters, tomato yield and improve fruit quality. This increase in tomato yield through the use of grafted plants could be attributed mainly to disease resistance and to better plant growth. In grafted plants, the rootstock's vigorous root system is often capable of absorbing water and nutrients more efficiently compared to non grafted plants and may serve as good supplier of endogenous plant hormones (Fernandez-Garcia *et al.,* 2002; Estan *et al.,* 2005). However, the rootstock effect varies greatly with scion cultivar and growing season (Lee, 1994). This mechanism has not been intensively investigated. The disease tolerance in grafted seedlings may be entirely due to the tolerance of stock plant roots to such diseases.

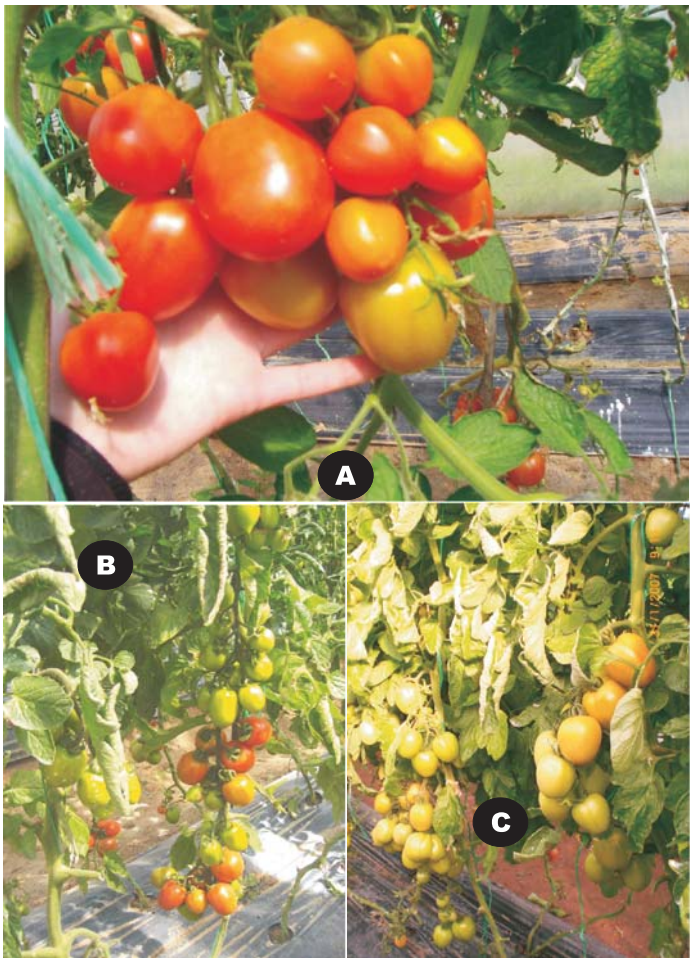

Fig. 1. A-Homogenous bunch from a single arm grafted plant. B-Fruits of similar size from a single arm grafted plant. C-A bunch of big sized fruit borne on a double arm grafted plant.

However, in actual plantings, adventitious rooting from the scion is very common (Lee, 1994). Plants having the root systems of the scion and rootstock are expected to be easily infected by soilborne diseases. However, seedlings having dual root systems often exhibit excellent disease resistance, almost comparable to those having only rootstock roots. This observation partially supports the previous report that substances associated with *Fusarium* tolerance are synthesized in the root and translocated to the scion through the xylem (Biles *et al.,* 1989). The activity of the substances related to disease resistance may vary during the development stages of the grafted plants (Heo, 1991).

On the basis of the results obtained in these experiments on tomato, grafting effectiveness seems to be determined not only by disease resistance but also by their impact on both production and fruit quality. The rootstocks Beaufort F_1 and Kemerit RZ, resistant to *FORL* and *FOL*, were also the best genotypes capable of significantly improving the productivity and fruit quality of tomatoes cultivars. Moreover, regardless of the used tomato cultivars, grafted plants gave the best results concerning plant growth, fruit yield and fruit quality, compared to non-grafted plants.

The tomato grafting proved to be an effective method to attenuate the impact of *Fusarium* wilt and *Fusarium* crown and root rot caused by *FOL and FORL*, respectively. Moreover, as grafted plants are expensive they could be trained in two arms to reduce seedlings number per unit area.

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The effect of upper-limit of soil water content on tomato and cucumber

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Abstract

This study was conducted to determine the optimum upper limit of soil water content (SWC) for tomato and cucumber from early stages after transplanting. Five different upper limits of SWC were tested at the lowest limit of 60% of field capacity (FC) for tomato and of 75% for cucumber. Stem growth, root viability, yield and fruit concentrations of vitamin C and total soluble solids were significantly affected by the treatment. The highest yield and best fruit quality was obtained at 85% of FC for tomato and at 90% for cucumber. This suggests that irrigating to FC does not necessarily result in higher yields and better fruit quality.

Key word: Upper-limit, soil water content, yield, fruit quality, tomato, cucumber

Introduction

Water supplies are limited worldwide (Postel, 1998). Therefore, it is necessary to explore efficient irrigation methods. Soil water content (SWC) is one of the improtant parameter used most often by small farmers in China (Blanke *et al.,* 2007) for determining irrigaiton requriements. Field capacity (FC) is viewed as an upper limit when irrigating based on SWC. But 80% of FC was better for lettuce compared to 98% (Dunkel, 1968). Maize yield decreased when SWC exceeded 90% of FC (Gao *et al.,* 2006). Better results for watering below FC could be because of higher soil aeration (Sidorova *et al.,* 1988).

The objective of this research was to explore the optimal upper-limit of soil water content for tomato and cucumber. We measured plant stem diameter, root viability, yield, and quality of fruit. The experiment was carried out in a greenhouse to minimise the adverse effects that frequently changing weather may have on plant responses.

Materials and methods

The experiment was conducted at the Horticulture Crop Test Unit, North West A&F University, Yangling (latitude 34°.21′ N, longitude 108°10′ E), China, from February to June 2005. Seedlings of fresh market `Qinfen' tomato and `Jingchun 2' cucumber were transplanted with plant and row spacing of 0.40 × 0.55 m on 28 March 2005 in two greenhouses. The soil was clay loam.

Irrigation was applied according to:

$$m=10 \times r \times h \times \beta_f (\beta_U-\beta_L) \frac{1}{\eta \times p}$$ (Wang *et al.,* 2000) [1]

Where m=irrigation amount (mm), r =bulk density (g cm^{-3}), h= the depth of wetting front (cm), η =efficacy of irrigation water use, β_f=field capacity (%), β_U=upper-limit of SWC (%), β_L= lower-limit of SWC (%), p =water density (g cm^{-3}). The values of these parameters are shown in Table 1.

There were five treatments with three replicates in a completely randomized block design. The five irrigation treatments started 18 days after transplanting and continued for 35 days. At each irrigation, 102.4, 76.8, 64.0, 51.2, 25.6 mm of water were applied to the five treatments for tomato and 38.1, 30.4, 22.8, 15.2, 7.6 mm for cucumber following Equation 1. There were 9, 10, 14, 14 for tomato and 4, 5, 6, 7 and 9 irrigations for cucumber, respectively. Furrow irrigation under plastic mulch was used every other row.

The soil moisture meter HH2 connected to an ML2x soil moisture sensor (Delta-T Devices Ltd., Cambridge, England) was used to measure soil moisture every other day at 10 a.m. at a soil depth of 20 cm. Three plants were tagged in each plot and diameter of the main stem, 5 cm from ground, was measured twice using a ruler accurate to 0.001cm (50-75, Shanghai Cany Precision Instrument Co. Ltd., China), at the beginning and the end of the experiment. Root viability was measured by the reduction of triphenyl tetrazolium chloride (TTC) method (Gao, 2000). Concentration of soluble solids was measured by the Anthracene Ketone method and that of vitamin C by Molybdenum blueness colorimetric method (Gao, 2000). Root samples were collected at the end of irrigation treatments. Fruit samples were randomly chosen at peak harvest. The data were analysed using the DPS procedure of Statistical Analysis System software. Treatment means were separated by t-test at $P \leq 0.05$.

Results and discussion

Stem diameter growth over 30 days, root viability, yield and vitamin C content and total soluble solids in fruit were significantly affected by irrigation treatments (Table 2). For tomato, the lowest values of these parameters were for the 70% treatment while the highest values were shared by the 85 and 90% treatment except for the root viability which was highest for the latter. In cucumber, the lowest values of these parameters was shown in the 80 or 100% of FC treatment and the highest were for the 90%. More irrigation water did not result in higher yield. The reason for the yield drop

Table 1. Soil parameters and treatment levels of irrigation water applied as % of soil field capacity (the parameters are explained in the text)

Experiment	r (g cm^{-3})	h (cm)	η (%)	P (g cm^{-3})	β_f (%)	β_L (%)	β_U (%)				
							T1	T2	T3	T4	T5
Tomato	1.2	60	90	1	32	60	70	80	85	90	100
Cucumber	1.37	40	90	1	25	75	80	85	90	95	100

Table 2. Effect of irrigation treatment (in terms of soil water as a percentage of field capacity (FC)) on stem diameter increase over 30 days (SDI), root viability (RV), yield, percentage yield increase compared to 100% of FC (PYI), vitamin C content (VC) and total soluble solids (TSS) in fruit

Treatment (% of FC)	SDI (cm)	RV (g kg^{-1} h^{-1})	Yield (kg ha^{-1})	PYI (%)	VC (mg kg^{-1})	TSS (g kg^{-1})
Tomato						
60-70	0.423c	0.9935c	55532.5c	-0.49	257.2c	45.2c
60-80	0.550b	1.0041bc	58540.4b	4.90	299.7b	47.3b
60-85	0.610a	1.0982bc	62812.5a	12.55	339.6a	53.4a
60-90	0.609ab	1.4888a	61352.5a	9.94	328.4a	50.7a
60-100	0.574b	1.2723b	55807.5b	0	271.1b	46.2b
Cucumber						
75-80	0.141c	0.5504c	79479.17c	-1.06	236.4c	20.9c
75-85	0.163b	0.8074b	88020.83b	9.57	248.1b	28.5b
75-90	0.185a	1.0756a	95104.17a	18.39	276.2a	37.4a
75-95	0.167b	1.0253a	90083.33b	12.14	252.6b	31.2b
75-100	0.134c	0.5194c	80333.33c	0	239.6c	29.2b

Means followed by different letters are significantly different at $P<0.05$ using Tukey's Studentised range test.

at 100% of FC may be inadequate oxygen concentration in the root zone (Bhattarai *et al.,* 2004.), and this can be explained by the values of root viability (Table 2).

It is a very popular management technique in China that the growth point on main stem is cut when tomato plant have the fourth or fifth inflorescence in soil culture style. So, water management was a key technique to change the growth centre from vegetation to reproduction for tomato to reduce the abortion at the early stage after transplanting (Zhang, 2001). Although, this experiment had been carried for only 30 days at the early stage after transplanting, it was very important for changing the growth centre and total yield of tomato; it can represent all growth stages of cucumber in this experiment, because the cucumbers were harvested during the treatment. Also, soil water content below field capacity may be more advantageous in regulating the relationship between underground and above ground plant growth at early stage. This needs to be explored further.

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Growth, nutrient uptake and nitrogen use efficiency of *Ficus hawaii* grown by nutrient film techniques (NFT) using different N-sources

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Abstract

Nutrient film technique was designed and used to grow *Ficus hawii* using different nitrogen sources, nitrate (calcium and potassium nitrate (N), urea (U) and ammonium nitrate (AN) in the same dose. Aim of the study was to investigate the most proper form of nitrogen, which gives the highest vegetative growth and nutrients uptake in the early growth of the plants. Results show that in general, AN gave the highest vegetative growth parameters expressed as plant height, number of branches plant^{-1}, leaves plant^{-1}, leaf area, fresh and dry weight. AN favoured apical growth, while U favoured lateral growth. Shoot/root ratio was highest in the AN treatment. Nutrients uptakes by the whole plant was much higher in the case of AN then U and N. Nitrogen use efficiency was highest in AN followed by U (more or less similar) and lowest in case of N.

Key words: *Ficus hawaii*, N use efficiency, N forms, nutrient film technique, nutrient uptake

Introduction

The nutrient film technique (NFT) is considered as a method for production of vegetables and ornamental plants. More than one type of this technique were designed and successfully used. The main differences between them are due to the aim of cultivation and availability of material. Hydroponics is defined as a distinct technique for plant growing where no root-supporting medium is used, whereas the other systems employ a rooting medium, either inorganic or organic.

Hydroponic systems increase the cultivation capacity per unit area. Also, they lead to decrease the cost of pesticides, water and fertilizers as well as infection effects. Successful application of these systems could minimize the pollution by the above mentioned compounds. Nitrate and ammonium are the major forms of N available for plant uptake. Although most plants can use either or both forms as a source of N, the degree of effectiveness of these two forms on plant growth is dependent on plant species and NH_4: NO_3 ratio and the concentration.

Numerous studies have shown that NH_4 as the sole source of N was deleterious to the growth of many plant species. However, addition of small amounts of NH_4 to NO_3 culture has been reported to increase growth of many plant species over that of NO_3 alone. The study was planned to design a (NFT) growth unit as well as to investigate the effect of different nitrogen forms on the growth and development of *Ficus hawii* L. plant.

Materials and methods

The study was carried out in the green house under the project "Micronutrients and other Plant Nutrition Problems in Egypt", National Research Centre (NRC) Dokki, Giza during the season 1999.

Growth unit design: The (NFT) system used for the study is illustrated in Fig. 1. The system was operated under fixed flow rate (45 L min^{-1}). It was made of dark blue plexiglas material. It was positioned with a gentle slope of (1%). Accordingly, the nutrient solution was flowed under the influence of gravity.

The system consisted of: a) trough as growth container or pot carrier, b) experimental pots which contained an inert growth media (peat-moss), c) non-metal pump, d) fixed pipe system, and e) the recommended nutrient solution according to Cooper (1979). Automatic pH, temperature, E.C. and humidity controller and monitoring system were used. The system is described in details below:

Trough (1): The channels were made from 10-mm metal free dark blue Plexiglas with the dimensions of 190 x 20 x 10 cm for length, width and height, respectively as shown in Fig. (1). To prevent growth of algae and other contaminating microorganisms, the channels were coated by black film of polypropylene (plastic mulch 40 micron thickness). This also led to minimize evaporation rate and photo-damage of roots.

Nutrient solution tank (2): Double face tank was made (80 x 40 x 20 cm) to have a final capacity of 60 L. The outer face of the tank was made from polypropylene (6 mm, gray color) to prevent contamination and light effect. The inner face was made from Plexiglas (3 mm, white color). The emerged plastic pump was installed in the tank centre. All connections inside the tank were made of polypropylene. The tank was located under troughs to allow the high flowing rate of drained nutrient solution to be recycled again.

Pump (3): An emerged plastic pump was used to avoid the metal concentration engaged by salt corrosion. The pump was running continuously with a given flow rate of 150 litre per-minute and

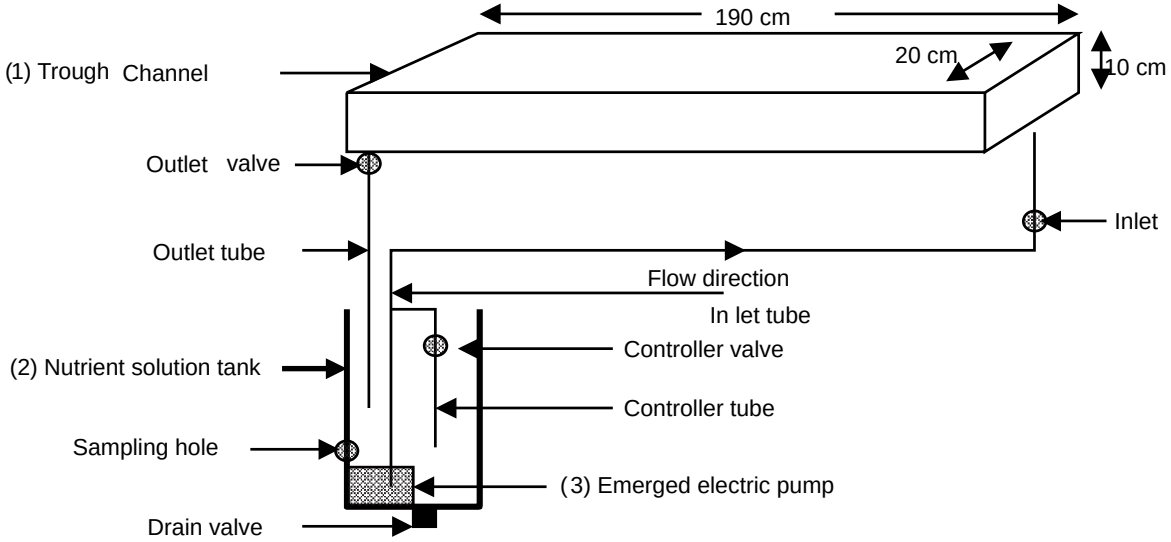

Fig.1. Side view of the plexiglas unit

was adjusted at 54 L minute^{-1}, for the type, carrying the nutrients solution from the tank to the head of channels. Flow rate was adjusted to be parallel with that of drainage rate, and also, to give a shallow solution of about 0.5 cm thickness.

Cultures were supplied by the nutrient solution using emerged centrifugal pump (Eheim 1250-Germany). Technical data of the pump used in the system are listed in Table (1).

Table 1. Technical data of the pump used (Eheim 1250)

Pump-out approx.	1200 (L h^{-1})
Delivery head	2.00 (m.w.c.)
Power consumption	28 (watt hr^{-1})

Pipe system (4): The nutrient solution was supplied through PVC pipe started at the highest end to allow the high flowing of nutrient solution. The drain of the nutrient solution was re-collected in the nutrient tank and re-cycled again by the emerged pump to the upper side. The content of nutrients and their balance were recorded. The feeding pipes were connected from the rear side to prevent the root damage by solution pressure, which is generated from the high flow rate.

Experiment: The experiment was initiated to investigate how various nitrogen sources affect plant growth, plant nutrient concentrations and nutrient uptake of *Ficus hawaii* . The experiment was conducted during January-April 1999 and three nitrogen sources *i.e.* nitrate, urea and ammonium nitrate were examined.

Nutrient solution

Treatment (1) - Nitrate (N): Nutrient solution was prepared according to Cooper (1979), containing the nitrogen as calcium nitrate and potassium nitrate at concentration of 200 mg L^{-1} in equal rates.

Treatment (2) - Urea (U): Nutrient solution contained the nitrogen as urea. K_2SO_4 and CaO were compensated for the initial amount of K and Ca from nitrate salts (treatment1).

Treatment (3) - Ammonium nitrate (AN): Nutrient solution contained the nitrogen as ammonium nitrate and additional equivalent quantity from K_2SO_4 and CaO, to compensate K and Ca in treatment 1.

Day temperature during the experiment ranged from 22 to 32^{O}C, relative humidity from 36 to 56%, and pH of all solutions was adjusted to pH 6.0-6.5, and conductivity of the nutrient solutions was measured two times every week. HCl or NaOH were added to keep pH in the range of 6.0-6.5. All the nutrient solutions were changed every two weeks. Solutions were aerated constantly; meanwhile de-ionized water was added to maintain the volume of the nutrient solution. Each treatment was replicated three times.

Planting: Terminal stem cuttings of *Ficus hawaii* 5-7 cm long were taken on 15 October 1998 and rooted in peat moss under low tunnel in the greenhouse. Four weeks later, selected rooted cuttings were transplanted to grow under green house conditions till the experiment started (January 1999) and then transferred to the solution culture (NFT system).

Growth parameters measured: At the end of the experiment, plant length, stem diameter, number of leaves, number of lateral branches, leaf area, fresh and dry weights for whole plants and their fractions, were measured.

Plant height (cm) was measured from peat-moss surface to the top of the plant at the end of the experimental period. Leaf area (cm^2 plant^{-1}) was measured by getting a disk area, disk dry weight and leaves dry weight (g plant^{-1}). Shoot/root ratio was calculated. The leaves on the main stem and branches were taken to determine fresh and dry weights.

Sampling for chemical analysis: Three plants from each replicate of each treatment were sampled at the end of the experiment for chemical analysis. Plants were divided to roots, stems and leaves. Each part was sequentially washed with running tap water then with 0.001 normal HCl followed by distilled water twice. Thereafter, they were air-dried at room temperature under gentle ventilation for 1 h and then, the samples were oven-dried at 70 ^{O}C for 24 hours in drying oven; and finely ground using a stainless steel mill with 0.5 mm mesh sieves, homogenized and kept in sealed polythene containers till analysis according to Chapman and Pratt (1978) and Walinga *et al.* (1989).

Chemicals analysis: The dried parts of the plant were used. Total nitrogen in plant was determined based on micro-Kjeldahl method according to Markaham (1942), using boric acid modification as

Table 2. Chemical composition of the nutrient solution (Cooper, 1979)

Elements	mg L^{-1}	Elements	mg L^{-1}
Nitrogen	200	Manganese	0.1
Phosphorus	60	Boron	0.3
Potassium	300	Copper	0.1
Calcium	170	Zinc	0.1
Magnesium	50	Molybdenum	0.2
Iron	12		

described by Ma and Zuazage (1942), under steam distillation using Buchii 320 unit, and was calculated as nitrogen percent. The wet ashing technique was carried out for extracting the other nutrients from plant tissues by digesting plant material using a mixture of nitric acid, sulphuric acid, perchloric acid (8:1:1) according to the method described by Chapman and Pratt (1978). Concentrations of the microelements Fe, Mn, Zn and Cu were measured by using atomic absorption spectrophotometer Perkin Elemer, 1100 B apparatus. Phosphorus was measured in digested plant material according to the method described by Jackson (1973). Potassium, calcium and sodium were measured in digested plant material using Flame photometer, Eppendorf. Magnesium was measured in digested plant material by using atomic absorption spectrophotometer Perkin Elemer, 1100 B apparatus according to Chapman and Pratt (1978).

Results of analysis were calculated on dry weight basis. Concentrations of N, P, K, Ca, Mg and Na were calculated as (%), whereas, Fe, Mn, Zn and Cu were calculated as ppm.

Statistical analysis: The experimental design was complete block with three treatments. Each treatment contained three replicates. Data were statistically analyzed using COSTAT computer statistical program and means were compared using L.S.D. at P=0.05 and P=0.01. The parameters *viz.*, number of leaves, number of branches, plant height, fresh and dry weights and chemical analysis of macro and microelements were analyzed.

Results and discussion

Morphological characters: Plant height was significantly affected by N-sources. The maximum plant height resulted in plants grew with AN. The increment of plant height with NH_4-NO_3 reached 30% more than that of using nitrates alone (control), or 32% than that of U treatment.

The number of branches per plant was also affected by the N-source. The highest value was obtained in plants supplied with U, followed by those grew in AN and the least value was recorded in N treatment. The differences in number of branches plant^{-1} were significant. The differences in leaf number plant^{-1} were significant. AN treatment increased number of leaves plant^{-1} by 71% over the N treatment and by 30% compared with U treatment.

Leaf area was significantly affected. The largest leaf area resulted for plants which grew in nutrient solution containing AN, being significantly higher than that of the plants grew in U and N alone. The percentage of increment in leaf area reached (81%) for plants grew in AN and 54% for plants grew in U, over the plants grew in a nutrient solution containing only N (Table 3).

In this connection, several authors mentioned that growth of different plants is greatly affected by the nitrogen source in the nutrient solution. Scoggins and Mills (1998) found that leaf area of poinsettias (*Eupliorbia puicherrima* Willd-Ex Klotz) was maximized with 25:75 and 50:50 NH_4: NO_3 treatments, respectively. Errehbi and Wilcox (1990) mentioned that the addition of small amounts NH_4 to NO_3 solution, up to 14 ppm improved plant growth of tomato.

On the other hand, Qasem and Hill (1993) found that the growth of tomato was reduced when ammonium or urea was the only sources of N. In our experiments AN produced plants taller than the other two treatments with more branches and leaves and leaf area plant^{-1}. It is interesting to note that U did not affect plant height compared with N, but produced more branches, leaves and leaf area, which indicates that U encourages the lateral growth more than the apical growth, which is in contrast to the effect of AN.

Fresh weight (g plant^{-1}): Table 4 show that the plant fresh weight was highest in nutrient solution contained AN, followed by U and NO_3. The stem, shoot (aerial growth) and roots fresh weight

Table 3. Effect of N sources on some morphological parameters of *Ficus hawaii* L. (7 days old)

N sources (200 ppm)	Plant height (cm)	Number of		Leaf area (cm^2 plant^{-1})
		Branches plant^{-1}	Leaves plant^{-1}	
Nitrate (N)	25.83	7.67	73.33	197.21
	100%	100%	100%	100%
Urea (U)	25.33	13.67	103.33	249.93
	98%	178%	141%	127%
Ammonium nitrate (AN)	33.67	13.33	125.33	356.15
	130%	174%	171%	181%
LSD (P=0.05)	4.24	1.99	11.53	54.53
LSD (P=0.01)	6.42	3.03	17.84	82.61

Table 4. Effect of N sources on fresh weight (g plant^{-1}) of *Ficus hawaii* L.

N sources (200 ppm)	Fresh weight (g plant^{-1})					Shoot : Root Ratio
	Leaves (L)	Stems (S)	Shoot (L+S)	Root (R)	Whole plant	
Nitrate (N)	14.24	4.49	18.73	4.98	23.71	3.76
	100%	100%	100%	100%	100%	
Urea (U)	18.29	5.08	23.37	4.89	28.38	4.78
	128%	113%	125%	98%	120%	
Ammonium nitrate (AN)	27.91	7.53	35.44	5.92	41.15	6.99
	196%	168%	189%	119%	174%	
LSD (P=0.05)	1.35	0.73	1.64	NS	2.62	1.88
LSD (P=0.01)	2.05	1.10	2.54	NS	3.97	NS

showed the same pattern when comparing growth of plants grown under the three nitrogen forms.

The AN treatment had a higher shoot/root ratio of fresh weight (6.99) than U (4.78) and N treatment (3.76) (Table 4). These differences were highly significant between AN and the other sources. Similar results were reported by Spiers and Braswell (1993) who found that shoot growth of muscadine grapes plants was greatest with NH_4NO_3. Santamaria *et al.* (1998) reported that rocket growth was inhibited by NH_4 nutrition, while it reached the highest values with the NH_4:NO_3 ratio 1:1.

Dry weight (g plant^{-1}): Table 5 indicates that dry weight for whole plant was highest for plants which grew in AN, followed by those grew in U and then N, which is the same pattern as the fresh weight. The highest shoot growth in terms of dry weight was obtained by plants which grew in a nutrient solution containing AN as N-source, as compared when nitrogen nutrient came from urea or from nitrate as sources for nitrogen.

The same trend was obtained for dry weight of shoots and whole plant. This leads to that AN treatment had a high shoot: root dry weight ratio (7.35) as compared to the N treatment (5.14). The average increment in dry weight per whole plant and different organs were: (leaves 78% , stem 46%, root 18%, shoot 69% and whole plant 61%) with AN over the plants received N treatment.

The above-mentioned results indicate that AN application increased plant growth, in terms of dry matter production. Shaviv *et al.* (1990) also reported that tomato plants grew on mixed ammonium and nitrate as N sources produced larger dry matter and protein yields than those grew on nitrate alone. Somda *et al.* (1990) found that the largest shoot and root dry weights were obtained when tomato plants were fed with 1:1 NH_4^+ NO_3^- ratio. Further, Qasem and Hill (1993) found that the growth of all species of tomato plant was reduced when ammonium or urea were the only sources of N. Santamaria *et al.* (1998) found that rocket growth was inhibited by NH_4 nutrition, while it reached the highest values with the NH_4^+ NO_3^- ratio 50:50. On the other hand, Feigin (1990) mentioned that the dry matter production was not significantly affected by the NH_4/NO_3 ratio on crop yield. Ganmore-Neuman and Hagiladi (1990) found that the ratio did not affect cutting yields of Pelargoniurn plants. Also, Aminuddin *et al.* (1991) reported that plant dry weight did not differ significantly between treatments of ammonium or nitrate.

Effect of N-source on nutrient status of Ficus hawaii plants

Nutrient concentration

Leaves: N, P, Ca, Na and Fe concentrations in leaf tissues increased in N compared to U or AN. While K, Mg, Mn Zn and Cu concentration in leaf tissues increased when U was the nitrogen source (Table 6).

Table 5. Effect of N sources on dry weight (g plant^{-1}) of *Ficus hawaii* L plants

N sources (200 ppm)	Dry weight (g plant^{-1})					Shoot / Root Ratio
	Leaves L	Stems S	Shoot (L+S)	Root R	Whole plant	
Nitrate (N)	2.64	1.01	3.65	0.71	4.36	5.14
	100%	100%	100%	100%	100%	100%
Urea (U)	3.21	1.00	4.21	0.69	4.90	6.10
	122%	99%	115%	97%	112%	119%
Ammonium nitrate (AN)	4.69	1.48	6.17	0.84	7.01	7.35
	178%	146%	169%	118%	161%	145%
LSD (*P*=0.05)	0.71	0.32	0.90	NS	0.96	2.04
LSD (*P*=0.01)	1.08	0.48	1.37	NS	1.46	NS

Table 6. Effect of N sources on elemental concentration in *Ficus hawaii* leaf tissues

N sources (200 ppm)	Macronutrient (%)						Micronutrient (ppm)			
	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Nitrate (N)	3.67	0.29	1.88	0.31	0.51	0.48	521	93	53	6
Urea (U)	2.31	0.28	2.86	0.30	0.59	0.37	478	141	76	11
Ammonium nitrate (AN)	2.40	0.26	2.67	0.27	0.56	0.35	375	125	66	14
LSD (*P*=0.05)	0.47	0.05	1.40	NS	0.09	0.08	115	NS	NS	4
LSD (*P*=0.01)	0.72	NS	NS	NS	NS	0.12	NS	NS	NS	6

Table 7. Effect of N sources on elemental composition in *Ficus hawaii* stem tissues

N sources (200 ppm)	Macronutrient (%)						Micronutrient (ppm)			
	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Nitrate (N)	2.47	0.31	2.87	0.17	0.77	0.37	327	150	108	12
Urea (U)	3.31	0.30	2.43	0.19	0.81	0.32	511	147	110	9
Ammonium nitrate (AN)	2.80	0.28	2.68	0.34	0.73	0.33	432	178	95	6
LSD (*P*=0.05)	0.28	NS	0.44	0.13	0.06	0.09	137	28	22	3
LSD (*P*=0.01)	0.42	NS	NS	NS	NS	NS	NS	NS	NS	4

Table 8. Effect of N sources on elemental composition in *Ficus hawaii* root tissues

N sources (200 ppm)	Macronutrient (%)						Micronutrient (ppm)			
	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Nitrate (N)	2.48	0.47	1.49	0.13	0.75	0.43	165	121	83	13
Urea (U)	2.46	0.38	1.89	0.15	0.76	0.35	238	156	88	12
Ammonium nitrate (AN)	2.47	0.35	3.12	0.12	0.75	0.24	318	136	75	11
LSD (*P*=0.05)	NS	0.12	1.09	NS	NS	NS	125	NS	NS	NS
LSD (*P*=0.01)	-	NS	NS	-	-	-	NS	-	-	-

Stem: The highest values of P, K, Na and Cu concentrations were found in plants grown in a nutrient solution containing NO_3 as a sole N-source. While, the highest values of N, Mg, Fe and Zn concentrations were obtained when plants were fed with a solution containing nitrogen either in U or AN forms (Table 7).

Roots: No specific trend could be detected for nutrient concentrations in response to the source of nitrogen in the nutrient solution. Nitrogen, phosphorus, sodium and copper contents recorded the highest values in root tissues of the plants, which were supplied with NO_3, as compared to the other sources. The highest concentration of Ca, Mg, Mn and Zn were found in root tissues of the plants which received U. The highest value of K and Fe was found with AN treatment (Table 8).

Nutrient uptake

Content of leaves: Table 9 indicate different responses of nutrient uptake by leaves of *F. hawaii*. The AN application gave the highest of all nutrient uptakes when compared to U or N treatment. The lowest values of nutrients uptake by leaves (except N) were obtained with N application treatment.

Content in Stems: Table 10 show that the highest values of most nutrient uptake in stems resulted from AN as a nitrogen source in nutrient solution when compared to U and N treatments. The lowest values of Na and Mn nutrient uptake resulted in plants which grew in nutrient solution contained U as nitrogen source. Whereas, the lowest N uptake value was found in plants which were supplied with N treatment,

Content in roots: Table 11 demonstrate different responses of nutrient uptake in roots of *F. hawaii*. The highest nutrient uptake values of N, K, Mg, Fe, Mn and Cu were found in AN treatment, as compared to the other treatments. The lowest uptake values except for K, Fe, Mn and Zn were observed when N was the nitrogen source in nutrient solution.

Content in shoots: Table 12 show that the nutrient uptake of shoots exhibit various responses to different nitrogen sources. The highest uptake of all measured nutrients resulted from AN treatment as compared to the other nitrogen sources. The application of N as nitrogen source resulted in the lowest values of most nutrients uptake, as compared to other sources.

Table 9. Effect of N sources on nutrient uptake by leaf tissues of *F. hawaii* L. plants

N sources (200 ppm)	D.M. (g)	Macronutrient (mg plant^{-1})						Micronutrient (ppm)			
		N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Nitrate (N)	2.64	95	8	49	8	13	12	1400	242	138	16
Urea (U)	3.21	74	9	82	10	19	12	1500	451	243	35
Ammonium nitrate (AN)	4.69	113	12	125	13	26	16	1800	586	310	66
LSD (*P*=0.05)	0.71	9.67	2.83	10.14	3.12	3.46	NS	NS	43.93	27.6	9.30
LSD (*P*=0.01)	1.08	14.66	NS	15.37	NS	5.24			66.56	41.81	14.09

Table 10. Effect of N sources on nutrient uptake by stem tissues *F. hawaii* L. plants

N sources (200 ppm)	D.M. (g)	Macronutrient (mg plant^{-1})						Micronutrient (ppm)			
		N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Nitrate (N)	1.01	25	3	29	2	8	4	327	150	108	12
Urea (U)	1.00	33	3	24	2	8	3	511	147	110	9
Ammonium nitrate (AN)	1.48	42	4	40	5	11	5	648	267	143	9
LSD (*P*=0.05)	0.32	7.11	NS	8.71	1.41	2	NS	30.4	16.2	15.1	NS
LSD (*P*=0.01)	0.48	10.77		13.19	2.14	NS		NS	24.59	22.99	

Table 11. Effect of N sources on nutrient uptake by root tissue *F. hawaii* L. plants

N sources (200 ppm)	D.M. (g)	Macronutrient (mg plant^{-1})						Micronutrient (ppm)			
		N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Nitrate (N)	0.71	17	3	10	1	5	3	116	85	58	9
Urea (U)	0.69	17	3	13	1	5	2	167	109	62	8
Ammonium nitrate (AN)	0.84	20	3	25	1	6	2	254	109	60	9
LSD (*P*=0.05)	0.33	NS	NS	3.46	NS	NS	NS	5.65	8.71	NS	NS
LSD (*P*=0.01)	0.50			5.24				8.56	13.19		

Table 12. Effect of N sources on nutrient uptake by whole plant *F. hawaii* L. plants

N sources (200 ppm)	Macronutrient (mg plant^{-1})						Micronutrient (ppm)			
	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Nitrate (N)	137	14	88	11	26	19	1843	477	301	37
Urea (U)	124	15	129	13	32	17	2178	707	415	62
Ammonium nitrate (AN)	175	19	190	19	43	23	2702	964	513	84
LSD (*P*=0.05)	7.74	2.0	7.56	2.0	4.32	2.0	163	19.8	23.4	5.65
LSD (*P*=0.01)	11.72	3.03	11.46	3.03	6.54	3.03	248	30	35.4	8.56

Table 13. Effect of N sources on nutrient uptake by shoot tissue *F. hawaii* L. plants

N sources (200 ppm)	Macronutrient (mg plant^{-1})						Micronutrient (ppm)			
	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Nitrate (N)	120	11	78	10	21	16	1727	392	246	28
Urea (U)	107	12	116	12	27	15	2011	598	353	44
Ammonium nitrate (AN)	155	16	165	18	37	21	2448	855	453	75
LSD (*P*=0.05)	8.44	2	7.11	2.83	5.29	2	34.9	11.12	8.48	8.16
LSD (*P*=0.01)	12.84	3	10.77	4.28	8.01	3.03	52.8	16.9	12.81	12.36

Table 14. N use efficiency mg dry matter produced/mg N in the plant

N-Sources	Dry weight (g plant^{-1})	N Content (mg plant^{-1})	Use efficiency (mg dry wt g^{-1})
Nitrate (N)	4.360	137	31.8
Urea (U)	4.90	124	39.5
Ammonium nitrate (AN)	7.01	175	40.1

Nutrient uptake by whole plant: Table 13 show that the nutrient uptake of whole plant exhibited the same trend. The highest nutrient uptake of all nutrients was found in AN treatment, as compared to U and N.

Nitrogen use efficiency (NUE): It is clear that AN treatment, which was superior to other treatments in almost all measured parameters showed also a higher use efficiency of nitrogen. The difference between NUE in this treatment and U was very negligible. However, the total fresh and dry weight accumulation in the urea treatment was much lower.

It could be concluded that AN is a better source of nitrogen for *F. hawaii* while urea enhances lateral growth with lower dry matter accumulation.

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Effects of grafted eggplants on allelopathy of cinnamic acid and vanillin in root exudates

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Abstract

Cinnamic acid and vanillin are the allelochemicals commonly exist in eggplant root exudates. With pot culture experiment, the effects of grafted eggplants on allelopathy of cinnamic acid and vanillin in eggplants root exudates were studied. The results showed that cinnamic acid and vanillin had allelopathic effects on eggplants, lower concentration of cinnamic acid and vanillin (0.1 mmol L^{-1} or 0.5 mmol L^{-1}) could promote the growth and physiological metabolism of eggplants, while higher concentration (from 1 mmol L^{-1} to 4 mmol L^{-1}) had slightly promotive or inhibitive effects on eggplants. Meanwhile, this study suggested grafting could relieve autotoxicity of cinnamic acid and vanillin, and significant difference in the regulation intensity for the autotoxicity was found between cinnamic acid and vanillin. Grafting decreased the amounts of cinnamic acid and vanillin, especially of vanillin. The maximum reduction amount of cinnamic acid reached 68.96%, and that of vanillin reached 100%. Under the stress of exotic cinnamic acid and vanillin, especially of exotic cinnamic acid, grafting relieved the autotoxicity of the two substances on eggplants. Compared with own-rooted eggplant, grafted eggplant had a higher plant height and a larger stem diameter, its leaf cholorophyll content increased by 5.26-13.12%, root electric conductivity and MDA content decreased, and root SOD activity enhanced. Grafting was found to be one of the most effective methods for relieving replanting problems caused by autotoxicity.

Key words: Grafting, root exduates, cinnamic acid, vanillin, autotoxicity

Introduction

Eggplant (*Solanum melongena* L.) is a popular vegetable crop worldwide. However, yield and quality reduced sharply annually owing to continuous cropping in the same field, and the adverse effect is considered as continuous cropping obstacle and may become a key problem restraining eggplants sustainable production. There are many causes for continuous cropping obstacle, such as soil-borne diseases, nutrition unbalance and allelopathy (Zheng *et al*., 2004).

Soil-borne diseases have been paid more attention in eggplant studies. Grafted eggplant root exudates could inhibit soil-borne diseases and induce beneficial microbe increase, which help to overcome the continuous cropping obstacle caused by soil-borne diseases (Zhou *et al*., 2001; Wang *et al*., 2005). When the previous crop root exudates or residues containing allelochemicals are in soil, allelochemicals accumulate in soil to a certain level, which inhibit the growth and yield of following crop in continous cropping *i.e.* autotoxicity (Fisher, 1980; Yu and Matsui, 1993; Wu *et al*.,1999; Yu and Matsui., 1999; Cao *et al*., 2001). Eggplant root exudates have a key role to cause continuous cropping problem, and its autotoxicity (Yu and Matsui, 1999; Ruan *et al*., 2003; Zheng *et al*., 2004). The cinnamic acid and vanillin are general allelochemicals detected in eggplant root exudates (Zhou *et al*., 2001; Wang *et al*., 2005b; Zhang *et al*., 2005), and they had allelopathic toxic effect on seed germination and seedling of eggplants (Wang *et al*., 2006). Therefore, it is of importance to conduct study on technique which overcome continuous cropping obstacle caused by autotoxicity.

In present investigation, we studied the effect of grafted eggplants on the amount of cinnamic acid and vanillin in root exudates to further understand the mechanism responsible for increased production by grafting by eliminating problems caused by continuous cropping

Materials and methods

Material preparation: The experiment was carried out in greenhouses at Vegetable Experiment Station, Shenyang Agricultural University. The commonly grown eggplant (*Solanum melongena* L.) cv. 'Xi'anlv' and a kind of wild eggplant [*Solanum torvum* (*S.tor*)] were used as scion and rootstock, respectively. Rootstock and scion plants were sown on January 1st and February 12th, 2006, respectively. When the rootstock plants reached 4-5 leaf stage, cleft grafting was done (Wang *et al.*, 2005a). Then, some grafted eggplants and 'Xi'anlv' were transplanted into 20.5cm dia container with substrate of perlite, turf soil and vermiculite (3:2:1), and irrigated by Hoagland nutrient solution twice a week and used for collecting root exudates of different growth stage. Other grafted eggplants and 'Xi'anlv' were transplanted into 18 cm diameter container with substrate of turf soil, soil and chicken manure (1:1:1) and watered with exotic cinnamic acid and vanillin, which were used for the two substances stress treatment. The grafted eggplants and self-root eggplants were referred to as *S.tor* and control, respectively.

Root exudates collection and cinnamic acid and vanillin contents determination: Root exudates at different growth stages were collected by soaking root. The growth stages including phases of bud, first fruit expanding and second fruit expanding, were referred as earlier, mid and post stage, respectively. The root exudates solution was adsorbed with XAD-4 resin produced by Sigma Company, and concentrated after dissolving with alcohol,

then cinnamic acid and vanillin contents were determined with Waters High Pressure Liquid Chromatography made in America (HPLC) (Wang *et al.*, 2005).

Cinnamic acid and vanillin solution: Cinnamic acid and vanillin solutions were made with concentration of 0, 0.1, 0.5, 1, and 4 mmol L^{-1}, respectively. Firstly, cinnamic acid was dissolved in alcohol , then diluted with distilled water, and finally regulated alcohol content to 1.5%. Vanillin was directly dissolved with distilled water, then also regulated alcohol content to 1.5%.

A week after transplanting, the plants were irrigated with cinnamic acid and vanillin solution respectively around root 10 cm once every 5 days. The irrigated amount was 100 mL each time. After a month, the growth and physiological metabolism indexes were investigated. Chlorophyll content, cell membrane permeability, MDA contents and SOD content was respectively determined with Zhaoshijie method (Xiong, 2003), relative electrical conductivity method (Zheng, 2006), Thiobarbituric acids method (TBA) (Omran, 1980) and nitroblue tetrazolium (NBT) Oxidatuion and Deoxidize Method (Li, 2000).

Statistical analysis: The data were statistically analyzed with Excel software and DPS software.

Results

Cinnamic acid and vanillin amounts: The cinnamic acid content in *S.tor* root exudates was also less than control, whereas vanillin content was markedly lower than control (Table 1). Compared with control, vanillin content in *S.tor* root exudates decreased by 95.44% at earlier stage and 100% at mid and post stage. Cinnamic acid content in *S.tor* root exudates was significantly less than control, with 68.96% reduction at earlier stage and 50% reduction at post stage. The results showed grafting reduced release of the two substances by eggplant roots, in particular, significantly reduced vanillin amount.

Growth response of eggplants: The effect of cinnamic acid and vanillin on eggplant height and stem diameter were basically identical in action trend, whereas different in action character and degree (Fig. 1 and Fig. 2). The results showed lower concentration of cinnamic acid and vanillin had promotive effects, while higher concentration had inhibitive effects on eggplants height and stem diameter growth. The effect of cinnamic acid on plant height and stem diameter were stronger than vanillin, and impact of two substances was more plant height than on stem diameter.

Physiological and biochemical responses of eggplants: The chlorophyll content increased gradually with cinnamic acid and vanillin concentration ranging from 0 to 0.1 mmol L^{-1}, and at 0.1 mmol L^{-1} reached maximum, afterwards, decreased with the concentration increasing (Fig. 3). The results showed cinnamic acid and vanillin of lower concentration had promotive effects on chlorophyll content, whereas the higher concentration had inhibitory effect.

Table 1. Effects of grafting on the contents of cinnamic acid and vanillin in eggplant root exudates

Phenolic acids	Treatment	Content at different growth stage (μg g^{-1})		
		Early stage	Mid stage	Post stage
Cinnamic acid	*S.tor*	0.009bB	0.090aA	0.290bB
	Control	0.029aA	0.060aA	0.540aA
Vanillin	*S.tor*	0.115bB	0.0bB	0.0bB
	Control	2.520aA	4.430aA	12.260aA

Note: Data are mean values of three repetitions.Capital letter and small letter indicate significance at P=0.01 and P=0.05, respectively

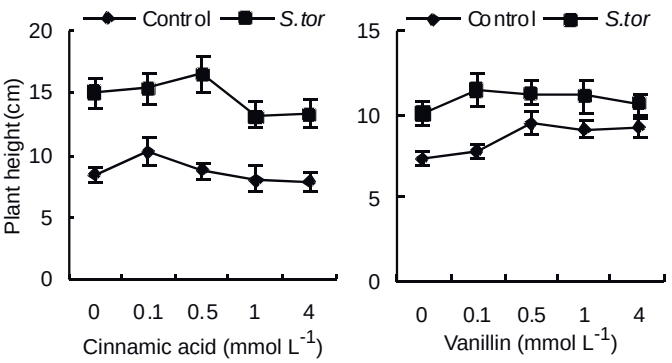

Fig. 1. Effect of cinnamic acid and vanillin on plant height of eggplant

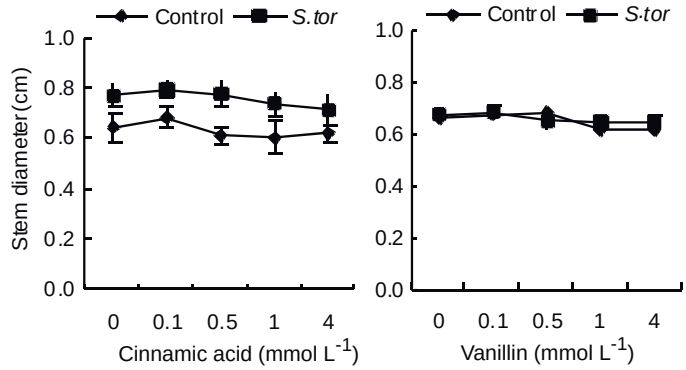

Fig. 2. Effect of cinnamic acid and vanillin on stem diameter of eggplant

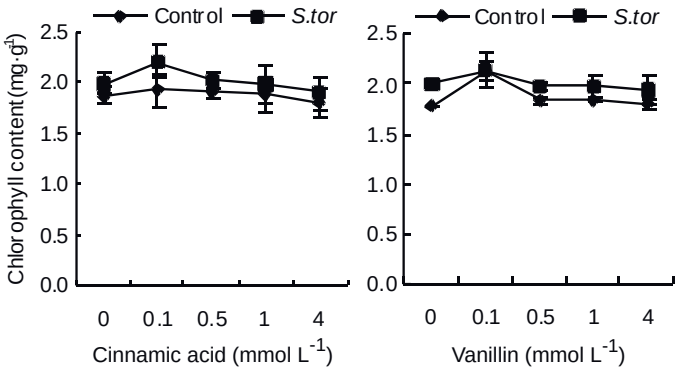

Fig. 3. Effect of cinnamic acid and vanillin on cholorophyll content of eggplant

Compared with control, chlorophyll content in *S.tor* increased by 5.53, 13.12, 5.94, 5.53, 5.26% at 0, 0.1, 0.5, 1, 4 mmol L^{-1} cinnamic acid treatment respectively, and increased by 11.05, -0.16, 6.60, 7.57, 7.73% at 0, 0.1, 0.5, 1, 4 mmol L^{-1} vanillin treatment, respectively. The result showed chlorophyll in grafted eggplant was generally higher than control, and grafting could maintain better chlorophyll content under cinnamic acid stress than vanillin stress.

The relative electrical conductivity in eggplant root decreased with cinnamic acid and vanillin concentration increasing from 0 to 0.1 mmol L^{-1}, and reached the minimum at 0.1 mmol L^{-1}, afterwards, the relative electrical conductivity increased as the concentration increasing (Fig. 4). MDA contents sharply increased at 0.5, 1, 4 mmol L^{-1} cinnamic acid treatment (Fig. 5). It reached

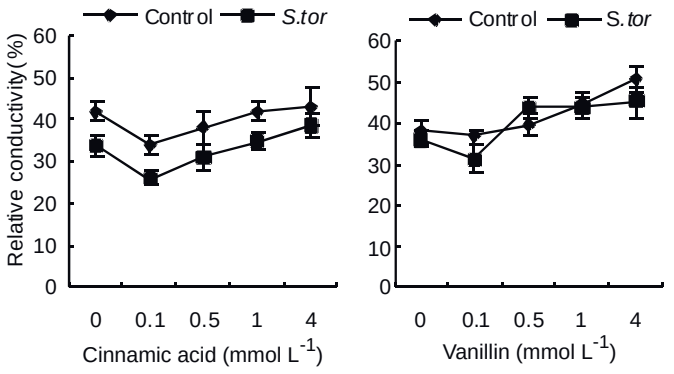

Fig. 4. Effect of cinnamic acid and vanillin on relative conductivity in root system of eggplant

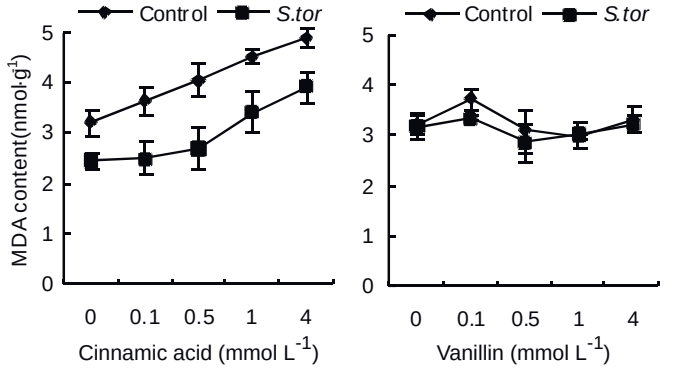

Fig. 5. Effect of cinnamic acid and vanillin on MDA content of eggplant

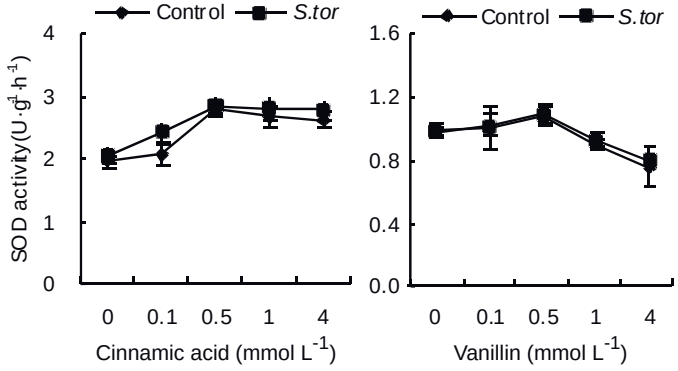

Fig. 6. Effect of cinnamic acid and vanillin on SOD activity in eggplant

the maximum at 0.1 mmol L^{-1} vanillin, and dropped significantly at 0.5 mmol L^{-1}, then increased again with concentration going up.

Compared with control, the relative electrical conductivity in *S.tor* reduced by 19.90, 23.63, 18.56, 16.97, 10.02%, and MDA content lowed by 23.19, 31.86, 33.17, 24.77, 20.04% at 0, 0.1, 0.5, 1, 4 mmol L^{-1} cinnamic acid treatment, respectively. Under the vanillin stress, the variation regularity of two indexes was not obvious. The results showed the relative electrical conductivity and MDA contents in grafted eggplants was markedly lower than control, which suggested that grafting could maintain or enhance resistance to cinnamic acid stress.

SOD enzyme activity increased with cinnamic acid and vanillin concentration ranging from 0 to 0.5 mmol L^{-1}, and began to declined as the concentration going up from 1 to 4 mmol L^{-1}, with more reduction under cinnamic acid than vanillin stress (Fig. 6). It indicated that the action of cinnamic acid on SOD enzyme activity was stronger than that of vanillin.

Compared with the control, the enzyme activity in *S.tor* increased by 1.99, 15.29, 1.06, 4.67, 5.45% at 0, 0.1, 0.5, 1, 4 mmol L^{-1} cinnamic acid treatment, respectively. Under vanillin stress, the enzyme activity in *S.tor* was similar to that in control. It showed that effect of grafting on SOD enzyme activity under cinnamic acid was stronger than vanillin.

Discussion

The action intensity of autotoxic substances on receptor plants had close relation with its concentration. The study showed cinnamic acid and vanillin also had allelopathic effect on eggplants, lower concentration (0.1 mmol L^{-1} or 0.5 mmol L^{-1}) could promote the growth and physiological and biochemical metabolism, while higher concentration (from 1 mmol L^{-1} to 4 mmol L^{-1}) had slightly promotive or inhibitive effects on eggplants. The result was accorded with the conclusion of cucumber and fir (Chen *et al.*, 2002; Wu *et al.*, 2002).

Our results suggested that autotoxicity mechanism of cinnamic acid and vanillin on eggplants were basically identical, while allelopathic intensity of cinnamic acid was stronger than vanillin. With the enhancing of cinnamic acid stress, MDA content and electrical conductivity increased remarkably, SOD enzyme activity and chlorophyll content initially increased and then decreased, plant height decreased gradually, but the change in stem diameter was not apparent. Under the action of vanillin, the change of MDA content was not apparent, electrical conductivity increased, SOD enzyme activity slightly increased initially, and then decreased remarkedly, chlorophyll content also followed the same trend. Plant height showed a gradual rise and the change in stem diameter was not conspicuous. From the above, cinnamic acid and vanillin disturbed cell membrane firstly and enhanced membrane permeability, after that influenced some enzyme metabolism, and finally growth and development. The result was in accordance with the conclusion drawn by Einhellig (1995) and Cao *et al.* (2001).

The allelochemicals variation and amounts belonged to quantitative inheritance controlled by polygene (Kong *et al.*, 2002), which depended on the common action of plant oneself and environment (Einhellig, 1996). Allelochemicals were affected by environment, with increase in quantity and enhanced intesity at adverse circumstances, while greatly reduced at favourable circumstances (Kong *et al.*, 1996; Kong *et al.*, 1997). Graft union is a new individual plant, and the interaction between rootstock and scion changes metabolism of the two parts such as substance synthesis, translocation, and transformation and so on (Zheng *et al.*, 2005; Qi *et al.*, 2006; Xu *et al.*, 2006). Through in grafting, eggplant oneself and ecological environment of rhizosphere improved (Wang *et al.*, 2005), which might influence the roots secondary metabolism, change the capability to release allelochemicals. The study showed grafting decreased the amounts of cinnamic acid and vanillin released from eggplants root, especially of vanillin. The former studies suggested grafted eggplant had better advantage than own-rooted eggplant in growth vigour, diseases resistance, increased production etc (Gao and Zang, 1998). In this study, we found grafted eggplants could maintain or enhance advantage in the growth and physiological metabolism under stress, especially for cinnamic acid stress.

The study suggested that grafting could overcome autotoxicity of cinnamic acid and vanillin, whereas there was difference between them. For vanillin, grafting significantly decreased the vanillin exudation amount from root, and for cinnamic acid, grafting relieved stress by maintaining or enhancing advantage in the growth and metabolism. Grafting is one of the effective methods for relieving replanting problems caused by autotoxicity.

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Low cost hydroponics devices and use of harvested water for vegetable and flower cultivation

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Abstract

Low cost hydroponics devices were designed using plastic trays and buckets. Cultivations of tomato, chilli, cauliflower and marigold cv. *Inca* were tested in these devices using rain water, pond water, tap water and distilled water for nutrient solution preparation. The vegetables were grown as multiple plant cultures in plastic trays and marigold cv. *Inca* in single plant culture in small buckets. Direct use of tap water and pond water created chlorosis in some plants that could be overcome by boiling of water before use. In rain water tomato and chilli plants performed the best. However, cauliflower curd yield was the best in tap water. Marigold cv. *Inca* bloomed well in all categories of water. Water qualities were the major factor for crop growth. Rainwater could be more safely used. The devices and procedures are recommended for the kitchen gardeners of the urban and soil stress areas.

Key words: Rainwater, tap water, pond water, distilled water, hydroponics

Introduction

Hydroponics is a age old practice. However, recently it has gained momentum due to a number of socioeconomic factors, soil stress and practice of hydroponics by many commercial growers (Schwarz *et al.,* 2004). Commercial growers have now adopted hydroponics method to produce different crops. Gardeners may grow flowers, ornamental plants, and vegetables by hydroponics. In areas where soil for cultivation is lacking or unsuitable for growth, hydroponics offers an alternative production system (Schmdit *et al.,* 2004). Since its initiation of use in large scale crop production, the technology was confined with some sophisticated high cost devices (Schwarz *et al.,* 2004). For more effective use of this technology, particularly in the soil stress areas of the developing countries, invention of low cost devices is necessary. The plant hobbyist in the urban areas also has the scope of using these low cost devices for more efficient production of flowers and vegetables (Schmdit *et al.,* 2004).

The important constraint in hydroponics is the necessity of good quality water. Although in hydroponics, the usable water could be comparatively better utilized in crop production than grown in soil. The cleaner the water, the greater is the opportunity to achieve maximum yields (Jensen, 1997). Although distilled water is comparatively pure but the cost of production is not economically viable (Schmdit *et al.,* 2004). The common water sources like well water, municipal water and pond water have separate problems of water quality due to their different nature of hardiness, presence of toxic elements and contamination by organic and inorganic substances (Mahmood, 2004). Whether rainwater harvesting can be used directly for crop production in hydroponics has got special attention in research in the recent times (Schwarz *et al.,* 2004). Water quality is mainly deteriorated due to contaminations with some inorganic chemicals; most of them are basically the macro or micro- nutrients of the plants. Also the requirement of these elements as nutrition varies from crop to crop. These contaminants could otherwise meet the nutritional requirements of some crops if their behaviour with variable nature of water qualities is properly studied (Valliant *et al.,* 2004). The objective of the study was to compare the performances of three major vegetables; tomato, chilli and cauliflower and a flower plant, marigold cv. *Inca* with four qualities of water namely, distilled water, rain water, pond water and tap water used separately in nutrient solutions by hydroponics system. The efficacy of the devices made by low cost materials could also be judged from this investigation.

Materials and Methods

The hydroponics device for growing tomato, chilli and cauliflower was designed following the principle of Gericke (1937). To develop this system high neck plastic tray of 45.5 cm length, 30.5 cm breadth, and 14 cm depth with a capacity of 11 L of water were used (Fig. 1 and 2). At the extreme lower edge of the plastic tray, one outlet was made which was connected with 0.5 cm diameter polythene pipe for drainage purpose. Thermocol sheets of 2-3 cm thickness were used to cover the upper part of the tray. Equal size apertures with a spacing of 11 x 11 cm were made on the cover sheet, through which the seedlings, collected from local nursery were inserted in such a way that their roots could sufficiently reach and immersed in the nutrient solution. Six plants were grown in each tray. The nutrient solution within the plastic tray was aerated with the help of aqua pump that supplied air through the polythene tubes. For growing instead of using plastic tray small plastic buckets of 14 cm height and 15.8 cm diameter having a capacity of containing 1.9 L of water were used following the same procedure. In each bucket a single *Inca* plant was grown (Fig. 5).

Four qualities of water were used in this experiment. Distilled water was prepared by glass distillation apparatus in the laboratory using the underground tap water as the basic source. Rainwater was harvested directly in the water tanks placed on the roof of the University Faculty Building in rainy season avoiding first few flashes. Pond water was collected from the pond situated in

front of the faculty building of the university. The tap water was the underground lifted water from the laboratory water supply. The chemical analysis of four water samples was done by the Water Testing Laboratory, Department of Agriculture Chemistry, BCKV. The nutrient solution was prepared following Hoagland and Arnon (1950). The nutrient solutions were refreshed as per the requirement of the plants keeping always the same composition of nutrients. The total amount of nutrients as well as water supplied to the plants was recorded.

Results and discussion

There was no problem in growing the plants of tomato, chilli and cauliflower in the multiple plant culture device of hydroponics used in the experiment. 5-6 plants of tomato and chilli and 3-4 plants of cauliflower could be grown well within a tray as shown in the Figs. 1, 2 and 3. Their yield performances varied due to the effect of different qualities of water, but not due to problems in the devices. The performance of marigold cv. *Inca* was best suited in single plant culture device as shown in the Fig. 5.

Chlorotic symptoms due to mineral nutrition deficiencies were noticed in the tomato plants grown in pond water solution and the chilli and cauliflower plants grown in both pond water and tap water solutions after 20-25 days of planting. In other treatments, including the *Inca* plants no such symptoms were noticed. At the same time precipitation was also noticed in the nutrient solutions of the respective tanks. To solve this problem, both pond and tap water were boiled for 30 minutes, allowed to settle down for few hours and then strained through muslin clothes prior to use. Water samples prepared in this way were further used for nutrient solutions. The chlorotic plants could revive their normal health within 7-10 days after refreshing in new solutions and the problem didn't arise further. Chemical analysis of four water samples used in this experiment is shown in Table 1. According to the crop irrigation water quality standards described by Ayers and Westcot (1994), none of the water samples crossed the level of severity that could damage crop growth. However, in tap water and pond water, the level of bicarbonate, calcium, magnesium, chloride, sodium, potassium and iron were much higher than in rain water and distilled water (Table 1). The high bicarbonate level indicated the higher temporary alkalinity level of water used in nutrient solution that made precipitation causing mineral nutrition deficiencies in the plants (Schwarz *et al.,* 2004). By boiling water prior to use, this temporary alkalinity level could be reduced by allowing precipitation of carbonate compounds with ions like calcium and magnesium present in the water. The water having reduced alkalinity level was thus made safe for both nutrient solution preparations as well as plant growth (Schwarz *et al.,* 2004). The distilled water used in this experiment was directly prepared from tap water by single distillation and as a result trace amount of different elements were retained (Table 1). The elements found in the rain water sample used in this experiment might be due to air pollution (Khare *et al.,* 2004).

The observations on further crop growth in four categories of water solutions showed that tomato plants performed well in all of them. However, variations in growth patterns were distinct among the treatments. At 120 days growth of the plants the total dry matter accumulation in the shoot of tomato plants, number of flowers/plant, average number of fruits/plant grown in rain water solution was much higher than that in other solutions (Table 2). The percentage of successful fruit bearing (62%) was also the highest in the plants grown in rain water solution. The total water requirement of the plants had not wide differences among the treatments however, the added nitrate nutrient in the solution was

Table 1. Chemical analysis of different water samples

Parameters	Water quality			
	Distilled water	Rain water	Pond water	Tap water
pH	6.75	5.99	7.19	7.41
Electrical conductivity (mmhos cm^{-1})	0.002	0.40	0.72	0.71
Bicarbonate (mg L^{-1})	0.083	0.133	0.337	0.279
Calcium (mg L^{-1})	0.80	20.04	41.68	71.34
Magnesium (mg L^{-1})	0.78	19.51	48.39	81.17
Nitrate (mg L^{-1})	0.78	1.50	1.78	1.66
Ammonium (mg L^{-1})	0.08	0.05	0.76	0.17
Phosphate (mg L^{-1})	0.09	0.26	0.25	0.48
Sulphate (mg L^{-1})	0.00	0.00	3.99	0.00
Boron (mg L^{-1})	0.00	0.038	0.032	0.026
Chloride (mg L^{-1})	1.875	0.625	10.621	8.125
Sodium (mg L^{-1})	1.25	2.50	23.10	18.10
Potassium (mg L^{-1})	0.125	0.750	5.500	3.250
Iron (mg L^{-1})	0.046	0.320	0.745	0.476
Manganese (mg L^{-1})	0.005	0.017	0.035	0.064
Zinc (mg L^{-1})	0.007	0.015	0.031	0.146
Copper (mg L^{-1})	0.100	0.001	0.00	0.001

Table 2. Growth parameters of tomato plants grown in four water solutions

Treatments	Dry weight of shoot (g)	Flowers per plant	Fruits per plant	Average fruit weight (g)	Total nitrate accumulation/ plant(g)	Total water requirement/ plant (L)
Distilled water	28.91	45.34	26.67	34.66	7.518	10.69
Rain water	38.37	46.50	28.50	35.42	9.111	9.94
Pond water	28.72	46.34	16.34	30.56	7.341	10.18
Tap water	28.70	42.00	16.83	30.36	7.734	10.04
LSD (P=0.05)	8.92	3.71	3.43	5.54	-	-

Fig. 1. Tomato plants grown in rain water solution. Fig. 2. Chilli plants grown in rain water solution. Fig. 3. Cauliflower grown in rain water solution showing browning symptoms in curd. Fig. 4. Normal cauliflower curd formation in tap water solution. Fig. 5. Marigold cv. *Inca* grown in a) rain water, b) distilled water c) tap water and d) pond water solutions.

much higher in the plants grown in rain water solution than in all other treatments (Table 2). For tomato plants, adequate supply of NPK and micro nutrients like Zn, Cu, and B is essential for dry matter accumulation, number of flowers, fruit quality and fruit size (Kallo *et al.,* 2003). Availability of these particular nutrients varied among the treatments and was better in rainwater solutions that was reflected in crop yield.

The variations in growth patterns of chilli plants were also distinct among the four treatments. At 165 days of growth, the total dry matter accumulation in shoot was much higher in the plants grown in rain water solution and distilled water solution than that of tap water and pond water solutions (Table 3). The plants grown in distilled water and rain water solutions had earlier initiation of flowering and fruiting and higher average number of fruits/plant than tap water and pond water solutions. The total water and nutrition added was highest in rain water solution followed by distilled water treatment (Table 3). Chilli plants prefer nitrate nitrogen and a proper balance of nitrate and ammonium nitrogen is recommended that might affect vegetative growth and delay in maturity (Despande, 2003). The common nutrients added to each treatment in this experiment had only nitrate nitrogen and no ammonium nitrogen (Hoagland and Arnon, 1950). However, the presence of ammonium in the pond water and tap water were initially much higher than rain water and distilled water (Table 1).

Table 3. Growth parameters of chilli plants grown in four water solutions

Treatments	Dry weight of shoot (g)	Initiation of flowering (days)	Initiation of fruiting	Flowers per plant	Fruits per plant	Total nitrate accumulation/ plant (g)	Total water requirement/ plant (L)
Distilled water	20.77	47	62	115.50	48.50	12.94	11.95
Rain water	22.49	51	66	96.17	45.50	14.47	13.15
Pond water	12.20	68	83	79.00	29.67	10.30	9.54
Tap water	11.37	71	86	73.67	34.50	8.89	8.28
LSD (*P*=0.05)	6.97	9.3	9.3	25.52	12.61		

This might have disturbed the necessary balance of nitrate and ammonium nitrogen in the solutions and affected the plant growth in these two treatments.

In case of cauliflower, within a period of 80 days growth, the total dry matter accumulation (35.64 g, excluding the curd) in the plants grown in tap water solution was much higher than the other treatments (20-25 g). The total water and nutrition added during the entire crop growth period were also much higher in these two treatments. Among the four treatments there were distinct differences in curd formations. The curds formed in the plants grown in rainwater and distilled water solutions showed browning symptom (Fig. 3). Such physiological disorders in cauliflower usually happens due to micronutrient deficiencies like magnesium, boron and molybdenum (Singh and Sharma, 2003). No such abnormalities in curd development were noticed in the plants grown in pond water and tap water (Fig 4). The highest curd weight (350g) was observed in the plants grown in the tap water. It appeared from the result that the nutrient solutions prepared by rain water and distilled water could not provide sufficient micronutrients. As the initial micronutrient content were high in tap water and pond water (Table 1) it could be properly utilized by the plants for normal curd formations.

Marigold cv. *Inca* could be grown well in the single plant culture devices. The plants came into flowering within 40-45 days after planting and continued blooming for next two months. There were no significant differences in the estimated vegetative growth parameters, root growth and flowering (20-25 in number) among the plants grown in different water treatments (Fig 5 a-c). There was no problem of precipitation in this culture by direct use of tap water or pond water. The results indicated that this plant had more tolerance to alkalinity stress as well as water quality status than the other plants used in this experiment.

In the low cost hydroponics devices designed for this experiment, different crops could be grown satisfactorily. It was estimated that from each plastic tray of 45.5 x 30.5 x 14 cm size, 5.8 kg tomato, 1.5 kg chilli and 1.75 kg cauliflower curd (excluding leaves) could be produced where the nutrients and water required were very minimum. Luxurious growth of flowers plants yielding maximum 25 flowers in a season could also be possible from a plastic bucket of 14cm height and 15.8 cm diameter. The system might be an ideal alternative for the horticulture hobbyists of the cities who usually practice soil pot culture of flower plants or roof gardens for vegetable productions. The composition of different categories of water used might vary from place to place that may affect crop growth (Mahmood, 2004). In this experiment rain water solutions gave better yield in all the crops except cauliflower where there was scope to add more micronutrients in the solution to avoid physiological disorders. Rain water, when harvested directly avoiding roof drainage contamination, could be more safely used for hydroponics (Pant *et al.,* 2002). In case of using pond and tap water, which are usually highly contaminated, crops' nutritional requirements and chemical compositions of water should be considered.

The present study indicates the successful way of low cost hydroponics which can be practiced by different cross sections of people. Apart from its commercial implications if such low cost hydroponics is used in a transparent container, although simple but that will definitely pave the way for the study of root system, the plant part which till date is poorly studied compared to other plant parts.

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Caffeine, phenol and protein contents of thirty-seven clones of Nigerian robusta coffee (*Coffea canephora* Pierre ex. Froehner)

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Abstract

A study was carried out to characterise thirty-seven *Coffea canephora* clones using three biochemical characteristics, namely caffeine, phenol and protein content. The phenol and caffeine contents were determined by gravimetric method, while protein was assessed by polyacrylamide gel electrophoresis (PAGE) of floral bud. Caffeine content among the clones ranged from 1.1 to 1.5% on dry matter basis (dmb). C36 a high yielding clone, had relatively low caffeine content, hence it is a suitable clone that could be included in any breeding programme for low caffeine coffee in Nigeria. All the Niaollou (M) clones had high caffeine content. Phenol content in the berry pulp of the clones ranged from 2.6 to 15.6%. Averaged over clones, phenol content of berry pulp (9.5 %) was significantly ($P < 0.05$) higher than leaf phenol content (4.5 %). The coefficient of variation for pulp phenol was high (35.3), thus indicating that, rapid response to selection for favourable phenol percentage might be feasible. The high level of phenol found in some clones may be valuable in breeding for resistance to some major diseases and insect pests of coffee. There were differences in the mobility and intensity of protein bands in the clones. The variation in the protein banding patterns of the different *C. canephora* clones observed provides further information on the existing genetic diversity of the coffee clones in addition to that provided by agro-botanical characters.

Key words: Caffeine, phenol, protein, *Coffea canephora,* Nigeria

Introduction

Coffee, a beverage crop, is the second most important commodity in the international market after petroleum (ICO, 2008). Among the important chemical compounds that are found in coffee, three of interests to breeders are caffeine, phenol and protein (ICO, 1999).

Caffeine (1, 3, 7-trimethylxanthine) is a methylated purine alkaloid (Vasudeva *et al.,* 1981) that is widely distributed in the family Rubiaceae (Bonner, 1950). Its presence in different members of the genus *Coffea* is well documented (Raju and Gopal 1979; Raju *et al.,* 1981). Caffeine ($C_8 H_{10} O_2 N_4$) is also found in kola, cocoa and tea. Caffeine stimulates the synthesis or release of the catecholamine, epinephine and norpinephrine hormones (better known as adrenalin and nor-adrenalin, respectively) into the blood stream (Nguyen-van Tam and Smith, 2001). This increases alertness, but may lead to loss of sleep. The stimulant is also not beneficial during pregnancy (Borea *et al.,* 2001). Consequently many consumers of coffee prefer decaffeinated coffee. Hence, demand for decaffeinated coffee is increasing rapidly in the world market. There is, therefore, an urgent need to develop caffeine-free coffee cultivars (Sreenath, 1997) in order to protect the income of coffee farmers. Caffeine content is greater in robusta than in arabica coffee (Raju and Gopal, 1979). Given that the direction of international market on caffeine content in *C. canephora* is towards its reduction (Ashihara and Crozier, 2001; Montagnon *et al.,* 1998); one of the breeding objectives for improved coffee quality in the world today is low or no caffeine content (Sreenath, 1997).

Phenol, when present in significant quantity in coffee, has been observed to be associated with resistance to leaf rust and coffee berry disease (Walyaro, 1983). Ram *et al.* (1982) observed significant positive correlation between phenolic compounds in coffee varieties and their resistance to leaf rust disease. The polyphenol content of coffee leaves has also been reported as a good criterion for evaluating breeding material and selecting donors for resistance to the insect *Aphis humili* (Romanenko, 1985).

The protein band variation from different crop plants is based partly on differences in the molecular weight of protein. Crop traits are the expression of genes, and proteins are the primary products of genes. Agro-botanical variation reflects diversity in genetic structure. Therefore, genetic diversity in a crop germplasm can be illuminated through the analysis of protein. Gottlieb (1971) reported that variation in banding pattern could be equated to variation in genes coding for various proteins. Protein electrophoresis therefore offers an efficient means of identifying and quantifying genetic variation in crop germplasm (Carrens *et al.,* 1997; Davis, 1964). The advantage of biochemical markers such as protein, is that they are genotypic markers and hence, will reflect the actual genetic distances between accessions and their common ancestors more accurately than phenotypic markers (Gepts, 1995). Berthou and Trouslot (1979) used isozyme analysis to separate the Guinean group from the Congolese group of *C. canephora* populations. The objective of this study, therefore, was to carry out biochemical characterisation of Nigerian *C. canephora* germplasm.

Materials and methods

The study was carried out at the Cocoa Research Institute of Nigeria, Gambari, Ibadan. The *C. canephora* clones studied and their geographical origins were: A110, A116 (Ghana robusta), C36, C90, C96, C105, C107, C108, C111 (Quillou robusta from

Gabon), E1, E77, E92, E106, E1 (Java robusta), G87, G129 (Uganda robusta), M10, M31, M36, M53 (Niaollou robusta from Republic of Benin), T4, T24, T45, T93, T116, T164, T169, T204, T314, T365, T921, T1049, T197, T155, T176, T395 and T220 (Java-ex-Gambari from Zaire). The clones were planted in 1966. The plants were coppiced in 1997 and 2000. The morphological differences among the "A", "C", "E", "G", "M" and "T" clones are described by Omolaja and Fawole (2004a). Three biochemical compounds namely phenol, caffeine and protein contained in each of the thirty-seven clones were determined.

Determination of caffeine content

Sample preparation, caffeine extraction and determination: Fruit used for caffeine determination were harvested at maturity and depulped using the wet processing method (Omolaja and Williams, 1997). Caffeine content was determined by the modified extraction methods of Horwits (1970) and Barre *et al.* (1998). Caffeine content was evaluated using 40 randomly sampled coffee beans per clone from freshly harvested berries. The samples were milled to fine powder using Moulinex electronic grinder and stored in plastic containers in a dessicator at 4°C until used. Each batch of beans was crushed for eight minutes. One g of dried coffee powder was weighed into a conical flask. Then 50 mL of 0.5N boiling or hot H_2SO_4 was added. The mixture was then refluxed on water-bath for 30 -50 minutes. Afterwards it was allowed to cool, then centrifuged and filtered. Forty mL of the mixture was taken into a separating funnel and 30 mL chloroform was added, and vigorously shaken. Two layers were formed: organic layer at the top and chloroform layer at the bottom. The chloroform layer was collected. Another 30 mL chloroform was added to the organic content remaining in the funnel and the mixture properly shaken. The chloroform layer was again collected. Then 40 mL of the chloroform fraction was dispensed into a pre-weighed 100 mL flat-bottom flask. This was distilled off at between 60-80°C. The distillate was dried in the oven to obtain caffeine, and the weight of caffeine was determined. The percent caffeine for each clone was obtained by dividing the caffeine weight by the initial weight of sample, and multiplying by 100. The extraction for each clone was repeated three times. Analysis of variance was carried out and means separation was done using Duncan's Multiple Range Test.

Determination of phenolic content

Sample collection, sample preparation and determination of total phenol: The procedure of phenolic content determination used in this study was modified methods of Bate-Smith (1962) and Odebode (1995). Fresh leaves and the pulp of seven months old mature berry were investigated for their phenolic content. The plant parts of each of the thirty-seven clones were randomly sampled. Fresh leaves and berry pulp weighing 100g were separately washed and dried in an oven at 50-60°C. The samples were milled to powder using Moulinex electronic grinder and stored in a conical flask in a dessicator at 4°C until used. A sample of 2 g of each of oven dried and milled leaves as well as berry pulp was taken into different conical flask containing 100 mL of 80% ethanol and boiled for eight hours. The ethanol was changed by decanting three times (3 x 100 mL) during the extraction of the total phenol. The ethanol extractions were then concentrated to 30-40 mL in a vacuum evaporator. The residue was refluxed for 1 hour, filtered hot and diluted to 100 mL with distilled water. Then the filtrate was dried in the oven at a temperature of 80°C. The percent phenol for each clone was determined from the weight of the dried residue. The percent phenol was calculated by dividing the weight of the dried residue with the initial 2 g of oven dried sample and multiplying by 100. The phenol extraction for each clone was repeated three times. Data of percent phenol for the leaves and pulp were separately subjected to analysis of variance, while significant differences among means were established by Duncan's Multiple Range procedure.

Electrophoretic analysis of floral bud protein: Unopenned floral buds collected from each of the thirty-seven *C. canephora* clones were used for the analysis. Five g of floral bud was used per clone.

(i) Protein extraction: Floral bud protein was extracted by grinding 1 g of the floral bud with mortar and pestle. 7.5 mL of 0.9% sodium chloride (NaCl) was added during grinding. The mixture was allowed to settle inside the test tube immersed inside ice for one hour (Illoh, 1990), thereafter centrifuged at 4000 rpm for 15 minutes. The clear supernatant was then poured into the test tube and stored inside the refrigerator. On the day of use, sucrose crystals (5 grains), three drops of each of 2% mercapto-ethanol and sodium dodecyl sulphate (SDS) were added to 1 mL of the supernatant in the test tube and the mixture was boiled for 15 minutes in a water-bath. After cooling down to room temperature, a drop of 0.05% bromophenol blue was added to the sample as front marker (Akinwusi and Illoh, 1995).

Preparation of gels is as indicated in Table 1. The loading of gels, electrophoretic run, staining, destaining, and measurement of the gels were carried out according to Omolaja and Fawole (2004b). The positions of different bands were noted. Each protein band was considered as a qualitative character, with presence coded as 1 and absence coded as 0 (Carrens *et al.,* 1997). The data were analysed for significant differences among clones with separation of means carried out by Duncan's Multiple Range Test.

Table 1. Preparation of gels for the electrophoresis of floral bud, anther, pollinated and un-pollinated styles' protein of *Coffea canephora* clones

Stock solutions/ components	Upper gel	Lower gel
Floral bud protein		
40% Acrylamide	1.00 mL	10.00 mL
Upper gel buffer	2.50 mL (0.5M; pH 6.8)	-
Lower gel buffer	-	7.50 mL (1.5M; pH 8.8)
Distilled water	6.33 mL	19.50 mL
10% Sodium dodecyl sulphate	1.00 mL	0.40 mL
10% Ammonium persulphate	0.05 mL	0.10 mL
TEMED (N N N 'N'-Tetramethyl ethylenediamine), 'Electran'	20.00 µL	50.00 µL
Anther, pollinated and un-pollinated styles protein		
Acrylamide	1.35 mL	10.00 mL
Upper gel buffer	2.50 mL	-
Lower gel buffer	-	7.50 mL
Distilled water	6.00 mL	11.20 mL
10% Sodium dodecyl sulphate	0.10 mL	0.30 mL
Ammonium persulphate	0.01 mL	0.30 mL
TEMED	0.03 µL	0.10 µL

Results

Caffeine analysis of thirty-seven *C. canephora* clones: The caffeine content of the clones ranged between 1.1 and 1.5% on dry matter basis (Table 2). Clones A116, C36, C90, C111, E130, T24, T116, T204 and T314 had caffeine contents that were significantly lower than the other clones ($P < 0.5$). Clones E106, M36 and T176 recorded the highest caffeine content of 1.5% dry matter basis, respectively. Clone C36 was among the clones that recorded the lowest caffeine content of 1.1%. All the Niaollou (M) clones had high caffeine content.

Phenolic content of thirty-seven *C. canephora* clones: The percent phenolic content in the berry pulp ranged from 2.6 (E130 and T104) to 15.6 (in T4), among the clones studied (Table 2). The percent phenolics of clones A110, C36, E1, E106, E130, M10 and T204 were significantly lower than those of the other clones at $P < 0.5$. Clones A116, M31, T365, T1049 and T4 had the highest percent of phenol. The average percent phenol in berry pulp was 9.5, which was significantly ($P < 0.05$) higher than what was obtained in the leaves (4.5%). The range of phenolics in the leaves was between 3.0 and 6.3%. The coefficient of variation for pulp phenolic content was high (35.3%), thus indicating that, rapid response to selection for favourable phenolic content might be feasible.

Electrophoretic analysis of floral bud protein in *C. canephora*: The protein band variations among the thirty-seven clones of *C. canephora* are presented in Table 3. Marked differences were observed in the number, combination and intensity of bands among the clones. In terms of mobility of bands, there were three categories: the fast (6.0 - 9.0 cm), the intermediate (3.0 - 5.9 cm) and the slow bands (0.1 - 2.9 cm.) (Table 3). The protein bands at 0.1 to 2.9 cm and 6.0 to 9.0 cm were exhibited by Java (E) and Java-Ex. Gambari (T) varieties. The Ugandan robusta (G) showed protein bands in all the regions, while Ghana robusta (A) exhibited unit band at only the fast region. The Quillou (C) showed thick bands at the fast region, but the Niaollou robusta (M) exhibited varied protein bands. While M10 has bands in fast region, M31 had bands in all the regions. M36 had no band. Of the thirty-seven clones, one (clone T116) has five bands, three has four bands, eight has three bands, twenty has two bands, four has one band while one has no band. Twenty-two clones had bands at the slow and fast regions, one clone at the slow and intermediate regions, while ten clones showed protein bands at only the fast region. The three categories of bands occurred at four different levels of intensities: very thick, thick, thin and faint. Majority of the " T" clones showed thin and faint bands.

Table 2. Phenol and caffeine contents of thirty-seven clones of *Coffea canephora* clones

Clone	Phenol (%)		Caffeine (%)
	Pulp	Leaves	
A110	3.3d	3.2b	1.4a
A116	15.0a	3.2cb	1.2c
C36	3.3d	4.1b	1.1c
C90	10.8b	4.3b	1.2c
C96	9.9b	3.0cb	1.3b
C105	10.8b	6.3a	1.4a
C107	7.7cb	4.1b	1.4a
C108	6.6c	3.6b	1.3b
C111	11.4b	6.2a	1.2c
E1	3.4d	4.1b	1.4a
E77	7.4c	4.1b	1.3a
E92	8.3cb	5.0a	1.3a
E106	4.4d	5.1a	1.5a
E130	2.6d	3.1cb	1.1c
G87	9.6b	3.8b	1.4a
G129	9.5b	3.6b	1.4a
M10	4.0d	4.3b	1.4a
M31	12.5b	3.4b	1.3a
M36	11.4b	5.1a	1.5a
M53	10.5b	4.7b	1.4a
T4	15.6a	5.4a	1.3a
T24	11.6b	5.2a	1.2c
T45	11.6b	5.1a	1.3a
T93	10.4b	5.0a	1.3a
T116	11.7b	5.4a	1.2c
T164	11.6b	5.6a	1.3a
T169	10.5b	4.9a	1.4a
T204	2.6d	4.6b	1.2c
T314	9.6b	4.6b	1.2c
T365	12.6b	4.6b	1.3a
T921	10.7b	5.2a	1.2c
T1049	12.6b	3.5b	1.3a
T197	8.4cb	3.4b	1.4a
T155	9.2b	4.8a	1.4a
T176	10.4b	5.2a	1.5a
T395	10.1b	5.1a	1.3b
T220	10.5b	4.5b	1.4a
Mean	9.51	4.54	1.31
CV (%)	36.3	19.1	8.4

Means with similar letters are not significantly different ($P < 0.05$) by Duncan's Multiple Range Test.

Discussion

The coefficients of variation among many of the three biochemical characters were low, indicating that the genetic base of the Nigerian *C. canephora* germplasm is very narrow. Studies on intra-specific variation in caffeine content in many *Coffea* species including *C. canephora* showed that caffeine-free coffee was yet to be found (Barre *et al.,* 1998). Low caffeine coffee is, however, increasingly being demanded in the international market (Sreenath, 1997). Heritage (2006) noted that robusta coffee has average caffeine content of 2.2%, while arabica coffee contains an average of 1.2%. The caffeine content among the clones studied ranged between 1.10 and 1.53%. Clone C36 is regular bearing, high yielding and relatively low in caffeine content, hence it is a suitable clone that could be included in any breeding programme for low caffeine coffee in Nigeria. The thirty-seven clones studied showed varying levels of phenol content. According to Bate-Smith (1962) high level of phenol in plants are associated with their tolerance/ resistance to some diseases and insect pests. Romanenko (1985) who noted an average of 4.3% phenol in the leaves of Asian coffee clones, equally observed that clones with high phenolic contents were resistant to attack by the leaf sucking insect *Aphis humilis*. The high level of phenol, in the range of 4.8 to 6.3%, found in some of the clones studied, indicates their potential as parents in breeding for resistance to some major diseases and insect pests of coffee.

All Ghana robusta ("A" clones) had fast protein bands between 6.0 and 9.0 cm. All the "E" and "T" clones however, had their bands in the slow and fast ranges of relative mobility, indicating that they share genetic identity. This is possible, as they had a common ancestry in Java. The Niaollou ("M" clones) robusta

Table 3. Relative mobility of protein bands of thirty-seven clones of *C. canephora*

Clone	Slow bands (0.1-2.9 cm)	Intermediate bands (3.0-5.9 cm)	Fast bands (6.0-9.0 cm)	Total number of bands
A110	-	-	1	1
A116	-	-	1	1
C36	-	-	2	2
C90	1	-	2	3
C96	-	-	3	3
C105	1	1	1	3
C107	1	-	2	3
C108	-	-	1	1
C111	-	3	3	7
E1	1	-	1	2
E77	1	-	2	3
E92	1	-	1	2
E106	1	-	1	2
E130	1	-	2	3
G87	2	1	1	4
G129	2	1	1	4
M10	-	-	2	2
M31	1	2	1	4
M36	-	-	-	-
M53	1	1	-	2
T4	2	-	1	3
T24	1	-	1	2
T45	2	-	-	2
T93	2	-	-	2
T116	1	-	2	3
T164	1	-	2	3
T169	1	-	1	2
T204	1	-	1	2
T314	1	-	1	2
T365	1	-	1	2
T921	1	-	1	2
T1049	1	-	1	2
T197	1	-	1	2
T155	1	-	1	2
T176	1	-	1	2
T395	1	-	1	2
T220	1	-	1	2

- Band absent

haddistinct protein bands in that their bands were spread in the slow, intermediate and fast mobility ranges. A previous report (Omolaja and Fawole, 2004a) on morphological traits corroborates the finding of the present study on the distinct characteristics of Niaollou robusta. Niaollou robusta is characterised by purple young leaves, purple immature berry, multi-petalloid corrola and with few tri-forked stigma, as against other clones. The high yielding clones most of which are found among the Quillou ("C") (Omolaja and Fawole, 2004a) have thick protein bands that are located in the fast range of mobility. Ugandan (G) clones are equally distributed in all the ranges: slow, intermediate and fast, but did not have thick bands.

The variation in the protein banding patterns of the different *C. canephora* clones observed in this study provides more information on the existing genetic diversity of the coffee clones. In the choice of parents for hybridization in robusta coffee in Nigeria, the clones that have the widest of variability in terms of bands' location are Niaollou (M) and Quillou (C), hence the two would be good candidates in *C. canephora* (robusta) improvement programme.

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Bunch covering impact on the ripening time, marketable yield and fruit quality of "Zaghloul" dates

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Abstract

This study targets to investigate the efficiency of date bunch covering treatments by using different bag types such as the polypropylene muslin, staved-plastic (polyethylene) or cecile tissue in comparison with uncovered bunches (control) in the same orchard (from the mid of July to mid of September) in Rossitta region (Rasheid), Behera province, Egypt. Quantity and quality of marketable yield for "Zaghloul" dates, beside the ripening time were assessed through two consecutive seasons. The main notice was, all kinds of used covers reduced the damage caused by birds, blights and wasps as well as no incidence of diseases was observed under the experimental covers. Polypropylene muslin treatment decreased the dropped fruits in both study seasons, consequently it increased the marketable yield. Fruits under the polypropylene muslin bags were late in the ripening. Date bunches under the staved-plastic covers were statistically superior than all other treatments regarding fruits quality and were early in the ripening. There were statistical differences in fruit quality traits and fruits ripening time according to the bunch cover types.

Key words: Polypropylene, dates, bunch covering, "Zaghloul", ripening time, fruit quality

Introduction

Bunch bagging is one of the earliest known operations during last century, for fruit protection against the damage caused by birds, blights and wasps as well as diseases (Nixon, 1932). For improving dates yield and fruit quality, bunch-covering alone (Elmer, 1964 and Salem *et al.*, 1977) or in combination with other treatments (Bliss *et al.,* 1950 and Darley and Wilbur, 1955) had been much investigated. Bunch covers including paper, cloth, plastic screen or large-mesh sheets, jute bags or date-leaf bags have been tested (Bliss *et al.,* 1949; Bliss *et al.,* 1950; Brown, 1955; Dowson and Aten, 1962; Elmer, 1964; Sharpless and Hilgeman, 1951 and Salem *et al.,* 1977).

The aim of the study was to investigate the efficiency of date bunch covering treatments by using different bag types such as the polypropylene muslin, staved-plastic (polyethylene) or cecile tissue with regard to ripening time, quality and nutrient content.

Materials and methods

The experiment was carried out for two consecutive seasons (2005 and 2006) in Rossitta region, Behera province, Egypt to study the impact of bunch covering treatments by using bags made of polypropylene muslin (Fig. 1), staved-plastic (polyethylene) (Fig. 2), or cecile tissue (Fig. 3) on the yield attributes (bunch weight, fruit weight, marketable yield and spoiled fruits); fruit quality traits (total acidity, TSS, total sugars and total protein) as well as minerals content of "Zaghloul" fruits dates in comparison with uncovered bunches. Four treatments were arranged and replicated five times. Each treatment consisted of five palms more than 15 years old with (9 : 1) leaf : bunch ratio. Palm trees were selected in symmetric morphology and bunch number was standardized at 8 bunches/palm. Bunches were covered for two months period from the "late Kimri" stage to the harvest time (from the mid of July to mid of September).

Analysis of variance was performed using completely randomized design (Steel and Torrie, 1980).

Results and discussion

Ripening time: The morphological observations on fruit bunches under staved-plastic and cecile tissue bags, indicated that fruits ripened 2-3 weeks earlier than fruits of un-covered bunches for 1st and 2nd seasons. However, fruits under polyproplyene muslin bags ripened 2 weeks later than un-covered bunch fruits in both the study seasons. It means that, the staved-plastic and cecile tissue bag treatments lead to raising the temperature around the fruit bunch, causing earlier fruits ripening in comparison with control (un-covered bunch). Contrarily, the polypropylene bags treatment leads to lowering the temperature around the fruit bunch causing delay in fruits ripening in comparison with control. Nixon and Reuther (1947) reported that the use of covers was found to have slightly delayed ripening of Khadrawy dates.

Yield attributes

Bunch weight: Bunches covered by polypropylene muslin bags were best in both the seasons followed by bunches covered by the staved-plastic bags, un-covered bunches and the bunches covered by cecile tissue bags (26.8, 23.7, 21.3 and 19.8 kg, respectively for the 1st season and 29.2, 24.5, 21.8 and 19.7 kg, respectively for the 2nd season). All differences among recorded values were significant (Table 1). These results were due to the decrease in fruit drop ratio for the polypropylene muslin bags treatment in comparison with all other treatments.

Fruit weight: Results of this trait were different than bunch weight trait. The fruits under staved-plastic bags were statistically heavy in comparison with all other treatments in both the seasons,

Fig. 1. Polypropylene muslin covers.

Fig. 2. Staved-plastic (polyethylene) covers.

Fig. 3. Cecile tissue covers.

followed by fruits under cecile tissue bags, then fruits resulted from un-covered bunches and fruits under the polypropylene muslin bags (32.8, 27.3, 20.8 and 18.7 g, respectively for the 1st season and 33.1, 28.2, 21.2 and 19.9 g, respectively for the 2nd season). All differences among recorded values were significant (Table 1). These results were due to the effect of temperature around the fruit bunches, which differed according to bags type. Nixon and Reuther (1947) reported that covering treatments had no significant effect on fruit weight of Khadrawy dates.

Marketable yield: Data in Table 1 indicate that the marketable yield of palm trees treated with the polypropylene muslin bag covers was statistically superior over all other treatments, followed by palms which received the staved-plastic bag covers treatment, while palms of control treatment (uncovered bunches) came third followed by cecile tissue bag covers treatment for both the seasons (196.3, 180.2, 157.8 and 145.2 kg, respectively for the 1st season and 189.5, 175.9, 153.1 and 147.3 kg, respectively for the 2nd season). All differences among recorded values were significant (Table 1). This result was due to the decrease of fruit drop ratio under the polypropylene muslin bags in comparison with all other treatments. Al-Bahrany *et al.* (1994) reported that the bunch covers significantly reduced the total unmarketable yield (fruit drop; bird and insects damage) leading to higher marketable yield.

Spoiled fruits: Results indicate that the cecile tissue bags treatment had statistically highest amount of spoiled fruits, followed by the staved-plastic bags (9.2 and 7.3 kg and 8.6 and 7.8 kg for the 1st and 2nd seasons, respectively). While bunches covered by the polypropylene muslin bags had statistically the lowest amount of spoiled fruits (3.8 and 4.1 kg for the 1st and 2nd seasons, respectively). The differences among all treatments were significant (Table 1). It means that the polypropylene muslin bags allow good aeration around the fruit bunch and reduced fruit spoilage. Contrary to this, cecile tissue bags and staved-plastic bags cause a high temperature around the bunches, so they increase the spoilage of fruits. It must be mentioned that, the reason of spoiled fruits is a physiological reason, not fungal. Bliss *et al*. (1949) reported that the physiological and fungal fruits spoilage occurred with bunches covering treatments. Nixon and Reuther (1947) reported that covering treatments were related with reduced sunburn of Khadrawy dates.

Table 1. Effect of covering treatments on yield attributes

Covering treatments	2005 season				2006 season			
	Bunch weight (kg)	Fruit weight (g)	Market. yield (kg)	Spoiled fruits (kg)	Bunch weight (kg)	Fruit weight (g)	Market. yield (kg)	Spoiled fruits (kg)
Uncovered	21.3	20.8	157.8	4.9	21.8	21.2	153.1	5.1
Polypropylene	26.8	18.7	196.3	3.8	29.2	19.9	189.5	4.1
Staved-plastic	23.7	32.8	180.2	7.3	24.5	33.1	175.9	7.8
Cecile tissue	19.8	27.3	145.2	9.2	19.7	28.2	147.3	8.6
LSD (*P*=0.05)	1.3	1.0	6.2	0.9	1.4	1.1	4.9	0.9

Table 2. Effect of covering treatments on fruit quality traits

Covering treatments	2005 season				2006 season			
	Total acidity (%)	TSS (%)	Total sugars (%)	Total protein (%)	Total acidity (%)	TSS (%)	Total sugars (%)	Total protein (%)
Uncovered	1.63	18.29	66.14	1.88	1.66	19.65	67.17	1.84
Polypropylene	1.82	15.47	61.58	1.39	1.85	15.69	60.99	1.41
Staved-plastic	0.98	24.13	81.73	2.11	0.99	23.89	82.08	2.09
Cecile tissue	1.04	22.60	78.65	2.20	1.01	22.41	79.90	2.18
LSD (*P*=0.05)	0.17	0.88	2.43	0.19	0.16	0.83	2.35	0.21

Table 3. Effect of covering treatments on mineral contents of fruits

Covering treatments	2005 season					2006 season				
	N (%)	P (%)	K (%)	Fe (ppm)	Mg (ppm)	N (%)	P (%)	K (%)	Fe (ppm)	Mg (ppm)
Uncovered	0.33	0.08	0.75	59.0	181	0.35	0.08	0.76	61.5	178
Polypropylene	0.26	0.05	0.60	48.5	164	0.24	0.06	0.62	47.5	170
Staved-plastic	0.36	0.10	0.94	67.5	198	0.38	0.09	0.92	66.0	185
Cecile tissue	0.38	0.09	0.80	67.0	193	0.38	0.10	0.79	65.5	180
LSD (*P*=0.05)	0.06	0.02	0.12	4.3	11	0.07	0.02	0.12	3.8	N.S

Fruit quality traits

Total acidity: Statistical analysis of data showed that the fruits under polypropylene muslin covers had highest total acidity percentage (1.82 and 1.85 % for 1st and 2nd seasons, respectively). On contrary, fruits covered by the staved-plastic bags had lowest total acidity percentage without significant difference in comparison to fruits covered by the cecile tissue bags (0.98 and 1.04 %, respectively for the 1st season and 0.99 and 1.01 %, respectively for the 2nd season). Fruits of uncovered bunches had medium values of total acidity percentage in two study seasons (1.63 and 1.66 % for 1st and 2nd seasons, respectively) (Table 2). It means that the fruits under staved-plastic bag covers had a good savor in comparison with fruits under polypropylene muslin bag covers. Al-Bakir *et al.* (1988) reported that bagging improves the fruit quality of "Zahdi" dates.

TSS: Statistical analysis of TSS (%) data indicated that staved-plastic bags treatment was superior over all other treatments (24.13 and 23.89 % for 1st and 2nd seasons, respectively) followed by fruits covered by cecile tissue bags (23.60 and 23.41 % for 1st and 2nd seasons, respectively). On contrary, the treatment of polypropylene muslin bags had statistically lowest TSS (15.47 and 15.69 % for 1st and 2nd seasons, respectively) in comparison to all other treatments for both the seasons. However, uncovered fruit treatment had a moderate TSS value in both the seasons (Table 2).

Total sugars: Data of Table 2 indicated that total sugars (%) of date fruits covered by the staved-plastic bag covers was statisticaly superior than all other treatments in both the seasons (81.73 and 82.08% for the 1st and 2nd seasons, respectively). However, no significant difference occurred in comparison with cecile tissue bags treatment in the 2nd season. Likewise, fruits under the cecile tissue bags were statistically superior than other two treatments in both the seasons (78.65 and 79.9% for 1st and 2nd seasons, respectively). Fruits under polypropylene muslin bag covers had statistically low total sugar (%) values in both the seasons (61.58 and 60.99% for 1st and 2nd seasons, respectively). It means that, the fruits produced under polypropylene bag covers had a sour savor in comparison with fruits produced from all other treatments. Al-Bakir *et al.* (1988) reported that bagging increased the total sugars of Zahdi dates.

Total protein: Total protein percentage was statistically affected by types of bunch covering bag. Fruits produced under the cecile tissue bags had a higher percentage of protein (2.20 and 2.18% for the 1st and 2nd seasons, respectively), followed by fruits under the staved-plastic bags (2.11 and 2.09% for the 1st and 2nd seasons, respectively) without significant difference. Contrary to this, fruits grown under the polypropylene muslin bags had a lower total protein percentage in both the seasons (1.39 and 1.41% for the 1st and 2nd seasons, respectively). While, fruits grown without covering treatments had a medium values of this trait in both the seasons (Table 2).

It is revealed from the results that the bunch-covering using staved-plastic bags had improved the dates fruit quality. Dowson and Pasiote (1972) reported that the bagging of Deglet Noor fruit bunches had improved their quality.

Fruits mineral contents

Nitrogen: Data tabulated in Table 3 indicated that nitrogen (%) in fruits related with the polypropylene muslin bag covers treatment (0.26 and 0.24 % for the 1st and 2nd seasons, respectively) was statistically lower in comparison with cecile tissue bags, staved-plastic bag covers and uncovered treatments which had nonsignificant differences in both the study seasons (0.38, 0.36 and 0.33 % for the 1st season, and 0.38, 0.38 and 0.35 % for the 2nd season, respectively).

Phosphorous: Staved-plastic bag covers, cecile tissue bag covers and uncovered (control) treatments had more P percentage in fruit in both the seasons (0.10, 0.09 and 0.08 % for the 1st season; and 0.09. 0.10 and 0.08 % for the 2nd season, respectively) without significant differences (Table 3). However, the polypropylene muslin bags treatment was related with the statistically low P percentage in fruit in both the seasons (0.05 and 0.06 % for 1st and 2nd seasons, respectively).

Potassium: The recorded K (%) values in Table 3 indicate that the staved-plastic bags treatment had highest value in comparison with all other treatments (0.94 and 0.92 %, for the 1st and 2nd seasons, respectively). While, the lowest values were observed in polypropylene muslin bag covers treatment (0.60 and 0.62 % for the 1st and 2nd seasons, respectively). Cecile tissue bag covers treatment was statistically not different than un-covered treatment in both the seasons (0.80 and 0.75% for the 1st season as well as 0.79 and 0.76% for the 2nd season, respectively).

Iron: The statistically superior values of Iron (ppm) were recorded in staved-plastic bag and cecile tissue bag treatments without significant difference in both the seasons (67.5 and 67.0 ppm for the 1st season; and 66.0 and 65.5 ppm for the 2nd season, respectively). While the lowest Fe (ppm) was recorded in the polyproplyene muslin bag covers treatment in both the study seasons (48.5 and 47.5 for the 1st and 2nd seasons, respectively), (Table 3).

Magnesium: The significant differences among magnesium (ppm) content were recorded only in the 1st season. The staved-plastic bag covers treatment was superior without significant difference over cecile tissue bags treatment (198 and 193 ppm, respectively). Magnesium (ppm) content in polypropylene muslin bag covers treatment was statistically lowest among studied

treatments (164 ppm) (Table 3). The differences among values of all studied treatments were insignificant for the 2nd season.

Nixon and Reuther (1947) reported that dry weight of Khadrawy dates determinations from composite samples in the different bunch treatments did not show differences that could be correlated with the type of covering used.

It can be concluded from the results that date bunch covering treatments influenced ripening time, quality and mineral contents of fruits signinficantly and bunches with staved-plastic covers were statistically superior than all other treatments regarding fruits quality and were early in the ripening..

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A study on adaptation of tomato ecotypes in northern latitudes under southern Iran conditions

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Abstract

Tomato hybrids and cultivars from northern latitudes are tolerant to temperature variations and are early maturing crops. In order to produce new cultivars for southwest of Iran, it is necessary and useful to study adaptation of genotypes in this area. The seeds of 74 cultivars from Moscow and 8 hybrids from Netherlands were germinated and then transplanted to Jiffy-pots under plastic tunnels before being transferred to the soil in the field. Growth habits, leaf and inflorescence forms, fruit weight, fruit number, yield in each harvest, total yield and earliness were recorded. There were differences among cultivars for all measured characteristics. Some cultivars had relatively good tolerance to high temperature, and could produce fruits at temperatures higher than 30°C. The tested varieties had different growth habits. Maximum yield was obtained from determinate types, M66, M63, M49 and M48. For most cultivars, the largest fruits were produced in the first harvest while the next harvests had smaller fruits. A negative correlation was observed for fruit numbers and average fruit weight. Also, some cultivars including M39, M46, M74, M40, and M35 exhibited early and more uniform yield per plant compared with control varieties. Some cultivars such as M48 and M66 had late maturity with higher yield as compared with control. The tested entries were classified on the basis of leaf shape, inflorescence, fruit number and weight. Maximum difference was between controls and M27.

Key words: Ecology, temperature stress, growth habit, tomato

Introduction

Tomato (*Lycopersicon esculentum* Mill) originated from And Mountains at North American coasts and domesticated in Mexico and northern America. High ability of this plant in adaptation to various climatic conditions helped this crop to be cultivated from equator line to North Pole (Atherton and Rudich, 1986).

According to several researches, temperature is one of the important factors affecting physiological reaction of tomato (Nakano, 2004). In the original climate of this plant, temperature changes between minimum of 15˚C at night and maximum of 19˚C at day time (Atherton and Rudich, 1986). Nautiyal *et al.* (2005) indicated that optimum temperature for growth and development of tomato is 26/20˚C during day and night. Some other studies indicate that desirable growth of this plant is obtained between a minimum of 15˚C and a maximum of 30-35˚C (Tarakanov and Mukhin, 1993). Some researchers reported that suitable minimum temperature in transplanting time is 17-19˚C in day and 10-12˚C at night. When temperature increases over 30˚C, pollen grains are destructed (Tarakanov and Mukhin, 1993) and reduction in fruit yield is observed (Sato and Peet, 2005). There are more than 6000 tomato cultivars over the world and every one has its own physiological and morphological characteristics. These cultivars show different reactions for temperature, light, and maturation time (Breshnov, 1952; Tarakanov, 1975).

High temperature has been a limiting factor for tomato fruit formation; and many efforts have been made to find appropriate cultivars for tropical areas (Sott *et al.,* 1995). South of Iran has been known as one of the important centers of vegetable production in the country, but its high temperature always has been a limiting factor for fruit formation of many vegetable crops in this region, especially that temperature rises rapidly, and prevents fruits formation, growth and development.

During last 25 years, tomato production in Iran has increased from 130 to 2975 thousand tons per year (Anonymous, 1977 and Anonymous, 1997). In order to increase the quantity and quality of tomato yield, it is necessary to produce improved cultivars, for each ecological region.

This study was conducted to study the adaptability and desirable characteristics of tomato ecotypes from northern latitudes, compared with existing cultivars in Iran, and introduce them for use in future to produce improved tomato cultivars.

Materials and methods

Eighty two tested genotypes were coded as follows: Samples which were from Academy of Agriculture Moscow, were coded with letter 'M' and a number from 1 to 74. Samples from Netherlands, *i.e.* 'Rs2661', 'Arfela', 'Parana', 'Royesta', and 'Dual Prido' (obtained from Royal Sluis Company) were coded as H5, H7, H8, H2, and H1, respectively. Samples from United States *i.e.* 'Florida', 'President' and 'Floramerica' were signed as H6, H4 and H3, respectively. The seeds were treated with Vitavax 75% fungicide against pathogens, and then 20 seeds of each genotype were germinated on wet filter paper in Petri dish at 24-26 °C on January 20th, 2000. 'Early Urbana' and 'Red Cloud' cultivars were used as controls. After radicle appearance, two germinated seeds were sown in Jiffy-pots and then irrigated. Jiffy-pots were filled with equal portions of sand, manure, and soil. In warm hours of the day, plastic tunnels were removed to bring about the field weather conditions for plants. By the end of the day, plants were covered again. Jiffy-pots kept under plastic

tunnels until open air condition was suitable for transplantation. On March 15, ten vigorous seedlings from every cultivar were planted to the field. Row spacing and plant spacing in rows were 110 and 35 cm, respectively. Maximum, minimum and daily average temperatures were recorded under plastic and open air. In order to adapt the seedlings to outdoor conditions, plastic covers were removed from plants three days before transplantation. Since May 22, five vigorous plants from each cultivar were selected for harvest. Selected plants were harvested five times with one week interval. Except for final harvest, fruits had turned red at harvest time. From each cultivar, at least five plants were studied for plant height, growth and developmental processes, inflorescence form, fruit maturity time, fruit number per each harvest, total fruit number per plant, total weight of fruit per plant, and average of fruit weight. Also, maximum and minimum of these characters were calculated for all genotypes. Continuous data were reported as means ± standard deviation (SD). The growth habit trends and correlation coefficients of studied characters were drawn and calculated by software STATISTICA, V.5.1. The studied cultivars were classified for growth habit, inflorescence form, leaf shape, harvested fruits number, and average fruit weight by Complete Linkage Method based on Euclidean distance (Johnson and Wichern, 1998). All variables had the same weight and the following formula was used:

$$d_{(x,y)} = \sqrt{(x_1-y_1)^2+(x_2-y_2)^2+\ldots\ldots+(x_n-y_n)^2}$$

Results

Temperature changes: Premature changes under and around plastic tunnels, and in the field are presented in Figs. 1 and 2. In first 30 growing days of seedlings under plastic tunnels, the average of maximum temperature was 31˚C, minimum temperature 10˚C, and the average daily temperature was 20˚C. The satisfactory growth conditions of some genotypes indicated that it is possible to keep tomato seedlings after germination in the field without heating sources. Emergence of the germinated seeds can take place, only by using plastic cover during winter without heating sources in Ahwaz region. 25 days after transferring the seedlings to the field; the average temperature was 19˚C which was suitable for growth and development of seedling. This temperature was suitable for growth and development of plants until 30 days after they were transferred. But after 62 days, the average temperature raised up to 30˚C. Maximum temperature in first 25 days raised rapidly, so that at the end of harvesting period it was over 40˚C. This high temperature caused the branches and leaves in many cultivars to dry out. Minimum temperature did not decrease below 10 ˚C. So, at harvest period, plants were not exposed to cold weather; instead, after two months, plants steadily exposed to warm weather (over 30˚C). Majority of cultivars showed very good resistance to temperature changes until their growth stages were finished. High fruit yields of some late maturing cultivars can confirm their relative resistance to hot temperatures. Generally, it can be concluded that climatic conditions of Ahwaz from sowing date of seeds (March 3) to harvesting the fruits (June 15) is suitable for tomato cultivation in this region.

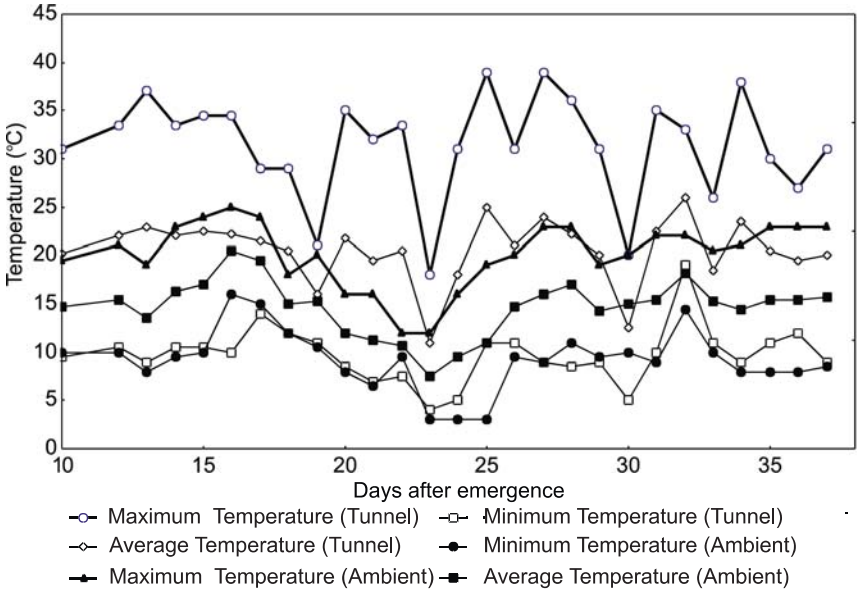

Fig. 1. Temperature variations under plastic tunnel and in open air.

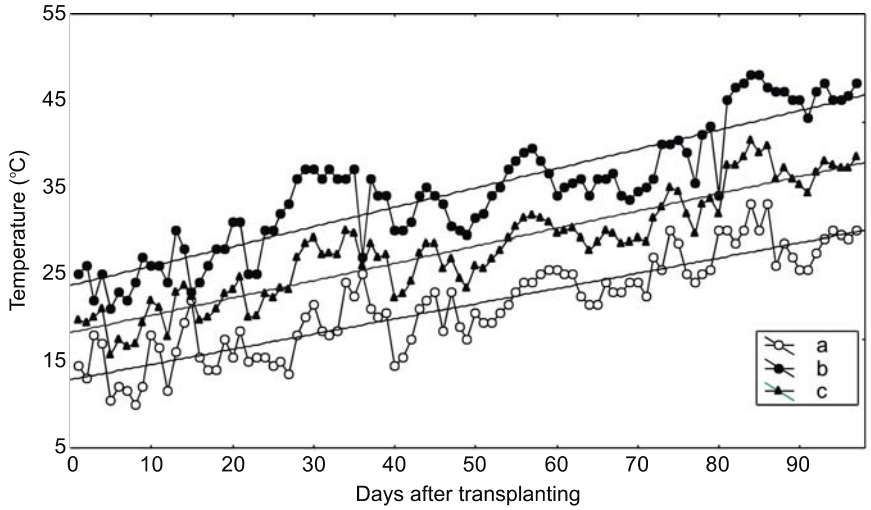

Fig. 2. Temperature variations in open air after transferring seedlings to the field
a= maximum. b= minimum. c= average

Growth habit: The growth habit of tomato cultivars can be determined by their continuous sympodium production (Rubatsky and Yamaguchi,1997). Tomato accessions were divided into three groups (Table 1). First group included cultivars with determinate growth habit, which in turn, by considering their main stem and axillary branches, they were divided to two subgroups. First subgroup had very short main stems; their main stem growth stops after appearance of 2nd to 4th flower cluster. Some of these cultivars were very early maturing than controls, and in some entries, axillary branches did not grow. These cultivars are suitable for mechanical harvest. They have small canopy, can be cultivated with high density, resulting higher productivity. Second subgroup of cultivars had determinate growth habit, but main stem had more growth, axillary branches grew and occupied more space in the field. This subgroup is not suitable for mechanical harvest, because they have low uniformity in maturation time. In these cultivars, some of axillary branches

Table 1. Classification of tomato cultivars according to their growth habit

Growth habit	Cultivars
First group	M18, M33, M34, M35, M36, M37, M38, M39, M40,M41, M42, M43, M46, M50, M52, M54, M57, M58, M60, M61, M62, M69, M70, M73, M74
Second group	M3, M4, M5, M6, M7, M23, M24, M25, M27, M30, M31, M32, M44, M47, M48, M49, M55, M56, M59, M63, M64, M65, M66, M72, M77, H1, H4, H7, M17, M19, Red Cloud, Early Urbana
Third group	M2,M8, M9, M22, M28, M20, M45, M67, M68, M78, H9, M10, M1, M12, M13, M14, M15, M21, M26, M29, M71, H2, H5, M16

have high fruit productivity. Cultivars of second and third groups have semi determinate and indeterminate growth habits. They are suitable for greenhouse cultivation.

Fruit weight and number: Studied ecotypes were classified according to their fruit weight. Fruits less than 40 g, were classified as very small, those between 40-69.9 g as small, fruits between 70-100 g as medium, and those that weighted more than 100 g as large fruits (Table 2). Tomato plant has two main yield components, number of fruits per plant and average fruit weight (Wein, 1997). The correlation between number of fruits per plant and average fruit weight is presented in Fig. 3. There was a negative correlation between number of fruits per plant and average fruit weight so that by increasing total number of fruits, average fruit weight was decreased. Correlation coefficient (R) was significant at 5% level in all cases (Ravinder and Cheema, 2004). Very wide range of genotypes was included in tested cultivars with different growth habits and fruit size which is presented in Tables 3 and 4 (Singh *et al.,* 2004).

In most cases, large fruits were obtained in first harvest of every cultivar, and smaller fruits in next harvests. But generally, number of fruits in first harvest was low although it was increased in next harvests. In the other word, there were negative and significant correlations between fruit number and average fruit weight, except for cultivars having average fruit weight less than10 g. By using Fig. 3, the yield potential of plants can be estimated.

These results confirm that many of tested cultivars have better potential to produce more fruit numbers, compared to standard cultivars.

Inflorescence: The morphology of tomato inflorescence is affected by genotype and also by environmental conditions (Seleciar, 1987). The division of main axis of inflorescence depends on plant genotype and environment. Usually, the first is produced from beneath the flower, axillary branch. Tomato inflorescences can be classified according to the number of flowering axes. Simple inflorescences have only one axis, therefore have limited number of flowers. Semi compound inflorescences have two or more axes. Sometimes, these inflorescences have more than 300 flowers. The studied cultivars in first year of experiment are presented according to this classification (Table 5).

Leaf shape: The size and shape of leaves were different in various tomato cultivars. Tomato plants generally have serrate leaves, but

Table 2. Classification of tomato cultivars according to their growth habits and fruit weight

Fruit weight	Growth habit		
	Determinate	Semi-determinate	Indeterminate
Large (>100 g)	H4, M32, M72, M47, Red Cloud, Early Urbana, H1, D4, F6, E5, H7	M22, M68,	M21, H2, H5
Medium (70-99 g)	M52, M56, H8, M63, M65, M42, M23, M3, M30, M73, M48, M64	M8, M9, D5, M11	
Small (40-69.9 g)	M36, M57, M25, M41, M37, M50, M7, M38, M14, M46, M5, M6, M18, M56, M17, M19 M36, M69, M62, M40, M34, M49, M13, M1	M2, M28, M67, M78, H9, M10, M14, M15, M12, M13	M26
Very small(<40 g)	M54, M58, M61, M39, M71, M74, M24, M35, M43, M31, M33, M27	M20	M71, M16, M29

Table 3. The minimum and maximum total fruit number per plant of various cultivars in different growth habit types and it's average in control cultivars

Harvest date after transplanting (days)	Growth habit						Control cultivar	
	Determinate		Semi- determinate		Indeterminate			
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Red Cloud	Early Urbana
117	0	10±2*	0	4±2	0	6±1	1±0	2±1
125	0	27±9	0	5±2	0	9±3	2±1	2±1
132	0	43±13	2±1	18±4	2±1	38±18	3±2	3±2
139	0	38±10	0	36±9	0	53±21	5±1	3±2
146	0	258±64	5±2	56±10	9±1	55±21	12±7	15±5
Total	6±3	332±80	11±6	76±40	17±5	163±35	23±8	26±11

* mean ± SD.

Table 4. The minimum and maximum fruit weight (g) per plant of various cultivars in different growth habit types and it's average in control cultivars

Harvest date after transplanting (days)	Growth habit						Control cultivar	
	Determinate		Semi- determinate		Indeterminate			
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Red Cloud	Early Urbana
117	2±1*	310±120	55±32	143±38	15±8	141±40	141±40	123±45
125	2±1	178±34	44±11	108±31	6±4	154±30	140±28	152±10
132	4±1	204±50	48±4	117±33	9±1	159±40	117±50	132±56
139	9±1	147±32	29±4	90±26	7±1	133±3	105±17	143±40
146	2±1	172±49	27±5	100±18	6±1	113±18	88±36	83±23
Total	3±1	146±35	21±6	168±40	6±3	128±33	102±20	103±25

* mean ± SD.

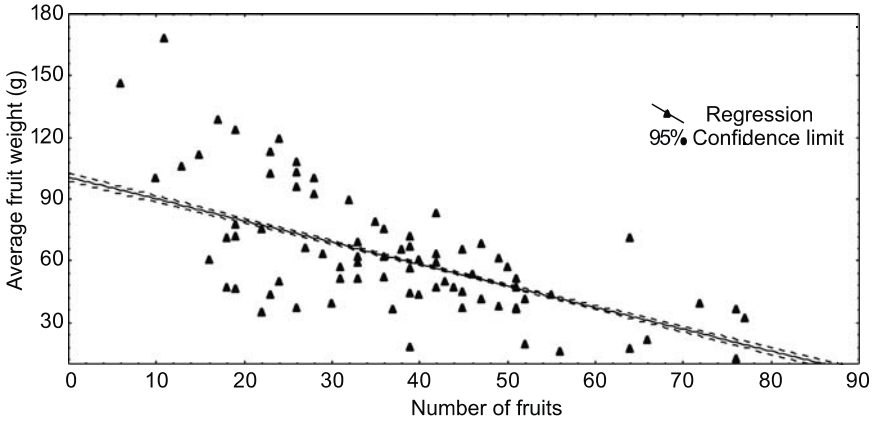

Fig. 3. Correlation between average fruit weight and number of fruits per plant

"*Grand Folium*" cultivars because of a recessive gene have leaves similar to potato plants. Tomato leaf was classified into 3 groups according to their shape as given below (Table 6).

Potato leaf shape: Leaf shape in this group was similar to potato leaves; sometimes, leaves are similar to pepper leaves.

Normal tomato leaves: In this group, leaves were similar to normal tomato leaves which are serrate types. Majority of studied cultivars were included in this group.

Carrot leaf shape: In this group leaves had maximum depth and they were similar to carrot leaves.

Fruit yield: The average fruit yield was different among studied entries, for each harvest and for total yield per plant (Table 7).

First harvest (117 days after transplanting): In early maturing cultivars, the first harvest had very wide range of fruit yield. The average yield per plant of some cultivars such as M9, M39, M74 and M73 were more than standard controls. Although some of these cultivars had good yield in this harvest, but their total yields were below controls. Maximum yield of first harvest was obtained from M39 which is an early maturing cultivar. Maximum number of fruits at first harvest per plant was related to M74 cultivar with average weight of one fruit as 42 g.

Second harvest (125 days after transplanting): In this harvest, like first harvest, cultivars had a wide range of yield with a 400 g difference between maximum and minimum yielding cultivars (Table 7). H1 cultivar did not differ significantly from Early Urbana (control). Some other cultivars including H5, H4, and M39 had fruit yield more than control. Comparing two control varieties, for second harvest, Early Urbana was earlier than Red Cloud, although they did not show any significant difference for total fruit yield per plant. Some of tested cultivars as compared with controls were earlier and had yielded more uniformly than controls. Some cultivars which had a good growing activity at the end of the season, were later maturing types compared with controls. M32, M31, M42, M23, M21, M22, H8, M55, M49 and M48 were included in this

Table 5. Classification of tomato cultivars according to their growth habit and inflorescence type

Inflorescence type	Growth habit		
	Determinate	Semi determinate	Indeterminate
Simple	H7, M1,M17, M18, M19, M23, M24, M25, M3, M30, M31, M32, M36, M37, M38, M4, M44,M45, M46, M47, M48, M49, M5, M52, M54, M67, M55, M59, M6, M60, M63, M64, M65, M66, M67, M68, M69, M7, M70, M72, M73, M74, M77, M78, Red Cluod, Early Urbana	M10, M1, M13, M14, M15, M2, M8, M28, M20, M45, M67, M68, M78	H2, H5, M21, M26, M29, M71
Semi-composite	H4, M33, M34, M35, M57, M58, M61, M62		
Composite	H1,M27, M39, M40, M41, M42, M43, M50, M56		M16

Table 6. Classification of tomato cultivars according to their growth habits and leaf shape

Leaf shape	Growth habit		
	Determinate	Semi-determinate	Indeterminate
Potato type	H1, M18, M78, M46, M57, M60, M62 M68, M65	M68, M78	
Common type	H4, H7, M17, M19, M23, M24, M25, M27, M3, M30, M31, M32, M35, M36, M37, M38, M39, M4, M40, M41, M42, M47. M48, M49, M5, M50, M52, M55, M56, M58, M59, M6, M61, M63, M7, M70, M71, M72, M73, M74, M76, Red Cloud, Early Urbana	H9, M11, M2, M8, M22, M28, M45, M67, M10, M1, M13, M14, M15	H2, H5, M16, M21, M26, M29, M71
Carrot type	M33, M43, M45,M44, M64		

Table 7. Total fruit weight (g) per plant of various cultivars in different growth habit types and it's average in control cultivars

Harvest date after transplanting (days)	Growth habit						Control cultivar	
	Determinate		Semi- determinate		Indeterminate			
	Minimum	Maxium	Minimum	Maxium	Minimum	Maxium	Red Cloud	Early Urbana
117	0	411±71*	0	386±200	0	141±30	152±40	262±80
125	0	417±130	0	316±103	0	385±146	350±44	363±220
132	0	1114±374	194±68	1205±380	75±42	533±150	340±180	359±150
139	80±40	2754±700	0	1046±250	0	901±560	508±120	430±150
146	0	2415±700	319±200	2250±650	585±255	1443±230	1009±700	1257±711
total	706±80	4535±800	1724±120	2911±655	871±194	2985±300	2350±800	2671±825

*- Plus and minus values are mean ± SD.

group. Maximum numbers of mature fruits were obtained from M33 and M27.

Third harvest (132 days after transplanting): Majority of cultivars could produce some fruits in this harvest. Fruits of early, some moderate, and late maturing cultivars were harvested for third time. The early maturing cultivars (such as M39) had lower yield in this harvest as compared with previous harvests. Late maturing cultivars (such as M48) when compared with fourth harvest had very low yield; but some of intermediate maturing cultivars (such as M73) had relatively good yield. In this stage both control varieties could produce some fruit. The yields of early maturing cultivars were reduced at this stage. Many cultivars had more yield per plant in this harvest, as compared with controls. These cultivars were M65, M29, H2, M30, M47, M66, M34, M54, M40, M12, M1, M37, M42, M14, M48, M49, M53, H5, M19, M8, M15, M32, M73, M51, M6, M52, M25 and M69.

Fourth harvest (139 days after transplanting): The amount of fruit yield in this stage was very variable between cultivars. Some cultivars that had relatively good yield in first harvest had minimum yield in this harvest. Cultivars such as M48 that produced reasonable fruit yield in third harvest, produced even more fruits in this stage. Maximum yield in this harvest was obtained from this cultivar. In this harvest, many cultivars produced more yields than controls. These cultivars were M49, M53, H5, H8, M55, M8, M35, M7, M78, M5, H4 and M41. The maximum yield in this stage was produced by cultivar M16.

Fifth harvest (146 days after transplanting): At this stage, all of red and green fruits from each plant were harvested at the same time. Maximum fruit yield was obtained from M66, which is a late maturing cultivar. Early maturing cultivars had minimum yield. Medium and late maturating cultivars had good production; and it seems that under suitable environmental conditions, one or two additional harvests from late maturing cultivars could be obtained. In this harvest, several cultivars produced more fruit yield than controls. They included M10, M22, H8, M5, M18, H19, M1, H7, M48, M52, M2, M9 and M3. Average yield per plant in Red Cloud was lower than Early Urbana, and in ranking entries, a number of cultivars were placed between them. Although, statistically there was not any significant differences between these controls.

Total yield: The amount of total yield in many cultivars, M48, M66, M49, M63, M8, M16, H5, H8, M65, H1, M22, M19, M15 and M73 was more than standard controls. Total yield of some cultivars such as M25, M3, M17, M9, H9, M54, M64, H7, M5, M47, M3, M78 and M7 were lower then Early Urbana and more than Red Cloud. The differences between yield per plant were very significant. Maximum fruit yield per plant was 4.53 kg and minimum 0.70 kg. The average fruit yield per plant for Early Urbana and Red Cloud were 2.67 and 2.35 kg, respectively. In cold area, tomato axillary branches are pruned in order to reserve more nutrition for fruits. But in warmer regions, in order to prevent fruits from sun radiations, these branches are not pruned. Usually, axillary's branches do not produce fruits or they have very limited fruit yield, in some of studied cultivars, axillary branches produced more yields than standard controls. This leads to increase the total yield per plant. This is a good sign of the presence of a high tolerance in some cultivars. However, if these fruits are not prevented from high solar radiation, the probability of sunburn will be increased.

Classification of cultivars: In order to have better evaluation and comparison between tested cultivars, they were classified by Euclidian method. As it can be seen it Fig. 4, minimum distance was between M3 and M64 entries. Maximum distance was between cultivars with high fruit numbers but with less weight *i.e.* cherry types and some other types. So that these distances between

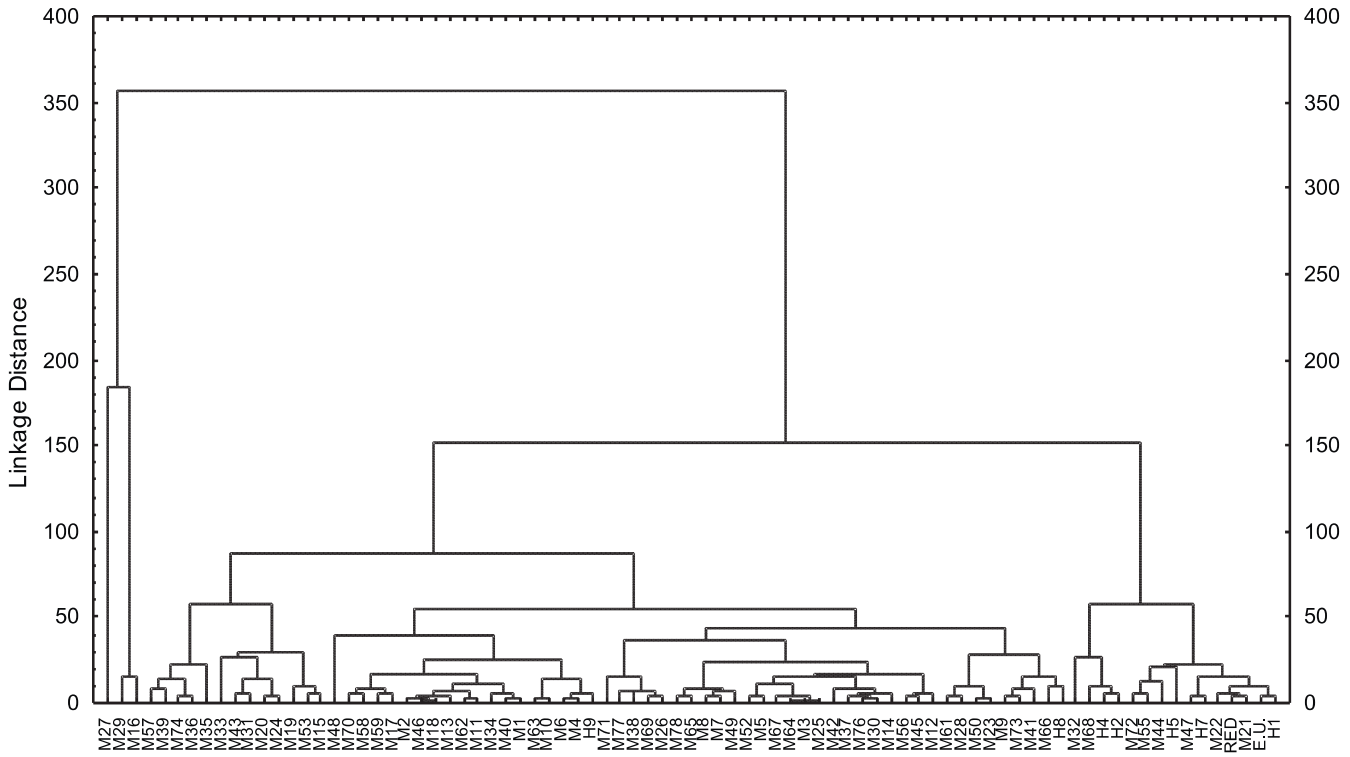

Fig. 4. Classification of tomato cultivars based on their growth habit, inflorescence type, leaf shape, number of fruits, and average fruit weight using Euclidian method

M27 and controls were 324 and 325 Euclidean units. For better understanding of this classification, 83 cultivars were divided into 7 groups. Minimum equal distance for group formation was 55 Euclidean distances (Nafar, 1985).

First group: This group had only M27 cultivar, which had semi determinate growth type with an average fruit weight of 2.6 g and 332 fruits per plant. So, it had maximum fruit number with minimum fruit weight.

Second group: In this group, only 2 cultivars, *i.e.* M29 and M16 were included. These were similar to previous group, with a difference that fruit weight increased but fruit numbers per plant decreased. The average fruit weight in this group was 7.85 g, with an average of 155.5 fruits per plant.

Third group: This group included M35, M63, M74, M39 and M57 which had super determinate type. Their average fruit weight was 32.74 g, and fruit numbers was 31 fruits per plant.

Fourth group: Cultivars, M15, M53, M19, M24, M20,M31, M43, M33, M58, M70, M48, M4, M6,M10, M63, M1, M40, M43, M11, M62, M13, M18, M46, M2, M17, M59, M64, M67, H9, M5, M52, M49, M7, M8, M65, M78, M26, M69, M38, M77, M71, M12, M45, M56, M14, M30, M76, M37, M42, M25 and M3 were included in this group. These cultivars had different growth habits but they had similar inflorescences. Their fruit weight was 49 g and they had 48.9 fruits per plant in average.

Fifth group: This group included H8, M66, M41, M73, M9, M23, M50, M28 and M61 cultivars. Average of their fruit weight was 75.6 g and they had 28.6 fruits per plant.

Sixth group: In this group, cultivars H2, H4, M68 and M32 were included. Average of their fruit weight was 131.7 g and they had 13.2 fruits per plant.

Seventh group: This group included M22, H7, M47, H5, M44, M55, M72, Red Cloud and Early Urbana cultivars. Their average fruit weight was 102.73 g and they had 22.1 fruits per plant in average.

Discussion

In this classification, more cultivars were included in the middle classes. By getting nearer to the end classes, the number of cultivars reduced, so that, the first class had only one cultivar and the last class had only 9 cultivars. The fruit weight of first class was low, the middle class had medium fruit weight and the last class had heavy fruit weight, respectively. But cultivars that placed in middle classes had higher yields, large fruits, and lower fruit numbers. This classification helps us to better recognition of distances between cultivars. If it is needed to select desirable cultivars with good characters, cultivars that are nearer to controls should be selected for quality improvement. Cultivars with large Euclidean distances can be used in breeding programs to make great changes in various characterizations of fruits, leaves, growth habit, inflorescence, and their adaptation to various conditions (Nuez *et al.*, 2004)

Different environmental stresses cause reduction in crop yield, so that in most cases, the plants under field stress can express only 10-20 percent of their real yielding potentials (Ghorbani and Ladan, 2005).

The first step to confront against temperature stress is using tolerant varieties which have been adapted to different climatic conditions (Giordano, 2005; Matsubara and Tanaka, 2005). Generally, tomato varieties from cold climates (which show relatively, good tolerance to low temperatures) are early maturing varieties (Ansari *et al.*, 2003). In south areas of Iran, like similar climates of the world, for spring cultivation, usually plastic tunnels for covering plants, cultivation in sandy soil, and greenhouse cultivation are used (Kurklu and Bilgin, 2002; Salokhe, 2005). But some times unexpected cold weather after removing plastic tunnels can cause real damages in plants which have been grown under tunnels. This results a severe reduction in fruit yield. Furthermore, because in these areas by starting April, the weather temperature increases significantly which causes in reduction of pollen grain population and bud retention, and finally severe reduction in fruit yield (Sato and Peet, 2005). Also, the leaves will get burned and carbohydrate production will be diminished. The observed differences between varieties in reaction to this stress because of their susceptibility levels to rapid variations of temperature (Sato and Peet, 2005).

There are great deals of variations between different tomato cultivars, especially those which grow in northern latitudes. These variations are morphological characters such as leaf and fruit shape, inflorescence type, fruit size, stem and leaf length, and fruit yield. Variations in physiological characters include maturity time, tolerance to temperature variation and fertility rates in hot conditions. By using this genetic variability, better tomato varieties can be developed.

In general, it can be said that some of tested cultivars have good characters, such as adaptability to environmental conditions, tolerance to a wide range of temperature (especially in reproductive stage), early maturity, and maturation uniformity of yield. But for some other suitable characters such as disease resistance, salinity tolerance and good storage capability, they are to be evaluated for additional characterstics. Thus, in the first stage, it is better to develop pure lines (Nautiyal, 2005), then to make crosses between cultivars that have different suitable characters to produce suitable cultivars for each region. Therefore, in this stage of breeding program, entries M48, M21, M55, M66, M8, M40, and H1 were selected, then, in order to produce next generation, seeds were collected from plants of these selected lines which produced maximum yield.

The study revealed that several evaluated cultivars had good tolerance to high temperatures, especially in fertilization and fruit formation stages. Some studied cultivars had a wide range of yielding potentials. In most cases, with increase in fruit numbers of cultivars, their average fruit weights were reduced. Classification of cultivars indicated that some cultivars with good fruit productivity are near standard controls by means of Euclidean distance method. Therefore, considering the measured characters, it is possible to select cultivars for hybridization and producing new lines and hybrids. In the present study, entries M48 and M64 showed a good tolerance to temperature changes in Ahwaz area and a desirable fruit yield, even more than other varieties. Therefore, these can replace local standard varieties.

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Effect of hot-water and cold treatments on reducing contamination in almond tissue culture

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Abstract

In this study, hot-water and cold treatments were used for eradication of explant contamination, and also the effect of plant growth regulators on shoot proliferation was evaluated. The explants were nodal segments of a late flowering almond cultivar 'Sharood 7'. Experiments were carried out in a complete randomized design with 25 replications. All hot-water treatments eliminated fungal contamination. The best hot-water treatment was 50°C in which 88% of explants were both free of contamination and necrosis followed by 76% at 47.5°C and 56% at 45°C. The best proliferation rate obtained in 1.5 mg L^{-1} BA in combination with 0.1 mg L^{-1} IBA (5.25 shoots per explant) which was significantly higher than 1 mg L^{-1} (2.65 shoots per explant). Cold treatments only (2 and 4 days in 4°C) delayed fungal contaminations for 7 days, so it was impossible to assess bacterial contamination.

Key words: Benzyladenin (BA), late-flowering, nodal segments, proliferation, hot water

Introduction

In plant tissue culture, contamination (bacterial and fungal) is a major problem for both commercial and research laboratories (Cassells, 2001; Leifert and Waites, 1992). Plants that appear healthy may contain bacteria (Debergh and Vanderchaeghe, 1998; Legaatt *et al.,* 1988). These latent bacteria results in variable growth, reduced shoot proliferation, reduced rooting, tissue necrosis and finally cause death of tissue (Leifert *et al.,* 1989; Leifert and Waites, 1992). Using various combination of sterilants such as ethanol, hypochlorites, mercuric chloride, benolate and fungicides did not eliminate all contaminants (Kowalski and Staden, 1998). According to some studies, hot-water treatment (HWT) and cold treatment (CT) are very efficient in reducing initial contamination (Hol and Van Der Linde, 1992; Kowalski and Staden, 1998). In agricultural practices, HWT were used on a large scale with bulbs, tubers and seeds (Grondeau and Samson, 1994) and with grapevine cutting (Waite and Morton, 2007). The results from our preliminary work showed that explants contaminations were not completely controlled using sodium hypochlorite alone and with combination of benlate. In addition, mercuric chloride in concentration of 200 mg L^{-1} removed fungal contamination but bacterial contamination appeared in second subculture after 8 weeks.

It has been indicated that the use of hot-water treatment in tissue culture is very efficient in reducing initial contamination of both bulbs (*Lilium*) and axillary buds of trees (*Acer*) (Langens-Gerrits *et al.,* 1998). Cold treatment of surface sterilized explant material of hardwood trees at 4°C for 2 or 4 days reduced or eradicated internal contamination (Kowalski and Staden, 1998).

Seasonal testing of plant material showed that contaminations were very low during winter months when night temperatures were around freezing (Kowalski and Staden, 1998). In the regions of cultivated almond in Fars province in Iran, late spring frost kills a lot of flower buds and even new branches each year. Chilling injury can be avoided using late flowering cultivars, so these cultivars are in more demand. The aim of this research was decontamination of nodal segments of a late flowering local almond cultivar 'Sharood 7' for rapid clonal micropropagation.

Materials and methods

Three years old grafted almond (*Prunus dulcis* L.) trees cultivar 'Sharood 7' were grown in greenhouse condition. Nodal segments of 1.5-2.5 cm length from fresh shoots were used as explant. The collected nodal segments were washed in a commercial detergent (Rica) for 30 min, and then they were dipped in 70 % (v/v) ethanol, afterwards treated with 10% (v/v) Golrang solution, a house bleach (contain 5.25% sodium hypochlorite) and then rinsed three times with sterilized distilled water. A series of explants were maintained at 4°C for 0, 2 and 4 days. Another series was given hot-water treatments of 40, 42.5, 45, 47.5, 50 and 52.5°C in Ben-Mary for one hour. Treated explants were cultured on Murashige and Skoog (1962) medium supplemented with 3% sucrose (MERCK, LGaA64271 Darm stadt, Germany) and solidified by 0.8% agar (MERCK, LGaA64271 Darm stadt, Germany).

After 8 weeks, the explants were transferred to MS medium supplemented with different concentrations of BA (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3 mg L^{-1}) and IBA (0.01 and 0.1 mg L^{-1}). The pH of all media were adjusted to 5.7 prior to autoclaving at 1.2 atm. pressure, 121°C for 15 min. All cultures were maintained at 25±2°C with a 16 h photoperiod of 35-40 µmol m^{-1} s^{-1} provided by cool white fluorescent lamps.

Each experiment was carried out as completely randomized design with 25 replications. Data were analyzed using SPSS (SPSS, Inc. Chicago.USA) and the means were compared using Tukey's test at P=0.05.

Results and discussion

Cold treatments (2 and 4 days at 4°C) delayed fungal contaminations for 7 days, thereafter fungi started to grow and covered the cultured vessels in few days, so it was not possible to evaluate bacterial contamination. In contrast, all hot-water treatments completely eliminated fungal contaminations. The results of hot-water treatments on bacterial contaminations, after 8 weeks of culture showed that with increasing water temperature from 40 to 52.5°C bacterial contamination rate decreased from 100 to 0%, respectively (Table 1).

Similar results were reported by Langens-Gerrits *et al.* (1998) on eradication of fungal and bacterial contamination in *in vitro* culture of *Lilium* bulb and *Acer* stem segments. Hot-water had a damaging effect on explant viability (Baker, 1962), so that, there was no explant alive after treating in 52.5°C hot-water. The best hot-water treatment was 50°C in which 88% of explants were both free of contamination and necrosis followed by 76% at 47.5°C and 56% at 45°C. During hot water treatment the explants were under partial anaerobic condition because of submerging which lead to production of acetaldehyde and ethanol (George,1993). In *Prunus* accumulation of acetaldehyde and ethanol was followed by rapid deterioration (Righetti *et al.*, 1990). In *Malus* tissue culture, ethanol inhibited shoot proliferation and rooting (De Klerk *et al.,* 1997). After 8 weeks of explants establishments, they were subcultured on MS medium with different plant growth

Table 1. Effects of hot-water treatments on bacterial contamination of almond explants of cultivar 'Sharood 7'

Water temperature (°C)	Contaminated explants (%)	Decontaminated explants (%)	Explant survival (%)
40.0	100a†	0f	0f
42.5	96b	4e	4d
45.0	36c	64d	56c
47.5	24d	76c	76b
50.0	8e	92b	88a
52.5	0f	100a	0f

†Means in each column with similar letter(s) are not significantly different at P=0.05 using Tukey's test.

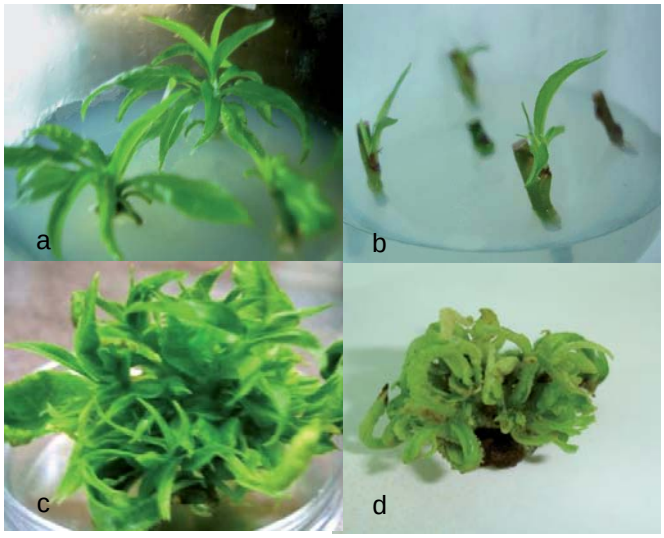

Fig. 1. Shoot proliferation in almond cultivar, ' Sharood 7'. a. bacterial contamination on the explants bases. b. contamination free explant and also necrosis explant c. shoot proliferation in 1.5 mg L^{-1} BA. d. shoot deformation in 2.5 mg L^{-1} BA.

Table 2. Effect of plant growth regulators on shoot proliferation and shoot length in second subculture

Plant growth regulators		Average shoot number per explant	Average shoot length per explant (mm)
BA (mg L^{-1})	IBA (mg L^{-1})		
1	0.01	2.65c†	4.87b†
	0.10	3.48bc	5.21ab
1.5	0.01	4.63ab	6.47a
	0.10	5.25a	5.73ab
2	0.01	4.61ab	5.45ab
	0.10	3.40bc	4.74b
2.5	0.01	4.19ab	4.32b
	0.10	3.83bc	4.46b

†Means in each column with similar letter(s) are not significantly different at 5% level of probability using Tukey's test.

regulators. In this culture period (5 weeks), the explants remained free of contamination. The best proliferation rate obtained in 1.5 mg L^{-1} BA in combination with 0.1 mg L^{-1} IBA (5.25 shoots per explant) which was significantly higher than 1 (2.65 shoots per explant) and 2.5 mg L^{-1} BA (3.83 shoots per explant) (Table 2).

In 2.5 mg L^{-1} BA, the shoots showed deformation with yellow colour (Fig. 1). This may be due to competition among shoots in absorption of growth regulator and nutrient from the medium (Ali *et al.,* 2003).

Hot water (50°C) treatment was very effective in reducing exogenous and endogenous contaminations of almond cultivar 'Sharood 7'. This technique provides a cheap, rapid, non-toxic and efficient method for removing bacterial and fungal contaminations.

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Health evaluation of cactus collection in botanical garden at Cluj-Napoca, Romania

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Abstract

In terms of artificial collections, cacti receive a specific microclimate, which ensures constant physical parameters leading to a low resistance and high susceptibility to attack by pests and diseases. The *Cactaceae* collection of the botanical garden "Alexandru Borza", Cluj-Napoca, Romania counts more than 4100 plants belonging to 115 genera. Following inventory collection, 4069 plants were studied. Preliminary assessment results that the radicular system of the best represented *Cactaceae* species is much worse than those stems. Genus: *Astrophytum, Aylostera, Echinocerus, Notocactus*, *Weingartia* had disease insidence of grade 2 (the area affected by 26-50%). The highest intensity of the attack was reported in the genus *Echinocereus* (47.24). In calculating the attack degree there was a greater uniformity in genus *Aylostera* (36.34), *Echinocereus* (37.46), *Rebutia* (37.83); *Weingartia* (33.37). In considerable stem attack by pahthogens, the highest attack frequencies were recorded in *Astrophytum* (51.75); *Ferocactus* (65.76) and *Notocactus* (58.18). The attack intensity, expressed in intensity degrees, reached value 2 (30.79) in the case of *Cleistocactus* genus, whereas the other genera remained under grade 1.

Key words: Cacti, *Cactaceae,* diseases, attack degree, attack intensity

Introduction

In Romania, in the patrimony of the 11 botanical gardens and in over 100 private collections, cacti represent a very important balance both numerically and qualitatively. The study of *Cactaceae* diseases in the botanical collections is very important because the specific growing conditions are totally different from natural habitats of these species. In greenhouse conditions, taxa agglomeration in small areas is a very important factor in triggering pest holes. Special conditions of temperature, humidity, light, growth substrate, often limited, are a part of these factors which differ from the optimal environment demand of *Cactaceae*.

Many authors (Miller, 1980; Rosciglione, 1980; Anson, 1982l; Chase 1982; Arnold, 1986; Nakhutsrishvili, 1986; Simay, 1987; Turner, 1992; Glang, 1993; Chavez, 1998; Anderson, 1999; Kobayashi, 2000; Swart and Kriel, 2002) have described multitude of cacti diseases. Feszt *et al*. (2006) has published diamensions of the viruses extracted from *Cactaceae* vegetative parts multiplied from Cluj-Napoca Botanical Garden collection.

Materials and methods

The *Cactaceae* collection used for the study is located in one of the side greenhouses of the greenhouse complex. In compiling the cactus collection, it was considered that the family should be represented by a range as high as found in nature. The *Cactaceae* collection from Cluj-Napoca botanical garden counts more than 4100 plants belonging to 115 genera, from the 241 total known, after the Backeberg system (Backeberg, 1962, 1966, 1968). From inventory collection, 4069 plants were checked of which 116 were dry. The remaining number of 3953 belong to 94 genera. The plants which at the moment of the inventory (2004) were younger than 3 years as well as some epiphyte genera as *Lepismium, Rhipsalis, Rhipsalidopsis, Zygocactus, Wittia*, which require subtropical climate conditions, being in other greenhouses than the ones reserved for xerophytes plants were not taken into consideration.

The inventory was made while transplanting the cacti. Transplantation was performed over a period of four months, from 04.11.2004 to 04.03.2005. Each plant has been reviewed separately, and data were recorded which include: Nr. - Current number; Gender, Species - gender, species, Prov. - provenience (from the individual seed or cuttings domestic or international exchange); Place - place of origin of plant material (+ or sowing or seedling year) Vig. - Vigor; Ch. - Changes in color; Ro. - Root (root integrity expressed in percentages); Stem. - Stem (stem integrity expressed in percentages) Sub. - Suber (grade suberizing percentage); Con. - Concave (morphological changes resulting from attack); Vex. - Convex (morphological changes resulting from attack); Cir. - Circular (morphological changes resulting from attack), Irr. - Irregular (morphological changes resulting from attack), Col. - Color (morphological changes resulting from attack), Vir. -Virosis; Myco. - Mycoplasma; Bac. - Bacterise ; Myc. - Mycosis; Mit. - Mites; Pad. – Lice; Het. - Heterodera; Com. - Comments.

Follow up observations were made to identify the suspected plants through further studies in laboratory. This way there were selected plants which: presented inlay symptoms; import plants of unknown origin; cacti which deviate by shape, color or appearance from normal: nonspecific discoloration, witches broom forms, fascia, crystal shapes, monstrous forms, spiral increases, flower deformations, *etc*.

The process of occurrence of diseases in plants included two main

points: the attack and the attack effect. Attack value is represented by frequency, intensity and degree of attack. Frequency attack (F) is the relative value of the number of plants (n) or members of plant attacked by a phytopathogen agent (virus, bacteria, fungus) reported to the number of plants or members. Data is calculated by the relationship: F= n x 100 / N.

Attack intensity (I) is the degree of coverage or attack expansion, reporting the attacked area with regard to the total area. To indicate the intensity attack scale ranks are used that can have a different number of ranking frames. In most cases, attack intensity is scale as 4, 5 or 6 frames. These frames correspond to certain percentage intervals of the intensity of the attack. The relative expression of attack intensity (I) is the relationship:

$$I = \sum (i \times F)/N,$$

where, i = grade or attacked area (%), F = number of cases with attack for every grade, N = total number of cases with attack.

Attack degree (AD) is expression of the attack's extension over the total number of plants observed. The value expression for this is given by the relationship: AD = F x I/100.

In order to compare the *Cactaceaes* health from the Botanical Garden with the situation from nature, respectively from the *Cactaceaes* place of origin, the notation scale of intensity attack indicated by James (1971) quoted by De la Torre (1987 (a), 1987 (b), 1988 , 2001) was used (Table 1).

Table 1. Attack intensity notation scale for *Cactaceae* diseases (De la Torre, 1988)

Affected surface (%)	Attack intensity degree
Healthy plant	0
1-25	1
26-50	2
51-75	3
76-100	4

The health of Mexican indigenous cacti plants, is affected with attack intensity degree like 2, 3 even 4 (de la Torre, 1988). In the collection of Botanical Garden Cluj-Napoca, Romania, the degree of attack intensity is not higher than 2. In the data processing the integrity of the radicular system and, separately, the integrity of the plant stem for a certain genus was pursued.

Results and discussion

Radicular system health: Regarding the radicular system, the smallest values of the attack degree were observed in *Cereus* (0.03); *Mammillaria* (4.9); *Cleistocactus* (4.8); *Eriocactus* (2.27); *Lobivia* (7.04); *Pilosocereus* (0.02). The minimal values of the attack degree for *Cereus* and *Pilosocereus* were due to a peculiar resistance to attacks provoked by pathogens agents of the radicular system. The species of the *Cereus* are probably preferred as stocks for genera with less sensitive radicular system (Table 2).

After the selection of plants through the inventory method and examination of health of every genus, sick plants were isolated and kept in an humid environment. After the mycelium burst, advent, emergence and eventual fructifications the material was prepared through standard procedures in order to be subjected for verification through light or electronic microscopy. Through these methods the following diseases were identified of the radicular system: dry rot or fusarium fading caused by many traits of *F. oxysporum* with a different pathology; root blight *P. parasitica*, or *Cactacee* stem's base caused by *P. cactorum*; another disease caused by *Rhizoctonia solani*; *Verticilium albo-atrum*.

In *Cactaceae* collection of the botanical garden, at the radicular system level, diseases caused by bacteriosys or mycoses as *Armillaria mellea, Sclerotinia sclerotiorum, Thielaviopsis basicola* were not prevalant as these diseases are generally encountered in apartment plants.

In the Botanical Garden collection, sick plants were infected by many species of *Fusarium*. Some were very aggressive, causing the plants' death manifested by a red-brown colour, sometimes aqueous discolouration. Other *Fusarium* strains had effects on integral cultivars, or on some portions from a culture, generally managing to cause mother plant's rot with remaining young portion alive. The most devastating effect was represented by *F. oxysporum* which caused root loss and rot progression on the central cylinder, to the top growth. If noticed in time, the rotten portion can be eliminated together with a part of healthy tissue. The resulted plant part can be rooted.

A non aggressive form of *Fusarium* causes slow tracheomicoza, where the plant can survive a couple of years, without normal growth. Normally, these plants can be saved if the sick portion is removed and the plants manage to root. Many times secondary compounds of fusarioze inhibit the synthesis of the hormones that results into inhibition of rooting. This way hormonal balance of the attacked plant is modified, which leads to inhibition of further rooting. These cases were observed in cacti that lived in latent period many years without having root. At globular cacti, normally when the rot of the radicular system is noticed, and the infection is generalized over parcel and the plant survives.

Fusarium can be present alone or through over infection by other pathogen agents, as *Pythium, Verticillium, Sclerotinia* can appear. Dependent on the association of pathogen organisms, which determine the destruction of the host plant tissues, this can colour differently, having as well a consistent difference from the normal. *Fusariozes* symptoms are described in literature. Attacked tissues rot from root to parcel towards the stem. The typical color of brown rot caused by *Fusarium* may acquire red, purple nuances, blackish with a characteristic mycelium smell.

Table 2. Evaluation of the attack caused by phytopathogen agents on the *Cactaceae* radicular system, Cluj-Napoca Botanical Garden

Genus	Number of studied taxa	Frequency of attack (F)	Intensity of attack (I)	Attack intensity degree	Attack degree
Astrophytum	114	81.57	32.80	2	26.75
Aylostera	119	92.43	39.32	2	36.34
Cereus	156	2.56	1.41	1	0.03
Echinocereus	116	79.31	47.24	2	37.46
Ferocactus	111	61.26	22.97	1	14.07
Gymnocalycium	310	59.03	22.90	1	13.51
Mammillaria	894	37.91	12.95	1	4.90
Notocactus	232	81.89	36.03	2	29.50
Parodia	213	57.74	23.61	1	13.63
Rebutia	252	98.80	38.29	2	37.83
Weingartia	100	94.00	35.50	2	33.37
Cleistocactus	63	28.57	16.82	1	4.80
Eriocactus	75	21.33	10.66	1	2.27
Lobivia	80	40.00	17.62	1	7.04
Pilosocereus	49	2.04	1.42	1	0.02

Table 3. Evaluation of the attack caused by phytopathogen agents on *Cactaceae* stem, Cluj-Napoca Botanical Garden

Genus	Number of studied taxa	Frequency of attack (F)	Intensity of attack (I)	Attack intensity degree	Attack degree
Astrophytum	114	51.75	10.87	1	5.62
Aylostera	119	16.80	4.36	1	0.73
Cereus	156	24.35	3.97	1	0.96
Echinocereus	117	19.82	5.51	1	1.09
Ferocactus	111	65.76	13.24	1	8.70
Gymnocalycium	310	18.70	5.16	1	0.96
Mammillaria	894	18.79	5.22	1	0.98
Notocactus	232	58.18	15.17	1	8.82
Parodia	213	24.41	6.57	1	1.60
Rebutia	252	1.58	0.23	1	0.0036
Weingartia	100	30.00	6.50	1	1.95
Cleistocactus	63	61.90	30.79	2	19.05
Eriocactus	75	42.66	8.26	1	3.52
Lobivia	80	55.00	9.75	1	5.36
Pilosocereus	49	2.85	20.00	1	0.57

This smell is perceived in heavily infected collections, usually when spraying. Unpleasant odour and an aqueous consistence is usually a sign of infection with bacteria and torulae.

An over infected fusarioza by *Verticilium alboatrum* gives a typical yellow tint. *Fusarium* in association with *Pythium* causes humid rot, which manifests through interior tissues liquefaction of the cactus and content drain in the soil. The epidermis of the cactus remains with a prick skeleton which normally falls laterally.

Stem health: In evaluation of the attack produced by phytopathogens on stem the highest attack frequencies were recorded in *Astrophytum* (51.75); *Ferocactus* (65.76); *Notocactus* (58.18).

Intensity attack expressed in intensity degrees reached to 2 value (30.79) in the case of *Cleistocactus*, the other genera remaining below the intensity of 1 degree. The highest values of *Astrophytum* and *Ferocactus* are owned to natural phenomena of suberizing of parcel as well as to anthracnose which trig and worsens suberizing. Some species of *Notocactus* have a greater suberizing capacity stem which has protection role and is of non pathogen origin. With regard to attack on stem, the frequencies were higher in *Astrophytum* (51.75); *Ferocactus* (65.76) *Notocactus* (58.18); *Cleistocactus* (61.9); *Eriocactus* (42.66); *Lobivia* (55). These high values are not exponential followed by high values of intensity and attack degree (Table 3).

The only exception was *Cleistocactus* genus, because of the multiple dry portions of branches which makes the stem to show high intensity and attack degree values. The grading scale undertaken after James (1971) quoted in De la Torre (1988) reached 2 value, while in the case of the other 14 genera it remained at the 1 value. In case of *Cleistocactus,* the causes of peak growth and branches which form the stem are of unknown origin. Causes can be of pathogen or non-pathogen origin. The non-pathogen origin is known as the physiological phenomenon, through which a plant guides reserve substances from the stems extremities to some favoured areas. Such phenomena are frequent, especially at the plants winter reponses when plants being in latent state temperature conditions are very high.

In such conditions, in case of long drought, plants have the capacity to auto eliminate certain portions through guiding reserve substances. In case of soil moisture increase, the shoot stem forms new sprouts near the mortified tissue. The pathogen origin of dried peak growth is normally owned to *Helminthosporium cactivorum.* This mycosis was not signaled in Cluj-Napoca Botanical Garden in the *Cactacae* collection.

In the assessment study of the attack with pathogen agents on stem, the lowest values regarding frequency were recorded in *Rebutia* (1.58) and *Pilosocereus* (2.85) . In *Pilosocereus* intensity was higher (20) (compared with low values of frequency and attack degree). *Rebutia* proved to be the most resistant to pathogen agents attack with 1.58 frequency, 0.23 intensity and 0.0036 attack degree. Attack degree having values below zero were scored in *Aylostera* (0.73); *Cereus* (0.96); *Gymnocalycium* (0.96); *Mammillaria* (0.98); *Rebutia* (0.0036); *Pilosocereus* (0.57). Higher values in evaluating attack intensity are owned to singular cases of antracnoze of *Astrophytum* and *Ferocactus*. In *Notocactus,* stem suberizing was of non pathogen nature. In *Pilosocereus*, high intensity was due to problems caused by

Table 4. Evaluation of the attack caused by phytopathogen agents on *Cactaceae*, Cluj-Napoca Botanical Garden

Genus	Number of studied taxa	Frequency of attack (F)		Intensity of attack (I)				Attack degree	
		Root	Stem	Root	Intensity grade	Stem	Intensity grade	Root	Stem
Astrophytum	114	81.57	51.75	32.80	2	10.87	1	26.75	5.62
Aylostera	119	92.43	16.80	39.32	2	4.36	1	36.34	0.73
Cereus	156	2.56	24.35	1.41	1	3.97	1	0.03	0.96
Echinocereus	116	79.31	19.82	47.24	2	5.51	1	37.46	1.09
Ferocactus	111	61.26	65.76	22.97	1	13.24	1	14.07	8.70
Gymnocalycium	310	59.03	18.70	22.90	1	5.16	1	13.51	0.96
Mammillaria	894	37.91	18.79	12.95	1	5.22	1	4.90	0.98
Notocactus	232	81.89	58.18	36.03	2	15.17	1	29.50	8.82
Parodia	213	57.74	24.41	23.61	1	6.57	1	13.63	1.60
Rebutia	252	98.80	1.58	38.29	2	0.23	1	37.83	0.0036
Weingartia	100	94.00	30.00	35.50	2	6.50	1	33.37	1.95
Cleistocactus	63	28.57	61.90	16.82	1	30.79	2	4.80	19.05
Eriocactus	75	21.33	42.66	10.66	1	8.26	1	2.27	3.52
Lobivia	80	40.00	55.00	17.62	1	9.75	1	7.04	5.36
Pilosocereus	49	2.04	2.85	1.42	1	20.00	1	0.02	0.57

singular cases of parcel infection and inferior stem portions with *Phomopsis sp.*

Preliminary assessment of the *Cactaceae* collection health results indicate that the radicular system of the best represented *Cactaceae* species is much infected than the stems. Six genera: *Astrophytum, Aylostera, Echinocerus, Notocactus, Rebutia, Weingartia,* assessed through grade scale reached 2 note (affected area 26-50%). The highest attack intensity was observed in *Echinocereus* (47.24). In calculating the attack degree, a bigger uniformity was noticed in *Aylostera* (36.34), *Echinocereus* (37.46), *Rebutia* (37.83) and *Weingartia* (33.37) (Table 4).

In the case of *Astrophytum,* although the attack frequency at the radicular system was higher (81%), intensity and attack degree were low. The analysis of different genus revealed differences in the values of the three parameters studied, which suggests specificity of receptivity and different resistance to the pathogen attack. Regarding the stem's health state, from the 15 genera that were best represented in the collection, only in the case of *Cleistocactus* genus, attack intensity of 2 degree was observed.

This study is the first of its kind aiming on the evaluation of the health of a *Cactaceae* collection in a Botanical Garden in the *Cactaceae* collection. In Cluj Napoca Botanical Garden, the health of the radicular system proved to be very weak in comparison with the health of the stem. The terminology borrowed in order to quantify radicular system integrity does not refer entirely to health or, better said, to the disease state of the roots, but reports to an ideal value of 100% healthy and implicitly 0% sick at the same time.

It can be concluded that in evaluating the roots health, a high value of intensity and attack degree does not necessarily represent a sick root but a weak rooting (below 50%). Within a genus there are very big differences between species, when it comes to the values of intensity and attack degree of the roots. These can be owned to genetic characterstics as well as to species features (revolving, superficial rootting) disturbing biological factors (fungus, heterodera, lice, *etc*) and their virulence or cultural mistakes which cause the loss of radicular body. Plants, with weak rooting or with pronounced sensibility, when grafted on an appropriate stock eliminate these inconveniences.

In case of the stem disease, intensity and attack degree are influenced by natural suberizing or anterior wound cicatrices (for example hail, lice stings, acarine, *etc*.). As in the case of the roots there were deformations from the ideal form of 100% healthy plant. While with advancement in age, many species natural suberization appears to play role in protection and development of mechanical resistance in old tissues. This phenomenon appears almost symmetrically on longitudinal plan and developes to the top of the plant. In addition to this phenomenon, in some cases fungus attack occurs, that usually evolves forward asymmetrically and radially from different points of the plant. The method is applicable and practical in assessing the health of other specialized collections in the botanical garden (orchid, bromeliads, carnivore and juicy plants).

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Nutrient content changes in strawberry plant parts at different development stages

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Abstract

The objective of the investigation was to study the effect of different development stages on distribution of mineral nutrients in the growing leaves, roots, petioles and fruits. Strawberry plants were grown in a greenhouse in perlite medium and fertigated with Hoagland solution. Mineral nutrient concentration was determined at three development stages *viz.*, flowering, fruiting and the end of fruiting. Also nutrient concentration was determined in different organs at fruiting stage. Our results show that nutrient uptake was variable at different development stages. Leaf and petiole were the main sinks for Ca at fruiting stage and also for Mg and K in petioles, Fe in root, Mn in leaf. Results indicated that plant have different uptake pattern at various development stages. Results on the element uptake by different organs at various development stage is indicative of their relative requirement at different stages.

Key words: Strawberry, nutrient, development stages, plant fragments

Introduction

Reducing fertilizer requirement is an important objective of sustainable production of many horticultural crops (Tagliavini *et al.,* 1996), including strawberry (*Fragaria* x *Ananassa* Dutch.). Also for optimal production, nutrient management is important. Knowledge about nutrient concentration in plant organs at various development stages is necessary. Plant parts have differential nutrient uptake at different developmental stages.

Plant tissue analysis provides a useful guide to efficient crop fertilization and also provide useful guide for sugesting which nutrient to be applied, at what rates, the best method and time of application. Interpretive guides for some crops, such as strawberry, are initial estimates and are subject to revision as more information is obtained. These interpretive guides are liable to continuous revision as more data are obtained relating plant nutrient concentrations and crop performance.

Probably the most important variable in plant analysis is the age of the plant at sampling. Also it is well known that strawberry is a perennial plant cultivated occasionally as annual crop, being transplanted in the summer of one year and yielding the following year in late spring-early summer (Faedi and Baruzzi, 2002). After fruit harvest, vegetative organs are usually removed and brought outside the field, so all nutrients taken up and not only those partitioned to fruits should be considered as net uptake (Mengel and Kirkby, 2001) and potentially have to be reintegrated by fertilizers. According to the above reason, it seems that knowledge about nutrient concentration in plant parts at different stages is necessary.

Information on nutrient concentration at different development stages in strawberry plant parts is limited. In this paper, nutrient concentration at different developmental stages in strawberry plants has been investigated for evaluating their requirements in relation to developmental stages.

Materials and methods

Plant culture and design: Uniform strawberry (*Fragaria ananassa*) plants (cultivars, Selva and Comerosa) which got sufficient chilling were brought from field nursery to a greenhouse in December. These plants were planted in 32 plastic pots filled with perlite in a semi-controlled greenhouse, at Bu-Ali Sina University (Iran) on 10 December, 2005. The pots where planted with a single plant. Temperature was 24/18°C (day/night) and relative humidity was 60-80% in greenhouse. The experimental design was randomized blocks replicated four times in groups and four plants per replication (16 plants per treatment). Each plant was fed by single dripper (80 drips in each four hours that were regulated by timer). Hoagland and Arnon (1950) solutions were used as nutrient solution.

Plant analyses: Plant samples were dried in oven at 70°C for 72 h. The dried leaves, roots and petioles were ground to powder using a pestle and mortar and stored in polyethylene bottles. 0.5g of each dried sample was ashed at 550°C in a porcelain crucible for 2 h. The white ash was taken up in 1 M HCl, filtered in to a 50 mL volumetric flask and made up to 50 mL with distilled water. The concentrations of K was analysed by Flame photometer; Ca, Mg, Zn, Fe and Mn were analysed by inductively-coupled plasma atomic emission spectrometer (ICPAES). Statistical analysis was made using analysis of variance by MSTATC software and the means were separated by Duncan's Multiple Range Test (DMRT) at P=0.05.

Results

Table 1 shows the mean concentration of potassium, calcium, magnesium (g kg^{-1} in dry matter), zinc, manganese and iron (mg kg^{-1} in dry matter) at three phenological stages (flowering, fruiting and growth stage after fruiting) in leaf tissues. Leaf calcium, magnesium, potassium and iron content in all leaf samples were significantly affected by different development

Table 1. Mineral nutrient content in the leaf tissue of strawberry (cvs, Selva and Comerosa) at three development stages

Treatment	Ca (g kg^{-1} DM)	Mg (g kg^{-1} DM)	K (g kg^{-1} DM)	Fe (mg kg^{-1} DM)	Mn (mg kg^{-1} DM)	Zn (mg kg^{-1} DM)
Selva						
Flowering	13.98abc	4.338b	40.25ab	125.5a	55.25a	32.07a
Fruiting	13.78abc	3.714b	30.00c	88.00ab	52.50a	26.72a
End of fruiting	16.78a	5.310a	30.25c	89.75ab	54.25a	25.60a
Comerosa						
Flowering	11.34c	3.656b	43.50a	125.0a	46.25a	24.13a
Fruiting	12.93bc	3.770b	38.00abc	75.75b	40.00a	27.27a
End of fruiting	16.20ab	5.908a	32.25bc	83.75ab	54.50a	29.36a

Means in each column followed by different letters are significantly different at *P*=0.05 by DMRT.

Table 2. Mineral nutrient content in the root tissue of strawberry (cvs, Selva and Comerosa) at three development stages

Treatment	Ca (g kg^{-1} DM)	Mg (g kg^{-1} DM)	K (g kg^{-1} DM)	Fe (mg kg^{-1} DM)	Mn (mg kg^{-1} DM)	Zn (mg kg^{-1} DM)
Selva						
Flowering	7.461a	2.127b	5.750a	574.7a	23.25a	23.27a
Fruiting	8.126a	3.235a	8.500a	205.7b	19.00a	21.64a
End of fruiting	9.113a	2.542ab	6.000a	301.0ab	14.00a	20.75a
Comerosa						
Flowering	9.007a	2.224b	7.250a	478.2ab	25.00a	21.43a
Fruiting	9.518a	2.607ab	8.000a	326.5ab	21.28a	22.74a
End of fruiting	10.00a	2.309b	6.500a	279.7ab	36.00a	20.23a

Means in each column followed by different letters are significantly different at *P*=0.05 by DMRT.

stages, but the concentration of manganese and zinc in leaves was not significantly influenced. Ca and Mg concentration in end of fruiting and K and Fe concentration in flowering stage was in highest amount.

Table 2 show the mean concentration of potassium, calcium, magnesium (g kg^{-1} in dry matter), zinc, manganese and iron (mg kg^{-1} in dry matter) at three phenological stages (flowering, fruiting and growth stage after fruiting) in root tissues. Magnesium and iron concentration in all root samples were significantly affected by development stages, but the concentration of calcium, potassium, manganese and zinc in roots was not significantly influenced. Mg concentration in fruiting and Fe concentration in flowering stage was of higher level.

Table 3 shows the mean concentrations of potassium, calcium, magnesium (g kg^{-1} in dry matter), zinc, manganese and iron (mg kg^{-1} in dry matter) at three phenological stages (flowering, fruiting and growth stage after fruiting) in petioles. Petiole calcium, magnesium, potassium, manganese and zinc concentration in all leaf samples were significantly affected by different development stages, but the concentration of iron in petioles was significantly not influenced. Ca and Mg concentration in end of fruiting and K and Zn concentration in flowering stage was in highest amount. Also minimum Mn was recorded at fruiting time.

Table 4 shows the mean concentration of potassium, calcium, magnesium (g kg^{-1} in dry matter), zinc, manganese and iron (mg kg^{-1} in dry matter) in four strawberry parts (leaf, root, petiole and

Table 3. Mineral nutrient content in the petioles tissue of strawberry (cvs, Selva and Comerosa) at three development stages

Treatment	Ca (g kg^{-1} DM)	Mg (g kg^{-1} DM)	K (g kg^{-1} DM)	Fe (mg kg^{-1} DM)	Mn (mg kg^{-1} DM)	Zn (mg kg^{-1} DM)
Selva						
Flowering	12.96bc	4.508bc	71.75a	62.50a	46.50a	21.69b
Fruiting	14.52b	4.779abc	42.50c	67.75a	14.90c	28.91b
End of fruiting	20.38a	5.804ab	59.25b	73.75a	23.42bc	39.46a
Comerosa						
Flowering	8.961c	3.812c	71.25a	63.25a	31.25b	26.83b
Fruiting	11.50bc	4.678abc	66.00ab	60.25a	12.20c	29.83ab
End of fruiting	13.82b	5.977a	60.50ab	83.75a	15.50bc	30.98ab

Means in each column followed by different letters are significantly different at *P*=0.05 by DMRT.

Table 4. Mineral nutrient content in various tissue of strawberry (Cvs, Selva and Comerosa) at fruiting stage

Treatment	Ca (g kg^{-1} DM)	Mg (g kg^{-1} DM)	K (g kg^{-1} DM)	Fe (mg kg^{-1} DM)	Mn (mg kg^{-1} DM)	Zn (mg kg^{-1} DM)
Selva						
Leaf	14.69a	3.927bc	36.00b	90.00b	35.50b	25.68ab
Root	9.385bc	2.788d	6.250c	310.0a	25.30bc	23.05ab
Petiole	13.38ab	5.217a	51.25a	50.00b	11.10c	29.95a
Fruit	2.892d	1.358e	45.75ab	28.25b	9.950c	12.36d
Comerosa						
Leaf	12.01abc	3.557bcd	32.00b	73.75b	57.00a	28.32ab
Root	8.260c	3.051cd	10.25c	222.3a	14.97c	21.33bc
Petiole	12.64ab	4.240b	57.25a	78.00b	13.50c	29.04a
Fruit	3.462d	1.773e	34.00b	35.00b	10.25c	16.02cd

Means in each column followed by different letters are significantly different at *P*=0.05 by DMRT.

fruit) at fruiting stage. The main sinks for Ca were leaf and petiole, for Mg and K is petioles, for Fe is root and for Mn is leaf. Fruit Zn amount was least in fruit samples.

Discussion

The most important aim of our investigation was to determine the uptake of various elements at different development stages. Our results show that Ca and Mg concentration was increased at end of fruit growth stage in leaf and petiole samples and the trend was similar to the results published by Tagliavini *et al.* (2005). It seems that Ca and Mg with progress of development stages increased in plant organs. Ca and Mg uptake is passive and semi active, respectively and water evapotranspiration is important for their uptakes. It seems that with decrease of plant growth, evapotranspiration from spatial organs was increased. Calcium is considered important for fruit firmness, in spite the fact that most Ca accumulates in plant organs other than the fruits (Albregts and Howard, 1978 and 1980). Results indicates similar Ca distribution pattern in plant organs.

K was in maximum amount at flowering stage in leaf samples and at fruiting time in root samples. Our findings confirmed results of Tagliavini *et al.* (2005). In leaves and petioles, K was in maximum amount followed by Ca and magnesium. These results show that K is important element for strawberry development. Potassium, followed by Ca and Mg were the nutrients absorbed most during the whole production cycle.

Results show that micronutrient content changes at different stages of plant development. Zn and Mn were proximally consistant with the change of development stages in root and leaf but Fe was inconsistant with change in development stages in both root and leaf samples. In petiole samples, Mn and Zn content varied with plant development. Also Fe was constant in petiole samples by progress of plant development. It seems that Fe requirement in flowering is maximum. Difference in various nutrient sink probably be because of various uptake mechanisms (active or passive) of each element. Nutrient needs should instead be defined as those amounts necessary to be absorbed to maximize a desired plant performance: under sustainable fruit production this performance cannot be identified only by a fruit yield but has to include nutritional quality of the plant food (Welch, 2002), and minimum or no risk of pollution to the environment.

In conclusion, these experiments indicate the pattern of root uptake of nutrient dynamics by strawberry plant. In practice, the knowledge of plant nutreint requirement might allow a precise control of nutrient supply especially if (1) flexible nutrient supply techniques, like fertigation, are adopted and (2) monitoring of nutrient availability in the nutrient solution and or in the plant is carried out.

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Growth, yield and productivity responses of okra-papaya mixture to intercropping in South West Nigeria

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Abstract

Field experiments were conducted between 2006 and 2007 at the University of Agriculture, Abeokuta, South Western Nigeria, to determine the growth and yield responses of okra (*Abelmoschus esculentus*) grown in orchards of two papaya (*Carica papaya* L.) varieties, 'Homestead Selection' and 'Sunrise Solo', at three different stages of papaya growth. Different sequences of okra sowing were; at three weeks before papaya (early), same time with papaya (simultaneous) and three weeks after papaya (late). Results showed that early and simultaneous introduction of okra performed significantly better than the late, with respect to plant height, number of leaves, leaf area, number of pods, pod weight plant^{-1} and total pod yield. All the okra intercrops experienced competitive effects that reflected in reduced yield more pronounced in Homestead Selection than in Sunrise Solo. The productivity efficiency index recorded intercropping advantages for the okra in mixture compared to the sole okra with a land equivalent ratio (LER) >1.0 while the area harvest equivalent ratio (AHER) was more descriptive of the trends observed among the sequences. In cv Homestead Selection, the highest profit margin (47.64 %) was recorded in the simultaneous papaya-okra intercrop, followed by early (44.57 %). A similar trend was observed in cv Sunrise Solo, where simultaneous and early okra introduction had a profit margin of 40.06 and 39.72%, respectively. Late sequence had the least profit margin in both papaya cultivars. .

Key words: *Abelmoschus esculentus* (L.) Moench., papaya, growth and yield, intercropping sequence, productivity efficiency indices, profit margin.

Introduction

Okra (*Abelmoschus esculentus*) plays important role in diets as it is regarded as an important draw soup in the tropical diet. Okra is rich in vitamins and mineral salts such as calcium, phosphorus, magnesium and iron and it is very valuable with regards to anti-carcinogenicity, human immunity promotion and ageing prevention (AVRDC, 1991). Vegetables occupy a valuable ecological niche in tropical agriculture and play a significant role in the eco-physiology of mixed systems (Olasantan, 2001). The planting of two or three crops concurrently or sequentially could have implications on the availability of limited natural resources. The resulting ecological relationships could be competitive or complementary in nature. However, the planting of several crops, which differ in height, root development and light requirement, allows for a more efficient use of solar energy, soil nutrient and water. Intercropping has been associated with such advantages as better utilization of growth resources, greater yield stability, soil protection, variability of food supply, increased return per unit area and insurance against crop failure (Beet, 1982). Szumigalski and Acker (2005) and Ofosu-Anim and Limbani (2007) reported that annual intercrops can enhance weed suppression and crop production compared with sole crops.

Fukai and Trenbath (1993) reported that intercrops are most productive when their component crops differ greatly in growth duration so that their maximum requirements for growth resources occur at different times. In 'additive' intercrops, where growth durations of component crops are similar, the crops compete more intensely for available resources but may nevertheless, be productive, particularly where growth resources are more completely captured than in corresponding sole crops. However, in 'replacement' intercrops where the non-replenished growth resources are utilized too rapidly, the less-competitive component may suffer greatly. Intercropping okra with papaya, which is more of replacement competition, specifically has to do with its compatibility in terms of favourable competition for soil nutrients, soil moisture and light. John and Mini (2005) observed favourable land equivalent ratio (LER), land equivalent co-efficiency (LEC), area time equivalency ratio (ATER), aggressivity values and total biomass production for the intercropping of okra with cucumber, implying their intrinsic advantages over sole crops. Okra intercrop in papaya has been observed to show effect of competition among the component crops (Aiyelaagbe and Jolaoso, 1992; Olubode *et al.,* 2005), but they also reported improved LER of the okra papaya mixtures. Calculated LER proved that plant growth resources were used 27 to 31% more efficiently by intercrop than the sole crop (Hauggaard-Nielsen *et al.,* 2003).

Intercropping is practiced with the sole aim of maximizing plant cooperation for maximum crop yield (Sullivan, 2001). Olasantan and Lucas (1992) had noted that canopy height is one of the important features that determine competition ability of plants for light. Palaniappan (1985) observed that when one component is taller than the other in an intercropping, the taller component intercepts major share of the light such that growth rates of the two components will be proportional to the quality of the photo-synthetically active radiation they intercepted. Muoneke *et al.* (1997) also reported that the taller okra plants obtained in intercrop with maize was a bid to display their leaves for solar radiation. Njoku *et al.* (2007) observed that intercropping

generally increased okra plant height while intercropping with TIS 2532P.1.13 sweet potato significantly increased the number of pods per plant of okra than intercropping with other sweet potato cultivars.

Previous works done on okra mixture with papaya varieties (Aiyelaagbe and Jolaoso, 1992; Olubode *et al.,* 2008) mostly considered the competitive effect when both components are grown concurrently but the time based sequential introduction of okra crop components to obtain the best time for highest profit was not considered. This experiment seeks to determine the best cropping sequence most suitable for okra papaya mixture and the crop responses of crops to alternate cropping sequence of components.

Materials and methods

The experiment was conducted at the University of Agriculture, Abeokuta, South Western Nigeria, (latitude 7°15´N, longitude 30° 25´E, altitude of 100m above sea level). Meteorological data for the experimental location and period are shown in Fig. 1. Composite analysis results of soil sampled are shown in Table 1. Two soil types dominate the location, *viz.*, the Iwo series and Apomu series (Smyth and Montgomery, 1962; FDALR, 1990). The Iwo series-Kandic Paleustalf (USDA, 1999), Ferric Luvisols (FAO/UNESCO, 1990), are generally well drained, fine sandy loam to sandy clay loam soils and Apomu series-Typic Transporthants (USDA, 1999), Eutric Regosols (FAO/UNESCO, 1990), are excessively drained sand to sandy loam. Analysis of the pacesetter organo-mineral fertilizer applied as 10 ton ha^{-1} basal application in the experiment is shown in Table 2.

The experimental design included two varieties of papaya and three times of introduction of crop components at the early, simultaneous and late sequence. The experiment was a randomized complete block design (RCBD) fitted into a split plot arrangement and replicated three times. The main plot was the papaya varieties while the sub-plot was the three times of introducing the components. The okra (*A. esculentus*) cv. V35, an erect and early variety was introduced into plots of papaya (*C. papaya* L.) varieties Homestead Selection, (a dioecious variety) and Sunrise Solo, an hermaphrodite, in three sequences of okra 3 weeks before papaya (early intercropping), okra same time with papaya (simultaneous intercropping) and okra 3 weeks after the planting of papaya (late intercropping).

Table 1. Characteristics of the soil used in okra papaya mixture in 2006

Depth (cm)	Particle size analysis			Chemical analysis					Exchangeable bases			
	Sand (%)	Clay (%)	Silt (%)	Soil pH (H_2O)	Organic carbon (%)	Organic matter	N (%)	P (ppm)	K (cmol kg^{-1})	Na (cmol kg^{-1})	Ca (cmol kg^{-1})	Mg (cmol kg^{-1})
0 - 15	61.20	22.00	16.80	7.78	1.08	1.86	0.081	6.74	0.31	0.35	3.48	0.52
16-30	73.40	14.30	7.30	6.90	0.44	0.76	0.055	10.05	0.15	0.43	1.66	0.18

Table 2. Analysis of Pacesetter Organo-mineral fertilizer used

OMF sample	N (%)	P (%)	K (%)	(%) Ca	Fe (mg kg^{-1})	Mg (mg kg^{-1})
	4.89	0.53	0.33	0.87	4.8	0.9

Source. Pacesetter purchased samples analytical results.

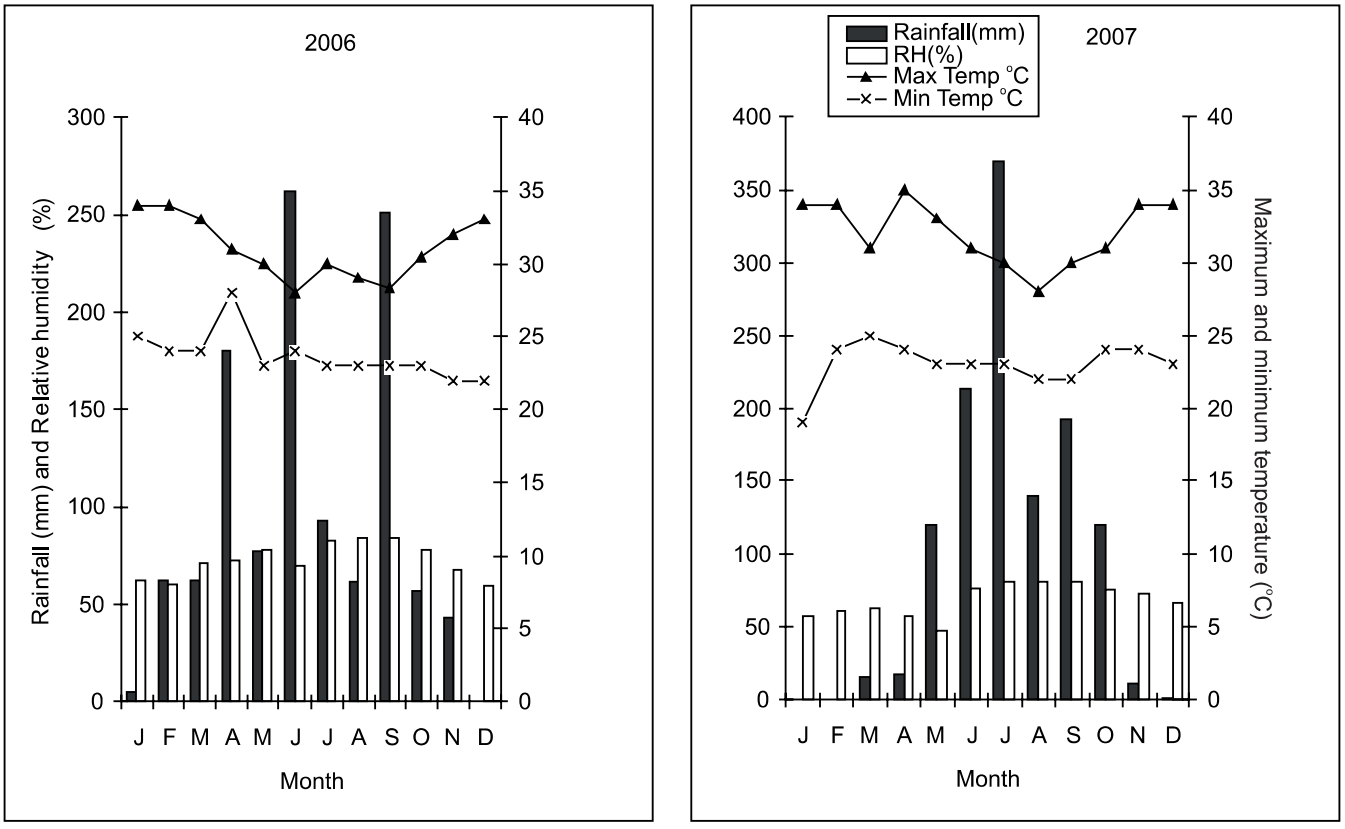

Fig. 1. Meteorological data for the experimental location for 2006 and 2007.

Three month old papaya seedlings were transplanted in August 2006 at a spacing of 2 x 2 m into holes of 60 cm^3 size while okra was spaced 0.5 x 0.3 m at the three sequences. The experiment was repeated in 2007 when the okra was sown into the alleys of one year old papaya orchards at the onset of rains. All plots were weeded manually while insect pests and diseases were controlled biologically using approaches reported by Lowell (1998).

Growth parameters estimation for the okra involved weekly measurement of plant height (cm), number of leaves and leaf area (cm^2) estimated by using leaf area formula described by Asif (1977), *i.e.* Y = 115X -1050 where, Y = leaf area (cm^2) and 'X' is the length of the midrib (cm $plant^{-1}$). Other parameters measured on okra include number and weight of pods, pod yield (ton ha^{-1}).

Growth parameters of papaya, measured fortnightly, were plant height, stem girth and leaf area using the formula described by Aiyelaagbe and Fawusi (1998) *viz.*, Y = 316.06- 47.09X, where Y = leaf area (cm^2) and X is the sum of the media midrib (cm $plant^{-1}$). Yield parameters of number of flowers, number of fruits and fruit weight (g $plant^{-1}$) were measured weekly. Net returns were calculated to determine the economic yield of the system. Intercropping efficiency was evaluated by comparing the productivity of a given area of intercropped land with sole crop using the competitive index of land equivalent ratio (LER) (Wiley, 1979). Other productivity indices like land equivalent co-efficiency (LEC) (Adetiloye *et al.,* 1983), area x time equivalency ratio (ATER) (Hiesbsch and McCollum, 1987), area harvest equivalency ratio (AHER) (Balasubramanian and Sekayange, 1990), relative crowding coefficient (RCC) (de Wit, 1960), aggressivity values (McGilchrist, 1965) and monetary equivalency ratio (MER) (Adetiloye and Adekunle, 1989) were compared with the LER.

Data collected were subjected to the analysis of variance procedures (SAS, 1990). Treatment means of each of the parameters measured were compared using the least significant difference technique (Steel and Torrie, 1980).

Results

Response of crop components to crop mixture: Okra and papaya growth and yield were significantly influenced by intercropping. Okra mixture with papaya significantly affected okra growth and yield parameters when compared with the sole crop (Tables 3 and 4). The okra performed significantly better under the Sunrise Solo than under the Homestead Selection papaya with regards to the leaf area (Table 5). Sole papaya was significantly better than papaya in mixture in plant height, leaf area and in the reproductive parameters like number of flowers, number of fruits and fruit yield (Table 6). Observed morphological differences in the papaya varieties are shown in Table 6. Plant height, stem girth and leaf area were higher in Homestead in the

Table 3. Growth responses observed for okra grown in mixture with papaya in 2006 and 2007

Cropping system/ sequence	2006				2007			
	Plant height (cm)	Number of leaves	TDW at 6 WAP (g)	Leaf area (x '000 cm^2)	Plant height(cm)	Number of leaves	TDW at 6 WAP (g)	Leaf area (x '000 cm^2)
Okra in Homestead								
Early	42.23	14.27	9.70	2.45	26.14	4.91	8.07	2.04
Simultaneous	39.75	13.43	9.13	2.30	24.60	4.62	7.60	1.92
Late	38.34	12.95	8.81	1.93	23.73	4.16	7.33	1.61
Mean	40.11	13.55	9.21	2.23	24.82	4.56	7.67	1.86
Okra in Sunrise								
Early	44.51	14.07	10.74	2.71	31.27	4.16	8.37	2.11
Simultaneous	41.35	13.07	9.97	2.51	29.05	3.87	7.78	1.96
Late	39.91	12.62	9.63	2.23	28.04	3.74	7.51	1.74
Mean	41.92	13.25	10.11	2.48	29.45	3.92	7.89	1.94
LSD(*P*=0.05)Var	1.17*	NS	NS	0.12*	2.13*	NS	NS	NS
Seq	2.48*	0.18*	0.57*	0.38*	3.75*	0.31*	NS	NS
Var X Seq	NS	NS	NS	NS	NS	NS	NS	NS

*- *P*= 0.05, NS-not significant, Var-Variety, Seq-Sequence, TDW-total dry weight, WAP-weeks after planting.

Table 4. Yield responses observed for okra grown in papaya cropping mixture in 2006 and 2007

Cropping system/ sequence	2006				2007			
	Number of pods	Weight per pod (g)	Pod weight $plant^{-1}$ (g)	Pod yield (ton ha^{-1})	Number of pods	Weight per pod (g)	Pod weight $plant^{-1}$ (g)	Pod yield (ton ha^{-1})
Okra in Homestead								
Early	5.30	14.30	75.87	3.79	3.10	3.42	10.6	0.53
Simultaneous	8.26	8.50	70.20	3.51	2.78	3.53	9.8	0.49
Late	7.92	7.65	60.60	3.03	2.35	3.83	9.0	0.45
Mean	7.16	10.15	68.89	3.44	2.74	3.59	9.8	0.49
Okra in Sunrise								
Early	5.32	14.62	77.80	3.89	4.12	2.67	11.00	0.55
Simultaneous	7.85	9.22	72.49	3.62	3.45	2.96	10.2	0.51
Late	8.22	8.52	70.00	3.50	3.98	2.51	10.0	0.50
Mean	7.13	10.79	73.43	3.67	3.85	2.71	10.4	0.52
LSD(*P*=0.05)Var	NS	0.56*	1.32*	0.21*	0.12*	0.47*	NS	NS
Seq	1.21*	2.13*	5.27*	0.40*	1.13*	0.74*	1.56*	0.046*
Var x Seq	*	NS	NS	NS	NS	*	NS	NS

**P*= 0.05, NS-not significant, Var-Variety, Seq-Sequence.

first year but in the second year, the stem girth and leaf area was more in Sunrise. Okra performed better under the Sunrise Solo due to less shading by the papaya canopy than the Homestead. Mixture with okra significantly influenced papaya growth as reflected in plant height, number of leaves and leaf area leading to growth and yield reductions.

Responses of crop components to cropping sequence: Cropping sequence significantly affected okra and papaya growth, yield determinants and yield. The early-introduced okra was best, followed by simultaneous okra and papaya. Both were though not significantly different but were significantly better than late introduction of okra into papaya. The okra in early sequence was highest in growth and yield performances under Sunrise Solo variety followed by early sequence under Homestead, simultaneous under Sunrise Solo and under Homestead, in that order. The late introduction of okra had the lowest growth and yield performances. Early introduction of okra into papaya orchard was superior in both years, with a significant difference for all the parameters in 2006 and all but yield in 2007 (Table 4). Both the component crops, papaya and okra experienced yield reduction at the reproductive phase of papaya (Fig. 2).

Productivity of the mixtures: The productivity efficiency index >1.0 was obtained for the mixture using LER. The LER values ranged between 1.06 for Homestead late sequence to 1.33 for Homestead simultaneous. Other indices like LEC, ATER and RCC gave similar responses. AHER gave a descriptive trend where Sunrise early sequence was highest with a value of 1.81 and Homestead late a value of 1.44. ATER was practically insensitive (Table 7) where a common value of 1.25 was obtained across board. Aggressivity was negative for okra and papaya was dominant over okra in the mixture. The crop productivity observed in the sequences showed a higher economic returns for the mixtures as shown in Table 8, where okra simultaneous with Homestead was highest with a profit margin of 47.64%, followed by early okra sequence in Homestead (44.57%) and okra simultaneous sequence in Sunrise (40.06%), while okra late sequence in Sunrise and Homestead in that order were least recording 34.99 and 33.62%, respectively.

Discussion

The observed growth and yield responses of okra in cropping mixture confirmed earlier findings by Palaniappan (1985), Olasantan and Lucas (1992), who reported that plant height is one of the important features that determine competitive ability of plants for light, while Muoneke *et al.* (1997) and Njoku *et al.* (2007) also confirmed that the taller okra plants obtained in intercrop with maize and sweet potato, respectively was a bid to display their leaves for solar radiation, hence intercropping generally increased okra plant height. The observed papaya

Table 5. Growth and yield responses observed for okra grown sole and in mixture with papaya in 2006 and 2007

Cropping mixture	2006					2007				
	Plant height (cm)	Number of leaves	TDW at 6WAP (g)	Leaf area (x'000cm^2)	Pod yield (ton ha^{-1})	Plant height (cm)	Number of leaves	TDW at 6WAP (g)	Leaf area (x'000cm^2)	Pod yield (ton ha^{-1})
Sole	38.65	14.74	18.33	2.56	4.30	41.74	13.93	13.75	2.44	4.04
Hs +okra	40.11	13.55	9.21	2.23	3.44	24.82	4.56	7.67	1.86	0.49
Ss +okra	41.92	13.25	10.11	2.48	3.67	30.0	4.0	8.03	1.80	0.52
Mean	40.23	13.85	12.55	2.42	3.80	32.19	7.50	9.82	2.03	1.68
LSD(*P*=0.05)	1.06*	NS	1.52*	0.31*	0.21*	3.17*	2.59*	1.13*	0.35*	0.33**

* *P*= 0.05, ** P= 0.01, NS-not significant, Hs-Homestead Selection, Ss-Sunrise Solo.

Table 6. The mean treatment effects of papaya vegetative and reproductive responses for the crop sequence with okra in 2006 and 2007

Cropping system/ sequence	Plant height (cm)		Stem girth (cm)		Leaf area (cm^2)		Number of flowers	Number of fruits	Fruit yield (ton ha^{-1})
	28 MAT	72 MAT	28 MAT	72 MAT	28 MAT	72 MAT	18 MAT	18 MAT	18 MAT
Py sole	80.72	246.17	3.26	15.60	12987	91838	78.07	58.54	33.63
Py + okra	67.28	199.80	2.16	13.26	9947	56104	70.74	48.49	26.97
Hs sole	83.77	252.17	3.97	16.22	15729	97217	67.28	48.44	41.17
Ss sole	77.67	240.17	2.55	14.98	10244	86459	88.85	68.63	26.08
Hs + ok Early	81.33	240.40	3.03	14.37	16590	59948	51.26	36.46	30.99
Hs + ok simult	72.67	193.37	2.44	12.51	13999	54120	63.22	41.29	35.10
Hs + ok Late	61.33	168.51	1.75	11.38	4550	41563	67.66	31.27	26.58
Ss + ok Early	72.33	238.10	2.34	15.48	6905	71763	81.59	60.81	23.11
Ss + ok simult	62.67	191.52	1.80	13.54	10436	61781	89.59	63.79	24.24
Ss + ok Late	53.33	166.90	1.60	12.30	7200	47445	71.10	57.32	21.78
Mean	67.28	199.80	2.16	13.26	9947	56103	70.74	48.49	26.97
LSD(*P*=0.05)									
Var	NS	NS	NS	NS	3674**	NS	4.37**	5.23**	5.03**
Intc	NS	28.77*	0.52**	NS	NS	19118*	3.24**	NS	1.08*
Seq	13.25*	56.29**	0.90**	NS	6073*	NS	3.86**	2.44*	2.11**
Var x Intc	NS	NS	*	NS	NS	NS	**	**	**
Var x Seq	NS	NS	NS	NS	*	NS	**	**	**
Intc x Seq	NS	NS	NS	NS	NS	NS	**	*	NS
Var x Intc x Seq	NS	NS	NS	NS	NS	NS	**	**	*

* *P*= 0.05, ** P= 0.001, NS-not significant, Py-Papaya mean, Hs-Homestead Selection, Ss-Sunrise Solo, ok-okra, Var-Variety, Intc-Intercrop, Seq-Sequence, MAT-months after transplanting.

Table 7. Productivity efficiency indices observed for okra-papaya cropping system.

Cropping sequence	Fruit yield (ton/ha)	Efficiency index						
		LER	LEC	ATER	AHER	RCC	Aggre-ssivity	MER
Homestead								
Early	31.23	1.28	0.39	1.25	1.75	3.38	-0.24	0.37
Simultaneous	35.10	1.33	0.41	1.25	1.77	5.33	-0.37	0.34
Late	26.53	1.06	0.27	1.25	1.44	1.30	-0.23	0.30
	30.95	1.22	0.36	1.25	1.65	3.34	-0.28	0.34
Sunrise Solo								
Early	20.54	1.32	0.42	1.25	1.81	4.22	-0.26	0.46
Simultaneous	21.56	1.32	0.41	1.25	1.78	4.68	-0.33	0.43
Late	19.36	1.22	0.36	1.25	1.66	2.66	-0.26	0.41
	20.49	1.29	0.396	1.25	1.75	3.85	-0.28	0.43
LSD(*P*=0.05)Var	8.79*	NS	NS	NS	NS	NS	0.035**	0.039**
Seq	12.94*	0.062**	0.037**	NS	0.074**	NS	NS	0.020**
Var x Seq	NS	**	**	NS	**	NS	**	**

LER-land equivalent ratio, LEC-land equivalent coefficient, ATER.-area x time equivalent ratio, AHER.-area harvest equivalent ratio, RCC-relative crowding coefficient, MER.-monetary equivalent ratio, *P= 0.05, **P= 0.001, ns-not significant.

Table 8. Profit margin calculated showing the profitability of each cropping system in the okra papaya sequence

Cropping sequence	Two year yield (ton ha^{-1})		Selling price kg^{-1} (₦)[a]		Yield values (₦)[a]	Production cost (₦)[a]	Profit (Naira ha^{-1}) (₦)[a]	Profit margin (%)
	Papaya	Okra	Papaya	Okra				
Sole								
	41.17	-	35.29	-	1,452,889	910,289	542600	37.35
	26.08	-	46.50	-	1,212,720	910,289	302,431	24.94
	-	8.34	-	125.00	1,010,000	639,250	370,750	36.71
Homestead + okra								
Early	31.23	4.32	35.29	125.00	1,642,107	910,289	731818	44.57
Simultaneous	35.10	4.00	35.29	125.00	1,738,679	910,289	828390	47.64
Late	26.53	3.48	35.29	125.00	1,371,244	910,289	460,955	33.62
Sunrise + okra								
Early	20.54	4.44	46.50	125.00	1,510,110	910,289	599,821	39.72
Simultaneous	21.56	4.13	46.50	125.00	1,518,790	910,289	608,501	40.06
Late	19.36	4.00	46.50	125.00	1,400,240	910,289	489,951	34.99

'a' denotes Naira (₦ 190.00 equivalent $1 US dollar).

growth and yield reduction in mixture with okra in this study corroborates earlier reports by Aiyelaagbe and Jolaoso (1992) and Olubode *et al.* (2008). This may have been due to competition by the components for limited growth resources. The taller height of okra in intercrop compared to sole and the shorter height of papaya component compared to the sole indicated signs of competition.

In the first year of okra sowing competition for soil nutrient was the likely to be critical factor as papaya was yet to have a wide canopy cover that could make competition for light critical, while at the second year cropping, it was clearly the problem of light interception which was advantageous to papaya but deleterious to okra growth and yield as a result of the relative heights of both crops. The near linear trend observed for the response of okra intercrops to time of introduction indicates the level of available nutrient and/or minimal light interference derived from the competition with papaya, which obviously affected the okra growth and yield. The okra introduced earlier had greater advantage and access to soil nutrient coupled with unhindered access to solar radiation for a greater part of the time which produced the significantly higher pod yield observed but caused more nutrient depletion to papaya compared to simultaneous and late introduction. The interaction observed under number of pods and pod weight showed the contributive effects of shading and nutrient availability to these parameters. Sunrise Solo had lower interference with okra growth and yield as the leaf area at the vegetative phase was lower compared to Homestead.

The intercrop competition effect was observed in the second year of papaya growth. Okra's improved growth and yield under Sunrise Solo and at the early sequence compared to other treatments could be as a result of higher light interception. The more than unity LER recorded under intercropping demonstrated higher yield advantage for the intercropped plots. In particular, papaya and okra in simultaneous planting gave higher LER of 1.33, implying that 33% more land would be required as sole crop to produce the equivalence of yield obtained under intercropped situation. LEC and ATER and RCC values followed trends that though not quite similar to that of LER but proved the higher productivity of the mixtures. AHER was more useful in apportioning productivity to the mixtures as recommended by Fukai and Trenbath (1993). The negative aggressivity value for okra shows that papaya was dominant while okra was dominated. The net returns also showed that Homestead mixtures with higher harvest index were more profitable than the Sunrise mixtures and the simultaneous planting followed by early okra introduction were more profitable than late sequence which was lowest.

In conclusion, papaya varieties would vary in their tolerance to intercropping as Sunrise Solo variety was found more suitable for good growth and yield of okra. For economic land utilization and crop productivity, okra papaya mixture with early introduction of okra is recommended. Okra introduced before the papaya component comes into full establishment and fruit bearing was satisfactory in growth and yield relative to sole okra. Intercropping advantage derived from the various indices used indicated that

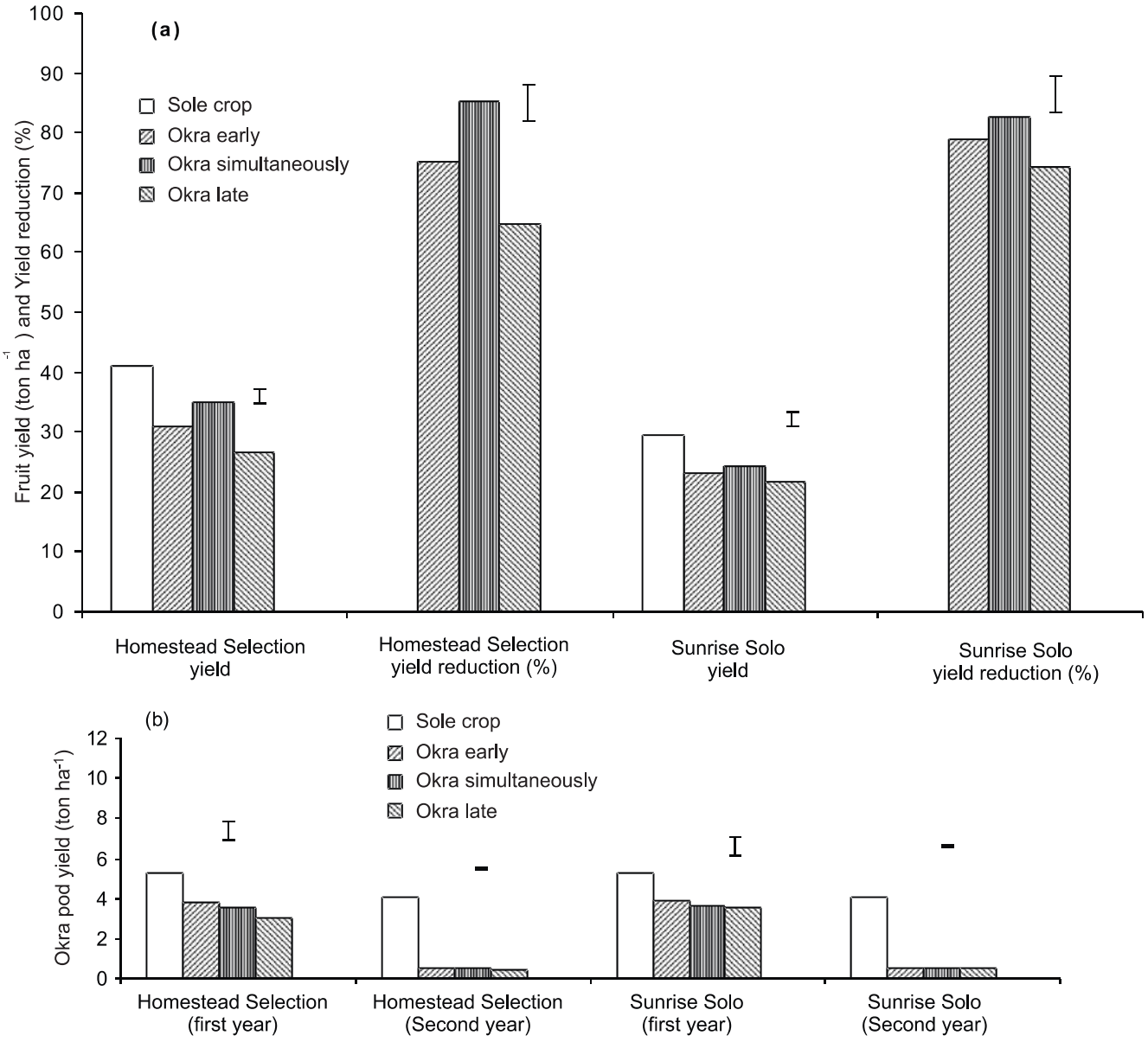

Fig 2. Fruit yield and percent reduction of component crops in papaya-okra mixtures showing (a) sole papaya varieties cv. Homestead (Hs) and Sunrise (Ss) and sole okra responses compared with papaya mixtures in early, simultaneous and late sequences, (b) okra in sequence with papaya varieties in 2006 and 2007. Vertical bars denote LSD (P=0.05).

okra-papaya mixture could be profitably grown.

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Constraints as perceived by the vegetable growers regarding the adoption of IPM technologies in cauliflower cultivation: An empirical study

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Abstract

The present study revealed that among all the various types of constraints perceived by the respondents regarding the adoption of Integrated Pest Management (IPM) technologies in the cauliflower (*Brassica oleracea* L. var. *botrytis*) production, the lack of knowledge of the respondents about the Economic Threshold Limit (ETL) concept (under the category of knowledge and information constraints) had the first rank closely followed by the lack of knowledge of the respondents regarding the bio-pesticides (under the category of knowledge and information constraints). The lack of knowledge of the respondents about the IPM techniques (under the knowledge and information constraints category) enjoyed third position, closely followed by lack of training of the respondents on the proper use of pesticides (under category of administrative and managerial constraints). The result clearly indicate that among six different categories of perceived constraints, knowledge and information constraints with a rank score of 2769 enjoyed first rank position, distantly followed by administrative and managerial constraints (with a rank score of 1586) in the second position, technological and communication constraints with a rank score of 1249 in the third position, socio-economic constraint (rank score of 828) in the fourth position.

Key words: *Brassica oleracea* L. var. *botrytis,* constraints, adoption of IPM technologies, cauliflower cultivation, knowledge and information constraints, rank score.

Introduction

Integrated Pest Management (IPM) is a scientific paradigm (Perkins, 1982) which is now of global significance. Its basic concern is with designing and implementing pest management practices that meet the goals of farmers, consumers and governments in reducing pest losses while, at the same time, safeguarding against the longer term risk of environmental pollution, hazards to human health and reduced agricultural sustainability.

While the philosophy and ideas of IPM are now widely accepted in the political and scientific arena, the practical implementation of IPM has proved far more difficult to achieve. Over two decades, attempts to develop and disseminate IPM technologies in the developing countries have met with limited success (Kiss and Meerman,1991; Yudelman *et al.*, 1998).

IPM was first developed in response to the environmental concerns about the abuse or over use of the chemical pesticides associated with the input-intensive agricultural systems of the developed countries. The traditional approach regarding the IPM technology development was to develop pest and disease control alternatives to reduce or eliminate the use of chemical pesticides. Here, the role of the agricultural extension system was to transfer and disseminate these technologies and practices directly to the farmers (Morse and Buhler, 1997). More recently, alternative approaches have been evolved for the small-scale farming systems of the developing countries. These approaches seek to combine indigenous farmer knowledge with scientific knowledge of cropping systems and pests to develop site appropriate IPM systems. Variously labeled as ecological or sustainable IPM (Schwab, 1995; Mangan and Mangum, 1998), these approaches are often described as being knowledge-intensive (Morse and Buhler, 1997). This new approach regarding IPM technologies require enhanced knowledge and understanding of the small farmers regarding the biological factors and ecological interactions (Dent, 1995).

Therefore, it is of utmost necessity to find out and analyze the field level constraints (as perceived by the farmers) in the adoption of IPM technologies especially in the vegetable cultivation in the third world countries where excessive and indiscriminate use of the pesticides were reported by various researchers like Rashid *et al.* (2003), IPM DANIDA Project (2004), Kim and Park (2005), Baral *et al.* (2006). Keeping these in mind, the present study was undertaken with following set of objectives: (i) to study the field level constraints (as perceived by the respondents) in the adoption of IPM technologies on the selected vegetable and (ii) to analyze the field level constraints (as perceived by the respondents) in the adoption of IPM technologies on the selected vegetable category wise.

Materials and methods

Site of the study: The Katwa-I block of Bardhaman District was purposively selected for the present study, since this block is famous for vegetable cultivation. Only one vegetable *i.e.* cauliflower was selected. This vegetable is the major crop of the winter season and grown in relatively more area than other vegetables.

All the villages, eighteen (18) in numbers falling under the five (5) kilometers radius of Katwa Town were selected for the present study, as these villages have sizeable population, who grew cauliflower commercially in more than 0.33 acre of farm land. At present, 150 such farmers were there. So, all the 150 vegetable growers were selected as the sample population of the present study.

Measurement of the degree of the perceived constraints: To measure the degree of constraints as experienced by the respondents in relation to the adoption of IPM technologies in cauliflower cultivation, the respondents were asked to indicate on a four point continuum about the extent to which each constraint was perceived as crucial factor for the adoption of IPM technologies in the selected vegetable. The scoring procedure was as follows:

S No.	Category	Score
1	High	3
2	Medium	2
3	Low	1
4	Not at all	0

Results and discussion

Field level constraints experienced by the respondents regarding the adoption of the IPM technologies in the cauliflower cultivation: The ranking (both category-wise and overall ranking) of various field level constraints as perceived by the respondents regarding the adoption of the IPM technologies in cauliflower cultivation is given in Table 1.

From the Table, it became clear that among the socio-economic constraints as perceived by the respondents regarding the adoption of IPM technologies in the cauliflower cultivation, lack of contact of the respondents with outside world held the first rank position followed by lack of financial resources, lack of education, small size of land holding, age and gender of the respondents. This was apparently because of more the contact of the respondents with outside world, more they would have exposures to various sources of information which would encourage the respondents regarding the adoption of IPM technologies for the cauliflower production.

Among the infrastructural constraints, lack of preservation and cold-storage facilities for the selected vegetable scored the first position followed by the lack of communication and transport. Lack of irrigational facilities held the third rank followed by lack of the vegetable market. Among the infrastructural constraints, lack of preservation and cold-storage facilities enjoyed the first position might be because of the fact that without proper preservation facilities, the cauliflower growers were under pressure to sell the harvested cauliflower crops immediately which in turn encouraged the cauliflower growers to apply the pesticides more for increased level protection from the pests and vis-à-vis increased yield of the cauliflower crops and thus the farmers tended to dissociate themselves from the adoption of IPM technologies in the cauliflower cultivation.

Table clearly shows that among the situational constraints regarding the adoption of IPM technologies in the cauliflower production, average distance between the fragments of the cultivated land held the first position, closely followed by fragmentation of the cultivated land. Fragmentation of the cultivated land and lack of supply of the pesticides in the market at the right time enjoyed the third and fourth positions, respectively.

From the Table, it became clear that among technological and communication constraints as perceived by the respondents, the inadequate / complicated description regarding the precautions to be taken in the case of toxicity related accidents in the written materials kept in the containers of pesticides enjoyed the first rank position, followed by the lack of proper Integrated Pest Management (IPM) technologies for the cauliflower crops in the second position. The quality of the printing of the written materials kept in the containers of the pesticides was in the third position and the colour used in the written materials kept in the containers of pesticides enjoyed the fourth position. The fact that given description regarding the precautions to be taken in the case of toxicity related accidents in the written materials kept in the containers of pesticides enjoyed the first rank position might be due to the cause that unclear, complicated, written in highly technical way of writing and below quality descriptions would compel the respondents to use the pesticides without any precaution in the cauliflower cultivation.

Among the knowledge and information constraints as perceived by the respondents, regarding the adoption of IPM technologies, the lack of knowledge of the respondents (vegetable growers) about the Economic Threshold Limit (ETL) concept of the cauliflower crops held the first rank position, closely followed by the lack of knowledge of the cauliflower growers regarding the bio-pesticides in the second position which was closely followed by the lack of knowledge about the Integrated Pest Management (IPM) techniques for the cultivation of the cauliflower crops. The lack of proper information on the judicious use of pesticides was in the fourth position. The fact that the lack of knowledge of the respondents (vegetable growers) about the Economic Threshold Limit (ETL) concept of the cauliflower crops held the first rank position among the knowledge and information constraints might be due to the cause that without the knowledge about the Economic Threshold Limit (ETL) concept of the cauliflower crop, the respondents were prone to use the chemical pesticides more in numbers, frequency and quantity in the cauliflower cultivation than suggested by the IPM experts.

It was apparent from the Table that among the administrative and managerial constraints as perceived by respondents, lack of training on the IPM technologies was most important, closely followed by non-availability of extension personnel in time and distantly followed by cheating by the sales agents and dealers of pesticide companies. In the fourth position, lack of loan sanctioning mechanism for the vegetable growers for cultivation of cauliflower crops was ranked. Among the administrative and managerial constraints, lack of training of the vegetable growers on the IPM technologies in the cauliflower production had the first position because of the fact that without proper training of the respondents regarding the IPM technologies, the respondents would be prone to misuse of pesticides in the cauliflower production.

Table 1 revealed that among all the constraints perceived by the respondents regarding the adoption of IPM technologies in the cauliflower production, the lack of knowledge of the respondents

Table 1. Ranking of various constraints (as perceived by the respondents) regarding the proper use of pesticides in the cauliflower cultivation

Sl. No.	Types of constraints	Rank score	Rank within a category	Overall rank
	A. Socio-economic Constraints			
01.	Lack of contact of the vegetable growers with outside world	306	I	V
02.	Lack of financial resources of the vegetable growers	195	II	XVI
03.	Lack of education of the vegetable growers	158	III	XXII
04.	Small size of land holding of the vegetable growers	150	IV	XXV
05.	Age of the vegetable growers	14	V	XXXXV
06.	Gender of the vegetable growers	05	VI	XXXXVI
	Total of Category	828		IV
	B. Infrastructural Constraints			
07.	Lack of preservation and cold-storage facilities for the selected vegetables	270	I	VII
08.	Lack of communication and transport	97	II	XXXVIII
09.	Lack of irrigational facilities	60	III	XXXXI
10.	Lack of vegetable market	57	IV	XXXXII
11.	Lack of proper plant protection implements	52	V	XXXXIII
	Total of category	536		VI
	C. Situational Constraints			
12.	Average distance between the fragments of the cultivated land of the vegetable growers	173	I	XVII
13.	Lack of mutual co-operation among the vegetable growers	169	II	XVIII
14.	Fragmentation of the cultivated land of the vegetable growers	138	III	XXVIII
15.	Lack of supply of the pesticides in the market at the right time	109	IV	XXXV
16.	Topography of land	51	V	XXXXIV
	Total of category	640		V
	D. Technological and Communication Constraints			
17.	Inadequate / complicated description regarding the precautious to be taken in the case of toxicity related accidents in the written materials kept in the containers of pesticides	236	I	VIII
18.	Lack of proper Integrated Pest Management (IPM) technologies for the selected vegetables	212	II	XIV
19.	The quality of the printing of the written materials kept in the containers of the pesticides	167	III	XIX
20.	The colour used in the written materials kept in the containers of pesticides	161	IV	XXI
21.	The quality of the pictures of the pests used in the written materials kept in the containers of the pesticides	151	V	XXIV
22.	Size of the letters of the written materials kept in the pesticide containers	114	VI	XXXIII
23.	Language of the written materials kept in the containers of the pesticides	105	VII	XXXVI
24.	The quality of the various diagrammatic representations used in the written materials kept in the containers of the pesticides	103	VIII	XXXVII
	Total of category	1249		III
	E. Knowledge and Information Constraints			
25.	Lack of knowledge of the respondents (vegetable growers) about the Economic Threshold Limit (ETL) concept of the selected vegetables	330	I	I
26.	Lack of knowledge of the vegetable growers regarding the bio-pesticides	324	II	II
27.	Lack of knowledge of the respondents about the Integrated Pest Management (IPM) techniques for the cultivation of the selected vegetables	323	III	III
28.	Lack of proper information on the judicious use of pesticides	233	IV	X
29.	Lack of knowledge of the vegetable growers regarding the process of diagnosis of the attacking pests	228	V	XI
30.	Lack of information regarding the pesticide application on the selected vegetables	201	VI	XV
31.	Lack of knowledge of the respondents regarding the proper handling procedure of the pesticides	157	VII	XXIII
32.	Lack of knowledge of the vegetable growers regarding the proper pesticide application procedure on the selected vegetables	145	VIII	XXVI
33.	Lack of knowledge of the vegetable growers about the ideal dose of the applied pesticides in the cultivation of the selected vegetables	141	IX	XXVII
34.	Lack of knowledge of the vegetable growers regarding the proper pesticide storage procedure to be maintained by the vegetable growers	137	X	XXIX
35.	Lack of knowledge of the vegetable growers regarding the proper way of disposing off of the date expired, unused pesticide containers	136	XI	XXX
36.	Lack of Knowledge of the vegetable growers regarding the ideal time of the day when the pesticide should be applied	129	XII	XXXI
37.	Lack of knowledge of the vegetable growers regarding the precautions to be taken when the pesticide application was on	113	XIII	XXXIV
38.	Lack of knowledge of the vegetable growers regarding proper way of disposing off of the empty containers of the pesticides	87	XIV	XXXIX
39.	Lack of knowledge of the vegetable growers regarding the ideal crop stage for the pesticide application	85	XV	XXXX
	Total of category	2769		I

Table 1 continued

Sl. No.	Types of constraints	Rank score	Rank within a category	Overall rank
	F. Administrative and Managerial Constraints			
40.	Lack of training of the vegetable growers on the proper use of pesticides	319	I	IV
41.	Non-availability of extension personnel in time	296	II	VI
42.	Malpractices by the sales agents and dealers of pesticide companies	235	III	IX
43.	Lack of loan sanctioning mechanism for the vegetable growers for the cultivation of the selected vegetables	226	IV	XII
44.	Lack of the agricultural extension mechanisms for the selected vegetables	218	V	XIII
45.	Problems created by the middlemen in the wholesale or the retail vegetable market	164	VI	XX
46.	Adulteration of the pesticides by the dealers of pesticide companies	128	VII	XXXII
	Total of category	1586		II

(vegetable growers) about the Economic Threshold Limit (ETL) concept of the cauliflower crops (under the category of knowledge and information constraints) enjoyed the first rank position closely followed by the lack of knowledge regarding the bio-pesticides (under the category of knowledge and information constraints). The lack of knowledge of the respondents about the Integrated Pest Management (IPM) techniques for the cultivation of the cauliflower crops (under the knowledge and information constraints category) enjoyed third position, closely followed by lack of training of the vegetable growers on the proper use of pesticides in the cauliflower production (under category of administrative and managerial constraints). It proved that the lack of knowledge of the respondents about the Economic Threshold Limit (ETL) concept of the cauliflower crops, lack of knowledge regarding the bio-pesticides, the lack of knowledge about the Integrated Pest Management (IPM) techniques for the cultivation of the cauliflower crops and lack of training of the vegetable growers on the IPM technologies in the cauliflower production put the most formidable hurdle regarding the adoption of IPM technologies in the cauliflower production.

Analysis of category wise field level constraints experienced by the respondents regarding the adoption of the IPM technologies in the cauliflower cultivation: Table 1 and 2 clearly indicate that among six different categories of perceived constraints regarding the adoption of IPM technologies in cauliflower cultivation, knowledge and information constraints with a rank score of 2769 enjoyed first rank position, distantly followed by administrative and managerial constraints (with a rank score of 1586) in the second position, technological and communication constraints with a rank score of 1249 in the third position, socio-economic constraint (rank score of 828) in the fourth position and it was interesting to note that situational constraints enjoyed the last position with only 589 rank score. The fact that the knowledge and information constraints held the highest position among different categories of constraints might be because of the reason that the respondents had very poor level of knowledge regarding the adoption of IPM technologies in cauliflower cultivation as well as they had very poor access to the sources of the information regarding the adoption of IPM technologies which is already indicated in the earlier sections. The above results also show that various administrative and managerial lacunae (incompetent extension services, inadequate monitoring of the markets etc.) on the part of the central and state governments contributed to a great extent to the low level of adoption of IPM technologies in cauliflower cultivation among the respondents. The results also revealed that the respondents were technologically ill equipped as well as there were various problems in the communication with the cauliflower farmers to adopt the IPM technologies in their cauliflower cultivation.

It is interesting to note that poor extension services, poor vigilance of the central and state governments on the pesticide market, inadequate and poorly governed vegetable markets etc. led the administrative and managerial constraints to the second position. The results clearly depicted a picture that the third biggest constraint before the respondents was technological and communication constraint regarding the adoption of the IPM technologies in the cauliflower production. This mean that the respondents were technologically ill equipped for adoption of the IPM technologies for the cauliflower crops and the communication channel for making the respondents aware about the technical specifications and information regarding the IPM technologies was poorly developed and to some extent ineffective in nature.

The socio-economic constraints like lack of contact of the respondents with outside world, lack of financial resources, lack of education, small size of land holdings etc. considerably affected the proper use of the pesticides by the respondents in the vegetable cultivation. As a result, socio-economic constraints came at the fourth position among the various categories of constraints regarding the adoption of the IPM technologies.

Table 2. Distribution of various categories of constraints as perceived by the respondents regarding the proper use of pesticides in the cauliflower cultivation

Sl. No.	Categories of constraints	Total rank score	Percentage	Rank position
1.	Knowledge and Information constraints	2769	36.40	I
2.	Administrative and managerial constraints	1586	20.85	II
3.	Technological and Communication constraints	1249	16.42	III
4.	Socio-economic constraints	828	10.88	IV
5.	Situational constraints	640	08.40	V
6.	Infrastructural constraints	536	07.05	VI
	Total	7608	100.00	

It is interesting to note that the infrastructural constraints came at last position with only 07.05 %. This means that there were little grass-root level infrastructural constraints related to the farming operations in general and the cauliflower cultivation in particular like lack of preservation and cold-storage facilities for the harvested cauliflower crops, lack of communication and transport, lack of irrigational facilities, lack of cauliflower market, lack of proper plant protection implements etc. posed little constraints to the respondents in relation to the adoption of the IPM technologies. This is a silver-line of hope among the otherwise bleak scenario.

The present study clearly revealed that the lack of knowledge of the respondents about the Economic Threshold Limit (ETL) concept was the limiting factor in adoption of IPM techniques closely followed by othe factors like lack of knowledge regarding the bio-pesticides, the lack of knowledge about the IPM techniques, closely followed by lack of training of the respondents on the proper use of pesticides. The present study also indicated that awareness enhancing and motivational messages delivered via traditional and mass media in support of an expanded extension service farmer training programme in vegetable IPM could be expected to reduce the constraints as perceived by the vegetable growers regarding the adoption of IPM technologies in cauliflower cultivation and contribute to safer and more profitable vegetable pest control.

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