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Pollen characteristics, pollination behaviour and pollinizer compatibility of some exotic and indigenous almond [*Prunus dulcis* (Miller) D.A. Webb] genotypes

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Key words: almond, compatibility, pollen, pollination behaviour, pollinizer,

Abstract: Pollination is the most critical and complex part of fruit production, particularly in cross pollinated crops like almond, and it is affected by pollen characteristics. In the present study 100% pollen viability was observed when stained by acetocarmine for all considered genotypes, except GP-17 (99%). Optimum stigma receptivity was observed for two days before anthesis, on the day of anthesis and one day afterwards all the stigmas remained receptive. In open pollination, maximum fruit set was noted in Primorskij (32.37%) with minimum in Makhdoom (17.96%). No fruit set was observed in any of the genotypes by self-pollination, confirming the self-incompatible nature of all tested genotypes. In cross pollination, fruit set was found between 0.00 and 42.84% for different cross combinations. Makhdoom, Pranyaj, Shalimar, Waris, Nonpareil, Waris, Waris and Shalimar yielded maximum fruits when cross-pollinated with IXl, Merced, Drake, Primorskij, Pranyaj, Nonpareil, Shalimar, Makhdoom and Waris, respectively. No pollen tube growth was observed in the style when genotypes were crossed with their own pollen. In crosses between IXL and Nonpareil, pollen tube growth was arrested in the styles. In compatible crosses, the pollen tube reached the base of the style after different times following pollination.

1. Introduction

Almond [*Prunus dulcis* (Miller) D.A. Webb], which belongs to the family Rosaceae, is an important edible nut with widespread popularity. Nuts are a rich source of energy and contain high amounts of fat, protein, minerals and vitamins. Presently, the United States of America is the leading producer of almonds and India ranks third as an importer of almonds from the USA after Spain and Germany. In India, almond cultivation is confined mainly to northern areas including the regions of Jammu and Kashmir and high hills of Himachal Pradesh. During 2006-2007, the area under almond cultivation was 16,404 ha with annual production of 15207 MT in Jammu and Kashmir (Directorate of Horticulture, personal communication), whereas in Himachal Pradesh the area was 5766 ha and the production was 1303 MT (Directorate of Horticulture, personal communication).

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Pollination is the most critical and complex part of fruit production, particularly in cross pollinated crops like almond. It involves a complex and sensitive sequence of events and interactions on a morphological, physiological and biochemical level. Climatic conditions and genetic factors of the cultivars have an important impact on pollination. The number of flowers which develop, their interaction and their position within the inflorescence as well as pollen compatibility or incompatibility are some of the determinants for fruiting. The self-incompatibility system in almond limits in-breeding and therefore fails to produce an adequate crop, as a consequence, almond requires pollen from some other compatible cultivar(s) for cross-pollination. Trees do produce abundant bloom but fail to set adequately due to lack of pollination and unfavourable weather conditions. Sometimes cultivars are self unfruitful, and require pollen from other compatible cultivars for fruit production. According to Socias i Company (1992) most almond cultivars are self incompatible and nearly 30% pollinizers are required to have an economic crop (Kester and Griggs, 1959) which necessitates the planting of more than one

cultivar with sufficient overlapping of flowering periods to ensure adequate cross pollination and fruit set. Some cultivar combinations also exhibit cross incompatibility (Talaie and Imani, 1998). Knowledge of the pollen characteristics and pollination behaviour of different cultivars is therefore an important prerequisite for the successful cultivation of almonds. Therefore, studies on pollen characters and pollination behaviour of almond genotypes were undertaken to find the compatibility groups between some exotic and indigenous selections of almond.

2. Materials and Methods

The study of pollen characteristics, pollination behaviour and pollinizer compatibility of almond genotypes was conducted on four introductions from the USA (IXL, Merced, Drake and Nonpareil), two from Ukraine (Primorskij and Pranyaj) and seven indigenous selections from India (Shalimar, Makhdoom, Waris, GP 10, GP 14, GP 17 and GP 19) at the Central Institute of Temperate Horticulture, Srinagar, Jammu and Kashmir during 2005-06 and 2006-07. The experimental site was located at a latitude of 34º 05' North and longitude 74º 50' East, and at an altitude of 1640 m above mean sea level. The plants were five to six years old and were laid out at a spacing of 4 × 4 m.

Meteorological data are reported in Appendix 1.

Pollen studies

Pollen viability was studied by staining with acetocarmine (2% solution) as per the method suggested by Das (1995). *In vitro* pollen germination in sucrose solution was studied in 5, 10, 12.5, 15 and 20% sucrose concentrations. Pollen tube growth was assessed for each genotype under a microscope after 24 hr of incubation at 22±2°C. The pollen grains with a pollen tube at least two times longer than pollen size were considered germinated.

Pollination studies

Stigma receptivity was studied in unopened, about-to-open buds and opened flowers with the help of a magnifying lens to visualize the presence of exudates (watery fluid) on the stigmatic surface indicating the stigma to be receptive. To monitor fruit set under open pollination, four shoots in all the directions were selected for each genotype and the number of flowers on each shoot was counted. Shoots were left open for natural pollination to occur. Percent fruit set was calculated at harvest. Under natural self pollination (by bagging) branches with flower buds were selected and all the opened flowers and shrivelled buds were removed. Numbers of buds at popcorn stage left on each shoot were counted. These shoots were covered with muslin cloth bags, tied at the lower end and properly labelled. After 35-40 days the bags were removed and percent fruit set was determined at harvest. For hand self-pollination flowers were emasculated at balloon or popcorn stage by removing the entire calyx and corolla, leaving only the pistil. Whole branches with emasculated buds were then covered with muslin cloth bags and properly tied and labelled. Pollination of the emasculated buds was done on the following or next day with the freshly collected pollen of the same genotype. The pollen was applied to the receptive stigma with the help of a camel hair brush. After pollination the bags were again placed onto the branches. For cross pollination studies, crosses were made in the nine genotypes (exotic and indigenous cultivars) in all the possible combinations. The procedure followed to determine the extent of cross pollination was the same as discussed for self pollination studies, except that the pollen used for crossing belonged to different cultivars. Fruit set was counted one month after pollination and percent fruit set was calculated as fruit harvested/flower pollinated × 100.

In-vivo pollen tube growth was studied according to the procedure given by Ortega *et al*. (2004). A sample of five pistils was collected at 24, 48, 72, 96 and 120 hr after hand self-pollination and immediately fixed in FAA: ethanol 500 ml; formaldehyde 100 ml; acetic acid 50 ml; distilled water 350 ml. Pollen tube growth in the style was viewed under fluorescent microscope (Olympus BX-40 model) and the extent of pollen tube growth penetration into the style at different intervals of time after pollination was recorded.

The data recorded were analysed statistically as per the methods described by Panse and Sukhatme (1978).

3. Results and Discussion

Almond has very low chilling requirements, so trees blossom in early spring, hence pollination and fertilization are negatively affected by the low temperature and rain which may prevail at that time. Low temperatures during the flowering period can prevent germination of pollen on the stigma or prevent development of pollen tubes in style. Further pollen can be washed away by rains and bees are not active at low temperatures. These factors can create some hindrance in the pollination and fertilization of almond, and subsequently affect fruit set percentage and final yield. Therefore, fruit set under open pollination is influenced by a number of factors such as genetic makeup of cultivars, nearness or distance from a compatible pollen source, prevailing weather conditions, bee activity, stigma receptivity, pollen germination, pollen tube growth and fertilization process.

Pollen viability

Pollen viability tested with acetocarmine (2%) in different almond genotypes revealed that all the genotypes had 100% pollen viability, except for GP 17 in which 99.00% pollen viability was recorded differing

significantly from the others. Similar higher values for pollen viability accessed through acetocarmine (2%) were observed previously by Das (1995). Under *in-vitro* pollen germination the percent germination varied significantly in different concentrations of sucrose (Table 1). A significant increase in pollen germination percentage was recorded up to 15% sucrose solution and thereafter it decreased significantly in 20% solution. Peak pollen germination was recorded in either 12.5 or 15% solution for most of the genotypes. In 5% sucrose solution pollen germination ranged from 12.67% in GP-19 to 76.90% in Waris. In 10% solution it varied from 40.40 to 87.83% for GP-14 and 'Nonpareil', respectively. This latter genotype gave values of 94.05, 96.45 and 90.05% germination in 12.5, 15 and 20% sucrose solution, respectively, whereas, GP 19 demonstrated low pollen germination of 12.67, 47.59, 72.54, 79.80 and 72.30% respectively at similar levels of sucrose concentrations. The variation in pollen germination in different genotypes under the same sucrose concentration may be attributed to their varied genetic constitution. Dhillon *et al*. (1982) in 'California Papershell' almond found the highest (80.36%) pollen germination in 20% sucrose solution. Eti (1994) found 10 to 15% sucrose concentrations quite suitable for almond pollen germination.

Stigma receptivity

In almond, the importance of the length of the period of flower receptivity to obtain good yield was first described by Griggs and Iwakiri (1964). The percent of flowers showing stigma receptivity at different durations before and after anthesis in various genotypes is presented in Table 2. Two days prior to anthesis stigma

Table 1 - *In vitro* pollen germination of different cultivars of *Prunus dulcis* (Miller) D.A. Webb genotypes in different sucrose concentrations. Values are expressed in percentage

Genotypes	Sucrose solution (%)				
	5	10	12.5	15	20
IXL	37.88 e	55.91 d	86.03 a	83.10 b	77.31 c
Merced	19.84 d	65.72 b	83.89 a	85.88 a	56.99 c
Drake	18.18 e	52.86 d	85.72 b	92.44 a	80.57 b
Primorskij	41.13 e	72.77 d	85.95 c	96.44 a	93.50 c
Pranyaj	45.19 d	64.73 b	88.01 a	87.17 a	59.10 c
Nonpareil	34.68 e	87.83 d	94.05 b	96.45 a	90.05 c
Shalimar	28.80 d	77.62 c	86.85 a	80.47 b	78.52 c
Makhdoom	16.90 e	55.41 d	81.05 b	84.00 a	64.53 c
Waris	76.90 c	78.13 c	91.30 a	92.71 a	86.08 b
GP-10	39.92 e	48.49 d	81.44 b	85.99 a	73.98 c
GP-17	31.92 e	53.21 d	80.05 b	82.66 a	70.99 c
GP-19	12.67 d	47.59 c	72.54 b	79.80 a	72.30 b
GP-14	23.98 d	40.40 c	75.81 b	79.75 a	75.32 b
$CD_{0.05}$					
	Genotypes (G)				3.27
	Concentration (C)				2.03
	Genotypes x Concentration (GxC)				7.33

Table 2 - Stigma receptivity in different almond genotypes

Genotypes	Sucrose solution (%)				
	-2 days	-1 day	0 day	+1 day	+2 day
IXL	52	76	100	100	82
Merced	68	72	100	100	76
Drake	72	88	100	100	68
Primorskij	32	64	100	100	76
Pranyaj	44	84	100	100	80
Nonpareil	56	92	100	100	72
Shalimar	36	60	100	100	88
Makhdoom	32	56	100	100	76
Waris	44	84	100	100	80
GP 10	36	60	100	100	72
GP 17	40	84	100	100	76
GP 19	28	72	100	100	72
GP 14	36	76	100	100	72

(-) Before anthesis, (0) On anthesis, (+) After anthesis.

receptivity varied from 28 to 72%; maximum receptivity was observed in Drake (72%) and the minimum value (28%) was observed for seedling selection GP-19. One day before anthesis, stigma receptivity varied from 60% in Shalimar and GP-10 to 92% in Nonpareil. In addition, it was observed that all the stigmas were receptive on the day of anthesis and remained so for the second day after anthesis; receptivity decreased thereafter for all genotypes. The work of Ortega *et al*. (2007) is in accordance with the present findings.

Pollination studies

Fruit set data under open pollination and self pollination for the years 2006 and 2007 is presented in Table 3 and revealed that it varied according to the genotype and the year. The analysis of variance showed that there were significant differences for all the genotypes as well as for the interaction among the genotypes under open pollination. The average effect of the year was observed to be significant as average set for 2006 (26.42%) was higher than that of second year (22.41%). The principal reason for this difference is that the average temperature was higher in March 2006 than it was in March 2007: in 2006 the minimum temperature was above 1°C for all the days while it was 0°C or less in ten out of first 17 days of March 2007. Low temperature along with snowfall on 12 and 13 March damaged the blossom of early flowering varieties like Makhdoom, Shalimar and GP-10. Maximum fruit set was recorded for 'Primorskij' (32.37%) which was at par with 'Pranyaj' (30.96%) and 'Drake' (30.51%) and significantly higher than 'IXL' (26.27%), 'Nonpareil' (26.53%), 'Waris' (25.17%) and 'Shalimar' (25.02%). Minimum fruit set was observed for cultivar Makhdoom (17.96%). Maximum fruit set in 2006 was recorded for cultivar Shalimar (32.99%) and was at par with 'Pranyaj' (32.25%) and 'Makhdoom' (30.15%) whereas, the minimum value was observed for Merced (21%). In 2007, 'Primorskij' had the highest fruit set (36.57%) and the minimum recorded was 5.77% for 'Makhdoom'; both these values differed significantly from all other genotypes. Low fruit set in early blooming cultivars, due to spring frost, was reported previously by Connell (2000). For commercial fruit production fruit set in almond must range between 25 and 40% of the initial number of flowers (Kester and Griggs, 1959). Low fruit set values for both the years of the present study can be further attributed to the non availability of supplemented pollinators (bee hives) during bloom for adequate pollination. Variation in fruit setting behaviour under open pollination was reported by Talaie and Imani (1998), Ak *et al*. (2001) and Socias i Company *et al*. (2005).

The degree of self compatibility in almond genotypes, assessed by observing fruit set following unassisted self pollination (bagging), revealed that there was no fruit set following bagging in any of the genotypes, thus indicating total self-incompatibility. Almond shows a gametophytic self incompatibility system (Socias i Company, 1992) controlled by a multiallelic locus, known as locus 'S' (Gagnard, 1954). This implies that the pollen tube of a flower of the same tree, the same cultivar and sometimes of certain other cultivars, will not grow down the style (Kester, 1969). In this regard, most almond breeding programmes have fostered the development of self-compatible cultivars to overcome the problems related to cross-pollination of a mostly self incompatible species such as almond

Table 3 - Fruit set of *Prunus dulcis* (Miller) D.A. Webb genotypes calculated for open and self pollination

Genotypes	Fruit set (%)					
	Open pollination			Selfing by bagging		
	2006	2007	Pooled	2006	2007	Pooled
IXL	27.20	25.33	26.27 c	0.00	0.00	0.00
Merced	21.00	21.28	21.14 de	0.00	0.00	0.00
Drake	26.93	34.09	30.51 b	0.00	0.00	0.00
Primorskij	28.16	36.57	32.37 a	0.00	0.00	0.00
Pranyaj	32.25	29.67	30.96 ab	0.00	0.00	0.00
Nonpareil	24.23	28.83	26.53 c	0.00	0.00	0.00
Shalimar	32.99	17.05	25.02 c	0.00	0.00	0.00
Makhdoom	30.15	5.77	17.96 f	0.00	0.00	0.00
Waris	28.70	21.65	25.17 c	0.00	0.00	0.00
GP-10	22.89	15.04	18.97 f	0.00	0.00	0.00
GP-17	24.28	19.42	21.85 d	0.00	0.00	0.00
GP-19	21.97	16.99	19.48 ef	0.00	0.00	0.00
GP-14	22.67	19.63	21.15 de	0.00	0.00	0.00
Mean	26.42	22.41	24.41	0.00	0.00	0.00
CD(0.05)	2.07	3.24				
Pooled CD 0.05						
G		1.85				
Y		0.72				
GxY		2.63				

(Social i Company, 2002). Our results are in accordance with a previous report of Kester *et al*. (1994) wherein a low level of fruit set was recorded following hand pollination in otherwise self-incompatible cultivars.

Cross pollination

Cross pollination is essential in almond orchards as most of the cultivars are self-incompatible. In order to guarantee good pollination, at least two cultivars must be inter planted which not only coincide in flowering time, but are also cross-compatible. As the commercial part of the fruit is the seed, a decrease in the number of pollinated flowers often results in crop reduction (Kester and Griggs, 1959). Thus rainy, windy or cold weather interferes with pollination by inhibiting bee foraging (Socias i company *et al*., 1996).

In the current work, nine almond cultivars were pollinated with one another in all possible combinations. The data pertaining to fruit set following cross pollination is presented in Table 4. The maximum fruit set value was recorded for cross-combination IXL x Makhdoom (42.84%) and was at par with Nonpareil x Pranyaj (39.69%), Pranyaj x Nonpareil (38.55 %), Primorskij x Waris (36.29%) and Waris x Shalimar (35.88%). Minimum fruit set was observed when IXL was crossed with its own pollen (0.95%). No fruit set was recorded in Merced, Makhdoom and Shalimar when they were pollinated with their own pollen. The data further revealed that when IXL was used as pollinizer, maximum fruit set was recorded in the cultivar Pranyaj (34.47%) followed by Waris (25.82%) and Primorskij (23.87%). When Merced was used as a pollinizer, maximum fruit set was observed with Pranyaj (34.71%) followed by Drake, Waris, IXL, Primorskij and 'Nonpareil' as 28.37, 21.88, 21.29, 20.94 and 14.09%, respectively when Merced was used as pollinizer. No fruit set was observed when Merced was pollinated by its own pollen. Fruit set ranged between 0.88 and 32.67% in different genotypes when Drake was used as pollinizer: the maximum was with Pranyaj (32.67%) which was statistically at par with fruit set in Nonpareil (29.10%) but differed significantly from IXL (25.19%), Waris (20.59%), Merced (19.69%) and Primorskij (18.79%); minimum fruit set (0.88%) was observed when Drake was pollinated by its own pollen. In addition, when Primorskij was used as a pollinizer, the maximum fruit set was observed with IXL (29.29%), at par with Nonpareil (27.44%).

Fruit set ranged between 2.44 and 38.55% in different genotypes when Nonpareil was used as pollinizer. Maximum fruit set was noted in Pranyaj (38.55%) followed by Primorskij (28.12%), Drake (27.28%), Waris (21.48%) and Merced (14.01%). Minimum fruit set was observed when Nonpareil was pollinated by its own pollen (2.44%), statistically at par with fruit set in IXL (3.05%) when pollinated with Nonpareil pollen. When Shalimar was used as pollinizer the highest

Table 4 - Fruit set (%) in *Prunus dulcis* (Miller) D A. Webb genotypes obtained in different inter-varietal crosses

Male \ Female	IXL	Merced	Drake	Primorskij	Pranyaj	Nonpareil	Shalimar	Makhdoom	Waris	Mean	$CD_{0.05}$
1XL	0.95	16.16	18.16	23.87	34.47	2.81	X	X	25.82	17.46	1.70
Merced	21.29	0.00	28.37	20.94	34.71	14.09	X	X	21.88	19.94	2.05
Drake	25.19	19.69	0.88	18.79	32.67	29.10	X	X	20.59	20.99	2.75
Primorskij	29.29	22.32	13.75	1.27	21.56	27.44	X	X	24.50	20.02	2.11
Pranyaj	13.72	32.24	12.64	19.37	1.57	39.69	X	X	24.35	20.50	3.30
Nonpareil	3.05	14.01	27.28	28.12	38.55	2.44	X	X	21.48	19.27	3.11
Shalimar	21.64	28.37	29.37	28.29	28.89	29.52	0.00	14.23	35.88	24.06	3.10
Makhdoom	42.84	23.95	28.55	31.60	28.44	26.06	23.33	0.00	25.13	25.46	2.81
Waris	24.14	18.76	20.70	36.29	35.91	31.90	28.97	15.85	0.91	23.71	3.59
Mean	20.24	19.42	19.97	23.17	28.53	22.56	17.43	10.03	22.28		
$CD_{0.05}$	2.22	1.32	2.38	2.19	5.16	4.90	2.29	2.09	1.77		
$CD_{0.05}$ Genotypes (G)	=	6.24									

X = crosses not attempted.

value of fruit set was observed in Waris (35.88%), which differed significantly from other cultivars. The data also showed that no fruit set was observed in cultivar Shalimar when pollinated by its own pollen. When Makhdoom was used as a pollinizer, the highest fruit set was recorded in IXL (42.84%) followed by Primorskij (31.60%). Drake, Pranyaj, Nonpareil, Waris, Merced and Shalimar had fruit set of 28.55, 28.44, 26.06, 25.13, 23.95 and 23.33%, respectively, which were at par with each other when pollinated with Makhdoom. No fruit set was observed when Makhdoom was pollinated by its own pollen, as was also the case when Waris was pollinated by its own pollen. The mean fruit set induced by different pollinizers ranged from 17.46 to 25.46%. Makhdoom, as a pollinizer, affected the highest average fruit set (25.46%), followed by Shalimar (24.06%), Waris (23.71%) and Drake (20.99%). The minimum fruit set value was observed when IXL (17.46%) was used as pollinizer. Among female parents, the maximum fruit set was observed in Pranyaj (28.53%), followed by Primorskij (23.17%) and Waris (22.28%). Cultivar Makhdoom (10.03%) had the lowest fruit set value as female parent when pollinated with different pollinizers. Cross-incompatibility of IXL with Nonpareil had been previously established (Gagnard, 1954) and the present study revealed low fruit set in IXL and Nonpareil crosses, thus supporting the findings. The rest of the cultivars showed optimum fruit set with crossing (13.72 to 42.84%) thus indicating cross-compatibility between the cultivars. The differences in fruit set may be due to various factors such as genotypic differences of the cultivars under study, response of genotypes to different pollen sources, ovary degeneration, unfavourable climatic conditions during flowering, flower sterility and heterostyly. Other workers (Talaie and Imani, 1998; Dalal *et al*., 2004) reported similar results.

In vivo pollen tube growth

In temperate tree crops the rate of pollen tube growth to the base of the style is quite low (Sedgley, 1982). The present studies, pertaining to *in-vivo* pollen tube growth, have revealed that in all the cultivars which were pollinated by their own pollen, pollen tube growth was arrested in the style. Observations regarding *in vivo* pollen tube growth are presented in Table 5 a and 5 b. The findings indicate that in all crosses where pollen of the same cultivar was used for pollination, the pollen tube failed to reach up to the base of the style. The observations revealed that in IXL the pollen tube reached the base of the style after 120 hr when crossed with pollen from Merced and Shalimar, whereas it took 96 hr with Pranyaj and Makhdoom. The pollen tube reached the style after 72 hr when IXL was pollinated by Drake, Primorskij and Waris, while the pollen tube failed to grow in the style of IXL when Nonpareil was used as pollen source. In Merced it was observed that the pollen tube reached the base of the style after 72 hr when crossed with Shalimar and Makhdoom; with IXL, Drake, Nonpareil and Waris it reached the same point after 96 hr. Primorskij and Pranyaj pollen tubes reached the base after 120 hr. Likewise, the study of Drake styles revealed that pollen tubes reached the base after 72 hr of pollination with Merced and Waris whereas with other cultivars it reached after 96 hr. The pollen tube of IXL, Nonpareil and Waris reached up to the base of styles of Primorskij after 72 hr of pollination; with Merced, Drake, Shalimar, Makhdoom and Pranyaj, it reached after 96 hr. It was also revealed that in Pranyaj the pollen tube reached up to the base of the style after 72 hr when pollinated with IXL, Merced, Drake and Shalimar while with others it took 96 hr. *In vivo* pollen tube growth in Nonpareil revealed that pollen of Drake Primorskij, Pranyaj and Makhdoom reached the earliest (i.e. 72 hr) after pollination, whereas with other pollinzers it took 96 hr to reach the base. In cultivar Shalimar it was observed that the pollen tube reached up to the base of the style after 96 hr when crossed with Makhdoom and with Waris it reached after 120 hr. Similarly, in Makhdoom the pollen tube reached up to the base after 120 hr and 96 hr when crossed with Shalimar and Waris, respectively. The pollen tube reached the base of the style at different durations in Waris. It took 72 hr for IXL, Drake, and Pranyaj pollen whereas, with Merced, Primorskij, Nonpareil, Shalimar and Makhdoom it took 96 hr after pollination. These findings, along with fruit set data, confirm the self incompatibility of cultivars under study. Similar results were observed by Ak *et al*. (2001). These authors found that the rejection of incompatible male gametophyte occurred on the stigma, as well as in the style. Similarly in pistils of Nonpareil and IXL none of the pollen tubes reached the base of the pistil when they were inter-pollinated, thus confirming the cross incompatibility between these two cultivars.

However, in the compatible pollination crosses, the pollen tube reached the base of the style after different durations of pollination. The difference did not affect the compatibility relationship of the pollinations. The observed differences must be mostly attributed to the interaction of weather conditions at the time of pollination and thereafter. Temperature is an important component for pollen tube growth and the most suitable temperature for pollen tube growth in almond is 12-13°C, and under these temperatures the pollen tube can reach the ovarium within three to four days (Loreti and Viti, 1984). Moreover, pistils may react differently to different pollen sources. Overall, it generally took three to five days for pollen tubes to reach the base of the pistil in otherwise compatible pollination. The present findings are in consonance with those of Ak *et al*. (2001) and Das and Kumar (2004) who reported that pollen tubes reached the base of pistils after four or five days of pollination in almond. Other *in vivo* pollen tube

Table 5 a - *In vivo* pollen tube growth in styles of *Prunus dulcis* (Miller) D.A. Webb genotypes obtained in different inter-varietal crosses

Female / Pollinizer	I X L						Merced						Drake						Primorskij						Pranyaj					
	Pollen tube penetration (Style length)					Compatibility status	Pollen tube penetration (Style length)					Compatibility status	Pollen tube penetration (Style length)					Compatibility status	Pollen tube penetration (Style length)					Compatibility status	Pollen tube penetration (Style length)					Compatibility status
	24 hr	48 hr	72 hr	96 hr	120 hr		24 hr	48 hr	72 hr	96 hr	120 hr		24 hr	48 hr	72 hr	96 hr	120 hr		24 hr	48 hr	72 hr	96 hr	120 hr		24 hr	48 hr	72 hr	96 hr	120 hr	
IXL	X	X	X	X	X	Incompatible	1/4	2/4	3/4	at the base		Compatible	1/4	2/4	3/4	at the base		Compatible	1/4	3/4	at the base			Compatible	1/4	2/4	at the base			Compatible
Merced	X	1/4	2/4	3/4	at the base	Compatible	1/4	X	X	X	X	Incompatible	1/4	3/4	at the base			Compatible	1/4	2/4	3/4	at the base		Compatible	1/4	2/4	at the base			Compatible
Drake	1/4	2/4	at the base			Compatible	1/4	2/4	3/4	at the base		Compatible	1/4	X	X	X	X	Incompatible	1/4	2/4	3/4	at the base		Compatible	1/4	2/4	at the base			Compatible
Primorskij	1/4	3/4	at the base			Compatible	1/4	1/4	2/4	3/4	at the base	Compatible	1/4	2/4	3/4	at the base		Compatible	1/4	X	X	X	X	Incompatible	1/4	1/4	2/4	at the base		Compatible
Pranyaj	1/4	2/4	3/4	at the base		Compatible	1/4	2/4	2/4	3/4	at the base	Compatible	1/4	2/4	3/4	at the base		Compatible	1/4	2/4	3/4	at the base		Compatible	X	X	X	X		Incompatible
Nonpareil	X	1/4	X	X	X	Incompatible	1/4	2/4	3/4	at the base		Compatible	2/4	3/4	3/4	at the base		Compatible	1/4	3/4	at the base			Compatible	1/4	1/4	3/4	at the base		Compatible
Shalimar	1/4	2/4	3/4	3/4	at the base	Compatible	1/4	3/4	at the base			Compatible	1/4	2/4	3/4	at the base		Compatible	1/4	2/4	3/4	at the base		Compatible	1/4	3/4	at the base			Compatible
Makhdoom	1/4	1/4	2/4	at the base	at the base	Compatible	1/4	3/4	at the base			Compatible	1/4	2/4	3/4	at the base		Compatible	1/4	2/4	3/4	at the base		Compatible	1/4	2/4	3/4	at the base		Compatible
Waris	1/4	2/4	at the base			Compatible	1/4	2/4	3/4	at the base		Compatible	2/4	3/4	at the base			Compatible	1/4	3/4	at the base			Compatible	1/4	2/4	3/4	at the base		Compatible

Table 5 b - *In vivo* pollen tube growth in styles of *Prunus dulcis* (Miller) D.A. Webb genotypes obtained in different inter-varietal crosses

Female / Pollinizer	Nonpareil						Shalimar						Makhdoom						Waris					
	Pollen tube penetration (Style length)					Compatibility status	Pollen tube penetration (Style length)					Compatibility status	Pollen tube penetration (Style length)					Compatibility status	Pollen tube penetration (Style length)					Compatibility status
	24 hr	48 hr	72 hr	96 hr	120 hr		24 hr	48 hr	72 hr	96 hr	120 hr		24 hr	48 hr	72 hr	96 hr	120 hr		24 hr	48 hr	72 hr	96 hr	120 hr	
IXL	1/4	1/4	X	X	X	Incompatible	-	-	-	-	-	-	-	-	-	-	-	-	1/4	2/4	at the base			Compatible
Merced	1/4	2/4	3/4	at the base		Compatible	-	-	-	-	-	-	-	-	-	-	-	-	1/4	2/4	3/4	at the base		Compatible
Drake	2/4	3/4	at the base			Compatible	-	-	-	-	-	-	-	-	-	-	-	-	1/4	2/4	at the base			Compatible
Primorskij	1/4	3/4	at the base			Compatible	-	-	-	-	-	-	-	-	-	-	-	-	1/4	2/4	3/4	at the base		Compatible
Pranyaj	1/4	3/4	3/4	at the base		Compatible	-	-	-	-	-	-	-	-	-	-	-	-	1/4	3/4	at the base			Compatible
Nonpareil	X	1/4	X	X	X	Incompatible	-	-	-	-	-	-	-	-	-	-	-	-	1/4	2/4	3/4	at the base		Compatible
Shalimar	1/4	2/4	3/4	at the base		Compatible	X	X	X	X	X	Incompatible	1/4	1/4	2/4	3/4	at the base	Compatible	1/4	2/4	3/4	at the base		Compatible
Makhdoom	1/4	3/4	at the base			Compatible	1/4	2/4	3/4	at the base		Compatible	X	X	X	X	X	Incompatible	1/4	2/4	3/4	at the base		Compatible
Waris	1/4	2/4	3/4	at the base		Compatible	1/4	2/4	3/4	3/4	at the base	Compatible	1/4	2/4	3/4	at the base		Compatible	X	1/4	X	X	X	Incompatible

(-) No observation recorded.
(X) No pollen tube growth observed.

growth studies in almond indicate that pollen tubes require two to four days or more to reach the ovule (Pimienta and Polito, 1983; Polito *et al*., 1996).

4. Conclusions

The present study has shown that all the considered genotypes had optimum pollen viability and confirmed their self-incompatible nature. Furthermore, examination of cross pollination has indicated that there is a potential to renew the declining almond industry of India by exploiting the existing diverse gene pool. Exotic varieties can be used for commercial cultivation or in future breeding programs to develop varieties suited to local conditions.

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APPENDIX I
Meteorological Data
March, 2006

Days	Temperature		RH (%)	RH (%)	Rain (mm)	Weather	
	Maximum	Minimum				7:30 hr	14:30 hr
	I	II	I	II		I	II
1	15.00	1.00	85.00	78.00	0.00	Clear	Clear
2	16.00	2.50	94.00	69.00	0.00	Clear	Clear
3	17.50	2.00	85.00	53.00	0.00	Clear	Clear
4	16.00	3.00	86.00	56.00	0.00	Clear	Clear
5	15.80	2.60	94.00	63.00	1.00	Clear	Clear
6	15.60	3.80	81.00	77.00	0.00	Cloudy	Rain
7	13.00	2.50	89.00	77.00	5.80	Cloudy	Cloudy
8	14.00	0.50	94.00	56.00	0.00	Clear	Cloudy
9	16.00	3.00	85.00	60.00	0.00	Cloudy	Clear
10	16.00	3.30	75.00	45.00	0.00	Cloudy	Clear
11	19.00	1.50	88.00	46.00	0.00	Clear	Clear
12	20.00	0.80	89.00	42.00	0.00	Clear	Clear
13	16.00	3.00	75.00	73.00	0.00	Clear	Rain
14	11.00	6.40	90.00	72.00	1.80	Cloudy	Rain
15	13.00	4.40	87.00	87.00	12.20	P. cloudy	Rain
16	11.00	0.50	86.00	74.00	7.40	Cloudy	Rain
17	14.00	1.20	81.00	60.00	2.20	Cloudy	Clear
18	16.50	0.40	78.00	67.00	0.00	Clear	Cloudy
19	9.00	5.20	78.00	74.00	0.00	Cloudy	Cloudy
20	6.00	5.00	94.00	92.00	13.00	Rain	Cloudy
21	14.00	5.00	94.00	58.00	4.80	Cloudy	Clear
22	17.50	3.40	87.00	53.00	0.00	Clear	Clear
23	18.50	4.60	73.00	46.00	0.00	Clear	Clear
24	11.50	4.50	75.00	69.00	0.00	Cloudy	Rain
25	11.40	5.50	95.00	66.00	8.20	Rain	Rain
26	15.00	6.50	75.00	68.00	1.60	Cloudy	Cloudy
27	14.50	6.50	92.00	68.00	4.20	Cloudy	Cloudy
28	16.50	5.60	85.00	56.00	4.00	Cloudy	Clear
29	20.00	3.00	95.00	39.00	0.00	Clear	Clear
30	20.50	4.00	75.00	39.00	0.00	Clear	Clear
31	21.60	9.50	60.00	38.00	0.00	Cloudy	Clear
	15.20	3.57	84.51	61.96	66.20		

Year 2006

Month	Temperature		RH%	RH%	Rain (mm)
	Maximum	Minimum			
January	4.07	-1.82	92.19	84.06	168.10
February	12.59	2.38	90.71	72.75	53.20
March	15.20	3.57	84.52	61.97	66.20
April	20.94	5.69	71.30	45.17	55.50
May	28.27	11.45	74.26	53.06	38.60
June	27.96	13.41	79.43	65.00	35.80
July	31.12	17.91	77.35	63.32	151.60
August	28.31	17.26	86.80	64.35	149.20
September	25.05	11.23	88.86	69.53	108.00
October	22.13	6.78	92.16	63.94	19.00
November	14.17	2.79	92.00	67.67	82.50
December	7.47	-0.54	93.06	76.23	94.90

March, 2007

Days	Temperature		RH (%)	RH (%)	Rain (mm)	Weather	
	Maximum	Minimum				7:30 hr	14:30 hr
	I	II	I	II		I	II
1	11.40	2.70	88.00	59.00	5.40	Cloudy	Cloudy
2	12.00	-1.20	100.00	43.00	0.00	Clear	Clear
3	10.00	2.50	73.00	64.00	0.00	Cloudy	Rain
4	10.50	1.00	97.00	75.00	3.60	Cloudy	Cloudy
5	13.50	0.20	94.00	47.00	3.00	Cloudy	Clear
6	14.50	-1.00	96.00	35.00	0.00	Clear	Clear
7	16.00	-1.00	90.00	35.00	0.00	Clear	Clear
8	17.00	-0.50	87.00	39.00	0.00	Clear	Clear
9	13.50	1.50	72.00	53.00	0.00	Clear	Cloudy
10	15.20	3.40	82.00	46.00	0.00	Cloudy	Clear
11	9.00	4.00	71.00	78.00	0.00	Cloudy	Rain
12	2.00	0.00	93.00	90.00	35.00	Snow	Snow
13	7.50	0.00	100.00	77.00	165.00	Snow	Clear
14	9.00	-1.50	93.00	64.00	5.40	Cloudy	Clear
15	11.50	-2.00	90.00	50.00	0.00	Clear	Clear
16	11.50	-2.60	69.00	55.00	0.00	Clear	Clear
17	10.00	-0.50	85.00	54.00	0.00	Cloudy	Cloudy
18	11.40	3.80	92.00	76.00	2.40	Cloudy	Cloudy
19	9.50	4.50	90.00	87.00	0.00	Cloudy	Rain
20	5.50	4.50	97.00	91.00	25.40	Rain	Rain
21	6.00	3.20	92.00	97.00	29.80	Cloudy	Cloudy
22	11.50	3.80	94.00	66.00	6.80	Cloudy	Clear
23	15.50	4.00	89.00	52.00	0.00	P. cloudy	Clear
24	16.50	3.40	75.00	53.00	0.00	Clear	Cloudy
25	18.00	1.80	73.00	46.00	0.00	Clear	Clear
26	19.40	2.40	62.00	49.00	0.00	Clear	Clear
27	22.50	3.50	79.00	45.00	0.00	Clear	Clear
28	22.50	3.50	61.00	42.00	0.00	Clear	Clear
29	23.50	5.00	65.00	38.00	0.00	Clear	Clear
30	25.00	6.60	73.00	42.00	0.00	Clear	Clear
31	25.00	8.50	80.00	38.00	0.00	P. cloudy	Clear
	13.73	2.04	83.93	57.61	281.8		

Year 2007

Month	Temperature		Rh%	Rh%	Rain (mm)
	Maximum	Minimum			
January	9.36	-2.95	88.8	53.80	8.90
February	11.11	1.8	90.03	64.43	50.50
March	13.74	2.04	83.94	57.61	281.8
April	24.99	6.87	70.90	39.23	1.40
May	25.13	10.52	77.32	53.29	44.50
June	28.54	14.55	75.80	55.27	49.70
July	29.90	16.86	82.00	56.58	57.60
August	29.79	16.53	81.84	57.39	46.40
September	26.88	12.35	85.43	58.97	23.20

Source (SASA).

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Radiation-induced chromosomal aberrations in grape phylloxera

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Key words: chromosome aberrations, grape phylloxera, irradiation, reproduction.

Abstract: Chromosomal aberrations in phylloxera females induced by different doses of gamma irradiation were detected. The results showed that the chromosomes of all tested embryos of irradiated phylloxera had aberrations, regardless of dose. When phylloxera nymphs were irradiated, the chromosomal number on the metaphase plate of some embryos' cells was increased. The result indicated that the chromosomal aberrations influenced the mortality, longevity and reproduction of phylloxera. Eight autosomal chromosomes were identified according to their length. Additionally, the karyotype of irradiated and unirradiated populations of local phylloxera strain was defined.

1. Introduction

Grape phylloxera, *Daktulosphaira vitifoliae* Fitch (Homoptera: Phylloxeridae), an aphid-like gall-forming parasite, is one of the most destructive insect pests of cultivated grape *Vitis vinifera* L. world wide. Grape phylloxera causes direct damage to grapevine by forming damaging root galls. These galls are metabolically active organs suited to match the nutritional requirements of phylloxera and can support populations with high reproductive rates. Granett *et al*. (1985) reported that there was frequent decline of commercial vineyards as result of this pest, and consequently losses of quality and yield of grapes.

The use of resistant rootstocks is the most common and effective means of managing phylloxera. Our previous studies showed that some rootstocks were more resistance than others to grape phylloxera (Makee *et al*., 2003). However, for unknown reasons, the resistance of some rootstocks may break down and farmers must replant vineyards (Granett *et al*., 1983; Song and Granett, 1990; De Benedictis and Granett, 1993). Therefore, additional ways to control this pest should be considered.

Sanitation and quarantine can be considered as required procedures to prevent the movement of this serious pest. Insecticides and hot water dip treatments are used as quarantine treatments (Granett *et al*., 2001). Ionizing radiation has been recognized as an alternative method for treating agricultural products to overcome quarantine barriers in trade (FAO, 2003). Irradiation treatment does not influence the quality of many commodities; it is reasonably safe to the consumers and environment. Previously, several authors have presented reviews of this subject (Hallman, 1998; Johnson and Marcotte, 1999; Hallman, 2000, 2001).

Irradiation has been successfully used for the control of many insect pests such as codling moth *Cydia pomonella* (L.), beetle *Prionolplus reticularis* White (Lester *et al*., 2000), apple maggot, *Rhagoletis pomonella* (Walsh), the borer *Eucosma notanthes* Meyrick (Lin *et al*., 2003), coconut scale *Aspidiotus destructor* Signoret (Follett, 2006), the weevil *Sternochetus mangiferae* (F.) (Follett, 2001), cigarette beetle, *Lasioderma serricorne* (F.) (Hu *et al*., 2002), and the rice weevil *Sitophilus zeamais* Motschulsky (Hu *et al*., 2003).

Makee *et al*. (2008) proposed that gamma irradiation could be economically very useful in quarantine treatments against phylloxera. The results showed that the percentage of matured phylloxera females and fecundity were markedly reduced when higher doses of gamma irradiation were used (Makee *et al*., 2008). However, to our knowledge efforts of associate performance parameters of irradiated phylloxera with chromosomal rearrangements have not yet been studied. Therefore, the purpose of the present research was to detect chromosomal aberrations in phylloxera females induced by different doses of gamma irradiation. The influence of such chromosomal aberrations on longevity and reproduction of phylloxera was examined. Moreover, determination of the number and length of autosomal and sex chromosomes was undertaken. Such

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study will allow definition of the karyotypes of irradiated and unirradiated populations of the local phylloxera strain.

2. Materials and Methods

Establishment of phylloxera colony

Grape phylloxera was originally collected from field-infested roots of the local grape varieties in southern parts of Syria. All insects were reared on fresh and healthy pieces of local grape variety Balady roots, 4-7 mm in diameter and 5-7 cm long as outlined in Makee *et al*. (2003). The experiments were conducted at 25±1°C with 70±5% RH, and 24 hr darkness. Egg sterilization was carried out as described by Makee *et al*. (2003). A Co^{60} source (Issledov Gamma Irradiator, Techsnabexport Co. Ltd., Moscow, Russia) delivering a dose rate of 60 Gy/min was used to treat the insects.

Effect of irradiation on matured females, fecundity, and oviposition period

New phylloxera eggs were placed on fresh root pieces and left until hatching. A group of three-week-old feeding phylloxera nymphs was taken. Nymphs were irradiated at different doses: 0-10-20 and 30 Gy (n= 25 nymphs at each dose). Irradiated and unirradiated nymphs were kept at 25±1°C with 70±5% RH, and 24 hr darkness.

A daily microscopic inspection of all phylloxera stages at each applied dose was carried out. The number of feeding nymphs, which were able to develop to adult stage, was observed to determine the percentage mortality at each dose. Female fecundity and longevity was determined at each tested dose.

Chromosome preparations

Mitotic metaphase chromosomes were obtained from 24 to 36-hr-old embryos. At each dose, 10 eggs were taken and placed in a 1.5 ml tube. The eggs in each tube were fixed in Carnoy's fixative (ethanol:chloroform:acetic acid 6:3:1) and shaken for 10 min. Then a drop was taken and transferred onto a clean slide. Shortly before drying, a drop of 60% acetic acid was added and macerated for 2-3 min with fine tungsten needles. Then the specimen was spread on the slide using a heating plate at 45°C to allow evaporation of the acetic acid. The preparation was then stained and mounted in lactic acetic orcein for 5 min; redundant stain was removed with a piece of filter paper. The cover glass was sealed with nail polish. Chromosome preparations were examined in phase contrast micrographs.

Chromosome measurement

The lengths of the chromosomes from 12 well spread orcein-stained metaphase chromosomes were measured in digital images using Digitizier software, version 1 (developed by the Mathematics Department, Atomic Energy Commission of Syria). Chromosome lengths were ranked for each cell nucleus and means and standard deviations (SD) were calculated. Relative chromosome lengths were calculated as percentages of the total length of all chromosomes in the diploid set.

3. Results

Effect of irradiation on mortality, longevity and fecundity

Our results show that the percentage mortality of irradiated phylloxera nymphs was significantly higher than that of unirradiated ones. A regression line was fitted to present the relationship between gamma irradiation and percentage mortality of phylloxera (Fig. 1), showing that the percentage mortality was positively correlated with dose (t= 7; P=0.05). The lowest percentages of mortality were recorded at 10 Gy, after which the percentages started to increase. Only about 16% of phylloxera nymphs were able to reach matured female stage at 30 Gy.

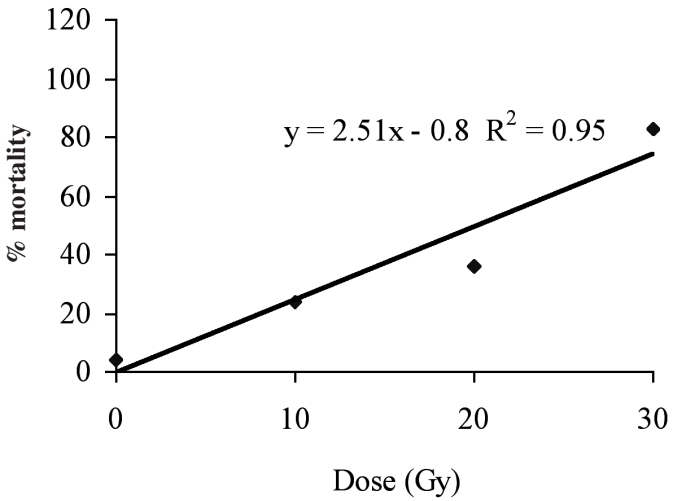

Fig. 1 - Effect of gamma irradiation on percentage mortality of grape phylloxera.

Table 1 illustrates that phylloxera longevity and number of eggs were considerably influenced by the applied dose (Table 1). The mean value for longevity and the mean number of eggs were significantly reduced by increasing the dose (F=75.67; df=3, 96; P=0.05 and F=106.78; df=3, 96; P=0.05, respectively).

Table 1 - Effect of different doses of gamma irradiation on mean number of eggs and longevity of phylloxera

Dose (Gy)	Mean no. eggs (±SE)	Mean longevity (d) (±SE)
0	60.0±4.0 a	14.6±0.75 a
10	24.5±2.7 b	10.0±0.88 b
20	10.7±1.0 c	7.0±0.69 c
30	0.7±0.2 d	0.6±0.01 d

Means followed by different letters (columns) are significantly different at $P< 0.05$ (Tukey HSD test).

Chromosomal analysis under a light microscope

From matured phylloxera females, 24 to 36-hr-old embryos, which contain a higher proportion of dividing cells, were taken to analyze the metaphase chromosomes. It was revealed that the wild-type metaphase karyotype of phylloxera consists of 10 chromosomes. There were eight autosomal chromosomes and sex chromosomes (XX) (Fig. 2 A). A karyotype of 2n=9 was also found (Fig. 2 B). Based on our observations, phylloxera metaphase chromosomes appeared like thick condensed rods. In some cells, an additional very small chromosome was detected (Fig. 3).

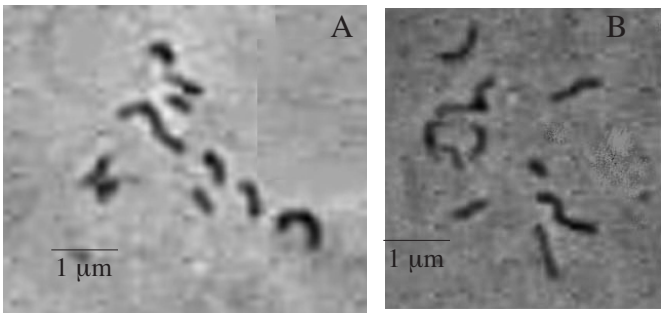

Fig. 2 - Normal metaphase kryotype from embryonic cells of Grape phylloxera, *Daktulosphaira vitifoliae* Fitch: A) 2n= 10; B) 2n=9 + small chromosome (the arrow).

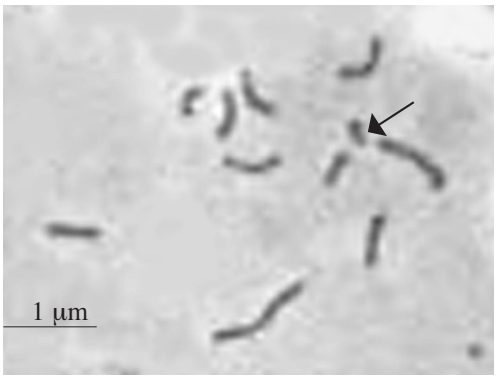

Fig. 3 - Normal metaphase kryotype from embryonic cells of Grape phylloxera: 2n= 10 + small chromosome (the arrow).

To study the effect of different doses of gamma irradiation on phylloxera chromosomes, 24 to 36-hr-old embryos were examined at each tested dose, and it was found that the chromosomes of all tested embryos of irradiated phylloxera had aberrations, regardless of dose. At all different doses, sticky chromosomes were observed in the embryo cells (Fig. 4). When 20 Gy was applied, inter-chromosome translocations were clearly visible (Fig. 5). However, increasing in the chromosomal number on the metaphase plate in some cells was noticed in embryos when phylloxera nymphs were irradiated (Fig. 6).

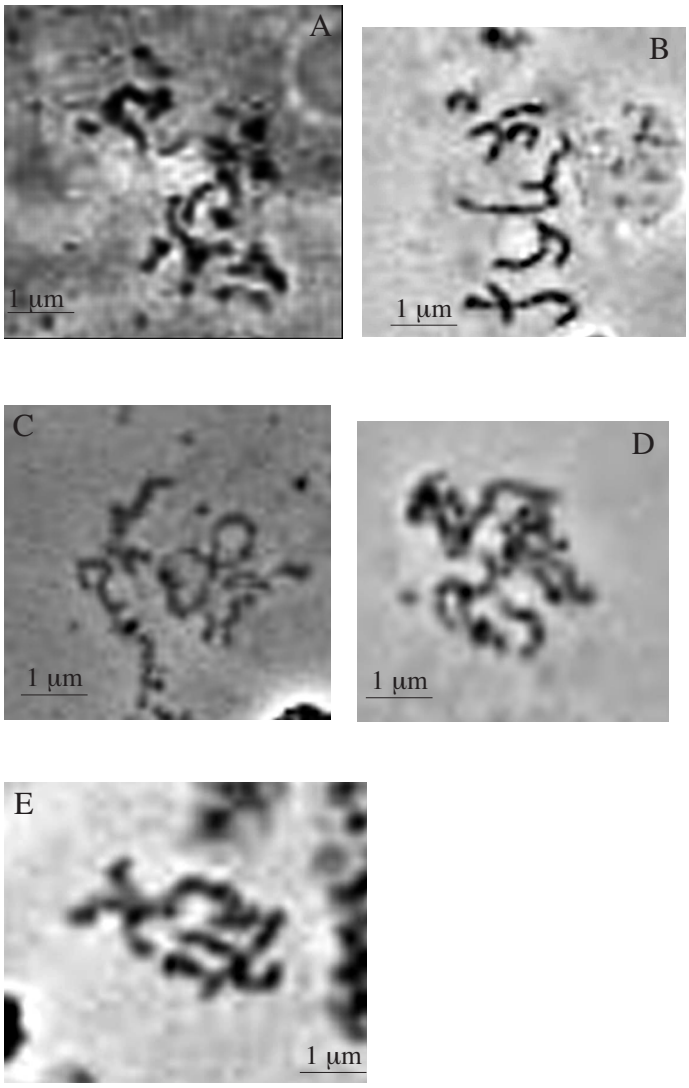

Fig. 4 - Mitotic metaphase chromosomes from embryos of treated Grape phylloxera with gamma ray, showing sticky chromosomes at deferent doses: A-B at dose 10 Gy; C) at dose 20 Gy, D-E) at dose 30 Gy.

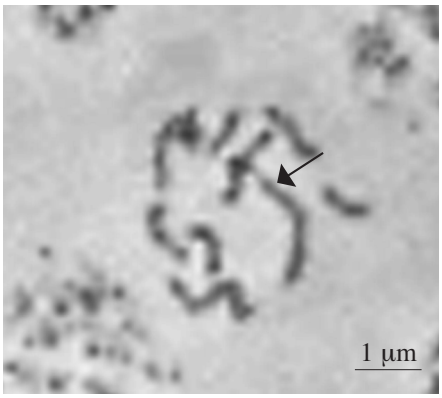

Fig. 5 - Orcein-stained preparations from embryos of irradiated phylloxera at dose 20 Gy showing mitotic metaphase chromosomes with a translocation on the large chromosome (the arrow).

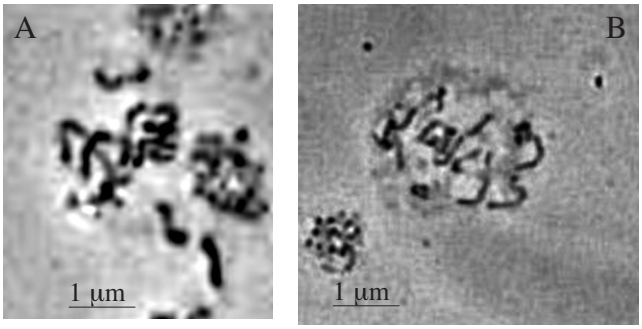

Fig 6 - Orcein-stained preparations from embryos of irradiated phylloxera showing: A) at dose 20 Gy: a cell with about 15 chromosomes which some of them are stuck together; B) at dose 30 Gy: a cell with 14 chromosomes.

Chromosomes measurement

It was possible to identify eight autosomal chromosomes according to their length when different metaphase chromosomes were investigated. Table 2 illustrates the mean length and relative length for each identified chromosome. The total mean length of complete metaphase chromosomes was 12.17±1.97 μm.

Chromosome pair no. 1 is an extra large chromosome with an average total length of 2.31±0.3 μm and relative length of 18.18%; chromosome pair no. 2 is a large chromosome with an average total length of 1.33±0.3 μm and relative length of 10.5%; chromosome pair no. 3 is a medium chromosome (1.12±0.65 μm); and chromosome pair no. 4 is a short chromosome (0.90±0.160 μm) (Table 2).

The sex chromosome (XX) was clearly recognized. The sex chromosome is the shortest chromosome in the metaphase complements with an average total length of 0.69±0.1 μm and relative length of 5.51%.

The mean length of the additional chromosome, which was observed in several cells, was only 0.5±0.1 μm.

Table 2 - The mean length and relative length (%) of phylloxera chromosomes

No. chromosome	Mean length (μm±SD)	Relative length (%)
1	2.31±0.3	18.18
1	2.31±0.3	18.18
2	1.33±0.3	10.5
2	1.33±0.3	10.5
3	1.12±0.65	8.81
3	1.12±0.66	8.81
4	0.90±0.16	7.1
4	0.90±0.16	7.1
X	0.69±0.1	5.41
X	0.69±0.1	5.41
Total mean length	12.17±1.97	100

4. Discussion and Conclusions

Several studies demonstrated that Dipteran, Coleopteran and Hemipteran species tend to be more radiosensitive than Lepidopteran species. However, considerable variation was noted among the species tested within these orders (Makee and Saour, 1999; Bakri *et al*., 2005; Follett *et al*., 2007). In fact, a few studies were carried out to determine the radioresistance of Hemiptera (scales, mealy bugs, aphids, and whiteflies).

A previous study showed that the egg hatch of phylloxera decreased when eggs were subjected to high doses of gamma irradiation and the percentage of matured phylloxera females significantly increased as older nymphs and lower doses were used (Makee *et al*., 2008). On the contrary, fecundity was markedly reduced when older nymphs and higher doses were employed. However, a relationship between chromosomal aberrations induced by irradiation and phylloxera biology was not determined in the study.

Phylloxera can be considered a cytogenetically exciting insect species because of abnormal features related to its cyclical parthenogenesis, and because it has holocentric chromosomes (chromosomes that lack a localized centromere). Phylloxera populations can consist totally of parthenogenetic (thelytokous) females. Several studies showed that grape phylloxera mainly reproduces asexually (*parthenogenesis*): an egg cell can develop into offspring without fertilization by a sperm. Thus, the offspring and its siblings are assumed to be genetically identical to the mother (Vorwerk and Forneck, 2006). Parthenogenetic reproduction of phylloxera has been observed in the field and can be easily maintained under constant conditions in the greenhouse or *in vitro*. This type of reproduction allows phylloxera populations to be replicated several fold, thus several asexual generations can be analyzed within a short period. Once a year, XX parthenogenetic phylloxera females, like aphids, produce one egg that develops as an XO male, having lost half its X chromatin during the single maturation division (Blackman and Hales, 1986; Blackman, 1987).

The current study has shown that phylloxera nymph mortality increased with irradiation (Fig. 1), confirming the results reported by Makee *et al*. (2008). Correspondingly, Dohino *et al*. (1998) found that the survival of aphids was significantly decreased when they were treated with doses of 400-600 Gy. The high death rate, especially when phylloxera nymphs were exposed to higher doses of gamma irradiation, can be attributed to the effects of the dominant lethal mutations induced in phylloxera nymphs' chromosomes by irradiation (LaChance, 1967). When low doses were applied, a small portion of irradiated nymphs successfully completed development and produced matured females, survival which was due to the holokinetic nature of phylloxera chromosomes. Irradiation can cause fragmentation but the resulting fragments are still able to move on the mitotic spindle so that chromosome breakage does not lead automatically to the loss of genetic material (Hughes-Schrad and Ris, 1941).

Our results reveal that the fecundity and longevity

of surviving matured females, irradiated as nymphs, were greatly impacted by irradiation (Table 1). Comparable results were reported when crawls and nymphs of mealybug, *Maconellicoccus hirsutus* (Green), were irradiated (Jacobsen and Hara, 2003). Therefore, at low doses some matured phylloxera females were recorded, but they laid only few eggs and lived for a short period of time. It could be that the induced chromosomal aberrations in irradiated nymphs prevent the normal process of mitotic division, which leads to egg production. Therefore, the matured females were unable to produce a normal number of eggs.

To study the effect of different doses of gamma irradiation on phylloxera chromosomes, 24 to 36-hr-old embryos were examined at each tested dose. It was noted that the chromosomes of all tested embryos of irradiated phylloxera had aberrations, regardless of dose. We noticed sticky chromosomes, inter-chromosome translocations, and increases in the chromosomal number on the metaphase plate in some cells. All these chromosomal aberrations in the embryos were expected as the phylloxera, like Lepidptera species, has holocentric chromosomes. It is reported that irradiation causes fragmentations and translocations in many species of Lepidptera (Traut *et al*., 1986; Makee and Tafesh, 2006, 2007). And because the chromosomes are holocentric when a break occurs, the fragments are usually not lost and can still be attached on the spindle. In the present study on phylloxera, a lot of chromosomal breaks occurred during the formation of eggs in the ova of the nymphs which gave sticky chromosomes and inter-chromosome translocations in the cells of the embryos. It can also be said that spindles were affected by the irradiation which caused an increase in the chromosomal number on the metaphase plate in some embryo cells.

In this work, the embryos of laid eggs varied greatly in their chromosomal rearrangements (Figs. 2, 3, 4 and 5). However, such rearrangements allow the formation of embryos but it is unknown if they will permit the development of embryos until egg hatch. In mealybug only embryos with an approximately normal amount of paternal chromosomal material were able to survive (Nelson-Rees,1962).

The current study has shown that the metaphase complement of the Syrian strain of the phylloxera female consists of 10 chromosomes, representing eight autosomal and two sex chromosomes, confirming the results presented by Forneck *et al*. (1999) and Maillet (1957). We found also normal karyotype with 2n= 9 in the embryonic cells coincidently. Forneck *et al*. (1999) found one karyotype containing 2n = 9 in the somatic cells of phylloxera and they interpreted it as a male sexual phylloxera, although they did not find any spermatides during their study, which leads us to think that maybe this deficiency in chromosome number is a kind of variety of the karyotype.

When defining the karyotype of six phylloxera populations from Germany, Forneck *et al*. (1999) noticed extra chromosomes. Similarly, in our study an additional very small chromosome was observed in some examined cells of the Syrian phylloxera strain. The detection of supernumerary chromosomes was reported in aphids as well (Blackman, 1976; Wilson *et al*., 2003). Such supernumerary or accessory chromosomes are not essential for the life of a species and are lacking in most of the individuals; they do not carry genes necessary for basic growth, but may have some functional significance such as to increase asymmetry chiasma distribution or increase variation by increasing crossing over and recombination frequencies.

In aphid, Blackman (1980, 1981) suggested that the differences in chromosome numbers might be due to dissociations or fusions involving elements of the normal diploid set, or to the presence of supernumerary B chromosomes. The centromeric activity of holocentric chromosomes, dispersed along its full length, allows the broken chromosomal fragments to segregate during mitosis (Ris, 1942). Moreover, Blackman (1980) proposed that thelytokous reproduction of aphids is a factor that permits karyotype variation within populations of the same species.

The phylloxera karyotype consists of 10 chromosomes. The total complement length is about 12Ìm and the chromosomes range in length from 0.7 to 2 Ìm.

When mitosis of *Agallia constricta* (leafhopper) was examined, the metaphase chromatin appeared to be a 2-3 Ìm wide (Rieder *et al*., 1990). Based on embryo metaphase, the chromosomes of phylloxera females could be sorted into five different size-dependent groups: extra long, long, medium, short and extra short (Table 2). However, in some cells a dot-like chromosome, that represents the additional chromosome, was observed. Forneck *et al*. (1999) classified phylloxera chromosomes into two classes: one pair of large chromosomes and four pairs of shorter chromosomes. Nevertheless, in their study they did not mention the exact length of each pair.

The X chromosome is the shortest one in phylloxera karyotype Forneck *et al*. (1999). However, Blackman *et al*. (2003) reported that in most aphids species the X chromosomes could be identified as the longest or second longest pair. On the contrary, in some aphids the X chromosome was the shortest pair (Blackman, 1986; Blackman *et al*., 2003).

The present study confirms the efficiency of cytogenetic techniques in analyzing the karyotype and chromosomal length of phylloxera, as well as tracing chromosome aberrations in irradiated phylloxera populations. This investigation is a contribution to the search for genetic variation of phylloxera behaviour and development from different populations and provides useful information that can be taken into account in pest management and quarantine measurements against phylloxera. However to apply irradiation technology more comprehensive studies are still needed.

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Growing spinach in a floating system with different volumes of aerated or non aerated nutrient solution

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Key words: hydroponics, hypoxia, leafy vegetables, nitrate accumulation, oxygen, *Spinacia oleracea* L.

Abstract: Vegetables grown in a floating system may encounter problems of hypoxia at root level, especially in the summer when temperature is high. Depending on the species, oxygen deficiency may cause a lower yield due to a reduction in water and mineral uptake by the plants. On the other hand, plants under oxygen stress may reduce nitrate accumulation, thus ameliorating produce quality. In the present work spinach was grown in summer and autumn in a floating system in different volumes (252, 126 and 60 l per m^2 of cultivated area) of aerated or non aerated nutrient solution. Aeration kept oxygen concentration at 7-8 mg l^{-1} while in the non aerated solution oxygen decreased gradually reaching at harvest, on average, values of 1.92 mg l^{-1} and 2.83 mg l^{1} in summer and autumn respectively. Such levels of hypoxia did not affect yield and did not reduce nitrate accumulation either. On the contrary, in the summer cycle leaf nitrate content was significantly lower when the nutrient solution was aerated. Reduction of the volume of the solution to 60 l m^{-2} of cultivated area induced a decrease in nitrate accumulation without negative effect on yield. No significant aeration x volume interaction was observed.

1. Introduction

A floating system is a soilless cultivation technique where plants are grown on alveolate or fissured polystyrene panels floating in tanks containing the nutrient solution (Lazzarin *et al*., 2001; Tesi, 2002). The system is particularly suitable for growing small-size, short-cycle species like leafy vegetables, especially if aimed at producing ready-to-use salads (Nicola *et al*., 2007). As in other hydroponic systems, plants grown in a floating system may encounter problems of oxygen deficiency (hypoxia) at root level, as roots themselves gradually consume the oxygen dissolved in the nutrient solution. Oxygen deficiency may cause a reduction in water and mineral uptake by the plants, with consequences on the development of their aerial part and therefore on yield (Morard and Silvestre, 1996). The problem is surely more urgent in the summer, since with higher temperatures the quantity of oxygen dissolved in a solution decreases and root respiration rate increases (Boisseau *et al*., 1988). In a floating system the stillness of the solution, that may favour the occurrence of hypoxia, is compensated for by the large volumes normally adopted (150-250 l per m^2 of cultivated area) and the shortness of the growing cycles. In any case, in order to avoid any risk, growers aerate the nutrient solution to enrich it with oxygen, thus incurring additional costs. On the other hand, plants under oxygen stress are known to increase the activity of nitrate reductase (Garcia-Novo and Crawford, 1973; Lambers *et al*., 1978; Veen, 1988), the key enzyme for nitrate utilization by plants (Campbell, 1988). Therefore, the reduction of oxygen concentration in the nutrient solution could actually control nitrate accumulation in hydroponically-grown vegetables, as suggested by Ferrante *et al*. (2003). In the present work oxygen trend during summer and autumn cultivation of spinach in a floating system equipped or not with a device to aerate the nutrient solution was studied and the possible repercussions on spinach yield and quality (namely nitrate content) were investigated. The aim of the work was to verify if, when the nutrient solution was not aerated (thus achieving a cost reduction), oxygen level was kept high enough to ensure spinach yield but at the same time decreased enough to control its nitrate accumulation. Furthermore, different volumes of solution were tested in order to verify if a cost saving may be achieved in the floating system by reducing water and nutrient consumption without negative effects on yield and produce quality, at least in spinach, and if such eventuality may be limited by oxygen concentration when the nutrient solution is not aerated.

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2. Materials and Methods

Growing conditions

Spinach (*Spinacia oleracea* L.) cv. Seven R was cultivated in a floating system in a glasshouse in Sesto Fiorentino (FI, Italy). Two cultivation cycles were carried out, one in the summer (sowing on 1 July 2008) and the other in autumn (sowing on 30 September 2008). Plants were grown in polystyrene 32.5x51.4x5 cm 160-alveoli trays (three seeds per alveolus). Trays floated in polypropylene 36x56x29 cm tanks, lined with a black (inside)/white (outside) polyethylene sheet, on 42, 21 or 10 l of continuously aerated or non aerated nutrient solution (252, 126 or 60 l of nutrient solution per m^2 of cultivated area, respectively). The bottom of the tanks containing 10 and 21 l of nutrient solution was raised in order to bring the cultivated panels at the same height as panels floating on 252 l m^{-2} of nutrient solution. Aeration was provided by aquarium aerators (air flux: about 2 l hr^{-1} per l of nutrient solution). The nutrient solution was composed as follows (macronutrients in mmol l^{-1}, micronutrients in µmol l^{-1}): 12 $N\text{-}NO_3$, 3.8 $N\text{-}NH_4$, 2.8 P, 8.4 K, 3.5 Ca, 1.4 Mg, 40 Fe, 10 Mn, 40 B, 5 Zn, 1 Cu, 1 Mo.

During the cultivation cycles the nutrient solution consumed by the plants was restored once (after 10 days and 14 days of cultivation in the floating system for the summer and autumn cycle, respectively) by adding tap water. Tap water was composed as follows (macronutrients in mmol l^{-1}, micronutrients in µmol l^{-1}): 0.08 $N\text{-}NO_3$, <0.0027 $N\text{-}NH_4$, traces of P, 0.9 K, 1.8 Ca, 0.57 Mg, 0.14 Fe, <0.04 Mn, 10.17 B, 0.09 Zn, 0.36 Cu, 0 Mo. At harvest, the overall consumption was on average 79.9 l m^{-2} in summer and 74.0 l m^{-2} in autumn, without differences due to the imposed treatments (aeration/volume). Plants were harvested on 23 July and 30 October 2008 for the summer and autumn cycle, respectively, when their developmental stage was suitable for ready-to-use salads (about 12 cm height). During the cultivation period, maximum, minimum and mean air temperature were 38.3, 19.9 and 27.5°C, and 34.3, 15.6 and 21.6°C, respectively in the summer and autumn.

Data collection

During the cultivation period, every two-three days oxygen concentration and temperature of the nutrient solution were measured using a portable dissolved oxygen meter HI 9146 (Hanna Instruments, Padova, Italy). At the same time, pH and electric conductivity (EC) were also checked by the portable pH and conductivity meter HI 991300 (Hanna Instruments, Padova, Italy).

At harvest, yield and produce quality were evaluated by collecting the following data: leaf and root fresh weight (FW, determined by weighing the whole production from each tank), leaf dry weight (DW, by oven-drying one sample of 100 g of fresh leaves per tank at 80°C until constant weight), leaf area (measured on five plants per tank using the leaf area meter LI-3000 by Li-Cor, Lincoln, NE, USA), leaf colour (estimated on 10 plants per tank using the Chlorophyll Meter SPAD-502, Konica Minolta, Tokyo, Japan) and nitrate content, which was measured spectrophotometrically on two samples per tank using the salicylic-sulphuric acid method (Cataldo *et al*., 1975).

Statistics

The treatments were arranged in a two-way randomized block design with three replications (one replication=one tank). Data were subjected to analysis of variance (ANOVA) and means were compared using the SNK test at P=0.05 level of significance.

3. Results and Discussion

Nutrient solution

Aeration of the nutrient solution affected pH and oxygen concentration, while it did not show any effect on EC and temperature (Fig. 1 and 2). In the aerated nutrient solution pH increased more than in the non aerated one, although without exceeding well-tolerated values for the species. Aeration kept oxygen concentration at 7-8 mg l^{-1} while in the non aerated solution oxygen decreased gradually reaching values below 5 mg l^{-1} after 17 days in summer and after 21 days in autumn,

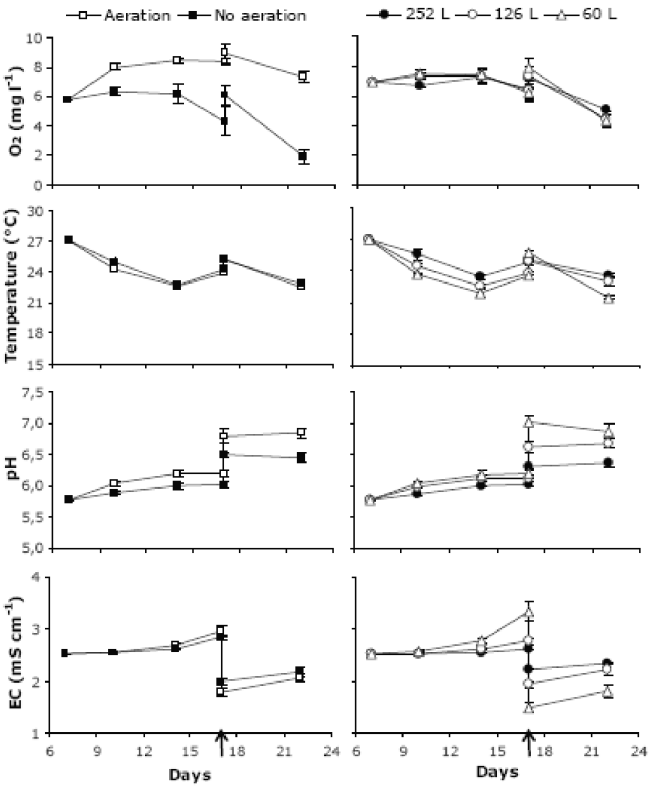

Fig. 1 - Effect of aeration (left) and volume (l per m^2 of cultivated area) (right) of the nutrient solution on its O_2 concentration, temperature, pH and EC during the summer cultivation cycle of spinach. Error bars (±SE) are shown when larger than symbols. Arrows indicate the date when the consumed solution was restored with tap water.

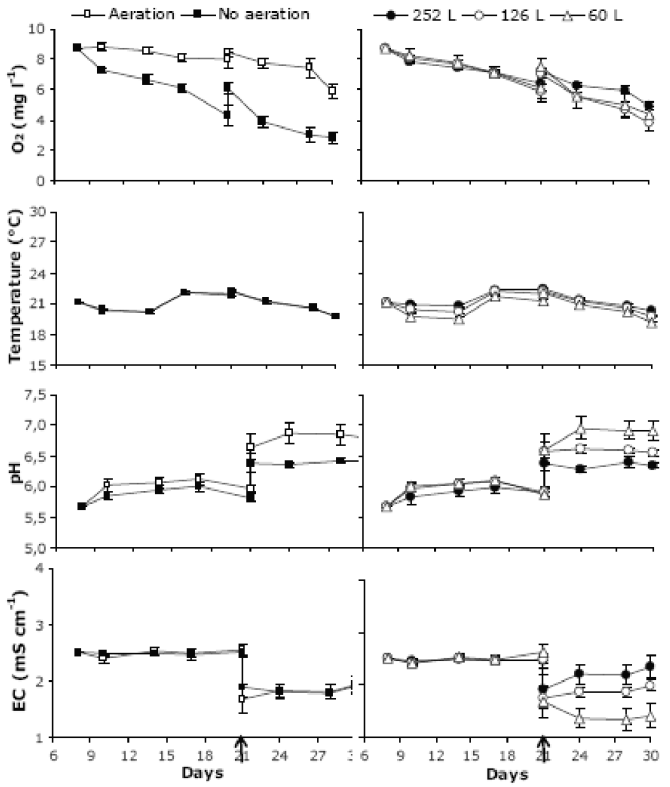

Fig. 2 - Effect of aeration (left) and volume (l per m^2 of cultivated area) (right) of the nutrient solution on its O_2 concentration, temperature, pH and EC during the autumn cultivation cycle of spinach. Error bars (±SE) are shown when larger than symbols. Arrows indicate the date when the consumed solution was restored with tap water.

that is five and nine days before harvest, respectively. Therefore, when the nutrient solution was not aerated, plants were subjected to sub-optimal oxygen concentrations only for a short period of their cultural cycle. At harvest oxygen level in non aerated tanks was on average 1.92 mg l^{-1} in summer and 2.83 mg l^{-1} in autumn.

No significant differences were noticed in temperature and oxygen concentration due to the volumes of the solution, while pH and EC showed variations that were greater when the volume was lower (Fig. 1 and 2). Those variations (a rise in pH and a decrease in EC) were due to the water added to the tanks to restore the solution consumed by the plants (about 8 l per tank in summer and 7 l in autumn), whose effect was inversely proportional to the starting volume of the solution.

Spinach yield and quality

In summer, temperature conditions unfavourable to germination in the first week after sowing (29.5°C as mean temperature) led to a lower plant density, justifying the lower yield in the summer cycle compared to the autumn one (on average 1.6 kg m^{-2} and 2.7 kg m^{-2} of fresh leaves, respectively). On the other hand, in summer the single plants showed a larger leaf area and more developed roots (Table 1 and 2).

Leaf and root production and leaf area did not show any significant differences due to the treatments in both the summer and the autumn cultivation cycles (Table 1 and 2).

Table 1 - Effect of aeration and volume (l per m^2 of cultivated area) of the nutrient solution on leaf production (leaf FW and leaf DW), leaf area and root production (root FW) in spinach grown in a floating system in the summer season

Treatments	Leaf FW (kg m^{-2})	Leaf DW (g m^{-2})	Leaf area (cm^2 plant^{-1})	Root FW (g m^{-2})
Aeration	1.6 a	85.9 a	55.3 a	162.7 a
No aeration	1.7 a	91.6 a	53.7 a	138.0 a
Volume l 252	1.5 a	94.5 a	50.5 a	134.0 a
Volume l 126	1.8 a	91.7 a	59.5 a	169.0 a
Volume l 60	1.6 a	80.0 a	53.6 a	148.0 a
Interaction aeration x volume	NS	NS	NS	NS

For each factor, values in each column followed by the same lower-case letter are not statistically different (SNK Test, P≤0.05).
NS = not significant.

Table 2 - Effect of aeration and volume (l per m^2 of cultivated area) of the nutrient solution on leaf production (leaf FW and leaf DW), leaf area and root production (root FW) in spinach grown in a floating system in the autumn season

Treatments	Leaf FW (kg m^{-2})	Leaf DW (g m^{-2})	Leaf area (cm^2 plant^{-1})	Root FW (g m^{-2})
Aeration	2.5 a	112.0 a	53.2 a	93.3 a
No aeration	2.8 a	127.7 a	45.2 a	84.0 a
Volume l 252	2.5 a	109.7 a	47.1 a	85.0 a
Volume l 126	2.9 a	130.2 a	50.9 a	93.0 a
Volume l 60	2.6 a	119.7 a	49.6 a	88.0 a
Interaction aeration x volume	NS	NS	NS	NS

For each factor, values in each column followed by the same lower-case letter are not statistically different (SNK Test, P≤0.05).
NS = not significant.

Spinach tolerance to hypoxia in a floating system was previously observed in spring (Lenzi *et al*., 2008; Baldi *et al*., 2009), in summer (Lenzi *et al*., 2008) and in autumn (Tesi *et al*., 2003 b); on the contrary, when cultivated in summer, spinach showed a reduction in yield due to oxygen depletion (Tesi *et al*., 2003 b) but in this case the length of the cycle was 40 days and plants were exposed to hypoxia conditions for almost twice as long compared to the present work, as well as with respect to the work described in Lenzi *et al*. (2008).

No effect on crop yield of root hypoxia in a floating system was detected in rocket (Ferrante *et al*., 2003; Lenzi *et al*., 2008; Baldi *et al*., 2009) and lamb lettuce (Ferrante *et al*., 2005). In head lettuce, roots were exposed to oxygen concentration of 2.1 mg l^{-1} without any significant effect on plant growth (Goto *et al*., 1996), while oxygen levels close to 0 mg l^{-1} during the last two weeks of cultivation caused an evident decrease in yield (Tesi *et al*., 2003 a).

As far as the quality aspect is concerned, the imposed treatments did not cause any differences in the colour of the product (SPAD values), while they showed some effects on nitrate accumulation in the summer cycle (Table 3). In previous experiments cultivation in a floating system without a device to oxygenate the nutrient solution reduced nitrate accumulation in lamb lettuce (Ferrante *et al*., 2005) and head lettuce (Tesi *et al*., 2003 a) but did not show any effect in spinach and rocket (Lenzi *et al*., 2008; Baldi *et al*., 2009). In the experiment of Ferrante *et al*. (2003) rocket showed a reduction in nitrate content when plants were subjected to anoxia conditions by bubbling nitrogen gas through the nutrient solution one week before harvesting.

In the present experiment spinach nitrate accumulation was even higher when the nutrient solution was not aerated, with statistically significant differences compared to the aerated nutrient solution in the summer season (Table 3).

Probably, to obtain a decreasing effect on nitrate accumulation by the onset of oxygen stress according to the mechanism suggested by Gracia-Novo and Crowford (1973) (nitrate used as an electron acceptor alternative to free oxygen), prolonged conditions of anoxia or severe hypoxia are necessary.

In experiment presented in this work, a decrease in leaf nitrate content was obtained also by reducing the volume of the nutrient solution, with statistically significant differences again in the summer cycle (Table 3). Such decrease, already observed both in spinach and rocket (Baldi *et al*., 2009), was not correlated to oxygen, as demonstrated by the non-significant interaction aeration x volume and by the fact that oxygen concentration in the solution did not change due to its volume (Fig. 1 and 2). Instead, since the lower volume underwent a stronger diluting effect from the water added to restore plant consumption, the nitrate decrease was probably simply due to a lower availability of nitric ions for plants. A similar result was obtained in spinach and other fresh-cut vegetables grown in a floating system by using low-concentrated nutrient solutions (Alberici *et al*., 2008; De Pascale *et al*., 2008).

In autumn, when environmental conditions are more favourable to plant nitrate accumulation (Maynard *et al*., 1976), nitrate content was on average higher than in the summer. As in the summer, nitrate was lower with aeration and with the lower volume, although the differences with respect to non aerated conditions and higher volumes, respectively, were not statistically significant (Table 3).

4. Conclusions

From the literature it appears that when vegetables are grown in a floating system without devices to enrich the nutrient solution with oxygen, root hypoxia may have or not a negative effect on yield depending on the species, the season and the length of the growing cycle. The season and duration of the cycle result in different hypoxia levels and more or less prolonged exposure to it. In spinach, in the case of short cycles (20-30 days), both in summer and autumn, hypoxia was not severe enough to influence yield even with low volumes of nutrient solution (up to 60 l per m^2 of cultivated area).

Extent and duration of oxygen deficiency are prob-

Table 3 - Effect of aeration and volume (l per m^2 of cultivated area) of the nutrient solution on SPAD and leaf nitrate content in spinach grown in a floating system in the summer and autumn seasons

Treatments	Summer cycle		Autumn cycle	
	SPAD	Leaf nitrate (mg kg^{-1} FW)	SPAD	Leaf nitrate (mg kg^{-1} FW)
Aeration	24.0 a	3610 b	23.6 a	4495 a
No aeration	26.2 a	4085 a	24.9 a	4814 a
Volume l 252	24.6 a	4090 a	23.9 a	4822 a
Volume l 126	25.1 a	3901 ab	23.7 a	4780 a
Volume l 60	25.7 a	3553 b	25.2 a	4361 a
Interaction aeration x volume	NS	NS	NS	NS

For each factor, values in each column followed by the same lower-case letter are not statistically different (SNK Test, P≤0.05).
NS = not significant.

ably the most important factors influencing also nitrate accumulation. While anoxia or severe hypoxia may reduce vegetable nitrate accumulation (Ferrante *et al*., 2003; Tesi *et al*., 2003 a), we found that in the case of short exposure to oxygen depletion up to 2-3 mg l^{-1} not aerating the nutrient solution may result in an even higher nitrate content in spinach leaves.

Therefore, although the necessity to aerate or not the nutrient solution in a floating system depends on the aim (yield/quality) and the specific situation, we support the use of aeration. Furthermore, reduction of the volume of the nutrient solution per m^2 of cultivated area, which did not cause any effect on spinach yield and reduced nitrate accumulation up to 60 l, appears an interesting strategy in order to save water and fertilizers and at the same time ameliorate produce quality.

Acknowledgements

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Photoselective shade nets reduce postharvest decay development in pepper fruits

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Key words: Capsicum annuum, postharvest, shelf life, storage.

Abstract: During two-year studies, we evaluated the influence of photoselective coloured shade nets on the quality of fresh harvested pepper fruits (*Capsicum annuum*) after prolonged storage and shelf life simulation. Pepper cultivar 'Romans' grown in a semi arid region under 35% pearl and yellow shade nets significantly maintained better pepper fruit quality after 16 days at 7°C plus three days at 20°C, mainly by reducing decay incidence during two consecutive years (2008 and 2009), compared to commercial black and red nets. No significant differences were observed in percentage of weight loss, firmness and total soluble solids in fruit harvested under the different coloured shade nets. The skin colour of fruit harvested under Pearl net was significantly lighter than that of fruit harvested under red and black shade nets and this fact can be associated with inhibition of fruit ripening during growth. After storability and shelf life simulation however skin colour was red to dark red under all shade nets. Pearl and yellow shade nets significantly reduced *Alternaria* spp. population in the field, which was evaluated with *Alternaria*-selective growing medium. The highest *Alternaria* population was found under red shade net. The significant low decay incidence in fruit harvested under pearl and yellow shade nets can be explained by the low inoculum level of *Alternaria* spp. in the field, and inhibition of fungal sporulation, and/or by a slowing of fruit ripening during its growth, reducing fruit susceptibility to fungal infection in the field due to the scattered light, its quality and the ratio between the light spectrum under the two shade nets.

1. Introduction

Sweet bell pepper (*Capsicum annuum* L.) is an important export commodity in Israel with more than 120,000 tons per year. Fruits may be green (unripe), red, yellow, orange, or brown when ripe. Peppers are rich in vitamins, minerals and dietary fibres, and are low in calories (Kevers *et al*., 2007) and they have become popular decorative items (Frank *et al*., 2001). However, to extend the export season with high pepper quality during the summer season, shade netting is necessary to protect agricultural crops from excessive solar radiation and pests (Shahak, 2008).

In recent years coloured shade netting (photoselective shade nets), designed specifically to manipulate plant development and growth, has become available. These nets can be used outdoors as well as in greenhouses. They can provide physical protection (from birds, hail, insects, excessive radiation), affect environmental modification (humidity, shade, temperature) (Perez *et al*., 2006), and increase the relative proportion of diffuse (scattered) light as well as absorb various spectral bands, thereby affecting light quality (Shahak *et al*., 2004). These effects can influence crops as well as the organisms associated with them. The effect of colour shade nets on plant development in crops, foliage crops, fruit trees and vegetables has been studied in recent years (Nissim-Levi *et al*., 2008; Shahak *et al*., 2008).

Bell peppers are grown commercially in semi-arid regions of Israel under black shade nets. Netting is frequently used to protect pepper plants from excessive solar radiation (shade-nets), environmental hazards (e.g. hail-nets), or pests (bird, or insect-proof nets) (Shahak *et al*., 2004). Shahak (2008) reported that productions of three cultivars of bell pepper were increased by 16 to 32% under pearl and red netting compared with equivalent black shade netting, or with open field production. In addition, Elad *et al*. (2007) reported that blue-silver, green and red nets were associated with lower levels of powdery mildew disease, caused by *Leveillula taurica* in pepper field experiments. Other studies have suggested differential effects of photoselective screens and shade nets on pest infestation and vector-borne viral diseases (Ben-Yakir *et al*., 2008). In a preliminary research conducted in 2007,

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Fallik *et al*. (2009) demonstrated the potential use of coloured shade nets to maintain the postharvest quality better of sweet pepper grown under red and yellow nets, during prolonged storage and shelf life.

Therefore, we undertook the two-year study presented in this work to evaluate, on a commercial growth scale, the influence of different coloured shade nets on the quality of fresh harvested bell pepper after prolonged storage and shelf life in order to understand, in part, the mode-of-action of those shade nets on harvested fresh produce.

2. Materials and Methods

Plant materials and coloured net shades

Red sweet bell pepper (*Capsicum annuum* L.) cv. 'Romans' was grown at the B'sor Experimental Station in the south-west of Israel (31.271° N; 34.389° E), using commercial cultivation practices (planting seedlings directly into the soil [~ 90% sandy regosol and ~ 10% arid brown soil], automatic drip fertigation), under four different coloured shade-nets, as follows: red, yellow, pearl and black (commercial shade net) with 35% relative shading (in PAR) (Polysack Plastics Industries, Nir-Yitzhak, Israel under the trade mark ChromaticNet), in four random replicates of 18 x 18 m each, all within one large horizontal net-house, 2.5 m high. Plants were planted during the third week of May, both in 2008 and 2009, and harvested as described below.

Light measurements under the nets

Measurements of light spectra, light scattering and microclimate parameters under the nets were taken as described previously by Shahak *et al*. (2008). Spectra of solar radiation outside and under the nets were measured by a spectroradiometer PS-100 (Apogee Instruments Inc. Logan UT, USA) which employs a light diffuser of 4 cm diameter above the 300 μm fiber optics. The diffuser was oriented along the sun beams. A round opaque plate of 6 cm diameter was held at 40 cm above the diffuser to block the direct light to measure the scattered light. Direct radiation was measured through a 4 x 45 cm tube placed on top of the diffuser. The radiation was measured three times at noon in the middle of August 2009 (Fig. 1).

Quality parameters

Fruit samples for storability and shelf-life were harvested five times each year, every three weeks, between the beginning of September and the end of November 2008 and 2009. Fruits (185±10 g) without defects or diseases were harvested without a calyx, at ~85% light red/red colour (~16% green 'cheek'), rinsed and brushed in hot water as described by Fallik *et al*. (1999) and packed in four 6.5 kg corrugated cartons. Fruit quality parameters were evaluated immediately after each harvest and at the end of 16 days storage at 7°C and relative humidity (RH) of ~ 94%, plus three days at 20°C (RH ~ 70-75%) as follows: Weight loss was expressed as percentage of weight loss from the initial weight of ten fruits. Fruit firmness was measured on ten fruit as described by Ben-Yehoshua *et al*. (1983). Each fruit was placed horizontally between two flat surfaces and a 2 kg weight loaded on top of the flat surface. A dial fixed to a graduated plate recorded the deformation of the fruit in millimetres. Full deformation was measured 16 s after exerting the force on the fruit, then the weight was removed and the residual deformation was measured 16 s later. Fruit was considered very firm with 0-1.5 mm deformation; firm = 1.6-3 mm deformation; soft = 3.1-4.5 mm deformation; very soft = above 4.6 mm deformation. Total soluble solids (TSS) were measured on the same ten fruits tested for firmness, by squeezing out juice from fruits onto an Atago digital refractometer (Atago, Tokyo, Japan) and taking readings. The development of fruit colour was expressed as colour index (CINX) – on a scale of 1 to 4 with 1 = light red with 10-16% green peel; 2 = light red; 3 = red; and 4 = dark red. The index was calculated as follows: (number of fruits at light red colour X 1 + number of fruits at light red X 2 + number of fruit at red colour X 3 + number of fruits at dark red X 4)/total number of fruits. Decay incidence was considered once fungal mycelia appeared on fruit pericarp and/or calyx. Decay was expressed as a percentage of the total initial fruit number.

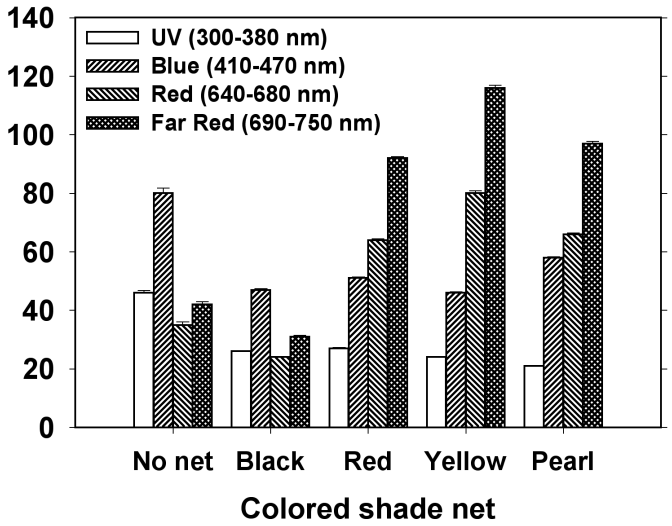

Fig. 1 - Influence of coloured shade nets on the scattered light under the different nets. Measurements were taken at noon in August 2009. Scattering relate to PAR (in μmol m^{-2} s^{-1}). The values represent integrals of the spectra over the following wavelength ranges: PAR: 400-700 nm; UV: 300-380 nm; Blue: 410-470 nm; Red: 640-680 nm; Far Red: 690-750 nm (Means of 3 replicates ± SE).

Evaluation of total microorganism population levels and Alternaria *spp. levels under coloured shade nets*

Population levels of conidia in the greenhouse envi-

ronment were evaluated according to Elad (1997) by exposing 90 mm Petri dishes, under each net, containing PDA (Potato Dextrose Agar, Difco) supplemented with chloramphenicol (Sigma chemicals, 50 μg/ml) at a height of 60 cm above the ground, in five different places (five plates) under each coloured shade net for 60 min. Plates were then incubated for four days at ~22°C and colonies were counted. Based on preliminary results obtained in 2007, the main decay-causing agent on pepper fruits after harvest was *Alternaria alternata*. Population levels of *Alternaria* spp. were evaluated by exposing 90 mm Petri dishes containing selective medium for Alternaria (Soon Gyu and Pryor, 2004), as described above. Colonies were counted after 10 days incubation at ~22°C. The plates were exposed horizontally 60 cm above the ground. Each evaluation was repeated three times from the beginning of October to the end of November.

Statistical analysis

All the results were analyzed using a one- or two-way Anova statistical analysis at P=0.05 using JMP 6 Statistical Analysis Software Program (SAS Institute Inc. Cary, NC., USA) (Sall *et al*., 2001).

3. Results

Light quality under different shade nets

Figure 1 shows the spectra of the scattered light under the different nets. The pearl net significantly reduced UV, while it significantly increased the blue light, thus significantly increasing the ratio between blue/UV compared to all other shade nets. The yellow net significantly increased the scattered red light compared to all other shade nets, while the blue light under this net was significant lower compared to red and pearl nets (Fig. 1). The three shade nets significantly increased the far-red scattered light compared to the commercial black net.

Quality evaluation

After 16 days storage at 7°C plus three days at 20°C, the major differences in fruit quality were found between 2008 and 2009 in weight loss and fruit firmness. However, TSS was similar in the two years (Table 1). In 2008, fruits lost less weight under the red net (2.9%), while under the yellow net weight loss was higher (3.5%). In 2009, percentage of weight loss under the four shade nets was similar (between 3.4 to 3.6%).

Immediately after harvest, fruits picked from the pearl net treatment were significantly lighter in their red colour index (2.33) than fruit picked from the commercial black or red shades (2.60 and 2.64, respectively) (Table 2). No significant differences were observed in fruit colour index between the yellow and pearl treatments. After 16 days at 7°C plus three additional days at 20°C, all fruits turned almost dark red, however fruits picked under the red shade were significantly darker (3.86) than fruits picked under yellow and pearl shades (3.76 and 3.75, respectively) (Table 2).

Decay incidence

In both years, decay incidence under yellow and pearl shade nets were significantly lower compared to fruit picked under the commercial black or red net (Fig. 2). In 2009, the average decay incidence was higher than in 2008. In 2008, the highest decay incidence was observed on fruit picked under black and red shade nets, which was significantly higher than on fruit picked under the yellow and pearl shade nets. In 2009,

Table 2 - The influence of coloured shade net on fruit colour index (CINX) development immediately after harvest and after 16 days at 7°C plus three days at 20°C (means of five experiments each year, with four 6.5 kg boxes per treatment)

Colored shade net	Immediately after harvest (1-4) [z]	After storage and shelf life (1-4)
Black	2.60 a [y]	3.80 ab
Red	2.64 a	3.86 a
Yellow	2.46 ab	3.76 b
Pearl	2.33 b	3.75 b

[z] Colour Index: 1= light red with 10-16% green peel; 2= light red; 3= red; and 4= dark red.

[y] Values within a column followed by the same letter do not significantly differ at P= 0.05 according to Duncan's multiple range test.

Table 1 - The influence of coloured shade nets on weight loss, firmness and TSS after 16 days at 7°C plus three days at 20°C (means of five experiments each year, with four 6.5 kg boxes per treatment)

	Weight loss (%) [z]		Firmness (mm) [y]		TSS (%) [x]	
Colored shade net	2008	2009	2008	2009	2008	2009
Black	3.2 ABb [w]	3.6 Aa	2.1 Ab	3.0 Aa	6.7 Aa	6.6 Aa
Red	2.9 Bb	3.4 Aa	2.3 Ab	3.0 Aa	6.4 Aa	6.4 Aa
Yellow	3.5 Aa	3.5 Aa	2.3 Ab	2.7 Ba	6.6 Aa	6.6 Aa
Pearl	3.3 Ab	3.6 Aa	2.0 Ab	2.9 ABa	6.5 Aa	6.4 Aa

[z] Percent weight loss from initial.

[y] Firmness - millimeter deformation (flexibility).

[x] Percent total soluble solids.

[w] Values followed by the same upper-case letter do not significantly differ between the treatments, while values followed by the same lower-case letter, do not significantly differ between the harvest seasons at p=0.05 according to Duncan's multiple range test.

a significantly lower decay incidence was observed on fruit picked under the pearl shade net, whereas the highest one was observed on fruit picked under the red shade net (Fig. 2).

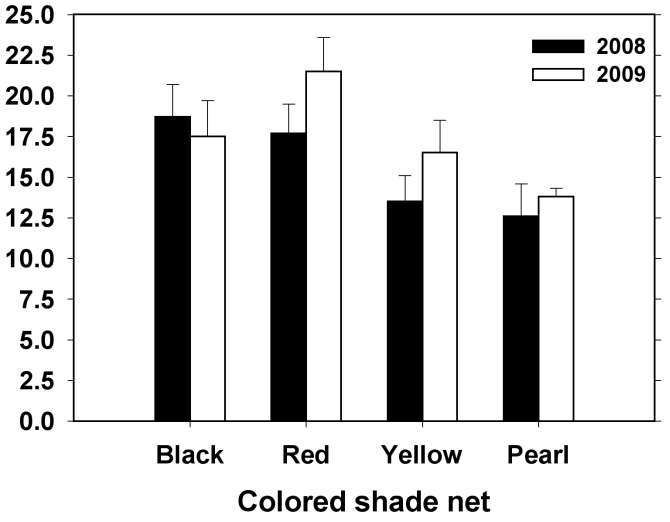

Fig. 2 - Influence of coloured shade nets on decay incidence in fruit of cv. Romans, in 2008 and 2009 after 16 days at 7°C plus three days at 20°C. (Means of five experiments with four 6.5 kg boxes per treatment ± SE).

Evaluation of total microorganisms population levels and Alternaria *spp. levels under coloured shade nets*

The epiphytic population of fungi evaluated on a regular PDA medium was lower under the pearl net, compared to the population that was counted under the red or black shade nets. However, no significant difference between treatments was found (Fig. 3).

Using a selective medium for *Alternaria* spp., a significant reduction in its population was observed under pearl and ellow shade nets (Fig. 4). The highest *Alternaria* population was found under the commercial black shade net (Fig. 4).

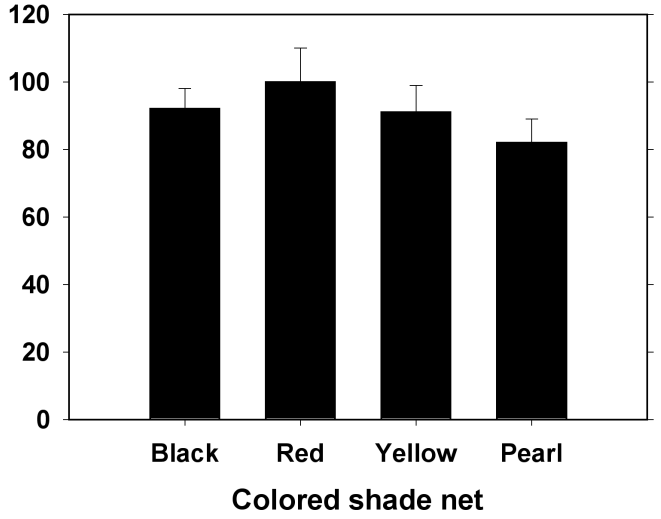

Fig. 3 - Influence of coloured shade nets on the epiphytic population of fungi detected under the nets (CFU/plate – means of 3 experiments conducted in 2009 ± SE).

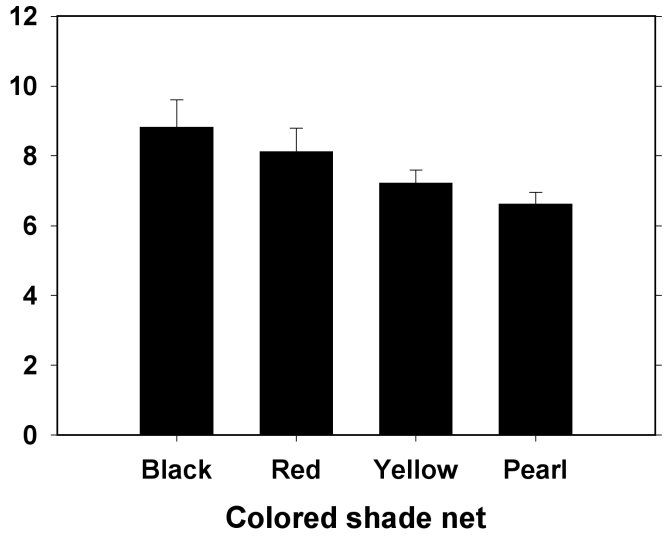

Fig. 4 - Influence of coloured shade nets on the *Alternaria* spp. population detected under the nets using selective medium (CFU/plate – means of 3 experiments conducted in 2009 ± SE).

4. Discussion

Photoselective coloured shade nets (ChromatiNets™) have been developed over the last decade to filter selected regions of the spectrum of sunlight, concomitantly with inducing light scattering, and are designed specifically to modify the incident radiation (spectrum, scattering and thermal components) (Shahak *et al*., 2004). Depending on the pigmentation of the plastic and the knitting design, the nets provide varying mixtures of natural, unmodified light together with spectrally modified scattered light. They are aimed at optimizing desirable physiological responses, in addition to providing physical protection to the crop, thus improving plant growth, flowering and yield and fruit quality (Rajapakse and Shahak, 2007; Shahak, 2008; Shahak *et al*., 2008).

The most prominent effect of the coloured shade nets was found on decay incidence in fruit harvested under pearl and yellow nets, without affecting fruit quality after prolonged storage and shelf life. It is well known that light plays an important role in plant growth. Red light induced resistance in broad bean against *Botrytis cinerea* (Islam *et al*., 1998) and *Alternaria tenuissima* (Rahman *et al*., 2003), and in rice against *Magnaporthe grisea* (Arase *et al*., 2000). Tabira *et al*. (1989) reported that continuous irradiation of visible light of 570-680 nm protected apple leaves by inducing insensitivity to *Alternaria alternata* apple pathotype. Disease suppression under red light was also observed in glasshouse-grown *Corynespore cassiicola*-inoculated cucumbers, and indicated that delay and suppression of Corynespora leaf spot of cucumber were due to induction of resistance in cucumber, and not to differences in environmental conditions or fungal populations between the two greenhouses (Rahman *et al*., 2009). We therefore speculate

that under yellow net, significantly more scattered red light penetrates into the plant and fruit (Fig. 1), which in turn inhibits fruit ripening as shown by fruit colour index (Table 2) and/or, indirectly, induces resistance against *Alternaria alternata* infection after harvest. Preliminary results have revealed high amounts of chlorophyll in fruit harvested under pearl and yellow nets and relatively low amounts of carotenoids, which might indicate slow fruit ripening (Fallik and Goren, unpublished).

In parallel, it was found that under the pearl and yellow shade nets the population of *Alternaria alternata*, the main decay-causing agent after harvest, was significantly low compared to commercial black nets. Light has profound effects on fungal biology. Growth and development of many fungal species are intricately regulated by light (Springer, 1993; Purschwitz *et al*., 2006). Fungal responsiveness to different wavelengths of light has been well documented, and blue light/UV and red/far-red light are two types of photoresponses primarily observed (Mooney and Yager, 1990; Griffith *et al*., 1994; Purschwitz *et al*., 2006, 2008; Olmedo *et al*., 2010). Blue light or a high ratio of blue to UV radiation was inhibitory to the sporulation of many important phytopathogens (Raviv and Antignus, 2004; Paul *et al*., 2005). Increases in the spectral ratios between transmitted light:Blue/near-UV was found to inhibit the sporulation of several isolates of the fungal pathogen *Botrytis cinerea* in tomato (Kotzabasis *et al*., 2008). Hence, the ability of the pearl and yellow shade nets to filter UV light and enrich the blue spectrum (Shahak *et al*., 2008) and the blue/UV (Fig. 1) may be involved in *Alternaria alternata* inhibition in the field, thus reducing inoculum levels and fruit infection during its growth and thereafter decay development during prolonged storage and shelf life (Barkai-Golan, 2001).

In conclusion, based on our results from two consecutive years of study which have shown a significant reduction in decay development on pepper fruit harvested under pearl and yellow shade nets, it seems that these two coloured shade nets influence both the pathogen in the field and the fruit ripening and its susceptibility to pathological deterioration after harvest.

Acknowledgements

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Fibrous root distribution in pineapple orange trees under semi-arid irrigated ecosystem

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Key words: Cleopatra (*Citrus reshni*), depth, radial distance, root excavation, root length density, rootstocks, Rough lemon (*Citrus jambhiri* Lush), Troyer citrange (*Poncirous trifoliate* x *Citrus sinensis* Osbeck).

Abstract: The root distribution pattern of 17-year-old pineapple orange trees budded on Rough lemon, Cleopatra and Troyer citrange rootstocks were studied by root excavation method at four radial distances, 0-75, 75-150, 150-225 and 225-300 cm from tree trunk, and at three depths, 0-15, 15-30 and 30-60 cm. Fibrous root length density (FRLD) and fibrous root length percentage differed significantly at various depths and radial distances among rootstocks. FRLD was closer to tree trunk on both horizontal and vertical planes. Root density decreased from 0.183 to 0.084, 1.051 to 0.238 and 0.238 to 0.095 cm.cm^{-3} from 0-15 cm to 30-60 cm depth within 0-75 cm radial distances from tree trunk in trees on Rough lemon, Cleopatra and Troyer citrange, respectively. Cleopatra contains the highest 0.231 cm.cm^{-3} FRLD as compared to 0.051 cm.cm^{-3} in Rough lemon and Troyer citrange. Troyer citrange has intensive lateral root development with 84% fibrous roots (FR) within 75 cm radial distance, whereas Rough lemon and Troyer has an appreciable amount up to 225 cm distance (extensive lateral). Cleopatra contained 57% FR in upper soil layer (0-15 cm) (intensive vertical). In Rough lemon and Troyer 54% FR are confined to lower depth 15-60 cm (extensive vertical root development). Troyer and Rough lemon had the same vertical, whereas Rough lemon and Cleopatra showed the same horizontal rooting pattern under arid irrigated ecosystem. Thus, irrigation depth and fertilizer placement should be critically rootstock specific.

1. Introduction

Citrus production depends not only upon soil, climate and high density planting but also rootstocks play an important role as different rootstocks have different intensities of root proliferation and penetration (Castle and Krezdorn, 1975; Neves *et al*., 2004; Morgan *et al*., 2007). Moreover, roots are the principal organ for absorption of nutrients and water from soil. Root system structure determines the volume of the soil accessible to the crop plant and it is important to maintain sufficient water and nutrient concentration within the soil occupied by the crop root system for optimal nutrient and water uptake (Kramer and Boyer, 1995; Scholberg *et al*., 2002). Increasing the density of fibrous root within a crop root system increases the amount of water and nutrients available to the crop (Eissenstat *et al*., 1999; Tinker and Nye, 2000).

The rootstock in turn can be influenced by the scion and soil environment. Performance of rootstock in a certain environment is related to total volume, configuration, lateral distribution and depth of the root system (Cintra *et al*., 1999). The root distribution pattern of a tree varies from region to region and from one rootstock-scion combination to another. Even a single rootstock-scion combination may differ in root distribution with a change in climatic condition. Mikhail and El-Zefhoui (1979) found that 79% of the total fibrous root of Valencia orange occurred in the first 60 cm of soil depth on sandy soil, whereas clay soil contained 94% in the same depth. Boman *et al*. (1999) reported that citrus production in deep sandy soils with a high volume irrigation system tends to cause the upper soil layer to dry out between long irrigation intervals and this condition favours deep rooting. Hipondoka *et al*. (2003) reported that most of the root activities in trees with regard to water uptake are performed near the soil surface in arid ecosystems of Africa. These differences in rooting pattern among rootstocks and soil environments are more likely to reflect the adaptation of plants to a given environment.

Since citrus growth and root distribution system is rootstock-dependent and may be modified as a result of changes in the root environment, a clear understanding of the root system is important to best deal with management practices such as irrigation and nutrient application and fixing the geometry in a particular ecosystem. Keeping in mind the above facts, the present investigation was carried out with the objectives to

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determine the rooting pattern of 17-year-old Pineapple orange budded on three rootstocks under an arid ecosystem of Punjab (India).

2. Materials and Methods

Study sites

The trial was conducted at the experimental orchard of the Punjab Agricultural University, Regional station, Bathinda located at 211 m above mean sea level, latitude 74° 58' E and longitude 30° 17', and average rainfall 400 mm/year. However 80% of the rainfall is received during the Southwest monsoon season (first week of July to mid September). The mean maximum temperature is 40-45°C in June with hot winds and minimum temperature is 4-5°C in January.

Soil characteristics

The soil samples collected from the experimental orchard at a depth of 0-30 cm were analysed for their physical and chemical properties. The soil type was loamy sand with clay content 13%, bulk density 1.5g/cc with moisture holding capacity of 40-45%, moisture at field capacity 25-28%. The pH of the site was 8.32 with electrical conductivity (EC) 0.2 dsm^{-1} and calcium carbonate 5-12%. The available N, P, K contents were 160-182, 13-17 and 320-346 Kg/ha, respectively.

Treatments

Mature pineapple orange trees budded on three rootstocks, i.e. Rough lemon (*Citrus jambhiri* Lush), Cleopatra (*Citrus reshni*) and Troyer citrange (*Poncirous trifoliate* x *Citrus sinensis* Osbeck), at a spacing of 6x6 m planted in 1990 were selected for the study. All three sets of five mature 17-year-old trees were grown under uniform cultural practices (i.e. irrigation with flooding); fertilizer application at 880 g Nitrogen and 440 g. Phosphorus/plant/year and mechanical weeding/hoeing were selected randomly in a randomized block design and examined for the root distribution system.

Sample collection

For each plant a circle with a radius of 3 m from the tree trunk was marked. This radius was further divided into four segments with 0-75, 75-150,150-225 and 225-300 cm radius. The circle circumference was divided into eight parts and one-eighth sections were excavated at three depths, viz. 0-15, 15-30 and 30-60 cm (Fig. 1). The roots of 15 plants were excavated with a jet of water at a pressure of 10-15 psi. The plants were exposed to a radial distance of 3 m from the trunk and down to a depth of 15 cm from the ground surface; exposed roots were painted red. The roots were then excavated to a depth of 30 cm (i.e. between 15-30 cm) and exposed roots were painted yellow. The roots were further excavated to a depth of 60 cm (i.e. between 30-60 cm) and these roots were kept as such to distinguish them from other roots. After the entire root system was exposed, the roots were collected from each segment of depth and radial distance separately and washed. The root diameter was measured with the aid of a vernier caliper and those having diameter < 0.2 cm were categorized as fibrous roots. The fibrous root length of each segment was measured using a meter scale separately.

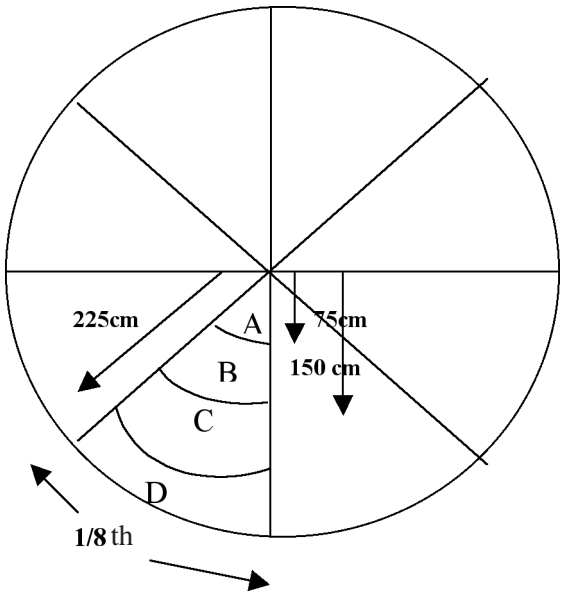

Fig. 1 - Scheme of the root sampling areas around the trunk (radial distances: A = 0-75 cm; B = 75-150 cm; C = 150-225 cm; D = 225-300 cm).

Data collection

We estimated root length per soil volume represented by the volume of soil calculated as the radial distance from the trunk by the depth increment (0-15; 15-30; 30-60 cm) for each of the 15 orange trees. The surface area of the ring of radial distance 0-75 cm (A) was determined by calculating the area of a circle with radius of 75 cm. The area of ring between radial distances 75-150 cm (B) was equal to the area of a circle with a radius of 150 cm minus the area of ring A. Similarly the areas of the other rings (C and D) were determined by subtracting consecutively the area of the adjacent smaller circle from the larger one. The volume of soil used to determine the estimated root length at each sampling location was the product of the area of each ring determined by the sampling distance and soil depth (0-15, 15-30 and 30-60 cm) then divided by 8 because only one-eighth of each ring was excavated. Fibrous root length density (FRLD) was determined by dividing the sample fibrous root length for each sampling location by their respective sample soil volume and expressed as cm $cm^{-3.}$

The root length percentage at various depth zones and radial distances from tree trunk was determined on the basis of total root length, irrespective of radial distance and depth respectively.

Statistical analysis

The experiment was set up in randomized block design with three sets and five replications. The FRLD and root length percentage of the three rootstocks at various depths and radial distances from the trunk were analysed by one-way ANOVA using Duncan's multiple range test ($P< 0.05$).

3. Results

Fibrous root length density (FRLD) was significantly different among the rootstocks. Therefore the FRLDs were pooled and analysed for interaction among rootstocks, soil depths and distances from tree trunk. Although the average FRLD to a 60-cm depth was statistically significant for Cleopatra (0.231 $cm.cm^{-3}$) in respect to Rough lemon (0.048 $cm.cm^{-3}$) and Troyer citrange (0.051 $cm.cm^{-3}$) which were otherwise at par (Table 1). A significant interaction of rootstock and depth suggests distinctly different root distribution patterns among the three rootstocks. Trees on Cleopatra had significantly greater FRLD than trees on Rough lemon and Troyer citrange, whereas the FRLD was not statistically significant between Rough lemon and Troyer citrange at all soil depths. FRLDs decreased significantly with every increase in soil depth in Rough lemon and Cleopatra, whereas with Troyer citrange FRLD was at par between 0-15 and 15-30 cm depth. The maximum FRLDs (0.067, 0.390 and 0.070 $cm.cm^{-3}$) were observed in the top 15-cm soil layer in Rough lemon, Cleopatra and Troyer citrange, respectively. A high proportion of fibrous root length (FRL) was found in the upper 0-15 cm soil containing 46, 57 and 45% of Rough lemon, Cleopatra and Troyer citrange, respectively, which also did not differ significantly. The proportion of FRL differed significantly with every increase in soil depth in Rough lemon and Cleopatra and at par in Troyer citrange at 0-15 and 15-30 cm depth. However trees grown on Rough lemon and Troyer citrange have more FRL (57%) deeper than 15 cm compared with trees grown on Cleopatra (43%), resulting in only 45% of Rough lemon and Troyer citrange root length at more than 15 cm depth. FRLD and percentage root length of Cleopatra differed significantly compared to Rough lemon and Troyer citrange at all depth zones, whereas Rough lemon and Troyer citrange were not significantly different.

Unlike soil depth, distance from trunk had more effect on distribution of fibrous roots among rootstocks (Table 2). Cleopatra had significantly greater FRLD at all 75-cm increments in radial distances from trunk in respect to Rough lemon and Troyer citrange rootstocks. Troyer citrange showed significantly more FRLD (0.173 $cm.cm^{-3}$) compared to Rough lemon (0.129 $cm.cm^{-3}$) at the 0-75 cm radial distance, whereas for greater radial distances, Rough lemon contained significantly more FRLD compared to Troyer citrange. FRLDs differed significantly with every increase in radial distance in Rough lemon and Cleopatra while in Troyer FRLDs at 75-150 and 150-225 cm radial distances did not differ significantly. The highest proportion of FRL was observed close to the trunk (i.e. 0-75 cm radial distance from trunk) in all the rootstocks. However, Troyer citrange showed maximum FRL (84%) within 75 cm radial distance whereas, trees

Table 1 - Pineapple orange tree mean fibrous root length density (FRLD) and percentage of root length in the radial distance up to 300 cm of the soil for rootstock and soil depth

Soil depths (cm)	Rough lemon		Cleopatra		Troyer citrange	
	FRLD ($cm.cm^{-3}$)	Root length 0-60cm (%)	FRLD ($cm.cm^{-3}$)	Root length 0-60cm (%)	FRLD ($cm.cm^{-3}$)	Root length 0-60 cm (%)
0-15	0.067 a	46.19 a	0.390 a	57.02 a	0.070 a	45.11 a
15-30	0.048 b	33.24 b	0.210 b	29.39 b	0.057 a	36.74 a
30-60	0.030 c	20.56 c	0.093 c	13.59 c	0.028 b	17.88 b
Average	0.048		0.231		0.052	

Fibrous root length density (FRLD) and root length (%) separation by Duncan's multiple range tests. Values followed by different letter within a column are significantly different (< 0.05) from other values in the same column. Mean (n=5).

Table 2 - Pineapple orange tree mean fibrous root length density (FRLD) and percentage of root length in the upper 60 cm of soil for rootstock and distance from the tree trunk

Radial distances (cm)	Rough lemon		Cleopatra		Troyer citrange	
	FRLD ($cm.cm^{-3}$)	Root length 0-300 cm (%)	FRLD ($cm.cm^{-3}$)	Root length 0-300 cm (%)	FRLD ($cm.cm^{-3}$)	Root length 0-300 cm (%)
0-75	0.129 a	66.54 a	0.642 a	70.31 a	0.173 a	84.20 a
75-150	0.031 b	15.87 b	0.139 b	15.23 b	0.017 b	8.26 b
150-225	0.022 c	11.39 c	0.097 c	10.58 c	0.011 bc	5.37 bc
225-300	0.012 d	6.19 d	0.035 d	3.87 d	0.004 c	2.15 c

Fibrous root length density (FRLD) and root length (%) separation by Duncan's multiple range tests. Values followed by different letter within a column are significantly different (< 0.05) from other values in the same column. Mean (n=5).

grown on Rough lemon and Cleopatra, showed 82-85% of FRL within 150 cm radial distance. The proportion of FRL beyond 75 cm radial distance was for Rough lemon and Cleopatra at par and significantly more than Troyer citrange. All rootstocks differed significantly for FRLD with every increment in radial distance, but Cleopatra and Rough lemon did not significantly differ in root length percentage and differ significantly compared to Troyer citrange.

However, the greatest FRLD in the top 15-cm depth ranged from 0.08 to 1.05 $cm.cm^{-3}$ soil at a distance of 300 cm or less for trees on Cleopatra, whereas FRLDs ranged from 0.016 to 0.183 and 0.006 to 0.238 $cm.cm^{-3}$ at the same depth and distance from trees on Rough lemon and Troyer citrange, respectively (Fig. 2). The figure illustrates that the fibrous roots are concentrated closer to the tree trunk (i.e. up to 75 cm radial distance and 0-15 cm depth). Beyond the radial distance of 75 cm there was a very sharp decrease in FRLDs in all rootstocks. The effect is more pronounced in Troyer citrange at 0-60 cm depth, followed by Cleopatra (15-60 cm) and Rough lemon (30-60 cm).

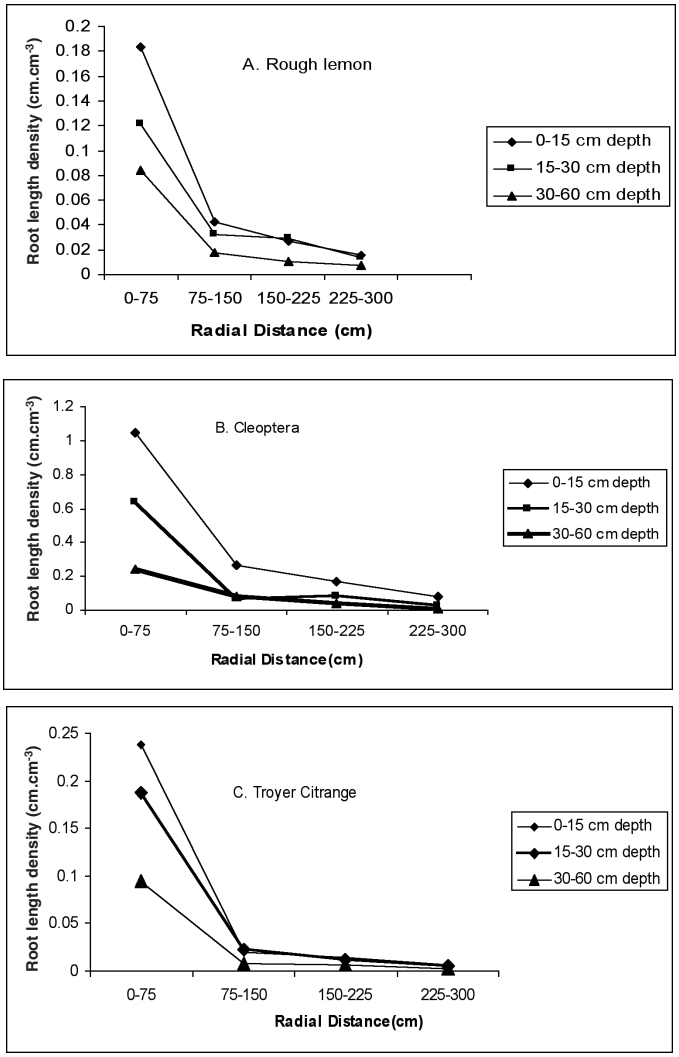

Fig. 2 - Changes in fibrous root length density as a function of soil depth from the surface and radial distance from the tree trunk for pineapple orange trees on (A) Rough lemon (n=5); (B) Cleopatra (n=5) and (C) Troyer citrange (n=5) rootstock.

4. Discussions and Conclusions

Fibrous root density was influenced by depth and distance from trunk and rootstock. However, fibrous root length observed was lower than earlier reports (Kaufman *et al*., 1972; Castle, 1980; Morgan *et al*., 2007) which may be due to sampling time in late spring because in citrus root growth is periodic; root activity declines during fall/winter with unfavourable environment and moisture stress condition then a spring growth flush takes place. Root activity then increases immediately after the cessation of shoot elongation in summer months. There is a gradual decrease in FRLD with depth and distance from tree trunk. However the FRLD's were highest near the surface and closer to trunk in all rootstocks (Castle, 1980; Kurien *et al*., 1991; Swietlik, 1992; Zhang *et al*., 1996). Cleopatra had more overall fibrous root length compared to Rough lemon and Troyer citrange, the latter which showed the same intensity. This may be due to differences in their rooting pattern or genetic make-up.

Cleopatra had more roots (57%) in the upper layer (0-15 cm) compared to Rough lemon and Troyer citrange (45% each), however Cleopatra rootstock showed only 42% fibrous roots between 15-60 cm while Rough lemon and Troyer citrange contained 54%. Hence Cleopatra may be classified as shallow rooted. Thakur *et al*. (1981) concluded that citrus is basically a surface feeder. Similarly, Avilan *et al*. (1985) reported that most Cleopatra roots (80%) were located in the top 30 cm of soil under the canopy of the tree. Similar results were reported previously by Zhang *et al*. (1996): root density was greater (75%) at 0-15 cm depth when field is flooded and nitrogen is spread and less than 10% at 30-60 cm depth in grapefruit on sour orange. Neves *et al*. (2004) found that 80% of the roots grow under 31 cm for African rough lemon and more root area was observed at lower horizon of the soil in P. trifoliata and C13 citrange as compared to Rough lemon and Sunki mandarin for Tahiti lime. Sharma and Chauhan (2005) found in apple nearly all fibrous roots above the 50 cm depth with very few roots between 75-100 cm.

Troyer citrange showed 84% fibrous root closer to tree trunk (0-75 cm) and at higher distance there was a very sharp decrease showing less than 10% at 75-150 cm distance, whereas, Rough lemon and Cleopatra has 82-85% FR within 150 cm radial distance with less than 10% fibrous root length beyond 225 cm. Thus Troyer citrange has an intensive lateral root development and Cleopatra and Rough lemon showed an extensive lateral root system. The maximum root growth in citrus takes place during summer months following rainy season, hence this may cause more lateral and less vertical root development due to the availability of water in the upper layer during active root growth period. In arid climates, higher root density are in irrigated compared to non-irrigated zones and effect

of irrigation is closer to tree trunk due to shading effect or lower evaporation under the canopy (Bielorai, 1985; Roth and Gardner, 1985; Morshet *et al*., 1989). Furthermore, Rough lemon and Cleopatra have a dense and large canopy in comparison to Troyer citrange, hence the dense and large canopy reduced soil water losses by evaporation forming a favourable environment for root development in the upper layer. Secondly, the largest part of roots are formed within the 0-15 cm depth, the most important layer for plant nutrient supply specially, phosphorus that stimulates root growth in layer fertilized with nutrients. Troyer citrange showed a reduced and somewhat upright growth of canopy hence more moisture loss under the canopy took place which make roots to divert to lower horizon for water uptake. These results are in accordance with those of Misra *et al*. (2003) in grape fruit budded on trifoliate orange. Carrizo citrange rootstock has intensive type root system and less lateral development in Hamlin rootstock (Castle and Krezdorn, 1975; Morgan *et al*., 2007). Similarly Castle (1980) and Cintra *et al*. (1999, 2000) found that Rough lemon and Cleopatra have large root system and rough lemon extensive lateral and vertical development. Kurien *et al*. (1991) reported that most root activity (75-80%) was confined within a radius of 80 cm and 24 cm in depth in acid lime on karna khatta (*Citrus karna*)

In this study, we have observed that FRLD distribution of pineapple orange trees grown on Rough lemon, Cleopatra and Troyer citrange rootstocks decreased with soil depths and lateral distances. The overall maximum FRLD was recorded in Cleopatra at all the depths and radial distances. The density of feeder roots was concentrated at a depth of 0-15 cm within 75 cm radial distance. Trees grown on Troyer citrange and Rough lemon showed an appreciable amount of FR up to 60 cm in depth and may be classified as plants with an extensive vertical root development, whereas, in Cleopatra and Rough lemon a noticeable amount of FR is confined up to 225 cm radial distance and hence can be considered as extensive lateral development. Trees on Troyer citrange have FR very closer to tree trunk (0-75 cm) i.e. intensive lateral roots. Cleopatra showed more roots in the upper soil layer (0-15 cm) and it can be considered as upper intensive root development. Therefore depth of irrigation and placement of fertilizer based on root distribution should be rootstock specific and deep ploughing should be avoided.

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Foliar application of molybdenum: effects on yield quality of the grapevine Sangiovese (*Vitis vinifera* L.)

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Key words: grapevine, foliar spray, Molybdenum, YANC.

Abstract: Field experiments with Sangiovese vines were carried out in the Chianti Classico region over a period of two years to examine the effect of molybdenum (Mo) foliar sprays on nutrient composition of leaves, petioles and berries, leaf gas exchanges, must composition, total yield, bunch size and pruning weight. Two Mo foliar doses and time sprays (Mox1: one application in early flowering; Mox3: three applications in early flowering, early fruit set and veraison) were applied. Basal sample of petioles, leaves and berries collected at fruit set (except berries), veraison and harvest for mineral analyses showed not relevant interactions between Mo and the main macro and micronutrients. Leaf gas exchanges monitored after the applications, as well as SPAD units, showed a higher activity in the Mox3-treated vines. Increased vigour was also confirmed by the slightly higher total yield, bunch size and pruning weight, as well as the delay in fruit maturation (lower sugar and polyphenol contents at harvest). No relevant discrepancy between Mox1 and the control was found, except for higher soluble solid and yeast-assimilable nitrogen contents (YANC) in the treated vines. YANC was positively influenced also in the Mox3 vines, however with no significant differences towards the Mox1 treatment. The application of Mo as a useful tool to stimulate nitrogen metabolism, as well as indications about dose and time of Mo application, are discussed.

1. Introduction

The transition element molybdenum (Mo) is a very rare but essential micronutrient for all organisms (Bortels, 1930; Fortescue, 1992), and understanding of its role and function in plants is progressing rapidly. Its importance for plants has been known for a long time (Coughlan, 1980), even though Mo itself seems to be catalytically inactive in biological systems until it is complexed by a special cofactor, the pterin (Mendel and Hänsch, 2002) which binds to diverse apoproteins. This latter compound is a unique pterin named molybdopterin or metal-containing pterin. In this form, it occurs in more than 40 enzymes catalysing many redox reactions, four of which have been found in plants (Hille, 1996; Kappl *et al*., 2002). One of these is nitrate reductase (NR) that catalyses the first step in nitrate assimilation, a pathway of key importance for plant nutrition. Nitrate reductase is the key-enzyme for nitrate assimilation while nitrogenase is found in nitrogen-fixing bacteria inside nodules of symbiotically growing species. The last step of abscisic acid biosynthesis is catalyzed by the molybdenum-enzyme aldehyde oxidase, and sulfite oxidase protects the plant against toxic levels of sulfite (Hänsch and Mendel, 2009).

Nitrogen (N) is assimilated into the cell as a fully reduced form, ammonia (NH_3). NH_3 may be obtained in free form (e.g. by fertilizers) and from the degradation of amino acids. If these sources are not available, then ammonia must be produced *via* nitrate (NO_3) reduction. When NR is a limiting factor, plant growth, development and protein synthesis by plants are reduced (Solomonson and Barber, 1990). Hence a shortage of Mo in the soil, even though plants' requirement for it is very low, or a mutational block of the cellular ability to use Mo leads to the loss of essential metabolic functions and can cause the death of the plant. Mo deficiency has been reported for many plant species including herbs, crops and trees (Gupta, 1997) and could determine poor NR activity (Hewitt, 1983), causing an inability to utilize N, with visible symptoms of chlorosis or yellowing of the leaves.

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In grapevine, N is essential in overall vine establishment and maintenance, fruit quality, and the conversion of grape juice to wine. In grape berries, N is found primarily as ammonium cations and organic compounds such as amino acids (proline, arginine, glutamine, glutamate, etc.), hexose amines, peptides, nucleic acids and proteins. There are two phases of intense N incorporation in the fruit: the first takes place during the two weeks before the "pea-size" stage of the berries; the second starts one month later at veraison and lasts an additional two weeks (Löhnertz, 1991). The amount of N in the clusters at harvest is approximately 40-44% of total available N of the entire plant (Alexander, 1957; Conradie, 1991; Weinbaum *et al*., 1984).

Furthermore, N compounds are required by yeast for the production of cell biomass and the synthesis of proteins and enzymes necessary for the biochemical process of fermentation. The readily (easily assimilable) fermentable N compounds in juice and must consist primarily of NH_3 and N available from the alpha amino acids present at harvest, particularly arginine (Bisson, 1991). Low levels of yeast-assimilable N content (YANC) in grape juice and must at harvest have been associated with sluggish and stuck fermentation and consequent undesirable levels of residual sugar in wines (Kunkee, 1991; Jiranek *et al*., 1995).

Slow and stuck fermentations are sometimes associated with grapes from vineyards composed of not vigorous plants or with high planting density, clearly characterized by scarce availability of N (Masi and Boselli, 2007). In some cases, despite plants having a sufficient level of N in their tissues, plant growth and crop yield show a clear N deficiency making it possible to hypothesize that most of the N in the plant is stored in non available forms. However, fertilizations with N are considered to be difficult, especially concerning choice of the right dose for optimum plant and yeast growth: even a low dose of N frequently causes excessive canopy growth and an unacceptable delay in fruit maturation being, at the same time, not sufficient in order to assure an optimal assimilable N concentration of the juice (Spayd *et al*., 1995). Assuming the inadequacy to reach optimal levels of available forms of N by chemical fertilization, Mo supply could be a possible solution since its key role in the activation of N metabolism could be compared to a N fertilization itself. Moreover, Mo application has been shown to increase fruit quality, sugar content (Rus'-Ko, 1979; Strakhov, 1988) and qualitative and quantitative composition of free amino acids (Veliksar, 1977) in grapevine. Mo is important in nitrogen nutrition of vines and has been suggested as a primary cause of millerandage in Merlot vines (Longbottom *et al*., 2004; Williams *et al*., 2004). It is thought that Mo directly affects the development of reproductive structures. Molybdenum is necessary for successful pollen tube growth, ovule penetration, and fertilization (Longbottom *et al*., 2004). Williams *et al*. (2004) showed that Mo increased the percent of coloured berries with one or more functional seeds and decreased the proportion of green berries, thus suggesting that Mo application affected pollination and/or fertilization, and thereafter berry development.

The present study, comprising two different levels and time of Mo supply, was undertaken to determine the effect of Mo on N metabolism by studying fruit and vegetative development in the grapevine Sangiovese (*Vitis vinifera* L.).

2. Materials and Methods

Plant material

The study was carried out in a six-year-old vineyard of Sangiovese *Vitis vinifera* (L.) cultivar, grafted onto *Berlandieri* x *Riparia* 420A, located in the Chianti Classico DOCG region (elevation about 380 m asl; weather was characterized by mild winter, hot and dry season during the summer and average yearly rainfall of 700 mm, with the highest levels in November and March). The vines were spaced 0.8 m within the row and 2.4 m between rows and pruned to a single cordon system with four spurs, with an average of eight buds growing off the cordon. Soil was rich in clay and "skeletal" material. Pre-planting studies showed that the soil chemical fertility was good for most of the elements except for N (Table 1). For that reason, every year, soil fertilizations rich in N (400 kg of NPK-fertilizer 12+10+20) had been executed since the vineyard was established; although tissue analysis did not show any mineral deficiency, neither for microelements nor macroelements (Fig. 1), light symptoms of N-deficiency were present on the vines at the moment the experiment was performed, consisting in low vigour and poor YANC must content in the previous vintages.

Treatments

During two consecutive years (2002-2003), the vineyard was divided into three homogeneous areas, of six rows each, for two Mo treatments and a control

Table 1 - Soil chemical analysis

Soil parameter	
pH (H2O)	8.2
Electrical Conductivity	126.5 µS ·cm^{-1}
Total Lime ($CaCO_3$)	7.6%
Active Lime ($CaCO_3$)	0.7%
Cation Exchange Capacity	18.5 mE·100g^{-1}
Organic Matter	0.9%
N	0.4%
Ca	1157 mg·kg^{-1}
Mg	134 mg·kg^{-1}
K (K_2O)	146 mg·kg^{-1}
P (P_2O_5)	12.5 mg·kg^{-1}
Fe	78.3 mg·kg^{-1}
Mn	85.7 mg·kg^{-1}
B	2.8 mg·kg^{-1}

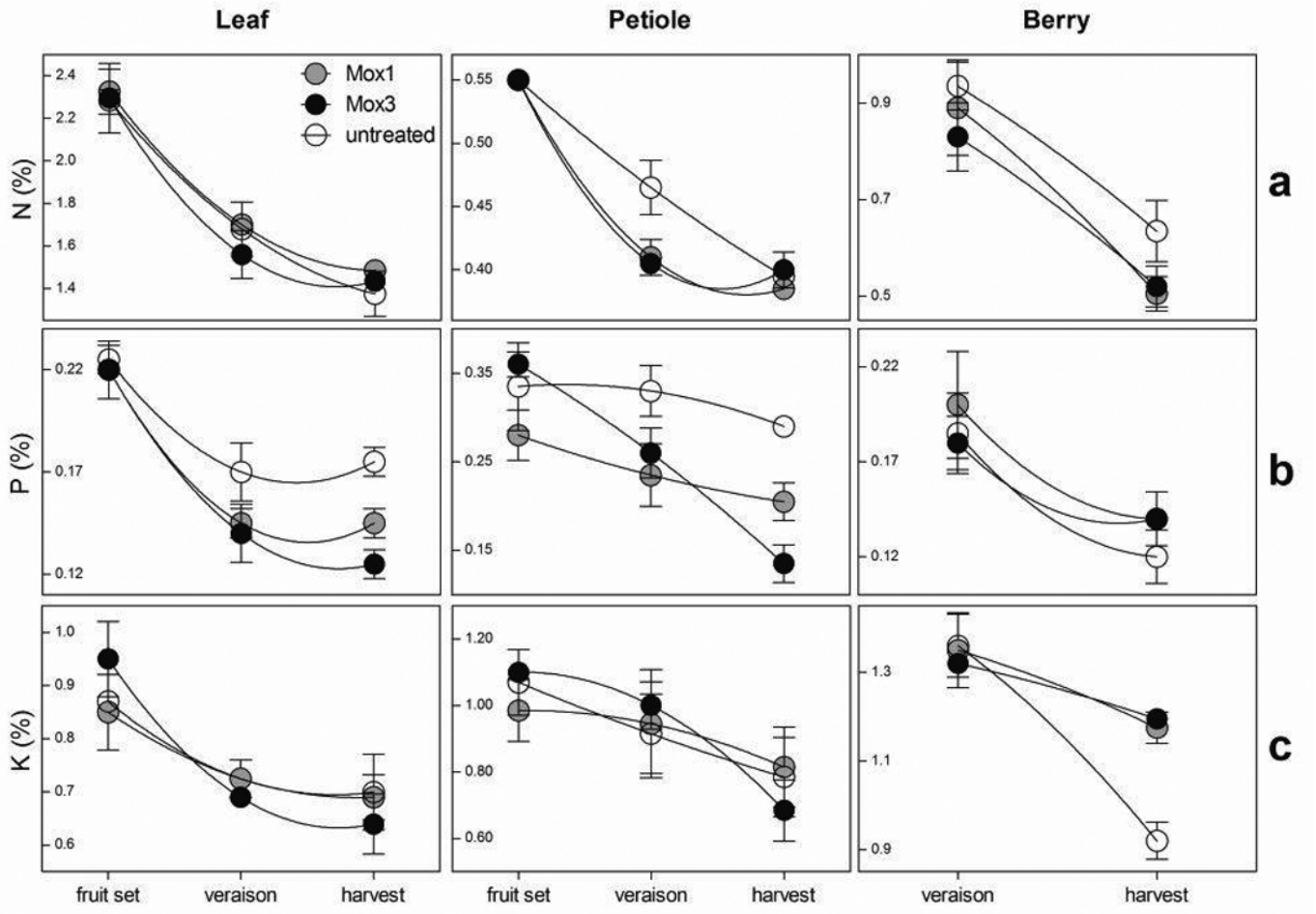

Fig. 1 - Primary macronutrient (N, P, K) concentrations in basal leaf, petiole and berry at fruit set, veraison and harvest in Mo-treated and untreated grapevine plants. Error bars reflect the LSD at $p \leq 0.05$. Mox1= Mo treatment in early flowering; Mox3= Mo treatments in early flowering, early fruit set and early veraison.

(untreated vines). Foliar applications were sprayed using 350 g·ha^{-1} of a custom-made Mo fertilizer (12% w/w) dissolved in 200 l of water. The first treatments provided a single application in early flowering (Mox1); the second provided three applications, respectively in early flowering, early fruit set and early veraison (Mox3). Recommended cultural practices of the vineyard were applied.

Data collection

Three basal leaf and petiole samples were collected (at fruit set, veraison and harvest); two berry samples were also collected from veraison to harvest. Each sample was the result of 50 to 60 organs harvested from random positions among the six rows of each treatment area. The material was rinsed with distilled water in order to take away any pesticide or fertilizer traces, then oven dried at about 80°C until thoroughly dry. Each dried sample was then ground and sent to the laboratory for chemical analysis of macro and microelements; the analyses were performed three times and results averaged.

Gas exchange measurements were taken after veraison on 25 replications obtained by selecting five leaves on five plants per treatment: the leaves were of the same age, spatial orientation and light exposure. Net photosynthetic rate (P_n) and transpiration rate (E) were recorded with a portable photosynthesis system (CIRAS-I, PPSystems, Hertfordshire, UK). Leaves were enclosed in a ventilated leaf cuvette and exposed to saturating irradiance (PAR≥1200 μmol·m^{-2}); concentration of CO_2 used for the measurements was 350 mg·l^{-1}. In addition, water user efficiency (WUE) was calculated as the rate of net photosynthesis per unit of transpired water.

According to the same statistical design, after veraison (and 30 days later) on the same plants and leaves leaf chlorophyll and nitrate content (Westerveld *et al*., 2003) was estimated non-destructively using a portable chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, N.J., U.S.).

At fruit set, veraison and harvest, berry samples were picked randomly from 10 plants per treatment, crushed, pressed and the juice analyzed for the yeast fermentable nitrogen content. The YANC is expressed as mg·l^{-1} of N as the sum of the assimilable nitrogen from ammonia plus the assimilable nitrogen from alpha amino acids.

At harvest, 300 berries were collected, weighed and then squeezed by hand and filtered through a strainer. Samples were harvested randomly from 10 plants (30 berries per plant) per treatment. The expressed juice was measured to determine total soluble solids (Brix) with a hand refractometer and titratable acidity by titration with 0.1 N NaOH to pH 7. Titratable acidity was expressed by $g \cdot l^{-1}$ of tartaric acid. Anthocyanin content of the must was performed *via* Glories indices (Glories, 1984). Indices evaluated were: total anthocyanins A_1 ($mg \cdot l^{-1}$); extractable anthocyanins $A_{3,2}$ ($mg \cdot l^{-1}$); extractability assay E_A (based on the ratio of the above two anthocyanin values); and extractability of the tannins contained in the seed M_P.

During the season, five plants per treatment were labelled; they were harvested separately in order to calculate average yield per vine and their pruning materials were weighed as an indicator of the vegetative vigour. The ratio of total yield to total pruning weight per vine, referred to as the Ravaz Index (Champagnol, 1984) was used as an indicator of balance between fruit and vegetative growth.

Statistical analysis of variance on the obtained data was performed with separation of the means by LSD test at 5%. No year effect was observed, thus data shown are the average of the measurements of two years.

3. Results

Tissue analysis showed in general no deficiency or excess in the levels. The effect of applied Mo on the concentration of the nutrients in basal petioles, leaves and berries was small and of little practical importance. Nitrogen (N) content of leaf, petiole and berry compared with control treatment was not influenced by Mo (Fig. 1 a). Leaf and petiole phosphorous (P) content tended to decrease in leaf and petiole after Mo supply, especially in the Mox3 treatment (Fig. 1 b), while berry content was not influenced. On the contrary, potassium (K) level was significantly higher in berries of Mo-treated plants (Fig. 1 c) at harvest. Calcium (Ca) content was not affected by any kind of treatment in all analyzed organs (Fig. 2 a), while petiolar and leaf manganese (Mn) tended to increase (Fig. 2 b), especially at harvest time and for Mox3 treatment. Moreover, for the same treatment, a higher and significant level of Mn was found in berries during both measurements (at veraison and harvest time). No significant differences were detected for the level of Iron (Fe) (Fig. 2 c).

CO_2 assimilation rate of Mox3-treated vines was significantly higher than those of the Mox1-treated and untreated vines (Fig. 3 top). Mox3 showed also relevant difference towards the other treatments in transpi-

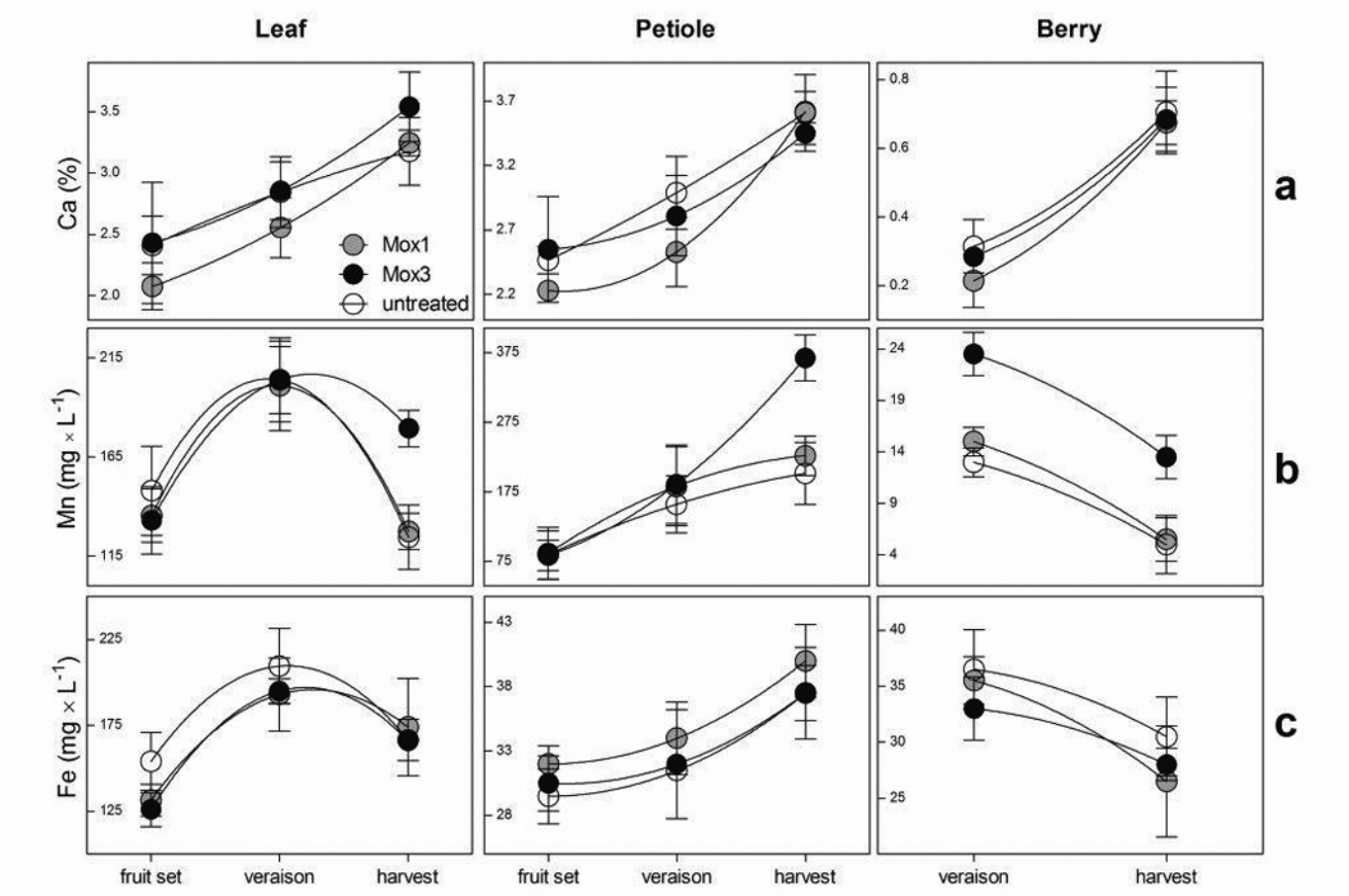

Fig. 2 - Secondary macro (Ca) and micronutrient (Mn, Fe) concentrations in basal leaf, petiole and berry at fruit set, veraison and harvest in Mo-treated and untreated grapevine plants. Error bars reflect the LSD at $p \leq 0.05$. Mox1= Mo treatment in early flowering; Mox3= Mo treatments in early flowering, early fruit set and early veraison.

ration rate (Fig. 3 middle); as a result of the higher transpiration rate, however, the Mox3 vines showed more critical water use efficiency values (Fig. 3 middle).

Accordingly, the SPAD unit measurements were significantly influenced by Mo treatments (Fig. 3 bottom). Mox3 showed the highest values in both sampling data; the values decreased for Mox1 treated vines after veraison, although remained higher than those of the control plants.

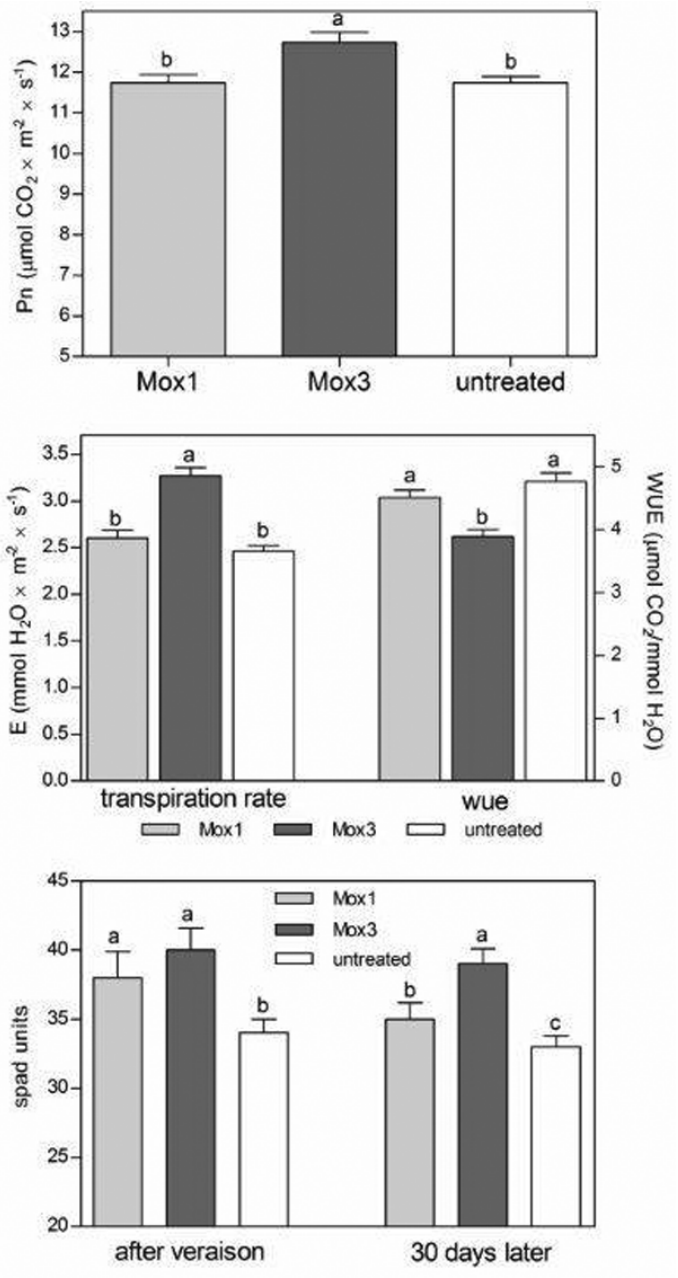

Fig. 3 - Top: assimilation rates measured after veraison in Mo-treated and untreated grapevine plants. Middle: transpiration rates and water use efficiency measured after veraison in Mo-treated and untreated grapevine plants. Bottom: SPAD units measured after veraison and a month later in Mo-treated and untreated grapevine plants. Error bars reflect the LSD at $p \leq 0.05$. Different letters correspond to significant differences ($p \leq 0.05$). Mox1= Mo treatment in early flowering; Mox3= Mo treatments in early flowering, early fruit set and early veraison.

The YANC content increased significantly after the applications showing the positive role of Mo on NR activity to provide an available form of N for the plant (Table 2). Furthermore, at veraison, after more than a month from the first (and single, for Mox1) application, both Mox1 and Mox3 berries had higher levels of YANC compared to the control ones. Surprisingly, no significant difference was found between the two Mo treatments, not showing a linear correlation between Mo dose and NR activity. In any case at harvest time, no effect was found for either of the Mo treatments: the difference tended to decrease during berry ripening, disappearing at harvest when both the treated and untreated vines had quite the same amount of available N.

Significant differences in must soluble solids (SS) analysis at harvest were observed between Mox1 treatment (higher value) and both Mox3 and control treatments (lower values). Titratable acidity (TA) and pH levels were similar for treated and untreated vines (Table 3). Total anthocyanin content was significantly greater in the untreated vines compared to the treated ones while extractable anthocyanins in Mox1-treated and control plants were similar and higher than in Mox3-treated vines (Table 3). Mox1-treated vines must extractability assay and M_P value suggest a better polyphenolic evolution during ripening. Mox3 treatment negatively influenced anthocyanin extractability and polyphenol evolution as confirmed by the highest value of M_P.

Table 2 - Yeast assimilable nitrogen content (YANC)

YANC (mg·l-1 N)	Fruit set	Veraison	Harvest
Mox1	96 a	66 a	68 NS
Mox3	92 a	62 a	64 NS
untreated	74 b	34 b	55 NS

Different letters within the same column correspond to significant differences ($p \leq 0.05$). Non-significant results are marked NS.
Mox1= Mo treatment in early flowering;
Mox3= Mo treatments in early flowering, early fruit set and early veraison.

Table 3 - Berry soluble solids (SS), pH, titratable acidity (TA), berry Glories indices (A_1: total anthocyanins; $A_{3,2}$: extractable anthocyanins; E_A: extractability assay; M_P: extractability of the tannins contained in the seed analysis results)

Technol. analysis	SS (Brix)	pH	TA ($g \cdot l^{-1}$)
Mox1	23.9 a	3.1 NS	7.3 NS
Mox3	22.1 b	3.0 NS	7.5 NS
untreated	22.6 b	3.0 NS	7.3 NS

Polyphenol. analysis	A_1 ($mg \cdot l^{-1}$)	$A_{3,2}$ ($mg \cdot l^{-1}$)	E_A (%)	M_P (%)
Mox1	1535 b	1007 a	34.4 b	29.6 b
Mox3	1437 b	706 b	50.9 a	40.5 a
untreated	2038 a	1005 a	50.7 a	32.0 b

Different letters within the same column correspond to significant differences ($p \leq 0.05$). Non-significant results are marked NS.
Mox1= Mo treatment in early flowering;
Mox3= Mo treatments in early flowering, early fruit set and early veraison.

The different ripening development between Mox3 and untreated vines probably has to be linked to the influence of Mo on the fruit and vegetative activity of the plant, suggested also by the average bunch weights (Fig. 4 right) and the total pruning weight, which were higher in the Mox3 treatment (Fig. 4 left): plants treated with higher dose of Mo had greater difficulty completing the ripening process within the same time as the other vines. No significant differences were however revealed with regard to total yield per vine (data not shown) and Ravaz Index (Fig. 4 left).

4. Discussion and Conclusions

The present study supports earlier suggestions that Mo affects the N metabolism in grape, whose unquestionable evidence is in the YANC analysis of the berry. The fact that the reaction of the plants to Mo applications was measurable, in terms of YANC, as early as a few days after the first treatments performed before fruit set gives interesting indications about its rate of speed. The absence of differences in YANC content between the two Mo treatments suggests that when the general plant availability of N is good (like in this case), higher doses of Mo result to be useless.

This hypothesis, therefore, is not confirmed by the analysis of the bunch weight and total yield per vine nor in the development of the ripening process, when relevant differences were found between the two Mo treatments, as well as a clearly positive correlation between the quantity of Mo supplied and the appearance of such phenomena, well known to be connected with an increased N-availability for the plant. In fact, Mo, especially when supplied at high rate, is able to increase plant activity (see results about gas exchange analysis) as the result of rendering the N of the plant more available for the metabolisms and, consequently, plants tend to be generally more vigorous.

Finally, important information about fertilization management can be underlined: a high dose treatment (Mox3) seems to be inadequate and sometimes contrary to the quality of production. Mox1-treated vines, on the other hand, did not show an influence of the treatment on vegetative and fruit balance nor on phenolic evolution and, interestingly, differentiated positively from the control regarding the sugar content of the berry and the YANC, even though its level at harvest was not optimal. Assuming the Mox1 dose and time optimal for quality, further analysis should be aimed at correlating the role of Mo on NR activity towards N availability in order to understand how to increase must quality.

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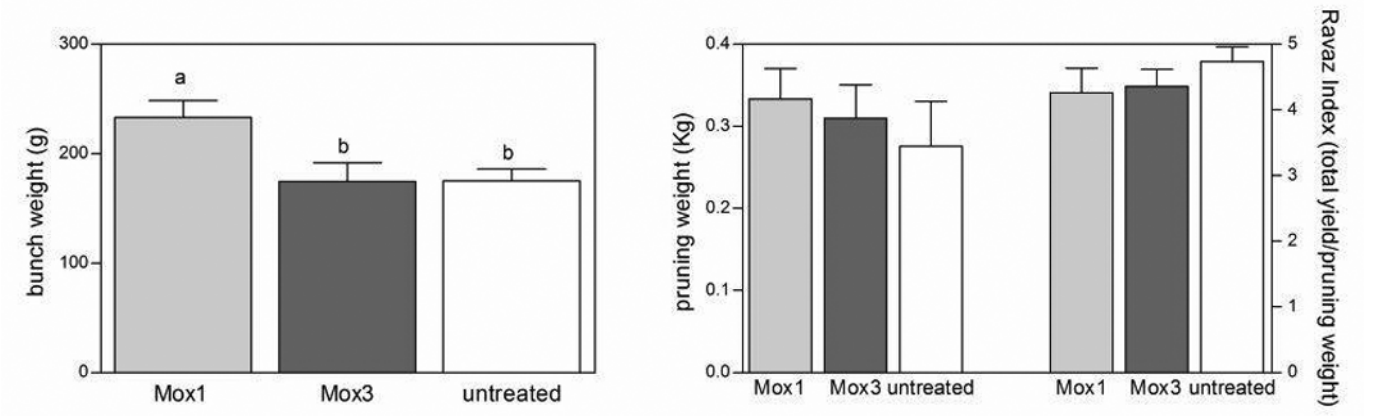

Fig. 4 - Bunch weight (left), total pruning weight and Ravaz index (right) measured in Mo-treated and untreated grapevine plants. Right: Error bars reflect the LSD at $p \leq 0.05$. Different letters correspond to significant differences ($p \leq 0.05$). Non-significant results are marked NS.

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Shoot-tip vitrification protocol for red chicory (*Cichorium intybus* L.) lines

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Key words: cryopreservation, genetic resources, PVS2, RAPD, red chicory, vitrification.

Abstract: Shoot tips from *in vitro* stock plants of red chicory 'Rosso di Chioggia' line were cryopreserved by one-step vitrification. After two days of cold-hardening on hormone-free MS medium and loading for 30 min in a mixture of 2 M glycerol and 0.4 M sucrose at 25°C, shoot tips were dehydrated with PVS2 vitrification solution at 0°C for 60 min and plunged directly into liquid nitrogen. The post-thaw survival of shoot tips was achieved 79% when was cultured on recovery medium containing 0.5 mM BA. Observed regrowth, after six weeks of culture in the same medium composition, was 100%. Rooted cryopreserved microshoots showed good quality when transferred to the greenhouse. Preliminary results proved that the genetic fidelity of the cryopreserved line was maintained. The same vitrification protocol was then applied to three other red chicory lines, 'Rosso di Treviso precoce', 'Rosso di Treviso tardivo' and 'Castelfranco'. A simple and effective protocol for the cryopreservation of red chicory shoot tips has been successfully developed as a result of this study.

1. Introduction

In Italy, the major production area of red chicory (*Cichorium intybus* L. var. intybus), covering about 9 thousand hectares, is located in the Veneto region, one of the most economically important areas for vegetable production. An ancient typology, 'Rosso di Treviso Tardivo', that was progenitor of the present varieties of red chicory, was introduced into Italy in the 15th century. Over time, growers selected those plants having good production, while in recent years this leafy vegetable has undergone intense selection and breeding work, and several improved typologies have been produced (such as 'Rosso di Treviso precoce', 'Rosso di Verona', 'Rosso di Chioggia', 'Variegato di Castelfranco', and others) (Veneto Agricoltura, 2002), which are highly appreciated for their quality and productivity.

At the "Po di Tramontana" experimental farm in Rosolina (Veneto Agricoltura, Rovigo, Italy) a specific breeding program to select high-performance lines has been continuing for many years. Every year after in-field evaluation, the most valuable lines are introduced and maintained *in vitro* by subculturing every three weeks. The stock plants obtained from these lines are transferred to the greenhouse to produce high quality seeds to be used for the production of high quality red chicory. The costs of stock culture maintenance and the risks of contamination and decay of lines can be reduced by introducing cryopreservation as a tool for a long-term preservation. Cryopreservation involves the maintenance of plant propagules at ultra-low temperatures (-196°C, LN): under these conditions, biochemical and most physical processes are completely arrested and as such, plant material can be stored for unlimited periods.

Cryopreservation studies have been reported for Belgian endive (*Cichorium intybus* L. var. foliosum, cvs. Flash, Rumba and Carolus) by controlled-rate freezing (Demeulemeester *et al*., 1992; 1993) and encapsulation-dehydration techniques (Vandenbussche *et al*., 1993). Controlled-rate freezing is regarded as the traditional approach to plant cryopreservation. Although controlled-rate freezing has been effective for cryopreserving differentiated tissues (Reed and Uchendu, 2008), reports on cryostorage using this technique are limited. One of the practical limitations of the controlled-rate freezing approach is the need for an expensive programmable freezer (Engelmann, 2000). However, applying traditional (controlled-rate freezing) and new procedures (one-step freezing technique), the first examples of "cryogenic banks" are available today in several countries (Reed, 2001; Sakai and Engelmann, 2007). At the same time, the development

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of a vitrification system has made it possible to increase the number of plant species that can be cryopreserved (Towill and Bajaj, 2002; Lambardi and De Carlo, 2003, 2009; Sakai *et al*., 2008).

In the present study, a vitrification/one-step freezing procedure was developed using 'Rosso di Chioggia' shoot tips, with exposure of explants to the vitrification solution and rapid cooling by directly immersing in liquid nitrogen (LN). This procedure was then applied to three different red chicory typologies. In addition, validation of this method using molecular markers is presented with regard to maintenance of genetic stability.

2. Materials and Methods

Plant material

Microshoots of red chicory 'Rosso di Chioggia (mp C7 4/212) were adventitiously induced from leaf portions through direct organogenesis (Fig. 1 a). The lines were obtained by isolating each adventitious shoot and transferring them onto a semi-solid MS (Murashige and Skoog, 1962) medium, supplemented with 30 g l^{-1} sucrose, 1.0 μM 6-benzyladenine (BA) and 7 g l^{-1} agar (proliferation medium). The pH of the medium was adjusted to 5.8 before autoclaving for 20 min at 121°C.

The shoot cultures were placed at 21±1°C under

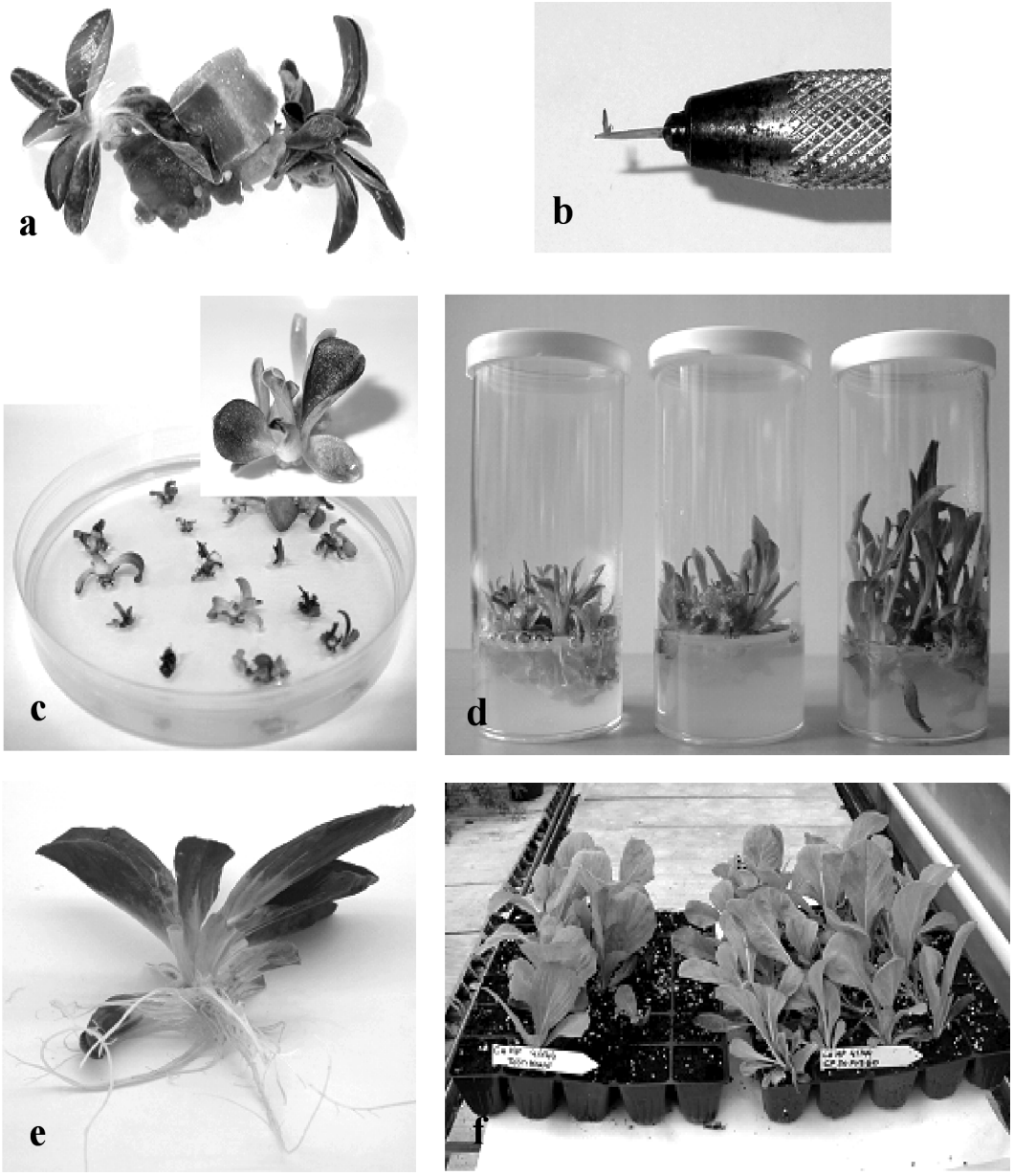

Fig. 1 - Cryopreservation of red chicory "Rosso di Chioggia" line (a-f). a - microshoots induced adventitiously from a leaf portion; b - a shoot tip just after excision from a shoot bud; c - shoot tip survival after three weeks and a well-formed shoot tip after cryopreservation with 60 min of PVS2 treatment (detail); d - regrowth in plastic cylinder on MS medium with 0.5 μM BA; e - cryopreserved rooted microshoot; f - plantlets from cryopreservation, after potting and acclimation in the greenhouse.

12-hr photoperiod conditions (60 μmol m^{-2} s^{-1} photosynthetically active radiation) and subcultured every three weeks (standard culture conditions). Short-day conditions avoided floral initiation.

Vitrification procedure

Before bud excision, stock plants were exposed to 4°C with an 8-hr photoperiod for a three-week cold-hardening period. Shoot tips (2-3 mm long), consisting of the apical meristem and 4-5 leaflets, were then excised from microshoots (Fig. 1b) under a stereo microscope in aseptic conditions, and were precultured on hormone-free MS medium supplemented with 0.09, 0.3 or 0.7 M sucrose for two days at 4°C under an 8-hr photoperiod to determine the effect of sucrose pretreatment on cryopreserved explant survival.

To induce dehydration, the shoot tips were loaded with a cryoprotectant (Loading Solution, LS; 2 M glycerol and 0.4 M sucrose) (Matsumoto *et al*., 1994) for 30 min at 25°C in 2-ml Nalgene® cryovials (10 shoot-tips per cryovial) and subsequently incubated at 0°C with the PVS2 (Plant Vitrification Solution 2; 30% w/v glycerol, 15% w/v ethylene glycol, 15% w/v DMSO in MS medium containing 0.4 M sucrose) (Sakai *et al*., 1990). In order to choose the longest possible exposure time of shoot tips to the vitrification solution while avoiding toxic effects, shoot tips were exposed to PVS2 for 30, 60, 90 or 120 min and evaluated for shoot tip survival. After dehydration, the samples were suspended in 0.6 ml of fresh PVS2 solution and rapidly frozen to –196°C by direct immersion in LN, where they were stored for at least 2 hr. For recovery, they were quickly rewarmed by plunging the cryovials into a 40°C waterbath (warming rate: about 150°C min^{-1}), unloaded from the PVS2 solution, washed for 20 min at 25°C in a liquid MS medium containing 1.2 M sucrose and finally plated onto the proliferation medium and maintained under standard culture conditions. Cryopreserved shoot tips were assessed for survival after three weeks.

In addition to proliferation medium, five different recovery media were tested to improve the regrowth of cryopreserved shoot tips: MS added with 0.5, 1.0 or 5.0 μM BA, MS with 1 μM Thidiazuron (TDZ) and MS with 0.5 g l^{-1} Activated Charcoal (AC). The role of AC has been discussed in many reports on plant tissue culture with different effects (Thomas, 2008).

Survival was defined as a percentage of green shoot tips after three weeks from thawing. Explants were transferred to plastic tubes (one shoot for each tube) and six weeks after LN treatment the percentage of plants demonstrating regrowth was assessed. Explants treated with PVS2 but not exposed to LN were used as controls.

In vitro *rooting of cryopreserved shoots*

Cryopreserved shoots were transferred to jars containing 100 ml of MS rooting medium with 20 g l^{-1} sucrose, 7 g l^{-1} agar and supplemented with 2.5 μM indole butyric acid (IBA). The jars were kept in a climatic chamber, at 21°C, under a light intensity of 40 μmol m^{-2} s^{-1} and a 12-hr photoperiod. After four weeks, the rooted microplantlets were transferred to *ex vitro* conditions. The plantlets were transferred to the greenhouse into pots with substrate containing both sterilised peat and sand in a ratio of 2:1. After five weeks, the plants were transferred to the field and their morphological stability was evaluated.

Cryopreserved chicory lines

The cryopreservation procedure, optimized for 'Rosso di Chioggia' shoot tips, was then tested on shoot tips from *in vitro* plants of three red chicory lines, 'Rosso di Treviso precoce' (TVP S5), 'Rosso di Treviso tardivo' (TVT) and 'Variegato di Castelfranco' (C90 S6'). All three lines are included in a program to safeguard and conserve red chicory in the Veneto region.

Encapsulation-vitrification procedure

'Rosso di Chioggia' shoot tips, cold-hardened at 4°C for two days, were encapsulated into 2% Na-alginate beads and treated with LS solution for 1 hr on a rotary shaker at 25°C. When this solution was removed, the beads were dehydrated by exposure to PVS2 for 2, 3 or 4 hr at 0°C, then placed in cryovials (five beads per cryovial) and plunged immediately into LN. Encapsulated shoot tips were thawed in a waterbath at 40°C for 3 min and placed on MS proliferation medium. Survival was assessed after three weeks.

Data analyses

A minimum of 30 samples were used for each treatment and each experiment was repeated twice. Statistical analysis of percentages was carried out by the χ^2 test or non-parametric statistical test, the Post Hoc Multiple Comparison Test (Marascuilo and McSweeney, 1977), both at $P \leq 0.05$.

Genetic stability assessments of cryopreserved plantlets

Random Amplified Polymorphic DNA (RAPD) analysis was applied on red chicory *in vitro* shoots to evaluate the genetic stability of cryopreserved and non-cryopreserved shoots (untreated control) collected from the same stock cultures. Genomic DNA was extracted from approximately 100 mg of leaves with a 'DNeasy Plant Mini Kit' (Quiagen). DNA concentration was determined using a spectrophotometer at 260 nm, and an aliquot of DNA was diluted to a working concentration of 20 ng μl^{-1}.

RAPD profiles were generated using 24 arbitrary 10-mers as primers, of which 10 primers were then selected for the reproducibility, the legibility and the

Table 1 - Nucleotide sequences of DNA primers used for RAPD analysis

Primer	Primer sequence
1253	GTT TCC GCC C
1247	AAG AGC CCG T
M2	ACA ACG CCT C
M3	GGG GGA TGA G
M10	TCT GGC GCA C
M13	GGT GGT CAA G
A1	CAG GCC CTT C
A5	AGG GGT CTT G
A9	GGG TAA CGC C
C7	GTC CCG ACG A

stability of the RAPD pattern: 1253, 1247, M2, M3, M10, M13, A1, A5, A9 and C7 (Table 1).

The amplification of DNA was performed according to Vettvori *et al*. (1996). Fragments were separated on 2% agarose gels by electrophoresis and visualized by ethidium bromide staining under UV light. For each primer, amplification reactions were repeated at least twice and only those having reproducible band partners were used. Minor fragments, which tend to be unstable in staining intensity, and therefore not reliable, were not considered.

3. Results

Influence of PVS2 times and preculture treatments

Prolonged exposure at 0°C to the vitrification solution appeared to be harmful for explants. Indeed, shoot-tip survival decreased after exposures of 90 min or more, while 30-min treatment was not enough to protect the explants during ultra rapid freezing (Table 2). When the incubation time was limited to 60 min, more than 72% of cryopreserved (+ LN) shoot tips survived after plating onto the proliferation medium (Fig. 1c). Incubation for 90 and 120 min in the PVS2 solution showed even a slight decrease in percentage survival of shoot tips without freezing (- LN). This result highlights the importance of incubation time with vitrification solution for shoot tip survival.

To enhance the shoot tips' osmotolerance to the vitrification solution, preculture treatments with different sucrose concentrations for two days at 4°C was applied before the LS and PVS2 treatments. However, frozen explant survival exhibited a significant decline when the shoot tips were pre-cultured on media containing high sucrose concentrations (Table 3), indicating that sucrose treatments are not appropriate to improve red chicory shoot tip survival.

All three red chicory lines can be successfully cryopreserved by loading shoot tips in PVS2 for 60 or 90 min, prior to freezing in LN. Among the lines, maximum explant survival ranged between 65% in 'Rosso di Treviso precoce' and 76% in 'Rossso di Treviso tardivo' and 'Variegato di Castelfranco', respectively with 60 and 90 min of PVS2 treatment (Table 4).

Table 2 - Survival of cryopreserved shoot tips of 'Rosso di Chioggia' after exposure to PVS2 for different time periods. Data were recorded three weeks after thawing (LN, liquid nitrogen)

PVS2 exposure time (min)	Shoot tip survival (%)[z]	
	- LN	+ LN
30	100 a	33.5 b
60	100 a	72.5 a
90	80 b	40.0 b
120	80 b	30.0 b

[z] In each column, percentages followed by different letters are significantly different at $P \leq 0.05$ by the post hoc Multiple Comparison test.

Table 3 - Effect of sucrose concentration in preculture medium (two days at 4°C) on 'Rosso di Chioggia' shoot tips cryopreserved following 60 min PVS2 treatment

Sucrose concentration (M)	Shoot tip survival (%)[z]
0.09	68.0 a
0.3	47.5 a
0.7	17.5 b

[z] Percentages followed by different letters are significantly different at $P \leq 0.05$ by the post hoc Multiple Comparison test.

Table 4 - Shoot tip survival percentage of three selected red chicory lines, following incubation and immersion in LN

Red Chicory Line	Loading time with PVS2 (min)	Shoot tip survival (%)[z]	
		- LN	+ LN
'Rosso di Treviso precoce'	60	80.0 a	65.0 a
	90	70.0 a	55.0 a
'Rosso di Treviso tardivo'	60	80.0 a	66.7 a
	90	90.0 a	76.7 a
'Variegato di Castelfranco'	60	90.0 a	50.0 a
	90	100 a	76.0 b

[z] For each select line and each column, percentages followed by different letters are significantly different at $P \leq 0.05$ by the χ^2 test.

Recovery media

The addition of BA to the recovery medium was found to be beneficial for post-thaw recovery of the shoot tips, even if with an increase of the concentration callus formation is stimulated. The highest post-thaw survival of 'Rosso di Chioggia' shoot tips was obtained with 0.5 µM BA (79%) (Table 5). When 1 µM TDZ was used, 33% of shoot tips survived, but after the first subculture (21 days), they stopped growing, after which no shoots developed. There was no survival of shoot tips on hormone-free medium supplemented with activated charcoal.

Table 5 - Effect of recovery media on 'Rosso di Chioggia' shoot tip survival (three weeks) and regrowth (six weeks) after thawing

Recovery medium	Shoot tip survival (%)	Shoot tip regrowth (%)[z]
0.5 µM BA	79.1 a	100 a
1.0 µM BA	47.6 b	95.0 a
5.0 µM BA	47.8 b	95.4 a
1.0 µM TDZ	33.3 c	0
0.5 g/l AC	0	--

[z] In each column, percentages followed by different letters are significantly different at $P \leq 0.05$ by the post hoc Multiple Comparison test.

A further increase of regrowth was easily achieved by transferring the explants in tubes containing proliferation medium with BA. The best concentration for the development of 'Rosso di Chioggia' chicory shoots was again 0.5 μM. Indeed, the plantlets obtained from this cytokinin treatment were generally taller and produced more shoot apices (Fig. 1d). After *in vitro* rooting (Fig. 1e), the microplantlets were transplanted into pots and successfully acclimated *in vivo* (Fig. 1f).

Field observations of the plantlets confirmed the morphological stability of acclimatized plants with the original mother plants (Fig. 2).

Fig. 2 - Experimental field, the arrow indicates the plot with cryopreserved 'Rosso di Chioggia' plants.

Vitrification vs encapsulation-vitrification

Cryopreservation by shoot-tip vitrification, using the PVS2 solution, was compared with the encapsulation-vitrification procedure in 'Rosso di Chioggia' line. The highest survival percentage of cryopreserved shoot tips was obtained by vitrification procedure (Table 6).

Table 6 - Shoot tip survival percentage of 'Rosso di Chioggia' shoot-tips after two different cryogenic procedures

Cryogenic procedure	Shoot tip survival (%)[z]
Vitrification	76.0 a
Encapsulation - vitrification	35.2 b

[z] Percentages followed by different letters are significant at $P \leq 0.05$ by the post hoc Multiple Comparison test.

The encapsulation-vitrification procedure proved to be less effective for the cryopreservation of the selected red chicory line. However, only a maximum of 35% explant survival was achieved when the longest treatment of the beads with PVS2 was applied; this percentage is markedly lower than that reported in the literature using an encapsulation-dehydration procedure in chicory (Vandenbussche *et al*., 1993).

Assessment of molecular stability by RAPD

To assess the genetic fidelity of plantlets regrown from cryopreserved shoot-tips, the RAPD patterns were compared with untreated samples of the same red chicory lines. Out of 24 primers screened, 10 selected primers produced clear and reproducible bands. The number of bands for each primer varied from five to twelve. Each primer generated a set of amplification products of a size ranging between 350 bp and 3000 bp.

Preliminary results proved that the genetic fidelity of the cryopreserved lines was maintained. For all 10 primers tested, RAPD fragment patterns of plantlets from cryopreserved shoot-tips did not show differences with respect to untreated shoots. Figure 3 represents amplified band patterns produced by two primers (1253 and 1247). Genetic fidelity was confirmed also by the other primers.

4. Discussion and Conclusions

'Rosso di Chioggia' shoot-tips cryopreserved using LS treatment for 30 min, PVS2 for 60 min at 0°C, and recovery on medium containing 0.5 μM BA resulted with a high survival rate (79%). This result is slightly inferior to that obtained in Belgian endive, cv. Flash (83%) where Demeulemeester *et al*. (1992) used a more complex procedure, consisting of cooling the explants to -40°C at a rate of 0.5°C min^{-1} prior to immersion in LN. In the present study, the vitrification protocol applied to different red chicory lines showed high survival percentages, even if tolerance to the PVS2 varied in different lines. Differences among lines can be considered to be genotype-dependent; these results are consistent with other experiences on cryopreservation in other plant species (De Boucaud *et al*., 2002; Kim *et al*., 2006; Benelli *et al*., 2009).

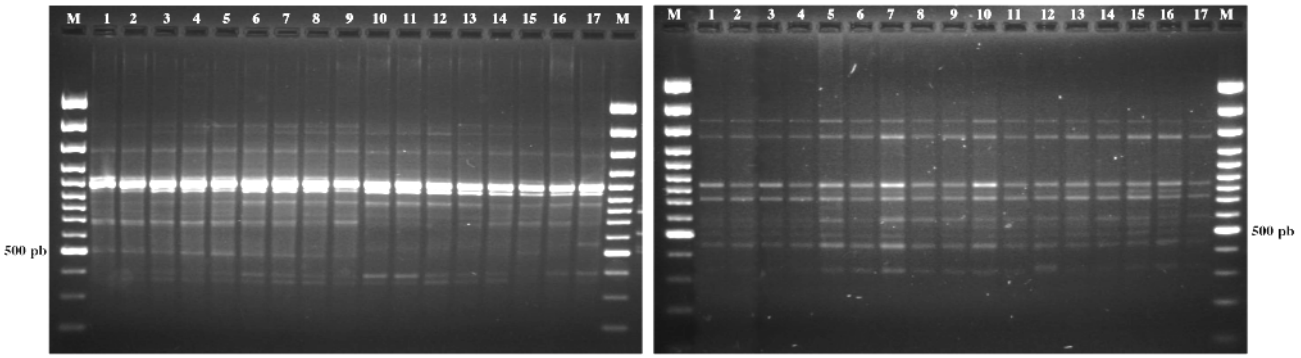

Fig. 3 - RAPD profiles generated with primers 1253 (left) and 1247 (right) from untreated and cryopreserved plantlets of 'Rosso di Chioggia' line. In both images, lanes M correspond to the 100-bp ladder; lanes 1-9 correspond to the amplification products from untreated samples, lanes 10-17 correspond to the amplification products from cryopreserved plantlets.

In many cases, preculture with sucrose can be very efficient to improve cryopreservation (Matsumoto *et al*., 1998), but such treatments applied on apical buds of several species did not induce increases in recovery after vitrification (Sakai, 2000). Sugar treatments can influence the membrane and protein composition (Ramon *et al*., 2002; Carpentier *et al*., 2005), influencing flexibility and permeability of the membrane. In red chicory shoot tips, preculture treatment on MS with 0.09 M sucrose for two days before PVS2 loading resulted in the best survival with respect to 0.3 and 0.7 M sucrose, but it did not improve the post-thaw survival percentage in the cryogenic procedure.

The type and concentration of growth regulators in the recovery medium is important for survival and regrowth of cryopreserved explants (Turner *et al*., 2001). Red chicory shoot tip survival (79%) was improved when the recovery medium contained 0.5 μM BA; callus formation was induced when the BA concentration was increased, in particular with 5 μM BA. Callus is not desired and represents a limitation in development of the cryopreserved explants. This phenomenon is observed in several species when a high concentration of BA was used (Wang *et al*., 2003). Demeulemeester *et al*. (1993) held the explants for one week on medium supplemented with plant growth regulators and then transferred them to hormone-free medium to avoid a callus phase in three varieties of chicory (Flash, Rumba and Carolus) after cryopreservation. This protocol was effective for restricting callus formation, but led to a decrease in survival percentage. In the present study, with the 'Rosso di Chioggia' line, we obtained the highest survival rate without callus formation by applying the lowest BA concentration and the best regrowth when the single cryopreserved shoot was transferred in tube.

Addition of activated charcoal in post-thaw recovery medium resulted deleterious for shoot tip survival and regrowth; whereas thidiazuron gave a low survival of explants after three weeks but no subsequent growth.

Rooted microshoots of 'Rosso di Chioggia', obtained from cryopreserved shoot-tips, exhibited high survival and vigour in the greenhouse.

Application of the encapsulation-vitrification procedure showed a limited survival of cryopreserved explants and further experimentation is needed to optimize the protocol for these red chicory lines.

A few genetic studies using molecular markers have been carried out on red chicory, mainly for the genetic characterization of commercial varieties (Barcaccia *et al*., 2003), and particularly RAPD markers were used to construct the genetic map of *C. intybus* (De Simone *et al*., 1997) to identify and to evaluate the phylogenetic relationships among cultivars of chicory (Koch and Jung, 1997; van Stallen *et al*., 2000, 2001; Barcaccia *et al*., 2003).

Assessment of the genetic stability of cryopreserved shoots can be performed with different techniques (Harding, 2004). In this study, RAPD markers were adopted because of their simplicity, rapidity and ability to screen a randomly large part of the genome.

Genetic stability was reported after RAPD analysis of cryopreserved plantlets and the untreated shoots. Comparison of the DNA patterns of control and frozen material did not reveal any variations caused by the cryoprotectant treatments or cryostorage.

The results obtained are confirmed by numerous studies that demonstrated that cryopreservation did not affect the genetic stability in various species, such as potato (Harding and Benson, 2000), grape and kiwi (Zhai *et al*., 2003), chrysanthemum (Martin and Gonzalez-Benito, 2005), *Citrus* spp. (Lambardi *et al*, 2007), and apple (Liu *et al*., 2008).

Moreover, the morphological analysis carried out in the field confirmed that there were no significant differences between plants derived from control and cryopreserved shoots.

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Review paper

Crop physiology of elephant foot yam [*Amorphophallus paeoniifolius* (Dennst. Nicolson)]

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Key words: corm, dormancy, sprouting, Elephant foot yam.

Abstract: ***Amorphophallus paeoniifolius*** **(Dennst. Nicolson), syn. *A. campanulatus* (Roxb.) BL. exDence (also elephant foot yam) is largely cultivated in the Philippines, Java, Indonesia, Sumatra, Malaysia, Bangladesh, India and China. In India, it is cultivated in the states of Andhra Pradesh, West Bengal, Gujarat, Kerala, Tamil Nadu, Maharashtra, Uttar Pradesh and Jarkhand. Sree Padma, Gajendra, Sree Athira (a hybrid), Bidhan Kusum and NDA-9 are some of the high yielding *Amorphophallus* varieties released for cultivation. The corm production potential of this crop is 50-80 t ha^{-1} and net economic return is about 2000 – 3000 US$ per ha. Plant growth and corm yield is influenced by the size of planting material (corms/cormels/corm pieces), plant spacing, nutrient management and water availability. Nevertheless, the production aspect of this crop is less understood as scanty research has been conducted in this crop. The available literature on growth and productivity of elephant foot yam is briefly described in this article.**

1. Introduction

Amorphophallus paeoniifolius (Dennst.), syn. *A. campanulatus* (Roxb.) BL. exDence (also elephant foot yam) is an herbaceous, perennial C_3 crop. It is basically a crop of southeastern Asian origin. It serves as a source of protein as well as starch. It has long been used as a local staple food in many countries such as the Philippines, Java, Indonesia, Sumatra, Malaysia, Bangladesh, India, China and southeastern Asian countries (Chandra, 1984; Sugiyama and Santosa, 2008). It is commercially cultivated due to its production potential and popularity as a vegetable in various Indian cuisines. In India, it is cultivated in Andhra Pradesh, West Bengal, Gujarat, Kerala, Tamil Nadu, Maharashtra, Uttar Pradesh, and Jarkhand states whereas in northern and eastern states, the wild, local cultivars grown are generally used for making vegetable pickles and medicine preparations for various aliments. The crop is also cultivated as an intercrop along with turmeric (Fig. 1) and under coconut (Fig. 2) or banana. In recent years, farmers in Bihar and Uttar Pradesh have also begun cultivation. Under improved cultural practices and high yielding varieties the production potential of this crop varies between 30 and 100 t ha^{-1} and the net profit (economic return) is about 2000 – 3000 US$ per ha (AICRP, 2004, 2005, 2006 a, b, 2007, 2008, 2009). This crop also offers export potential in India since it is not commercially cultivated in other

Fig. 1 - Elephant foot yam as an intercrop with turmeric. Arrows indicate elephant foot yam plants.

Fig. 2 - Elephant foot yam under coconut.

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countries (Misra and Shivalingaswamy, 1999; Misra, 2000; Misra *et al*., 2001). In India, 'Sree Padma', 'Gajendra', 'Sree Athira' (a hybrid), 'Bidhan Kusum' and 'NDA-9' are some of the high yielding *Amorphophallus* varieties released for cultivation (AICRP, 2006 a). The corms are usually eaten as a vegetable after boiling or baking and are rich in calcium, (50 mg g^{-1}), phosphorus (34 mg g^{-1}) and vitamin A (260 IU g^{-1}). The leaves are used as a vegetable by local tribes in India because they contain a high concentration of vitamin A (Rajalakshmi *et al*., 2001). Elephant foot yam plants grow well in medium to light soils (coarse-textured sandy soils) with adequate amounts of organic matter because they prefer well-aerated soils. The crop can tolerate temporary flooding, but anaerobic water logging causes corm rot. In Kerala, elephant foot yam is planted in February and harvested during November-December under rainfed conditions. In Andhra Pradesh, the crop is planted during September-October and harvested in June (winter season crop) or planted in June and harvested in January (rainy season crop) under irrigated conditions. In West Bengal, the crop is planted in October and harvested in June under irrigated conditions.

This review summarizes the available literature on growth and productivity of elephant foot yam.

2. Shoot characteristics

The new shoot (leaf) sprout emerges from the cut corm pieces or full corm used as planting material (Plate 1 A and B). The time of emergence (sprouting) of new shoots depends on the dormancy status of the planting material. If the planting material has completed its dormancy before planting, then the new shoot sprout will emerge as soon as it is planted. Leaf emergence is delayed when the apical buds of seed corms are damaged or cut pieces of corm are planted. Leaves were found to emerge earlier when whole corms were planted than when cut corms were planted, irrespective of corm size (Sen *et al*., 1996). When whole corms, bud portions or upper half sections were planted, buds sprouted 2-3 weeks after planting. However, buds started to sprout 4-7 weeks after planting when vertical 1/2, 1/4 and 1/8 corm sections and lower half corm sections were planted (Sugiyama and Santosa, 2008). Once the sprout is initiated, further development of new shoots may be completed within 30 days (Plate 2 A to F). Leaves are basal, compound, pinnate, solitary and erect. Leaves are medium to very large in size. The plant develops leaves by using preserved carbohydrates in seed corms (planting material) and then daughter corms (new corms) enlarge by using

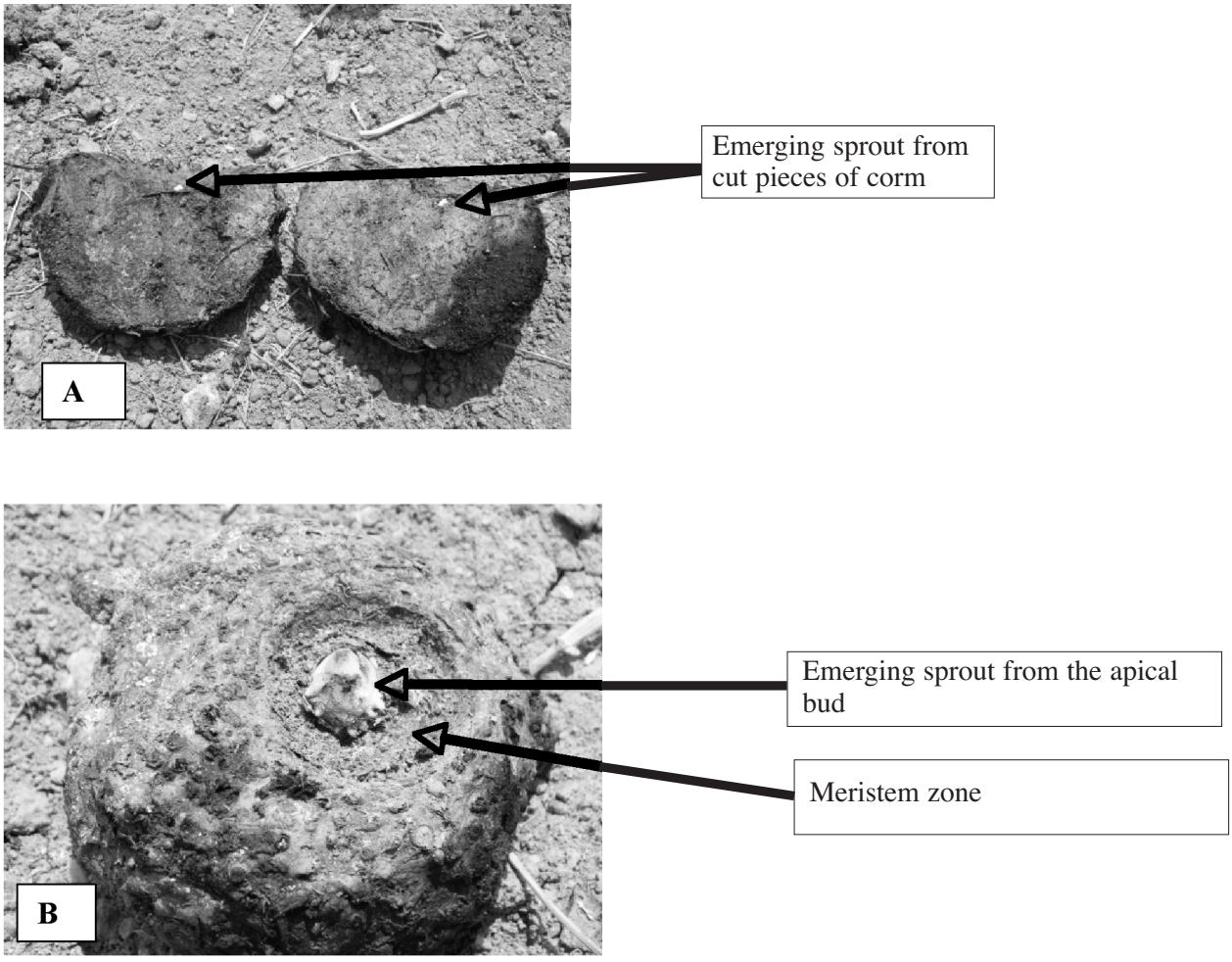
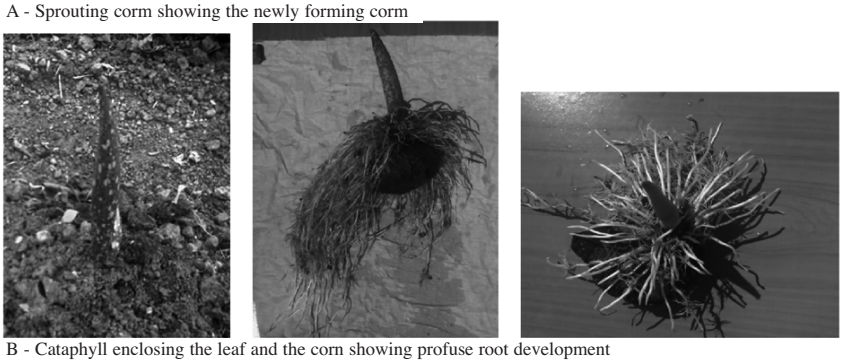


Plate 1 -New sprout emerging from cut corm pieces (A) and full corm (B) of elephant foot yam before planting.

A - Sprouting corm showing the newly forming corm



B - Cataphyll enclosing the leaf and the corn showing profuse root development

C - Early stage of leaf emerging out from the cataphyll

D - Leaf emerging out from the cataphyll

E - Spreading canopy

F - Full plant.

Plate 2 - Different stages (A-F) of leaf development from the sprout in elephant foot yam.

assimilates synthesized by the leaves. In general, *Amorphophallus* corms have one apical bud, which exists inside the cavity in the head part of seed corms. Three or four small cataphylls existing in the head part of corms cover the apical bud in which the first leaf primordium has already differentiated at planting. The cataphylls elongate concomitantly with leaf development. Possibly, they protect a leaf from damage by soil impedance during development. Furthermore, subepidermal cells of cataphylls may contain needle-like crystals of calcium oxalate which presumably offer protection to a young leaf from damage by pests. Cataphyll size depends on corm size and plant age. The cataphylls wither after leaves become mature. Leaves are composed of a petiole (pseudostem) and three rachises with many leaflets. The number of leaves which develop during the growing season is dependent on corm age. During a growing season up to 12 leaves may be produced successively. As such, more than two leaves may coexist at the same time. The number of leaves is

also determined by the size of planting materials. Plants originating from small corms (10 g) produce three to eight leaves, while large corms (500 g) usually produce one or two leaves during a growing season. Under field conditions, weeds grow much before shoot development from planted corms because of corm dormancy and delay in sprouting. Under weedy conditions, leaves are submerged under weeds (Fig. 3) and the number of leaves, total leaf area, leaf thickness and fresh masses of corms decreases markedly (Santosa *et al*., 2006 c). When preflowering and post flowering corms with similar fresh masses were planted both types of corms sprouted at about the same time; however, leaf sizes (length of petioles and rachis) were larger in preflowering corms than in postflowering corms (Sugiyama and Santosa, 2008). Up to 150-250 leaflets may be produced per leaf and this may vary among accessions.

Fig. 3 - A heavily weed-infested elephant foot yam field.

The leaf area of any one of the three lobes of *A. campanulatus* leaves showed a highly significant correlation (r = 0.93 to 0.97) with total leaf area (Patel and Mehta, 1987). The number of stomata in the lower epidermis increased from 10.22 per unit area at 50 days after planting (DAP) to 17.78 per unit area at 150 DAP (Gopi *et al*., 2008). A stoma has two adjacent cells surrounded by four subsidiary cells (Plate 3 A and B). The leaf area index increased with time and reached a maximum (6.1) at 120 DAP at a planting density of 140 x 10^3 plants ha^{-1} (Das *et al*., 1997). On the other hand, the LAI reached 4.4 and 5.4 at a planting density of 100 x 10^3 and 120 x 10^3 plants ha^{-1} respectively. Petioles (pseudostem) look like the stems of normal plants and are cylindrical in morphology. In general, large petioles indicate that the corm is also large. Depending upon the variety, plant spacing or size of planting material used, the mean shoot length varied between 47.3 and 122.5 cm (Mukhopadhyay and Sen, 1986; Ravindran and Kabeerathumma, 1991; Sen and Das, 1991; Goswami and Sen, 1992; James George and Nair 1993; Geetha, 2001; Suja *et al*., 2005, 2006; AICRP, 2004, 2005, 2006, 2007, 2008, 2009; Saraswati *et al*., 2008). Increases in N application from 50 to 150 kg ha^{-1} increased shoot length by 11%, (Mukhopadhayay and Sen, 1986) or did not increase shoot length and girth (Geetha, 2001), while increases in K application from 50 to 150 kg ha^{-1} did not have any significant effect on shoot growth (Mukhopadhayay and Sen, 1986; Geetha 2001). Regardless of plant spacing, an increase in size of planting material increased plant (pseudostem) height; plant height was maximum (84.6 cm) when 1 kg cut corm piece was used as planting material. Closer plant spacing (60 x 45 cm) increased plant height (53.8 cm) more than wider plant spacing (90 x 90 cm) (James and Nair, 1993). Plants produced from whole seed corms were taller than those produced from cut pieces of corm of the same size. This may be due to early sprouting and better root ramification (Sen and Das, 1991).

Canopy spread was found to vary between 70.2 and 143.8 cm (Ravindran and Kabeerathumma, 1991; Sen and Das, 1991; Goswami and Sen, 1992; James and Nair, 1993; AICRP, 2004, 2005, 2006 a, b, 2007, 2008, 2009). Regardless of plant spacing, increases in the

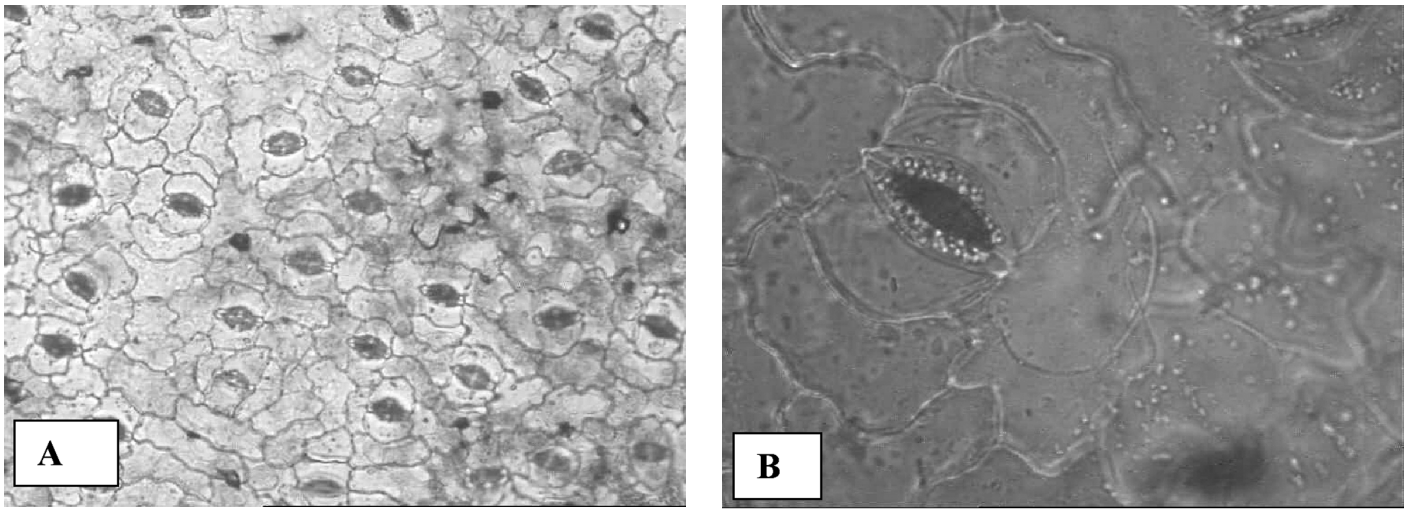


Plate 3 - A and B Stomata in elephant foot yam leaf. A) Stomata on the abaxial leaf surface (10x10). B) Single stoma with subsidiary cells (40x10).

size of planting material increased canopy spread and canopy spread was maximum (132.7 cm) when 1 kg cut corm piece was used as planting material at wider plant spacing (90 x 90 cm) (Sen and Das, 1991). Canopy spread was greater in plants raised by planting whole seed corms than in plants produced from cut pieces of corms of the same size. This was presumably due to early sprouting and better root ramification (Sen and Das, 1991).

Biomass production of shoots (leaf and pseudostem/petiole) increased up to 120 and 150 DAP respectively and declined thereafter, whereas corm dry weight and total dry matter production (TDMP) showed a steady increase up to maturity. The corm dry-matter production (CDMP) per ha increased with increases in planting material size or plant density and the highest CDMP (25.6 t ha^{-1} and 19.4 t ha^{-1} respectively) was observed at six months after planting (MAP) by using 250 g cut corm pieces as planting material or with high plant density (14 plants m^{-2}) (Das *et al*., 1997). Crop growth rate (CGR) increased gradually up to 120-150 DAP and sharply declined at maturity as crop growth ceased. However, the relative growth rate (RGR) continued to decrease with crop age and was the highest at the early growth stage (Das *et al*., 1997). The leaf area increased with increases in planting material size or plant density and the highest leaf-area index (5.4) was observed between 4 and 5 MAP by using 250 g cut corm pieces as planting material or with high plant density (14 plants m^{-2}) (Das *et al*., 1997). Similary CGR increased with increase in planting material size or plant density and highest CGR (25.3-32.2 g $m^{-2}day^{-1}$) was observed at 5 months by using 250 g cut corm piece as planting material. The CGR was 22. 4 g m^{-2} day^{-1} at a plant density of 14 plants m^{-2} (Das *et al*., 1997). Treating corm pieces from the bottom portion of corm with growth regulators thiourea, KNO_3 and GA_3 effectively influenced the growth characters and GA_3 gave the maximum corm yield (Das *et al*., 1997).

3. Plant growth regulators

Application of triazole compounds (systemic fungicides) triadimefon (TDM), paclobutrazole (PBZ) and propiconazole (PCZ) through soil drenching increased total root length (by 8.85-75.92%), dry weight of whole plant (by 71.44-84.91%), intercellular CO_2 concentration (by 25.12-27.91%), leaf thickness, number of spongy and palisade cells, number of chloroplasts per cell, net photosynthetic rate (P_N) (by 15.7-28.92%) and water use efficiency (WUE) (by 56.81-87.9%) as compared to untreated control plants. In contrast, total leaf area, transpiration rate (T_R) and stomatal conductance decreased (Gopi *et al*., 2005, 2008, 2009).

4. Root characteristics

Roots grow out from the surface of newly developing daughter corms at the base of the pseudostem through the remnants of the cataphylls concomitantly with leaf emergence. These roots extend horizontally and are densely distributed at a shallow depth of the top 15-30 cm soil depth. The roots are cylindrical and 2 to 5 mm thick. Roots grow more than 1 m in length under adequate soil moisture conditions or under adequate rain and are known as “rain roots”. Under dry soil conditions, the root length decreases to less than 30 cm length. The transverse section (T.S.) of root shows about 25 layers of thin walled parenchymatous cortex cells surrounding a central stellar portion with eight protoxylem points (Plate 4 A and B).

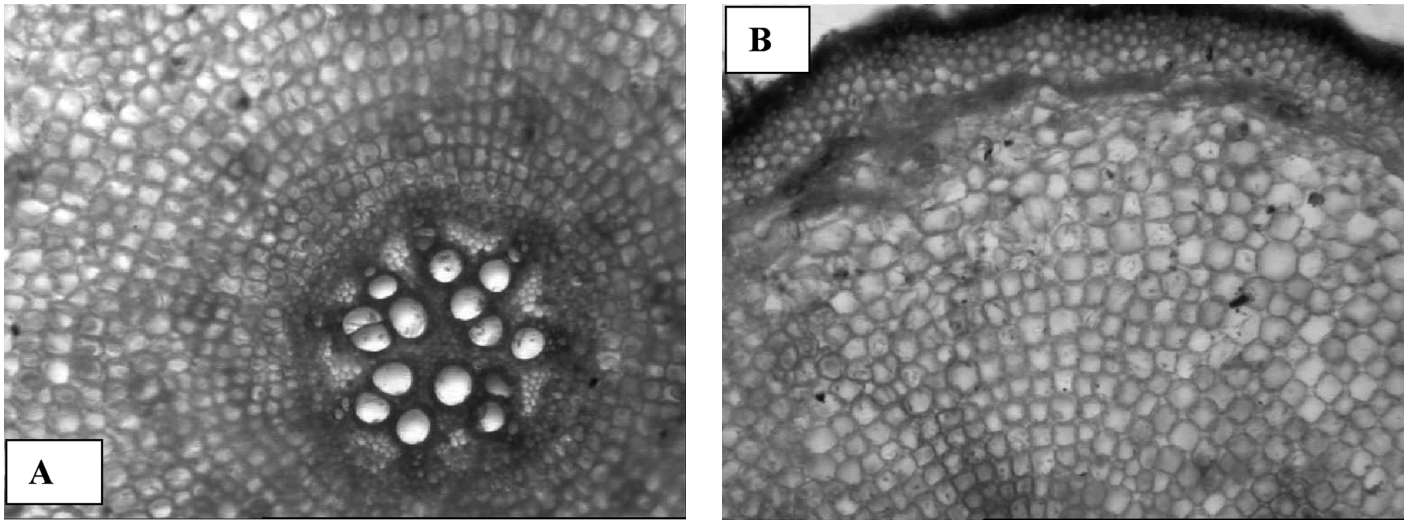


Plate 4 - A and B root anatomy of elephant foot yam (4x10). A) T.S. of root showing stele. B) T.S. of root showing cortex.

5. Corm development and yield

A new daughter corm is formed at the region between the petiole (pseudostem) and seed corm when a sprout grows out from the corm (Fig. 4). Then, roots appear from the surface of new corm and attain a maximum dry mass at 90 DAP. The daughter corm begins to enlarge after a leaf has fully expanded (one to two months) and remarkable enlargement occurs later. The dry mass of seed corms (planting material) decreases gradually, finally decomposing within three months after the new shoot sprouts and develops. After the corm has been initiated, it continuously grows and bulks as long as there is adequate moisture in the soil. Morphologically, the corm is a shortened stem with compressed nodes and internodes. There are many small lateral buds (about 1 mm in height) and one large lateral bud (5-15 mm in height) arranged in a definite pattern in concentric nodes of corms. The number of lateral buds per node ranges from 13.0 to 43.3. The number of lateral buds is larger in the middle region of corms than in the head and bottom regions. About 20% of visible lateral buds develop into cormels in the head and middle regions of corms, while about 8% of visible buds develop into cormels in the bottom region. Therefore, the middle region of corms produces a larger number of cormels than other regions (Sugiyama and Santosa, 2008).

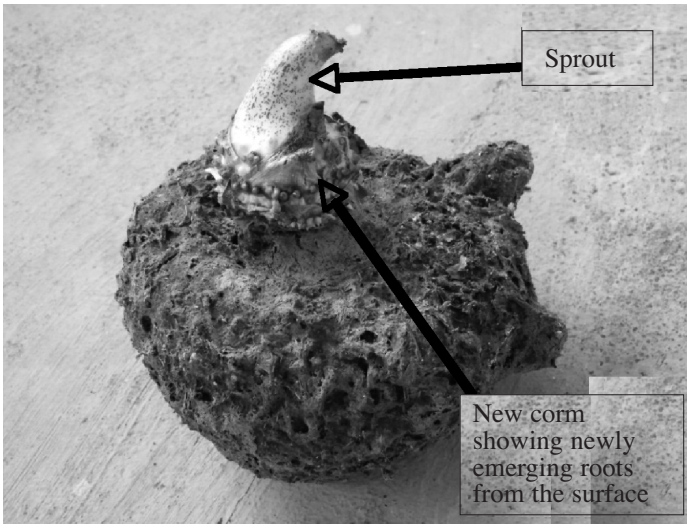


Fig. 4 - New corm formed at the base of the apical sprout before planting.

Corm growth rate (corm bulking rate) increased steadily between 1 and 5-6 MAP. Maximum corm bulking rate (7.2-8.2 g plant^{-1} day^{-1}) was observed during the fifth or sixth MAP (Mukhopadhayay and Sen, 1986; Nair *et al*., 1991). Corm bulking efficiency (final corm weight or size as compared to that planted) follows four rules of thumb: 1) at identical plant spacing, corm bulking efficiency decreases with increases in planting material size in both cut pieces and whole corm (Table 1); 2) at a given constant planting material size, corm bulking efficiency increases with increases in plant spacing (Table 2); 3) corm bulking efficiency is greater in the case of whole corm than cut pieces of corm used as planting material (Table 3); and 4) under rainfed conditions, using constant size of planting material, corms harvested from a particular field or a plot show gradient sizes (Table 4). Nevertheless, the proportion of gradient sizes may narrow under the best management and soil conditions. Thus, corms of desired size can be produced by using appropriate sized planting material and plant spacing. Production of full corms of 1 kg size is suitable for home consumption because cut corms may perish rapidly.

Increasing the level of N from 100 to 200 kg ha^{-1} or K_2O from 75 to 150 kg ha^{-1} increased the plant height

Table 1 - At identical plant spacings, corm bulking efficiency decreases with increase in planting material (cut pieces or full corm) size

Size of corm planted (g)	Plant spacing	Size of corm harvested (g)	Corm bulking efficiency	Reference
Cut pieces				
100	50 x 50 cm	513	5.1	Rajib *et al*., 2007
100	60 x 20 cm	500-700/1000	5-7/10	Nedunchezhiyan, 2006, James and Nair, 1993
200	50 x 50 cm	659	3.3	Rajib *et al*., 2007
250	60 x 45 cm	740	3.0	James and Nair, 1993
250	50 x 50 cm	840	3.4	Sen and Das, 1991
300	55 x 50 cm	810	2.7	Ghosh *et al*., 2008
500	60 x 45 cm	1063	2.1	James and Nair, 1990
500	50 x 50 cm	950	1.5	Sen and Das, 1991
500	55 x 50 cm	1320	2.6	Ghosh *et al*., 2008
750	60 x 45 cm	1017	1.4	James and Nair, 1990
750	50 x 50 cm	1230	1.6	Sen and Das, 1991
Full corm				
250	50 x 50 cm	1360	5.44	Sen and Das, 1991
500	50 x 50 cm	1480	3.0	
750	50 x 50 cm	1800	2.4	
1000	50 x 50 cm	2530	2.5	
400-500 g [(z)]	90 x 90 cm	3-4 kg	6-10	Nedunchezhiyan, 2006
1 kg [(z)]	1.2 x 1.2 m	5 kg	5	Ravindran (pers. comm.)
5 kg [(z)]	1.5 x 1.5 m	15 kg	3	Nedunchezhiyan (pers. comm.)

[(z)] Corm bulking efficiency in farmer's field under the best management practices.

Table 2 - At a given constant planting material size, corm bulking efficiency increases with increase in plant spacing

Size of corm planted (g)	Plant spacing (cm)	Size of corm harvested (g)	Corm bulking efficiency	Reference
100	50 x 30	279	2.8	Rajib *et al*., 2007
100	50 x 40	373	3.7	
100	50 x 50	513	5.1	
200	50 x 30	389	1.9	
200	50 x 40	510	2.6	
200	50 x 50	656	3.3	
250	60 x 45	740	3.0	James and Nair, 1993
250	90 x 45	927	3.7	
250	90 x 90	1143	4.6	
300	40 x 40	810	2.7	Ghosh *et al*., 2008
300	55 x 50	1005	3.4	
300	65 x 60	1265	4.2	
300	70 x 70	1450	4.8	
300	90 x 85	1585	5.3	
500	40 x 40	1170	2.3	Ghosh *et al*., 2008
500	55 x 50	1320	2.6	
500	65 x 60	1610	3.2	
500	70 x 70	1800	3.6	
500	90 x 85	1970	3.9	
500	60 x 45	1063	2.1	James and Nair, 1993
500	90 x 45	967	1.9	
500	90 x 90	1533	3.1	
750	60 x 45	1017	1.4	James and Nair, 1993
750	90 x 45	1290	1.7	
750	90 x 90	1880	2.5	

Table 3 - Corm bulking efficiency is greater in the case of whole corm than the cut pieces of corm used as a planting material (Sen and Das, 1191)

Size of corm planted (cut pieces) (g)	Size of corm harvested (g)	Multiplication ratio	Size of whole corm planted (g)	Size of corm harvested (g)	Corm bulking efficiency
250	840	3.4	250	1360	5.4
500	950	1.5	500	1480	3.0
750	1230	1.6	750	1800	2.4
1000	1740	1.7	1000	2530	2.5

and corm bulking rate (Sen *et al*., 1996). Increases in N application from 50 to 150 kg ha^{-1} increased corm growth (corm bulking rate) by 10.6-27.6% during the six month growth period (Mukhopadhayay and Sen, 1986). The effect of N was more pronounced during the initial growth period than during the later growth period. The increase in corm bulking rate due to increase in N application from 50 to 150 kg ha^{-1} was highest (27.6%) during the four month growth period but declined to 15.3% and 10.6% during the fifth and sixth MAP respectively. The increase in N application from 50 to 150 kg ha^{-1} increased the mean corm weight per plant by 21.3%. The corm yield per ha increased by 20% with an increase in N application. The corm yield was 84.6 and 102.3 t ha^{-1} with N at 50 and 150 kg ha^{-1} application respectively. Increases in K application did not significantly increase corm growth, mean corm weight per plant and corm yield per ha. However, N and K had significant interactive effects on corm growth (corm bulking rate), mean corm weight per plant and corm yield per ha and this appears to be mainly due to N (Mukhopadhayay and Sen, 1986). Shoot height, basal shoot (pseudo-stem) girth, and dry matter accumulation in shoot increased and reached a peak at 120 DAP. Corm and total (shoot and corm) dry matter increased up to 150 days and declined thereafter. Maximum shoot height (85.2 cm), shoot girth (16.4 cm), shoot dry matter (6.63 t ha^{-1}) and corm yield (67.83 t ha^{-1}) were obtained with the application of 150 kg ha^{-1} N and K in two splits (Verma *et al*., 1995). Treating planting material (corms) with 2% *Azotobacter* solution at the time of planting and application of 9.0 kg ha^{-1} of culture mixed with 40 kg of soil at the root zone of the crop along with 150 kg N ha^{-1} resulted in high corm yield (64.9 and 62.2 t ha^{-1} respectively) (Mukhopadhayay and Sen, 1999).

Table 4 - Under rainfed conditions, with use of constant size of planting material, corms harvested from a particular field or a plot shows gradient sizes

Size of harvested corms	Proportion (%)
2-2.5 kg	1-2
1.5-2.0 kg	5
1.0-1.5 kg	5
900 g	5-6
700 g	6-7
600 g	7-8
500 g	15
400 g	12
300 g	10
200 g	10
100 g	10
50 g	10

Corm yield varied between 30.9 and 85.4 t ha^{-1} depending upon the variety, cultural practices (particularly plant spacing) and manurial practices (Mukhopadhayay and Sen, 1986; Nair *et al*., 1991; Ravindran and Kabeerathumma, 1991; Goswami and Sen, 1992; James and Nair, 1993; Kundu *et al*., 1998; Geetha, 2001; Suja *et al*., 2005, 2006, 2007, Suja and Sundaresan, 2008 a, b). Corm yields between 39.6 and 98.9 t ha^{-1} were obtained due to application of 100-200 kg N and 100-150 kg K_2O_5 each per ha (Nair *et al*., 1991; Sen and Das, 1991; Kundu *et al*., 1998). Application of farmyard manure at a rate of 30 t ha^{-1} increased the fresh mass or corms by 15 %, while application of N at 150 kg ha^{-1} increased yield by 6.5% (Patel and Mehta, 1984). Kabeerathumma *et al*. (1987) reported that 100 kg ha^{-1} N, 38 kg ha^{-1} of $^{P}2^{O}5$ and 267 kg ha^{-1} of K_2O were removed from the field every year when 33 t ha^{-1} of corms were produced. Organic farming (FYM at 35 t ha^{-1} + green manuring with cowpea to generate 2 0-2 5 t ha^{-1} of green matter + neem cake at 1 t $ha^{-1}$and ash at 3 t ha^{-1}) increased corm yield by 25.37% (62.67 t ha^{-1}) as compared to traditional method (farmer's practice, FYM 25-30 t ha^{-1} + and ash at 3 t ha^{-1}) (49.99 t ha^{-1}) and by 19.21 % as compared to conventional method (FYM 25 t ha^{-1} + NPK @ 100: 50: 150 kg ha^{-1}) of cultivation (52.57 t ha^{-1}) (Suja *et al*., 2005, 2006, 2007, Suja and Sundaresan, 2008 a, b, 2009).

The corm yield was significantly influenced by the size of seed corm and higher yields were recorded from planting materials of 1 kg size (Sen *et al*., 1984; Asokan, 1984; Sen and Das, 1991). Increasing the size of planting material from 2 50 g to 1 kg increased mean corm weight per plant from 0.75 to 1.74 kg whereas the corm yield per ha increased from 21.6 to 77.34 t (Sen *et al*., 1984; Asokan, 1984; Sen and Das, 1991; James and Nair, 1993; Das *et al*., 1995). Comparatively more corm yield was obtained by planting whole seed corms: about 45% greater than the corm yield obtained from cut pieces of corms of the same size (Table 5 and 6). This was presumably due to early sprouting and better root ramification (Sen and Das, 1991). Nevertheless, a seed corm size of 400 - 500 g at 90 x 90 cm spacing would be ideal for economic cultivation of elephant foot yam (James and Nair, 1993; Yadav *et al*., 2008). For production of small size (< 1 kg) corms for home use, planting materials of 100-300 g may be used (Das *et al*., 1995; Mondal and Sen 2004; Rajib *et al*., 2007). To prevent decay after planting due to the presence of several soil borne pathogens, cut corm pieces are dipped in cow dung slurry mixed with mancozeb (0.2%) + monocrotophos (0.05%) for 10 min and surface dried under shade for 24 hr before planting. Biofertilizers and other beneficial microorganisms may be added to the cow dung slurry for high productivity (Nedunchezhiyan *et al*. 2006). It was found that planting depth affected plant growth and yield (Santosa *et al*. 2004 a). Deeper planting of seed corms led to deformation in daughter corms. At a depth of 30 cm, most corms were elongated or became pyriform. Therefore it is desirable to have corms at a depth of 10 cm below the soil surface (Sugiyama and Santosa, 2008). The multiplication ratio in *Amorphophallus* could be enhanced to 1:15, from the conventional 1:4, by adopt-

Table 5 - Effect of seed corm size on shoot length, canopy spread, mean corm weight and corm yield

Seed corm size (g)	Shoot length (cm)	Canopy spread (cm)	Mean corm weight (kg)	Corm yield (t ha^{-1})
Cut corm piece (g)				
250	62.6	85.8	0.84	37.4
500	72.3	89.0	0.95	42.2
750	81.0	114.8	1.23	54.7
1000	84.6	132.7	1.74	77.3
Whole corm (g)				
250	69.1	88.6	1.36	60.5
500	75.4	99.7	1.48	65.8
750	88.8	117.9	1.8	80.0
1000	96.8	134.9	2.53	112.4
CD (0.05)	0.8	0.8	0.06	

Source: Sen and Das, 1991.

Table 6 - Effect of seed corm size on shoot length, canopy spread, mean corm weight and corm yield

Seed corm size (g)	Shoot length (cm)	Canopy spread (cm)	Mean corm weight (kg)	Corm yield (t ha^{-1})
250	36.5	99.8	1.14	14.1
500	40.4	97.1	1.53	18.9
750	48.7	114.4	1.88	23.2

Source: James and Nair, 1993.

ing the minisett technique developed in CTCRI (James *et al*., 2004). Minisetts produced corms in the range 600 g to 1.5 kg. Treating setts of corm pieces from the bottom portion of corm with GA_3 (200 ppm) resulted in maximum corm yield (Das *et al*., 1997). Various integrated nutrient management practices (combination of inorganic fertilizers, organic manures and biofertilizers) and weed management practices enhanced plant height, canopy spread, corm size, and corm yield per ha (AICRP, 2004, 2005, 2006 a, b, 2007, 2008, 2009).

The mean starch content of *Amorphophallus* corm varied between 9.2 and 23.8% and the increase in N or K application did not have a significant effect on starch content (Mukhopadhayay and Sen, 1986; Geetha, 2001). Organic practices favoured starch content of elephant foot yam corm (Suja *et al*., 2005, 2006, 2007; Suja and Sundaresan, 2008 a, b). The starch content was found to range from 3.6 to 11.5% on a fresh mass basis in Indonesian accessions (Santosa *et al*., 2002), and from 7.0 to 14.3% in Indian accessions (Moorthy *et al*., 1994). Little variation was noted in the average size of starch granules (9-13 µm) and amylase content (22-24%) among different accessions (Moorthy, 2002).

6. Corm dormancy

Amorphophallus corms exhibit dormancy for about three to five months after harvest. As a result, planting and harvesting are done at a particular time of the year. *Amorphophallus* is propagated by corms as such or by cut corm pieces having a part of apical meristem. Sprouting percentage was greater (98%) with top cut portion of corm than the cut corms from the lower half of the mother corm (Dhua *et al*., 1988; Nedunzhyan and Mohankumar, 1997; Mondal and Sen, 2004; Santosa *et al*., 2006 b). The bottom portion of the corm is not generally used as planting material due to its lower sprouting efficiency (Dhua *et al*., 1988; Nedunzhian and Mohankumar, 1997; Mohankumar and Ravi, 2001). Therefore, a greater portion (about 25%) of the harvested produce is again lost as source of planting materials. Also, the apical bud from the corm can be excised and used as planting material. Removal of the apical bud results in development of one or two adjacent buds within two weeks which also can be excised and used as planting material (Fig. 5). Ethrel or ethephon was reported to induce early sprouting in *Amorphophallus* corm (Dhua *et al*., 1988; Bala and Indira, 1992). Treating cut pieces of corms from the lower half with chemicals significantly improved sprouting, subsequent growth and yield. Among the different chemicals used, thiourea, potassium nitrate and CCC were effective in promoting sprouting. Thiourea (200 ppm) and KNO_3 (1000 ppm) and kinetin (5 ppm) increased corm sprouting by 24.3-92.0, 17.8 and 13.4% respectively as compared to control (Table 7) (Dhua *et al*., 1988; Kumar *et al*., 1998). However, mean corm weight was greater in plants from corms treated with thiourea (100 ppm), potassium nitrate (KNO_3) (500 ppm) and CCC (0.02 ml l^{-1}) yielding 722, 821 and 806 g per plant respectively (Dhua *et al*., 1988). However, corm yield per ha did not increase significantly in plants from corms treated with chemicals, as compared to plants from untreated corms. Exposing the whole corms to smoke for 6 h per day for six weeks increased sprouting by 58.3% as compared to untreated corms presumably due to ethrel in smoke. Similarly exposing the corms to high temperature (32-45°C) increased sprouting by 83.3% as compared to untreated corms (Mohankumar and Ravi, 2001; Archana *et al*., 2009). Pre-harvest, foliar application of potassium nitrate (2%) and thiourea (1%) had greater influence on breaking dormancy and inducing early sprouting (Bhagavan *et al*., 2008). This may be due to an increase in the availability of sugars as a result of an increase in respiration at higher temperature. Compared to smoke and heat treatments, soaking corms in different chemicals [KNO_3, thiourea, ammonium sulphate (NH_4SO_4)] for a short period (20-30 min) for 1-2 hr had no significant effect on inducing early sprouting (Mohankumar and Ravi, 2001). However, treating the apical portion of corm (after removing the apical bud) with thiourea and subsequently wetting the apical portion for a period of 10 days induced early sprouting with more sprouts (Archana *et al*., 2009). Darkness had an adverse

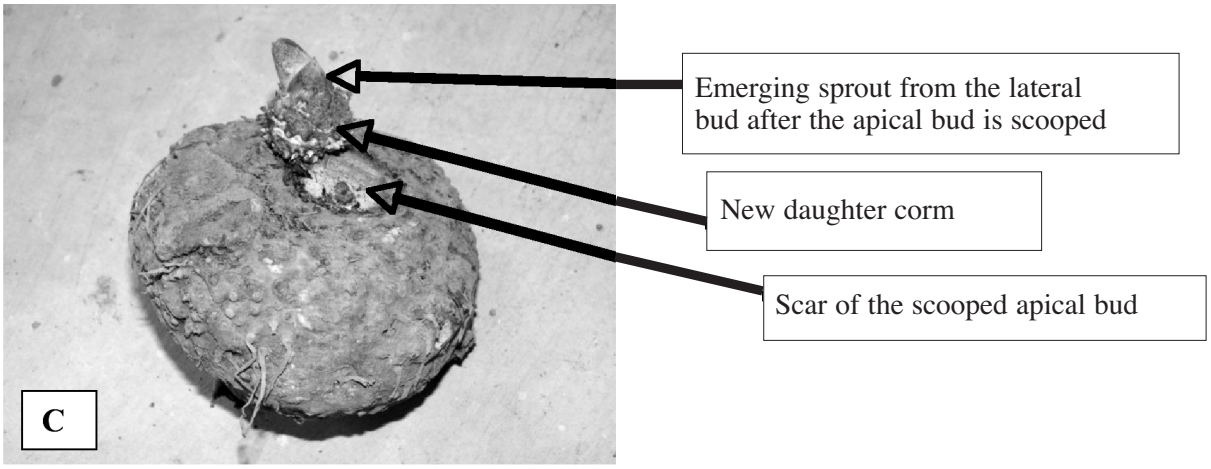


Fig. 5 - Emerging sprout from the lateral bud after the apical bud is scooped.

Table 7 - Effect of chemicals on sprouting of corm, mean corm weight and corm yield

Treatments	Sprouting (%)	Mean corm weight (g)	Total corm yield (t ha^{-1})
Thiourea (100 ppm)	73.3	721.6	28.5
Thiourea (200 ppm)	91.1	488.0	31.3
KNO_3 (500 ppm)	82.2	820.6	30.9
KNO_3 (1000 ppm)	86.6	528.6	28.9
Ethrel (0.025 ml l^{-1})	75.5	434.3	18.9
Ethrel (0.125 ml l^{-1})	73.3	679.6	29.3
Kinetin (5 ppm)	82.2	390.0	24.5
Kinetin (10 ppm)	75.5	410.6	16.7
CCC (0.02 ml l^{-1})	84.4	806.3	32.4
CCC (0.1 ml l^{-1})	68.8	563.6	19.7
Control (soaked in water)	68.8	587.3	30.9
Control (unsoaked)	66.6	540.6	22.2
Top cut portion	97.7	722.6	36.7
CD (0.05)	16.7	218.3	11.6

(Source: Dhua *et al*., 1988)

effect on sprouting (Kumar *et al*., 1998). In *A. konjac*, abscisic acid (ABA) and ferulic acid were extracted from the dormant corms and exogenous application of ABA (10 mg l^{-1}) and ferulic acid (400 mg l^{-1}) inhibited sprouting and growth of the terminal buds of non-dormant corms, suggesting that ABA and ferulic acid are inhibitors of sprouting of dormant corms (Sun *et al*., 1996). Corms are acrid before dormancy, but it decreases after dormancy (Santosa *et al*., 2003).

7. Ecological requirements

Elephant foot yam grows well under tropical, warm, humid conditions with maximum day-time temperature ranging between 25 and 35ºC, minimum night-time temperature ranging between 20 and 25ºC and annual rainfall ranging between 1000 and 3000 mm spread over a period of about six to eight months. It grows well in sandy loam or sandy clay loam soil with good drainage and pH of 6.0 to 7.0. It can also be grown in laterite soil (with about 40-50% gravel) but heavy clay soil is not suitable for this crop. Soil with high organic matter favours good crop growth and corm yield. Planting material (whole or cut pieces of corm) is planted shallow in pits of 60 x 60 x 45 cm size dug out in well-ploughed soil. The top soil dug out is then mixed with farm yard manure or compost (2.0-2.5 kg per pit) and the mixture is put back into the pit prior to placing the planting material over it. The planting material is placed vertically in the pits and is then covered with soil and compacted lightly.

8. Response to shade

Elephant foot yam tolerates shade conditions. Therefore, it can be intercropped between young trees. Corm yield decreased by 66 % when light intensity was reduced to 2 5% of full sunlight (Pushpakumari and Sasidhar, 1992). On the contrary, Santosa *et al*. (2006 a) reported that the fresh biomass of corms increased with a decrease in light intensity; 75 % shading produced the largest corms and 0% shading produced the smallest. Under full sunlight necrosis and curling at either the edge or the tips of leaflets occurred causing 25 % loss of the crop. No damage was observed in the 25, 50 and 75 % shading. However, shading treatments significantly decreased the leaf number. The short life span of leaves might enhance the production of new leaves resulting in a larger number of leaves under full sunlight. Shading treatments significantly affect the length of petioles and rachis. Plants developed the shortest petioles under full sunlight but the longest under 75 % shading.

9. Effect of water deficit stress

Little research work has been done on the response of *Amorphophallus* to water deficit stress. Soil moisture status does not influence sprouting but further development of new shoot depends on adequate soil moisture. Elephant foot yam plants produce large corms and yield more when the water supply is adequate (AICRP, 2008). About 1000-1500 mm of rainfall per year is optimum for the crop. Many plants enter dormancy earlier than usual when the rainy season is shorter than four months and supplementary irrigation is necessary for high productivity under the same conditions. Plants produced a larger number of leaves under frequent watering (one-, three- and five-day intervals) than under seven- and 15-day intervals; the third leaves were produced in treatments up to seven-day intervals, but neither the second nor the third leaves were produced with 15-day intervals. Furthermore, frequent watering produced large leaves and extended their life span compared to less frequent water-

ing (Santosa *et al*., 2004 b). A decrease in the dry mass of seed corms was more evident with frequent watering, suggesting that reserved carbohydrates in seed corms are not easily metabolized under a limited water supply. The ratios of dry mass of daughter corms to that of seed corms are 6.1, 1.1, 0.6, 0.4 and 0.2 at one-, three-, five-, seven-, and 15-day intervals, respectively. The high ratios under frequent watering treatments could be ascribed to the fact that the soil water availability affects not only the utilization of dry matter in seed corms but also the production and translocation of photoassimilates into daughter corms (Sugiyama and Santosa, 2008). The roots dried earlier than usual when the soil water content decreased to less than 40 % of field capacity (Santosa *et al*., 2004 b) and the crop tolerates water deficit stress conditions for about 30-60 days but prolonged stress may affect corm yield (Santosa *et al*., 2004 b). In green-house conditions, plant growth was not affected when plants were watered at one-, three- or five-ay intervals. Nevertheless, infrequent watering (watering at seven- or 15-day intervals) reduced corm yield and forced the corms to enter into dormancy. Soil moisture conservation methods like mulching induced a higher percentage of early sprouting, greater canopy spread, plant height, greater mean corm weight and corm yield (Mohankumar *et al*., 1973). In India, mulching the field with paddy straw resulted in maximum plant height (78.2 and 88.2 cm respectively), girth (14.1 and 14.4 cm respectively) and corm yield (47.44 and 56.74 t ha^{-1} respectively) as compared to control (AICRP, 2004, 2006 a, b). Also cowpea live mulch produced greater yield (41.72 t ha^{-1}) than control (AICRP, 2006 a, b). Maximum corm yield was also obtained by black polythene mulching (82.48 t ha^{-1}) and straw mulch (64.82 t ha^{-1}) ranked second (AICRP, 2004). Although the corm yield and net return in the straw mulch treatment was lower than polythene mulch, the cost:benefit ratio of straw mulch (1:3.18) was greater than polythene mulch and other treatments (AICRP, 2004). Mulching with sesame leaves also resulted in better corm yield (41.8 t ha^{-1}) than straw and black polythene mulch (AICRP, 2004, 2006 a, b). Paddy straw mulch also resulted in greater corm yield (13.8 t ha^{-1}) than control (AICRP, 2004, 2006 a, b). When considering mulching with straw, black polythene or cowpea, corm yield was significantly greater only in the first two cases (11.69-14.12 t ha^{-1}) whereas live cowpea mulching significantly reduced corm yield (5.68 t ha^{-1}) compared to control (7.98 t ha^{-1}) (AICRP, 2004, 2006 a, b). Maximum corm yield (44.3 t ha^{-1}) was recorded with the application of 100% recommended dose of fertilizer (RDF) along with flood irrigation and the yield (43.5 t ha^{-1}) was on par with the application of 100% RDF plus irrigation at 100% CPE (AICRP, 2009). Corm yield was significantly reduced when irrigation was less than 100% CPE (AICRP, 2009). Finally, the corm yield of elephant foot yam was greater (37.3 t ha^{-1}) under micro-irrigation (drip-irrigation) at 60% CPE daily for the first 15 days and then on alternate days for the next 15 days, at 80% CPE between two and six months and then at 60% CPE between seven and eight months, than under surface irrigation (26.4t ha^{-1}) (Nedunchezhiyan *et al*., 2008).

10. Seed dormancy

Successful seed production has been reported in *Amorphophallus* (Arakeri, 1950). A seed dormancy of five to six months has been reported in this crop (Arakeri, 1956). Exposing seeds to running water for six days resulted in greater sprouting (55.5%) than in control (2.7%). However, exposing seeds to water for more than six days led to a lower percentage of sprouting (Rajendran and Hrishi, 1976).

11. Future thrust

Since the whole corm and cut corm pieces are used as planting material, a large portion of harvested produce is used for propagation. Therefore, the development of plantlets through *in vitro* culture of apical/lateral buds (Irawati *et al*., 1986; Archana *et al*., 2009; Unnikrishnan and Mohan, 2009) should be further refined and exploited for planting material production.

Furthermore, more detailed investigation of the physiological aspects of growth and productivity of Amorphophallus needs to be developed in the following areas:

Effect of photoperiod and temperature on leaf area development, crop growth rate, stomatal characteristics, photosynthetic rate, root development and rooting pattern, corm development and bulking rate, light interception, dry matter production and partitioning (harvest index), varietal variation in these aspects and physiological factors limiting corm yield.

Effect of exogenous application of growth regulators such as benzyl adenine and other such growth promoters on maximizing corm yield.

Since *Amorphophallus* needs a long duration (8 months growing period) for maximum corm yield, studies on factors controlling corm bulking could reveal the physiological basis for developing rapid bulking, short duration varieties.

Studies to determine water, light and thermal degree-day requirements and the effect of water deficit stress, high temperature (>35^{o}C) (heat stress), salinity and shade on growth and productivity.

Factors controlling corm dormancy, breaking of dormancy and sprouting and related gene expression.

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Short note

Performance of tomato under greenhouse and open field conditions in the trans-Himalayan region of India

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Key words: greenhouse, Ladakh, open field, *Solanum lycopersicon*, tomato.

Abstract: Production of tomato is limited by harsh climate and a short growing season in the trans-Himalayan Ladakh region of India. The performance of five tomato genotypes was compared under polyhouse and open field conditions. The study revealed that the performance of all tested tomato genotypes is far superior in the polyhouse, as compared to open field conditions, for all the considered characters. 'Shivalik' performed best with respect to yield characters followed by 'Pusa Rohini' under polyhouse conditions. However, in the open field, 'Pusa Rohini' showed the highest values, followed by 'Shivalik'. Cultivation of tomato under the polyhouse produced 136.12% more yield per ha and 188.93% more fruits per plant compared to open field cultivation. Therefore, tomato cultivation under protected conditions is advised for Ladakh growing conditions, employing specific polyhouse-responsive varieties.

1. Introduction

Tomato (*Solanum lycopersicon* L.) is available throughout the year in India. However, in the state of Jammu and Kashmir, with the exception of the Jammu region, it is mostly confined to the summer season. In the trans-Himalayan Ladakh region, production of tomato is limited by climate and a short growing season. Ladakh has a harsh climate and extreme temperature fluctuations ranging from -37°C to +38°C. In Ladakh, tomato can be grown in open conditions but yield remains poor with low quality and it remains weather-dependent. Therefore, protected cultivation is a feasible answer for successful cultivation of tomato in this region. Singh and Asrey (2005) also recommended that cultivation of tomato in a greenhouse would help obtain high productivity and better return. Therefore, it is useful to study tomato production potential in the Ladakh region with respect to yield and horticultural traits under protected conditions (preferably in a zero-energy polyhouse) in comparison to the open field.

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2. Materials and Methods

The experiment was conducted under naturally ventilated polyhouse and open field conditions at the Experimental Farm, Stakna (Leh) of the Regional Agricultural Research Station (SKUAST-K) located at 3319 m amsl with latitude 33°58.551' NS and longitude 77°41.995'EW. The climate of the area is typically dry temperate. Five genotypes including four hybrids (PH-5, Shivalik, Jaya and Naveen 2000^{+}) and one OP variety (Pusa Rohini) were transplanted in a naturally ventilated polyhouse and the open field. Planting distance was 60 x 30 cm. The design of the experiment was Factorial RBD and material was replicated thrice. Individual data of each location were also subjected to statistical analysis in RBD to have more authentic information with regard to tomato genotypes. Data recorded on 13 characters were subjected to statistical analysis as per Snedecor and Cochran (1967).

3. Results and Discussion

There were significant differences among tomatoes grown under polyhouse and open field condition for all the characters, except for locules per fruit, confirming thereby the certain role of polyhouse in the cultivation of tomato in the trans-Himalayan region. Similar

results were obtained by Singh and Asrey (2005) as they found excellent tomato crops in polyhouses compared to the outside environment. Significant differences were observed among tomato genotypes pooled over locations for number of fruits per plant, yield per plant, yield per ha, fruit length, TSS and locules per fruit. However, no polyhouse x genotype interaction was noted. Comparative performance of tomato genotypes is presented here.

Plant characters

In polyhouse and open conditions, 'Naveen 2000+' had significantly higher plant length. Results are in conformity with those of Singh and Singh (2000) under open field conditions. 'Naveen 2000+' also had a significantly greater number of harvests, followed by 'Pusa Rohini' in open field conditions. Under the polyhouse, the number of harvests ranged from 9.50 to 10.25. Cargnelutti *et al*. (2004) obtained 11-14 harvests under plastic greenhouse. The polyhouse had a significant effect on all the plant characters under study (Table 1). Ganesan (2002) also reported better plant height in polyhouses as compared to open field conditions.

Yield characters

Yield per plant, yield per ha and number of fruits per plant were highest in 'Shivalik' followed by 'Pusa Rohini' under greenhouse conditions (Table 2). Gualberto *et al*. (2007) also recorded significant differences for yield per ha. However, in open field conditions, 'Pusa Rohini' gave the highest value for all three characters, followed by 'Shivalik'. However, the differences were non-significant for yield per ha. The performance of 'Pusa Rohini' was the best, followed by 'Shivalik', for all these characters when data was pooled from the different locations. The performance of tomato was statistically superior in polyhouse cultivation compared to open conditions. Ganesan (2002) observed similar trends for yields per plant in polyhouses respect to open field conditions.

Fruit characters

The highest recorded value for fruit length under polyhouse and open conditions, as well as for pooled data, came from 'Naveen 2000+' however this genotype was at par with 'Jaya' and 'Shivalik' in the polyhouse (Table 3). Eklund *et al*. (2005) recorded fruit weight of 147.35 g, fruit length of 57.67 mm, fruit diameter of 69.75 mm and 5.25 locules per fruit for an elite hybrid in a controlled protected structure. All these values were higher than those found in the present study with the probable reason being fluctuating environment at fruit development as the present experiment was conducted under a naturally ventilated polyhouse. In open field and pooled data, 'Naveen 2000+' had statistically superior fruit length. TSS was statistically the highest in 'Pusa Rohini' under polyhouse conditions and in pooled data, while in open conditions 'Shivalik' exhibited the highest TSS, which was at par

Table 1 - Performance of tomato genotypes under polyhouse and open conditions for plant characters

Location / Varieties	Characters											
	Plant height (cm)			Stem girth (mm)			Days to first harvest			No. of harvesting			Harvest duration (days)		
	L1	L2	Mean	L1	L2	Mean	L1	L2	Mean	L1	L2	Mean	L1	L2	Mean
Pusa Rohini	91.12	77.50	84.31	10.25	6.25	8.25	91.5	95.25	93.37	10.25	6.25	8.25	68.50	39.00	53.75
PH-5	98.12	56.25	77.19	9.50	5.50	7.50	89.5	96.25	92.87	9.50	5.50	7.50	67.25	35.50	51.37
Shivalik	125.70	96.25	111.0	10.00	6.00	8.00	89.5	93.75	91.62	10.00	6.00	8.00	68.75	38.25	53.50
Jaya	122.90	95.50	109.2	10.00	5.75	7.88	91.00	99.00	95.00	10.00	5.75	7.88	71.50	36.75	54.12
Naveen 2000+	169.20	115.00	142.1	10.00	6.50	8.25	88.50	94.00	91.25	10.00	6.50	8.25	74.50	39.50	56.88
Mean	121.40	88.10		9.95	6.00		95.65	90.00		9.95	6.00		70.05	37.80	
$CD_{0.05}$ (L)	10.43			1.14			3.30			0.51			4.07		
$CD_{0.05}$ (G)	29.95	12.63	NS	NS	NS	NS	NS	NS	NS	NS	0.67	NS	NS	NS	NS
$CD_{0.05}$ (LxG)	NS			NS			NS			NS			NS		

L1= Poly house.
L2= Open field.

Table 2 - Performance of tomato genotypes under polyhouse and open conditions for yield characters

Location / Varieties	Characters								
	No. of fruits per plant			Yield per plant (kg)			Yield per ha (Q)		
	L1	L2	Mean	L1	L2	Mean	L1	L2	Mean
Pusa Rohini	25.03	13.00	19.02	1.243	0.657	0.95	690.42	365.14	527.8
PH-5	22.64	4.49	13.56	1.066	0.213	0.64	592.36	118.34	392.8
Shivalik	26.81	9.96	18.39	1.282	0.537	0.91	712.22	298.20	505.2
Jaya	20.72	7.36	14.04	0.994	0.354	0.67	551.25	196.39	373.8
Naveen 2000+	22.34	8.97	15.65	0.983	0.462	0.72	545.83	256.39	401.1
Mean	25.31	8.76		1.113	0.444		618.40	261.90	
$CD_{0.05}$ (L)	1.61				0.087			57.34	
$CD_{0.05}$ (G)	3.95	2.76	2.53	0.237	0.148	0.137	131.43	NS	90.65
$CD_{0.05}$ (LxG)		NS		NS			NS	NS	

L1= Poly house.
L2= Open field.

Table 3 - Performance of tomato genotypes under polyhouse and open conditions for various fruit characters

Location / Varieties	Characters														
	Fruit weight (g)			Fruit length (mm)			Fruit diameter (mm)			TSS (°B)			Locules/fruit		
	L1	L2	Mean	L1	L2	Mean	L1	L2	Mean	L1	L2	Mean	L1	L2	Mean
Pusa Rohini	78.00	60.06	69.03	48.97	44.51	46.74	52.23	47.63	49.99	6.75	5.35	6.05	3.0	3.0	3.00
PH-5	78.10	54.09	66.09	44.75	40.47	42.61	57.18	48.10	52.64	5.10	5.10	5.10	3.5	3.0	3.25
Shivalik	67.40	59.23	63.32	49.61	46.49	48.05	48.97	48.43	48.70	5.40	5.55	5.48	3.0	2.5	2.75
Jaya	71.05	57.86	64.45	49.59	47.36	48.47	51.89	48.08	49.98	5.95	5.10	5.53	3.0	3.0	3.00
Naveen 2000+	74.70	57.22	65.96	53.74	52.82	53.28	49.27	45.45	47.36	5.05	5.40	5.23	2.25	2.5	2.38
Mean	73.85	57.69		49.33	46.33		51.91	47.56		5.65	5.30		2.95	2.80	
$CD_{0.05}$ (L)	11.49			2.35			3.40			0.12			NS		
$CD_{0.05}$ (G)	NS	NS	NS	4.52	4.70	3.72	NS	NS	NS	0.26	0.30	0.18	0.56	NS	0.37
$CD_{0.05}$ (LxG)	NS			NS			NS			NS			NS		

L1= Poly house.
L2= Open field.

with 'Naveen 2000+' and 'Pusa Rohini'. The statistically lowest number of locules per fruit in polyhouse conditions was recorded for 'Naveen 2000+', while in pooled data 'Naveen 2000+' and 'Shivalik' were at par.

Performance improvement

Perusal of data in Table 4 reveals that mean yield per ha, number of fruits per plant, fruit weight, plant height, harvest duration and number of harvests were 136.12, 188.93, 16.16, 37.80, 85.32 and 65.83% more, respectively, under polyhouse conditions compared to the open field. These findings demonstrate the suitability, as well as economic feasibility, of polyhouses in the trans-Himalyan Ladakh region for tomato cultivation. Gualberto *et al*. (1998) also reported 40-45 % higher marketable yield in greenhouses than with open field conditions. Growth and yield attributes were also recorded as poor in the open field condition.

Therefore, it may be concluded that naturally ventilated polyhouses are a good and less expensive option for tomato cultivation in the trans-Himalayan region to obtain higher yield, number of fruits per plant and longer harvest duration. Varieties like 'Shivalik' and 'Pusa Rohini' are responsive to protected cultivation in this region and may be used for cultivation after further testing to increase the return per unit area.

Table 4 - Percent improvement in tomato performance under polyhouse versus open conditions for economic characters

Genotype	Percent increase in					
	Yield per ha	Number of fruits per plant	Fruit weight	Plant height	Harvest duration	No. of harvest
Pusa Rohini	89.08	52.54	29.87	17.57	75.64	64.00
PH-5	400.56	404.23	44.39	74.44	89.44	72.73
Shivalik	138.73	169.18	13.79	30.60	79.74	66.67
Jaya	180.79	181.52	22.80	28.69	94.56	73.91
Naveen 2000+	112.89	149.05	30.55	47.13	88.61	53.85
Mean	136.12	188.93	16.16	37.80	85.32	65.83

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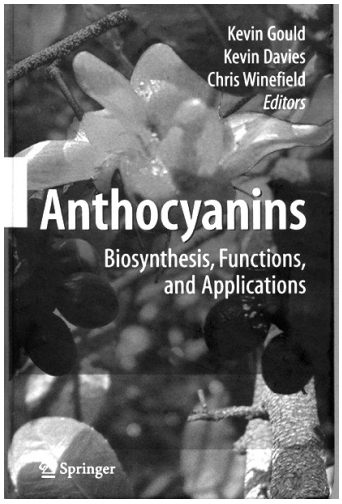
BOOK REVIEWS



ANTHOCYANINS. BIOSYNTHESIS, FUNCTIONS, AND APPLICATIONS. *Gould K., K. Davies,* and *C. Winefield* (eds.) Springer Science+Business Media, LLC, New York, NY, USA, 2009. pp. i-xviii + 329. ISBN 978-0-387-77334-6. USD 149.00. GBP 79.00. € 99.95.

Plant colours are attracting not only because they provide spectacular views of nature, but also for their functional roles. It is well known that nothing in nature is made by chance. Anthocyanins, molecules of plant secondary metabolism, are those natural pigments giving predominant contributions to the painted word. Their appearance on external portions of plant organs seems to be a kind of language to communicate with the animal kingdom, with the aim of attracting or repelling in order to facilitate plant reproduction and diffusion or plant defence, respectively. Learning about the beneficial effects of anthocyanins in plants, humans have discovered how beneficial anthocyanins can be to their health as well.

Considerable literature in plant sciences is devoted to understanding anthocyanin-involved mechanisms, however much remains as yet undisclosed. The book *Anthocyanins. Biosynthesis, Functions, and Applications* edited by Kevin Gould, Kevin Davies and Chris Winefield is certainly a useful reference for all researchers involved in the many multidisciplinary studies of these natural pigments and represents a valuable collection of research results and needed future work for this rapidly expanding field.

The different aspects of anthocyanin properties covered by this book include antioxidant activity and photoprotection, the role in plant defence mechanisms, the function in fruits and flowers. Extensive chapters are dedicated to plant cell cultures for the biosynthesis of anthocyanins and their biotransformation by microorganisms. In addition, the biochemical pathways of reactions occurring *in vivo* for the stabilization of anthocyanins and the characterization of new anthocyanin-derived compounds as food colorants are also considered. Finally, the last chapter deals with the phytochemical role of anthocyanins to promote human health.

Being an outsider to the plant science academic world, but working in spectroscopic monitoring of vegetation for about 20 years, I thoroughly enjoyed reading this volume and warmly recommend finding a place for this book on everyone's desk.

Giovanni Agati

LA CIVILTÀ DELLE ACQUE TRA MEDIOEVO E RINASCIMENTO. The culture of water between the Middle Ages and the Renaissance. *Calzona A.* and *D. Lamberin* (eds.). Collana *Ingenium*, vol. 14. Edizioni Leo S. Olschki, Firenze, 2010. Volume I and II pp. xviii + 718, 11 figures, and 83 plates, 22 of which in colour. ISBN 978 88 222 5969 1. € 78.00.

These two volumes contain the Proceedings of the international meeting of the same name held in Mantua 1-4 October 2008 and include presentations by 37 authors of national and international level. The works address the literary, philosophic, historic, political-economic and social, historical-artistic and architectural, and scientific and technical-engineering issues relative to the management and significance of freshwater in Italy from the Middle Ages to the Renaissance.

The works are grouped into five sections: literary and imaginary; political and economic management; art, architecture, landscape and territory; engineering, infrastructures, science and criticism; gardens, parties and spectacles. A final section deals with the magical, thermal, and nutritive aspects of the waters. The overall complex provides a true treatise on the subject matter, making these volumes valuable not only as a guide for those directly involved in the sector but they can also be a source for consultation for others who have personal interest in the topic.

Various illustrations and tables enrich each paper and there are numerous bibliographic citations, offering the reader the possibility to go into greater depth.

This subject is of considerable historical-cultural importance in Italy and both the meticulous work and the efforts by the various contributors – some of whom present their life work – attest to just how much there is to know about fresh waters in our country. These two volumes are therefore of great interest and usefulness and are an important contribution to the literature available on the topic and will surely be helpful for not only technical workers and students but also an increasing number of enthusiasts.

Francesco Ferrini

SAN ROSSORE NELLA STORIA: UN PAESAGGIO NATURALE E COSTRUITO. San Rossore in history: both a natural and built landscape. *Panattoni R*. Collana giardini e paesaggio, vol. 27. Edizioni Leo S. Olschki, Firenze, 2010. pp. xxxii + 230. 2 figures and 32 plates in colours. ISBN 978-88-222-6023-9. € 27.00.

This work by Rita Panattoni was awarded the *Premio Verbania, Editoria e Giardini* in 2009.

The title of the volume is, in itself, significant: the subject is not the history but rather the park of San Rossore. This difference may not seem important but the title of any publication should define the whole, which in this case is the role played by this park in the history of the region.

The book opens with an essay on the evolution of the botanical context by Fabio Garbari who underlines the extraordinary environmental value of this area which lies somewhere between being a garden and a landscape, and contains a total of 335 pages divided into five parts: the ancient San Rossore; San Rossore of the Medici family; San Rossore of the Lorena family; San Rossore of the House of Savoy; the post-World War II period, the 1950s. Overall the work effectively illustrates the story of the transformation of the original *Selva dei Tomboli Pisani* into the current San Rossore estate.

The earliest information about the San Rossore area – covering an area of 4800 hectares between the Serchio and Arno rivers, composed of woodlands and pine forests, beaches, dunes, fields and marshlands and approximately 12 km of coast – dates back to about 1000 AD. Up until the beginning of the 16th century this vast area was utilized for hunting and fishing or for the cultivation and harvesting of wood. It was the Medici family who began the work of transforming the estate, first as renters (in the 16th century) and later as owners. Subsequently ownership passed to the Lorena family, who used the estate frequently and added improvements. In the mid-1800s, when Tuscany became a part of the new Kingdom of Italy, the House of Savoy came to San Rossore and they occupied the estate for long periods of the year. After the War and the fall of the monarchy, San Rossore became the property of the President of the Republic.

The volume, which contains an ample list of bibliographic references and numerous illustrations in black and white and in color, is an important addition to the vast literature on the evolution of historical landscapes not only from a scientific and technical profile but also from an historical and cultural point of view. This approach thus offers the reader meaning and knowledge about the important and multiple values of the Park of San Rossore which has a role in creating an image of Tuscany.

Francesco Ferrini

PAESAGGIO RURALE: STRUMENTI PER LA PIANIFICAZIONE. STRATEGICA. *The rural landscape: tools for strategic planning. Agnoletti M*. Edagricole, Bologna, 2010. Figures 220. pp. xvi + 348. ISBN 978-88-506-5226-6. € 39.00.

Almost always legislation regarding a particular topic is distributed among various laws and decrees, which generally are based on or are handed down from previous periods and are more or less modified as needed. As a consequence, consultation of the regulations pertaining to a subject of interest can be often difficult and laborious and sometimes one has the feeling of not having investigated fully or having interpreted incorrectly, especially when the regulations are apparently contradictory (a not uncommon occurrence). Fortunately, Prof. Agnoletti has prepared this volume which represents a clear and precise compendium of the *Piano Nazionale di Sviluppo Rurale* 2007-2013 (PSN).

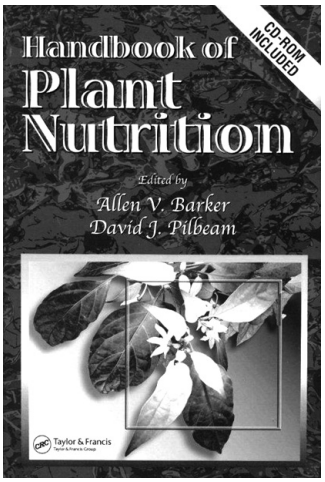
The text is divided into six sections: 1) The evolution of the rural landscape from Unification until today; 2) The evolution of forest landscapes from Unification until today; 3) Spatial characteristics and structural dynamics: the system of Tuscan monitoring; 4) The current structure of the Italian landscape; 5) The rural landscape in territorial politics; and 6) Strategies and actions in order to add value. The appendix contains a section dedicated to analysis methods and management processes.

The *Piano Nazionale di Sviluppo Rurale 2007-2013 (PSN)* has included landscape amongst its strategic objectives, underlining its role in the new agricultural policy and the transformations which have occurred in terms of understanding the importance of this resource as well as that of the grower and the rural territory. This initiative is in line with the activities set out by the European Landscape Convention and with landscape planning promoted by the new *Codice dei Beni Culturali* involving workers in the sector, public decision-makers and universities. The meaning of landscape and the perception of it has, in fact, changed over time. It is the essential element in defining a development model that is particularly suitable to the rural context, representing not only the cultural identity of our country but also economic and environmental values. However it is necessary to elaborate a cultural and scientific proposal that is appropriate for the rural setting, which takes into account the fact that landscape is the result of a combinationof social, economic and environmental factors in space and time where Man plays a central, not marginal role. The current volume proposes and adds to the materials utilized to develop a picture and definition of the strategies for landscape by the Ministry of agricultural, alimentary and forestry policy, illustrating approaches to analysis and providing additional material for integrated planning.

The text includes illustrations, tables, graphs and an ample list of bibliographic references which offer the reader a key to acquiring greater depth of knowledge in the particular aspects of the subject matter.

This work is of considerable scientific importance and the book can be of great interest for researchers and students in the field of environmental studies, as well as anyone who deals with landscape on a number of different levels.

Francesco Ferrini



HANDBOOK OF PLANT NUTRITION. ***Barker A.V.*** **and** ***D.J. Pilbeam*** **(eds.) Taylor and Francis Group. CRC Press, New York, NY, USA, 2007. pp. XVI + 614 + CD-ROM. ISBN 0-8247-5904-4. US$ 157.95.**

The increasing demand on the world food supply, coupled with concern over the use of chemical fertilizers, has determined an interest towards the practice of precision agriculture, has led to a better control and monitoring of plant nutrition to maximize the rate of growth, the yield of crops as well as their nutritional value.

This handbook covers principles of plant nutrition from a historical standpoint to current knowledge of the requirements of crops for certain elements and the beneficial effects of others.

The book consists of twenty chapters, each one dedicated to an essential macro or micronutrient or beneficial element. More in details, each chapter, written by eminent researchers from across the world, gives historical information on the specific nutrient, explaining why it is either essential or beneficial for the plants; moreover an explanation of how appearance and composition of plants can be used to assess nutritional status is given, as well as recommendations on fertilizers that can be applied to remedy nutritional deficiencies.

This handbook, including a CD-ROM containing more than 40 illustrations in full colour, can be considered of great value, and for this reason recommended, to growers, agricultural consultants, agronomist and plant scientist, providing a practical easy-to-use reference for determining, monitoring, improving the nutritional needs of plants. The graphical presentations of plant interactions with nutrients and beneficial elements, and the straight-forward explanations of how nutrient deficiencies arise are especially useful to those seeking knowledge of plant nutrition.

Francesco Paolo Nicese

ORTICOLTURA MEDITERRANEA SOSTENIBILE. ***Tesi R.*** **Pàtron Editore, Bologna, 2010. pp. 504. ISBN 978-88-555-3062-0. € 42.00.**

The original work of Romano Tesi is inspired and basically derives from the interesting connection among some important facts. First of all, the Mediterranean Basin is the area of origin and varietal differentiation of many important vegetables, but it also allowed the selection of many others imported from Asia and America during the Colonization Era. To this is added the fact that the countries bordering the Mediterranean Basin are the most important producers and consumers of those products. In the context of this mixture of cultures and traditions the well-known "Mediterranean diet" has developed, which is considered worldwide as a bright example of the highest dietary level.

"Orticoltura Mediterranea" is a new and updated book which gives particular attention to the changing market needs. Thus, in addition to general and specific aspects of the traditional horticulture, the book focuses into the composition and nutritional properties of vegetables, together with a outlook on the principal aspects of integrated production and organic farming.

The book is divided into two parts. The first part covers the horticulture in the Mediterranean Basin, which is deeply described in the following eight chapters: 1) *L'orticoltura mediterranea* (Mediterranean horticulture); 2) *Classificazione degli ortaggi* (Classification of vegetables); 3) *Tipi di orticoltura e sostenibilità* (Sustainability of the different kind of horticulture); 4) *I sistemi colturali* (Cultivation systems); 5) *Qualità dei prodotti orticoli* (Quality of horticultural products); 6) *Mezzi di protezione* (Protection devices); 7) *Sementi e vivaismo orticolo* (Seeds and horticultural nursery); 8) *Gestione dell'azoto e dell'acqua di irrigazione nel pieno campo* (Nitrogen and irrigation water management in the field horticulture). The second parts is dedicated to the monographic description of more than one hundred horticultural crops, with broad and updated insights.

Due to its modern setting, the rich collection of tables, the presence of numerous coloured or black and white images in which the scientific knowledge of the author can be easily recognized, the book may serve to a wide group of readers, from the student to the teacher, from the expert to the manager.

Enrico Rinaldelli