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Seed contents of *Coriandrum sativum* in Jordan Valley

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Key words: Coriandrum sativum, fertilized seeds and global warming, petroselinic acid.

Abstract: The aim of this research was to determine seed contents of Coriander (*Coriandrum sativum*). Research was conducted in the Jordan Valley at 150 m below sea level and in northern Jordan at 200 m above. Analyses of fertilized seeds showed that they contain 14.9% protein while non fertilized seeds had a significantly lower content (4.7%). The seeds contain 7.4% oil, which can vary according to strain. The fatty acid composition varied significantly among the seeds from the selected locations. Petroselinic acid was significantly the most concentrated fatty acid (80.10%). This acid (C18:1) can be split to produce C6 (adipic acid) and C12:0 (lauric acid) molecules. Adipic acid is used for the manufacture of a wide range of polymers including high grade engineering plastics; at present it is derived from mineral oil by a process that damages the ozone layer and contributes to the releases of gasses such as N2, which affect global warming. Petroselinic acid is abundant in *C. sativum* and could be an alternative, more environmentally friendly raw material for use in industry.

1. Introduction

Coriander (*Coriandrum sativum*) is a culinary and medicinal plant of the Umbelliferae, and is an annual herb originally from the Mediterranean area; several authors have indicated *Coriandrum sativum* as a wild plant. Linnaeus (1780) reported that *C. sativum* also occurs as a weed in cereals. Alefeld (1866) mentioned that *C. sativum* was a common weed spread from southeastern Europe to southern Russia. Stoletova (1930) reported on wild *C. sativum* from Armenia. All parts of the plant have a strong odor, from which the plant takes its name. Cultivation of *C. sativum* is widespread, but it is planted on a small scale only; it is cultivated as a summer or winter crop. In Jordan, it is found in gardens rather than in large fields, while in Germany there are many landraces of *Coriandrum sativum* (Diederichsen, 1996).

Description of the plant

The plant can reach heights of 20 to 80 cm. The stem is more or less erect, branched sometimes with several side branches at the basal node. Each branch finishes with an inflorescence. The color of the more or less ribbed stem is green and sometimes turns to red or violet during the flowering period. The leaves alternate, and the first ones are often gathered in a rosette. The leaves are of two types: lower ones with leaflets and upper ones divided into narrow linear segments (Diederichsen, 1996). The *Coriandrum sativum* flower has five irregular-shaped petals, five stamens, five sepals, and two styles. Flowering starts with the primary umbel. The first umbels to bloom have hermaphrodite flowers, with possibly a few staminate ones (Diederichsen, 1996). The inner flowers of umbellets are staminate. The central flowers are circular, with small inflexed petals. The color of the petals is pale pink or sometimes white. The umbels of higher order usually contain more staminate flowers than the first ones, and their flowering period is shorter (Diederichsen, 1996).

In a single flower, the five filaments of the staminate are located between the five petals. After the flower opens, the white filaments are visible between the petals. Under optimum conditions, many different insect species are pollinators or visitors of *C. sativum* umbels (Diederichsen, 1996), and the species that pollinate the plants depend on the area of cultivation. Flowering and pollination biology of *C. sativum* is typical of that for umbelliferous plants, according to Bell (1971). Depending on the weather conditions, two to three days after opening of the first flower, the pollen sacs open and spread the pollen. McGregor (1976) showed that selfing of *C. sativum* is impossible but Glukhov (1955) showed that it is partially self-fertile. He suggested that geitongamy is common and cross is possible. Bees are beneficial to *C. sativum*: Glukhov (1955) reported that when they were excluded only 49.4% of the seeds set, but when they were present 68.3% of the seeds set. Bogoyavlenseii and Akimenko (1966) associated seed yields with greater insect visitation.

Use of Coriandrum sativum

This plant is of economic importance since it has been used as a flavoring agent in food products, perfumes and

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cosmetics. Moreover, the essential oils of the fruits and various extracts from *C. sativum* have been shown to possess antibacterial (Burt, 2004; Cantore *et al.,* 2004; Kubo *et al.*, 2004), anticancerous and antimtagenic (Chithra and Leelamma, 2000) properties and the plant has been used in medicine for thousands of years. In Jordan, the primary product is the fresh green herb of *C. sativum* used for its specific flavor, which is completely different from that of the ripe fruits. In other countries the fruits are used as a spice and vegetable.

Oleum (1993) stated that Russia produces high quality *C. sativum* oil, with a linalool content of 55%.

Bauer (1942) found that *Coriandrum sativum* attains its greatest yield of volatile oil (0.9%). The fatty oil of *Coriandrum sativum* is of interest because of the high level of petroselinic acid.

2. Materials and Methods

Research sites

The research was conducted in Jordan at two different locations. The first is located 150 m below sea level. This area is humid with warm temperatures in winter, and dry and hot in summer. The other location is 200 m above sea level. It is characterized by rainy, cold winters and dry, mild summers (Fig. 1). Both locations have produced relatively high biodiversity in wild plants and bees. The Jordan valley, latitude 22° 40' 0'' and longitude of 35° 30' 0'', is located in Jordan, a part of the Middle East (Fig. 2) and extends down the entire flank of Jordan 50 km from Amman; it is the country's most distinctive natural feature. The northern segment of the Jordan valley, known in Arabic as the Ghor, is the nation's most fertile region. It contains the Jordan River and extends from the northern border down to the Dead Sea. Several degrees warmer than the rest of the country, its year-round agricultural climate, fertile soils, high winter rainfall and extensive summer irrigation have made the Ghor the food bowl of Jordan. According to MD (2002), the mean maximum and minimum temperatures are 29.9°C and 16.98°C, respectively, with rainfall of around 77-392 mm over 44.84 rainy days yearly. Over the last 30 years, mean relative humidity has been 72.45% in winter and 48% in summer. The Jordan valley is subjected to ground frost nearly 2.5 days yearly.

Fig. 1 - Jordan Valley overview of vegetation covers.

Coriandrum sativum *plantation*

Coriandrum sativum seeds, obtained from local markets (landraces), were planted at locations A and B on 5 November 2007. The rows were 20 m long with 1 m between rows. Water was supplied daily by drip irrigation, and extra fertilizers (N P K) were applied. Both locations were kept weed-free by cultivation and hand weeding.

Determination of seed content

To determine the seed content of *C. sativum*, 400 g of seeds were collected. Oil extraction and preparation of seeds involved the following: drying of seeds, crushing, and extraction. Solvents such as carbon dioxide and propane were used to facilitate oil extraction. Gas liquid chromatography was used to determine the seed content. Seed content analyses were performed at The National Center for Agricultural Research and Technology Transfer (NCARTT) chemist's lab, Amman, Jordan. The protein content of *C. sativum* was analyzed in fecundated and non-fecundated seeds using the international standard method (SOP: 130M01-006) (Table 1).

Table 1 - Analyzed constituents for *Coriandrum sativum*

	Standard method used
Seed constituent	
Ash	SOP: 130M01-009
Moisture	SOP: 130M01-010
Oil	SOP: 130M01-001
Proteins	SOP: 130M01-006
Trace elements	
Mg - Mn - Cu	SOP NO :131M02-005
Ca - K	SOP NO :131M02-002
Oil constituent	
Fatty acids	COI/T.20/Doc. No. 24 (2001) – AOCS Ch 2-91 (1997)

The seed contains the essential oils based on the analysis applied (Table 2).

3. Results

Seed content

Seeds for testing (for fatty acid composition, essential oils and mineral contents) were chosen from both locations. Moisture content was not significantly different among treatments. Protein values were significantly different among pollination treatments and ranged from 13.01 to 15.78%. Oil content was low in all seeds and varied from 5.61 to 7.40%. Ash values ranged between 6.26 and 6.51% (Table 2). Oleic acid was significantly the most concentrated 80.10% (Table 3). All mineral contents

Location A: 150 m (below sea level).

Location B: 200 m (above sea level).

Fig. 2 - Research sites (53, 15, 30, 30, are the Main International Highway).

Table 2 - Essential oil component and percentage in seed of *C. sativum*

Main component	Total essential oil (%)
1. Linalool	66.7
2. Alpha-pinene	9.8
3. Gamma-terpinene	8.3
4. Geranylacetate	3.3
5. Camphor	3.0
6. Geraniol	1.9

Table 3 - Main components and mineral contents in seed of *C. sativum*

Seed content	
Moisture	8.30 %
Ash	6.31 %
Protein	15.78 %
Oil	7.40 %
Magnesium	0.34 %
Calcium	0.40 %
Potassium	1.46 %
Manganese	18.80 mg/kg
Cupper	11.60 mg/kg

Table 4 - Fatty acid content in seed of *C. sativum*

Fatty acid	Trivial name	Systematic name	Percentage
C 14:0	Myristic acid	Tetradecanoic acid	0.10
C 16:0	Palmitic acid	Hexadecanoic acid	3.33
C 16:1	Palmitoleic acid	*cis*-9-Hexadecenoic acid	0.42
C 17:0		Hexadecanoic acid	0.03
C 17:1		Desaturation of *cis*-9-Hexadecenoic acid	0.04
C 18:0	Stearic acid	Octadecanoic acid	0.88
C 18:1, n-7	Vaccenic acid	*cis*-11-Octadecenoic acid	80.10
C 18:2	Linoleic acid	*cis*-9, 12-Octadecadienoic acid	14.63
C 18:3	α –Linolenic acid	*cis*-9, 12, 15-Octadecatrienoic acid	0.29
C 20:0	Arachidic acid	Eicosanoic acid	0.09
C 22:0	Behenic acid	Docosanoic acid	0.03
C 20:1	Gadoleic acid	*cis*-9-Eicosenoic acid	0.04
C 24:0	Lignoceric acid	Tetracosanoic acid	0.02

varied significantly among seed samples; manganese and copper were the most prevalent minerals. Linalool acid is the common essential oil in Coriander and was determined to be 66.7%. Fatty acid composition varied significantly among the seeds from the selected locations. (Table 4) The fatty oil of Coriander is of considerable interest because of the high level of petroselinic acid, which has potential non-food applications in oleo chemistry.

Protein percentage in Coriandrum sativum *seeds*

The protein percent in treated fecundated seeds in *Coriandrum sativum* was 14.9% of the total dry mass, whereas in non-fecundated seeds it was 4.7%.

4. Conclusions

Coriander is an annual herb and is common in the Middle Eastern and Mediterranean cuisine, in which the fresh leaves and dried seeds are the most commonly used parts of the plant. Chemicals extracted from Coriander have also been used as a traditional treatment for diabetes and hyperlipidemia (Chithra and Leelamma, 1997; Gary and Flat, 1999).

Little is known about the metabolic origin of petroselinic acid (18:1), which is an unusual fatty acid that occurs primarily in seeds of the Umbelliferae plant families (Tsevegsuren *et al*., 2004).

Petroselinic acid is of potential industrial significance because of unsaturation at C-6. Through chemical cleavage at its double bond, petroselinic acid can be used as a precursor of both lauric acid, which is a component of detergents and surfactants, and adipic acid, which is the monomeric component of nylon (Avato *et al*., 2001). Adipic acid is used for the manufacture of a wide range of polymers including high grade engineering plastics and it has a global market in excess of 2.5 million tons worth over £1 billion.

Many previous studies have shown that petroselinic acid is the major constituent of the seed oils of many species of Umbelliferae such as parsley or coriander and ranges from 15% up to 85% (Cahoon *et al*., 1992; Tsevegsuren *et al*., 2004). Keeping with this, the present study illustrated that the major component of the seed oil of coriander tested was petroselinic acid (18:1) and it represented to 80% of the total fatty acid content.

Recently, derivatives or polymers from under-utilized fatty acids such as petroselinic acid have been regarded as a new raw material, representing an important oleochemical material for the food, cosmetics, chemistry and pharmaceutical industries (Avato *et al*., 2001). Additional recent uses include the use as a green vegetable by some ethnic groups and flavoring for dishes and foods such as pickles and sauces.

Although many studies have focused on the seed content in *Coriandrum sativum,* the percent of protein of the total dry mass in fecundated and non fecundated seeds has not been pointed out. In the present study, the protein percent in treated fecundated and non fecundated seeds for *Coriandrum sativum* was low. Protein content in fecundated seeds is more than in non-fecundated seeds, which can be explained by the enzymatic activity of protein synthesis in complete fertilized ovary (embryo) as compared to empty ovary (gamete). In fact, cell division in the fecundated seeds needs more protein in order to complete division.

Finally, further studies are needed to fully determine the seed contents of *Coriandrum sativum*, and to explore the feasibility of growing coriander on a large scale in Jordan. We also believe that the application of advanced plant breeding, together with extensive biochemical studies, could result in more environmentally friendly, high oil, and high petroselinic acid coriander varieties.

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Pollination of *Nigella sativa* L. (Ranunculaceae) in Jordan Valley to improve seed set

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Key words: Black Cumin, Jordan, style movement.

Abstract: In Jordan, pollination is one of the problems faced by plants under plastic houses, in open fields and in off-season planting. Therefore this study was conducted in Jordan to investigate the role of pollinators and to investigate the systems of pollination in *Nigella sativa* species grown at two different altitudes, 150 m under sea level and 200 m above sea level. Up to now little attention has been paid to the events associated with pollination such as seed set, and to address this deficit, we examined six pollination treatments of the selected plant species. Field work was conducted, repeated and recorded from 2005 to 2007 in Jordan. Controlled pollinations were carried out in selected individual's plant at the time of maximum stigma receptivity and anthesis. *N. sativa* flowers had anthesis intervals which last for five days, then followed by stigmatic receptivity which last for few hours. Plants are pollinated trough outcrossing and complete selfing to insure the reproductive assurance. However, self-pollination was occurred due to style movement. The observations confirmed that a mixing mating including a combination of out-crossing and selfing is a better strategy than selfing alone.

1. Introduction

Black cumin, *Nigella sativa* (Ranunculaceae), is an annual herbaceous plant. The genus *Nigella* is represented in 20 species of Mediterranean-western Asian origin (Dantuono *et al*., 2002). Only *N. sativa, N. damascene* and *N. arvensis* are of interest in Jordan; *N. sativa* is the only species planted by farmers. There is no accurate data about planted area, but the annual production for the year 2005/2006 was 3-5 tons (personal communication). *N. sativa* is a hermaphroditic species with determined flowering patterns, starting with the flower terminating the main shoot and ending with the flowers on the lowermost branches. In the natural forms, flowers are delicate, and usually colored pale blue and white, with 5-10 petals and characterized by the presence of nectaries. The androecium comprises a large number of stamens, which shed their pollen as the filaments curve outward during the male phase. The gynoecium consists of up to five completely united follicles, each with a long, indehiscent style and composed of a variable number of multi ovule carpels, developing into a follicle after pollination, with single fruit partially connected to form a capsule-like structure. Seeds are generally small in size (1-5 mg) dark grey or black (Filippo *et al*., 2002). The fruit is large and its inflated capsule contains numerous seeds. *N. sativa* is extensively used in traditional medicine for healing various respiratory disorders from Morocco to Pakistan and in southern Europe (Filippo *et al*., 2002). The seeds have been widely added as a spice to a variety of foods such as bread, yoghurt, pickles, sauces, and salads for flavoring. They are also used in Jordanian traditional folk medicine for some respiratory, gastrointestinal, rheumatic and inflammatory disorders (Nafisy, 1989; Zargari, 1990; Amin, 1991). *N. sativa* seeds have been reported to contain essential oil, fixed oil, flavonoids, saponins, alkaloids, and proteins (Zargari, 1990; Burits and Bucar, 2000; Al-Ghamdi, 2001). Pollination studies of *N. sativa* are very limited in the literature. Lloyd (1979) showed that *N. sativa* is self pollinated without mentioning the mechanism; Zohary (1983) showed that *N. sativa* is capable of setting seed without being cross pollinated. The flowers of *N. sativa* are visited by honeybees (Ricciardelli and Oddo, 1981).

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2. Materials and Methods

The research considered specific plant species (landraces) of *N. sativa*, which were planted on-site at different elevations: location A, 150 m below sea level; and location B, 200 m above sea level. *N. sativa* was obtained from botanical gardens in Jordan (NCARTT). The seeds were planted in hills 30 cm apart on 5 November 2005. The rows were 20 m long, with 1 m between rows. Water was supplied daily by drip irrigation and extra fertilizers (N P K) were applied. Black plastic mulch was used. Each plant was represented by three rows per

location. Missing hills were replanted when necessary. The plants were thinned to two plants per hill when they were at two- to three-leaf stages. The two locations were kept weed-free by cultivation and hand weeding.

The time of stigmatic receptivity was determined with the aid of a dissecting microscope. The direct test of receptivity was an assay that detects the presence of stigmatic peroxides. To determine receptivity, the stigmas were treated with hydrogen peroxide 3%: small air bubbles that form by maturation of the stigma indicate that the flower is in the female phase (Dafni and Maues, 1998). To determine receptive periods, 50 flower buds per plant of each species were marked, 10 flower buds of the same age were bagged a day before the opening of flowers during the anthesis period. On the following day, 10 flower buds were taken to the laboratory in order to check for stigma receptivity.

The timing of anthesis was checked in the field using a hand magnifier. After bending, anther capsules were observed with the naked eye. The mechanism of pollen release is described based on direct observations in the field. Any rupturing of the capsule causes pollen to release where it is verified by anther dehiscence.

In order to observe pollinator visitation tour, the number of visits per bee was estimated by counting the number of visits with anther or stigma contact from the beginning of pollination to fertilization. The counts were conducted every 15 minutes for a period of eight hours on a daily basis during flowering period.

Controlled pollinations were carried out on selected individual plants at the time of maximum stigma receptivity and anthesis. Pollinated flowers were observed periodically for fruit set. The reproductive success of the studied species was assessed by performing a spontaneous self pollination, manual self and cross pollination treatments. Following the initiation of the first flower bud, flowers were selected randomly and tagged: 180 flower buds of *N. sativa* in each location. Thirty flower buds were marked for each pollination treatment. Pollination treatments were performed from February to March 2006 to determine the best pollination treatment in each locations. In order to conduct geitongamy and xenogamy pollinations, all stamen organs of each flower were removed using special scissors (emasculation). The flowers were pollinated using pollen from freshly dehisced anthers from male flowers (of the same plant) by using a fine brush for geitongamy pollination treatment and from another plant for xenogamy pollination treatment (cross pollination). The flowers were left exposed to any insect as occurs in nature for open pollination treatment. To test the bagged self pollination, flower buds, bagged till the end of pollination stage, were left untreated and uncovered again in order to avoid any negative impact on their germination. In order to check forced self pollination on the same hermaphrodite flower, flowers were bagged till the last day of the male stage. The flowers were pollinated using pollen from freshly dehisced anthers from male to female flowers on the same hermaphrodite flower by using a fine brush. With regard to emasculation, flower buds of nearly the same age were selected in order to remove male flowers to investigate the differences between the role of pollinator and the role of plant, by numbering of fruit set. The anthers were removed with a pair of tweezers and were left to pollinate by pollinator. If an emasculated flower sets fruit, then it must have received pollen from a pollinator. However, if an emasculated flower fails to set fruit, a pollinator will have had no role in fertilization.

Changes in the relative positions of anthers releasing pollen and the styles was also documented. A total of 30 flower buds were monitored during the study period using a hand magnifier. A single flower from this group was monitored from the morning to the end of the day. Each flower was scored for the number of anthers on the flower, the number of anthers dehisced, the position of the dehisced anthers and the positions of anthers relative to the stigma. The length of anther and style were measured using a special caliber. Representative photographs were taken of flowers at each stage.

Thirty marked flower buds were selected to count the number of ovules in order to determine the standard number of ovules in the stigma. The number of ovules per capsule were counted, averaged for both locations and the average number was used as a reference in the calculation.

Data were analyzed as complete randomized design with three replicates. Comparisons between means were made using least significant difference (LSD) at 0.05 probabilities lend (SPSS). For statistical data, standard descriptive statistics were performed for each of the following quantitative parameters: the number of produced fruits, the number of seed for each stigma, the number of ovules, the number of chambers per capsule, the number of non fecundated seeds and the total number of fecundated seeds. Mean number of buds and stigmas of plants, standard deviation, and differences between pollination treatments in terms of seed set per fruit were calculated. The statistical program package SPSS was used. Insect visits were standardized by calculating the number of visits per flower per plant. These data were summarized over the season by taking an average of the observations. Minimum and maximum value was observed and graphical analyses were applied.

3. Results

Anthesis and receptivity

Styles are the first floral organ to emerge and extend, followed by extension of the stamens. When the style has almost straightened, the anthers began to dehisce. After the dehiscence of anthers about half an hour when it is considered as the first day for pollen shedding till fifth day, the male stage activated between 8:30 AM to end of the day and anthers were sink down. The male

phase is initiated a few days before the stigmas become receptive and male stage lasted for five days. By the fifth day of the male stage, female stage started during this day, stigmatic peroxides tests indicate that receptivity occurred between 8:00-13:00 PM and for one day only. Male and female stages synchronized in the last day of the flowering period (Fig. 1). The weight of pollen was 0.064 mg/flower, whereas the volume of nectar was 0.13 µl. Affluent floral rewards (both nectar and pollen) during the male phase of the flowers.

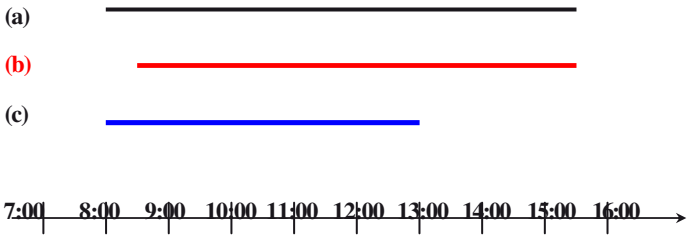

Fig. 1 - Blooming stages of *Nigella sativa*, a) flowers opening, b) anthesis and c) receptivity stage.

Movements of stigma and anthers

The male and female organs at bud stage are presented in (Fig. 2 a) At onset of the male stage, all the stamens stand erect (Fig. 2 b). They curve outwards one by one, roughly in whorls and strictly reflecting the order of initiation (Fig. 2 c). When the anthers reach a horizontal position, the pollen is released (Fig. 2 d). Then, the stamens sink down. An anther takes 4-7 hours to empty its contents. The stamen movement is not continuous, but it is divided into three phases. In the first phase (12-14 hours) the lower part of the filament inclines slightly, while the upper part curves more strongly, so that the anther is brought into a horizontal position. After reaching this position, movement comes to a standstill. The second phase - towards the ends of the male stage, the styles of the five carpels usually curve down (Fig. 2 e) and twist (Fig. 2 f). This ensures that in the female phase the stigmatic crests, whose bends were making an angle of 45°, continue to make a right angle with ovary to run down nearly the whole length of the style to touch the top of the anther at several points (Fig. 2 f).

The third stage, in which the stamen sinks down, is much shorter than the previous ones (4-6 hours). Finally, the empty anthers curve up. This is a purely passive movement, apparently without any function. After uptaking the pollen, the stigma is pollinated (Fig. 2 g), and then the stigma inclined upwardly erect as the order of initiation and makes an angle of 180° with the ovary (Fig. 2 h). The maximum style length reached 1.73 cm, whereas the maximum anther length was 1.72 cm. This indicates the equal length of style and anther.

Pollination

Season one. Location A. N. sativa' flowers produced a non significant number of ovules under all treatments conditions with an average of 96±0.5, as shown in (Table 1). Generally, all flowers under the different treatments produced seeds (Table 1). Open pollinated flowers produced significantly higher seeds as compared with other treatments 74.9±1.4. Hand cross, hand geitongamy and hand forced self ranked secondly in seed set and produced a nonsignificant differences between them with a seed set average of 82.9±1.6, 73.5±1.5 and 78.9±1.6 respectively. A non-fecundated seed production is also a common feature of *N. sativa'* flowers under the different treatments. In the first location, open pollination occupied the lowest average of non-fecundated seeds all over other treatments (Table 1). Hand cross, hand geitongamy and hand forced self ranked secondly in producing a non-significant fecundated seeds with an average of 12±1.7, 21.6±1.6 and 18±1.4 respectively. There were significant differences (P≤0.05) in the percentage of seed set between treatments (Table 1). Seed set percentage after open pollination (86.8% seed) was significantly higher than all other treatments (P≤0.05). Non significant differences were found between the average percentage of seed set when hand cross, hand geitongamy and hand forced self was used on flowers (79.8%, 75.4% and 81% respectively).

Location B. N. sativa' flowers produced a non significant number of ovules under all treatments conditions with an average of 91.1±0.5, as shown in (Table 2). Generally, all flowers under the different treatments produced seeds (Table 2). Open pollinated flowers produced significantly higher seeds as compared with other treatments 82.9±1.5. Hand cross, hand geitongamy and hand forced self ranked secondly in seed set and produced a nonsignificant differences between them with a seed set average of 72.4±1.4, 70±1.3, and 77.5±1.1 respectively for the first location. Characteristics of producing fecundated seeds in the second location were fairly constant in value and regulated mainly by treatments conditions. A non-fecundated seed production

Table 1 - Seeds set after different pollination treatments in *Nigella sativa*, location A. Season one

Treatment of pollination	No. of ovules/capsule	No. of fecundated seed/capsule	No. of non fecundated seed/capsule	Percentage of seed set/ capsule
Open	93.3±0.5 A	74.9±1.4 a	19.0±1.5 A	86.8±1.2 A
Hand cross	95.6±0.7 B	82.9±1.6 b	12.0±1.7 B	79.8±1.3 B
Hand geitonogamy	95.1±0.6 B	73.5±1.5 b	21.6±1.6 b	75.4±1.2 B
Hand forced self	96.0±0.5 b	78.9±1.6 b	18.0±1.4 B	81.0±1.4 B

a and b are symbols related to difference in comparison.

Fig. 2 - a) Plant at bud stage; b) The stamens stand erect; c) first phase of stames movement: The stamens curve outwardly in whorls; d) Pollens releasing; e) First phase of style movement: the styles of the usually five carpels curve down; f) Twisting point of style with anther; g) The stigma is pollinated; h) The stigma inclined upwardly erect.

is also a common feature of *N. sativa'* flowers under the different treatments. In the first location, open pollination occupied the lowest average of non-fecundated seeds (Table 2) all over other treatments. Hand cross, hand geitongamy and hand forced self ranked secondly in producing a non-significant fecundated seeds with an average of 18.7±1.3, 25±1.5 and 18.1±1, respectively.

There were significant differences (P≤0.05) in the percentage of seed set between treatments (Fig. 3). Seed set percentage after open pollination (87% seed) was significantly higher than all other treatments (P≤0.05). Non significant differences were found between the average percentage of seed set when cross, hand geitonogamy and hand forced self was used on flowers (79%, 73% and 80% respectively).

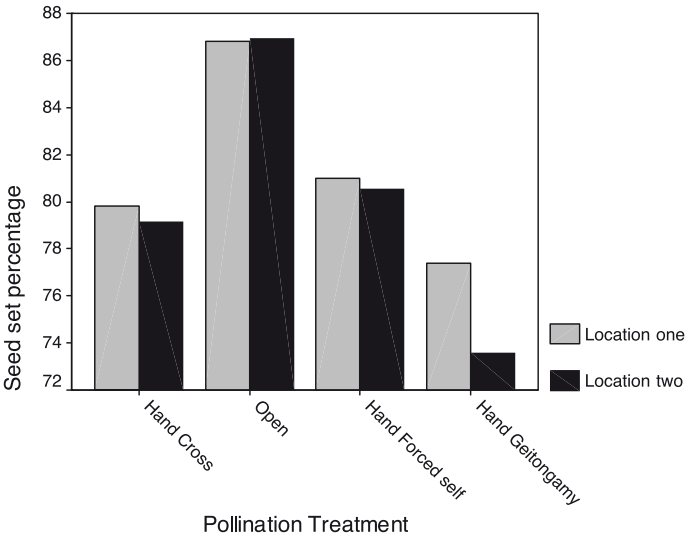

Fig. 3 - *Nigella sativa* seed set percentage upon pollination treatments in location A and B season one.

Season Two. Location A

N. sativa' flowers produced a non significant number of ovules under all treatments conditions with an average of 92.3±1.42, as shown in (Table 3). Generally, all flowers under the different treatments produced seeds (Table 3). Open pollinated flowers produced significantly higher seeds as compared with other treatments in both locations 83.4±0.67. Hand cross, hand geitongamy and hand forced self ranked secondly in seed set and produced a non-significant differences between them with a seed set average of 74.6±0.68, 73.6±0.67, and 79.7±0.32 respectively for the first location. Bagged self pollinated flowers ranked thirdly and produced 44.1±0.75 seeds. The lowest seed set was recorded in the case of emasculated flowers with an average seed production of 12.4±0.33. Hand cross, hand geitongamy and hand forced self ranked second. A non-fecundated seed production is also a common feature of *N. sativa'* flowers under the different treatments. In the first location, open pollination produced non-significant fecundated seeds with an average of 12±1.7, 21.6±1.6 and 18 ±1.4, respectively.

There were significant differences (P≤ 0.05) in the percentage of seed set between treatments (Fig. 4). Seed set percentage after open pollination (87% seed) was significantly higher than all other treatments (P≤ 0.05). Non significant differences were found between the average percentage of seed set when hand cross, hand geitongamy and hand forced self was used on flowers (79%, 78% and 83%, respectively). Nearly half of the produced set seed in bagged flowers with an average of 47%. Emasculated flowers (13%) recorded the lowest seed set from other treatments with significant difference.

Location B. N. sativa' flowers produced a non significant number of ovules under all treatments conditions with an average of 97.2±1.67, as shown in (Table 4). Generally,

Table 2 - Seeds set after different pollination treatment in *Nigella sativa* location B. Season one

Treatment of pollination	No. of ovules/capsule	No. of fecundated seed/capsule	No. of non fecundated seed/capsule	Percentage of seed set/capsule
Open	95.6±0.48 a	82.9±1.5 a	12.8± 1.5 A	87.0±1.3 a
Hand cross	91.1±0.50 b	72.4±1.3 b	18.7± 1.3 b	79.0±1.2 b
Hand geitonogamy	95.0±0.55 b	70.0±1.3 b	25.0±1.5 B	73.6±1.0 b
Hand forced self	96.0±0.60 b	77.5±1.1 b	18.1±1.0 b	80.0±0.99 b

a and b are symbols related to difference in comparison.

Table 3 - Seeds set after different pollination treatment in *Nigella sativa* location A. Season two

Treatment of pollination	No. of ovules/capsule	No. of fecundated seed/capsule	No. of non fecundated seed/capsule	Percentage of seed set/capsule
Open	96.8±2.19 a	83.4±0.67 a	13.4±1.93 d	87±1.67 a
Hand Cross	96.0±2.31 a	74.6±0.68 b	21.4±2.39 c	79±1.98 b
Hand geitonogamy	93.0±2.81 a	73.6±0.67 b	21.1±1.81 c	78±1.46 b
Hand forced self	97.2±1.67 a	79.7±0.32 b	17.6±1.77 c	83±1.54 b
Bagged self	94.2±1.50 a	44.1±0.75 c	51.9±0.73 b	47±1.10 c
Emasculation	97.6±1.67 a	12.2±0.33 d	85.4±0.33 a	13±0.46 d

a, b, c, and d are symbols related to difference in comparison.

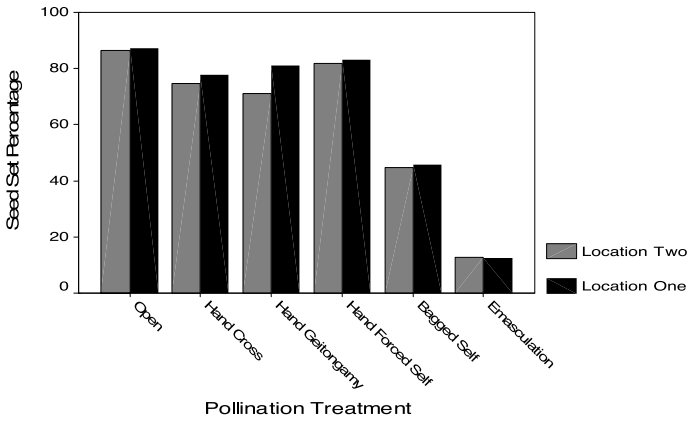

Fig. 4 - *Nigella sativa* seed set percentage upon pollination treatments in both locations, season two.

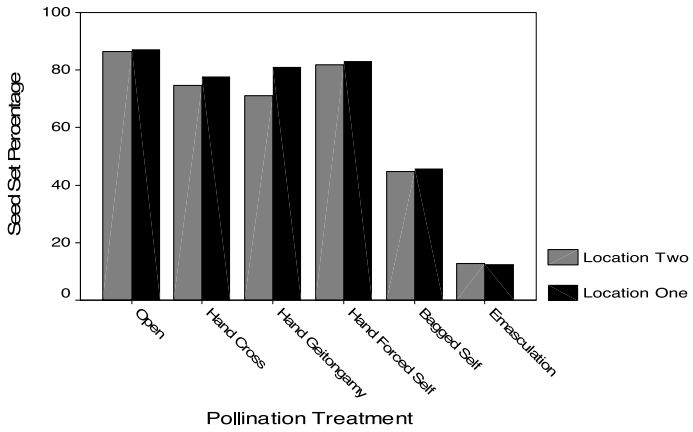

Fig. 5 - *Nigella sativa* seed set percentage upon pollination treatments in both locations, season two.

all flowers under the different treatments produced seeds (Table 4). Open pollinated flowers produced significantly higher seeds as compared with other treatments in both locations 82.4±0.57. Hand cross, hand geitongamy and hand forced self ranked secondly in seed set and produced non-significant differences between them with a seed set average of 71.8±0.57, 67.9±0.62, and 78.6±0.5, respectively. Bagged self pollinated flowers ranked third and produced 43±0.74 seeds. The lowest seed set was recorded in the case of emasculated flowers with an average seed production of 12.4±0.5. Open pollination occupied the lowest average of non-fecundated seeds 13.7±1.76 (Table 4) all over other treatments. Hand cross, hand geitongamy and hand forced self ranked second, a non fecundated seed production is also a common feature of *N. sativa'* flowers under the different treatments in producing a non-significant fecundated seeds with an average of 20.2±1.57, 25.1±1.62 and 14.5±1.6, respectively. There were significant differences (P≤0.05) in the percentage of seed set between treatments (Fig. 5).

Seed set percentage after open pollination (87% seed) was significantly higher than all other treatments (P≤0.05). Non significant differences were found between the average percentage of seed set when hand cross, hand geitongamy and hand forced self was used on flowers (78%, 73% and 85% respectively). Nearly half of the produced set seed in bagged flowers with an average of 46%. Emasculated flowers (13%) recorded the lowest seed set from other treatments with significant difference.

Behavior of honey bee visitors

During our observation, honey bees were the only visitor and pollinator that visited *N. sativa* in the morning around 7:00 A.M. Every flower had one bee at least. Each bee spent different time with an average of 12.5 s for nectar collecting, 8 s for pollen collectors. The only diurnal visitor and pollinator were honey bees. Honey bees were frequent visitors to *N. sativa* in the Jordan Valley. The honey bee had same behavior in the two locations. In the evening, no pollinators were found in the flowers in both sites. The major pollinator was honey bees. *N. sativa'* flowers' mean visit rates for the three replicates in both locations were 14.9 and 14.6 daily visiting tours, respectively. The ultimate activity during the three replicates was approximately from 9:30 to 12:30 in both locations. 33% of the total bees observed were pollen collectors, while the rest 67% were nectar collectors (Table 5). Honey bees

Table 4 - Seeds set after different pollination treatments in *Nigella sativa* location B. Season two

Treatment of pollination	No. of ovules/capsule	No. of fecundated seed/capsule	No. of non fecundated seed/capsule	Percentage of seed set/ capsule
Open	96.1±1.76 a	82.4±0.57 a	13.7±1.76 d	87±1.56 a
Hand cross	92.3±1.42 a	71.8±0.57 b	20.2±1.57 c	78±1.28 b
Hand geitonogamy	93.1±1.68 a	67.9±0.62 b	25.1±1.62 c	73±1.22 b
Hand forced self	93.1±1.53 a	78.6±0.50 b	14.5±1.60 c	85±1.39 b
Bagged self	93.6±1.50 a	43.0±0.74 c	50.6±1.71 b	46±1.10 c
Emasculation	94.9±1.9 0a	12.4±0.50 d	82.5±2.01 a	13±0.62 d

a, b, c, and d; are symbols related to difference in comparison.

Table 5 - Behavior of honey bees and their bearings for *Nigella sativa*

Behaviors of bees	Average spending time/flower (second)	Landing on			Departure of			Percent of bees according to their bearings
		Petals	Anthers	Twisting	Petals	Anthers	Twisting	
Nectar collector were observed	12.5	•			•			67%
Pollen collector were observed	8.0			•			•	33%

visiting tours were conducted in two stages during five days. Anthesis period took place in the first four days, and anthesis and receptivity periods were in the fifth day. Visiting tours were for functional nectar collecting. On the first day, honey bees landed on petals and then collected nectar during circular stepping upon petals, without getting directly exposed to anthers. On the second till the fourth day, the same behavior occurred. The pollen grains fell down upon bees back from the horizontal anthers during circular motion. On the fifth day, receptivity period began, in which the styles inclined towards anthers, and then the styles twisted themselves around the anthers. Honey bees were landing directly on this synapse (not on petals). After that, they left and flew to another flower.

4. Conclusions

The male phase is initiated a few days before the stigmas become receptive, where the anthesis duration remains for five days

Full flowering started with the appearance of bright blue petals. Male stage started as the anthers started to shed their pollen, since the first day till fifth day, the male stage activated between 8:30 A.M. to the end of day. The viability of one anther remained during one day then started to sink down. It is interesting to point out that anthers remain active for five days, which leads to synchronize the receptivity period in the fifth day. Because the flowering period for *N. sativa* coincides with good temperature in April in Jordan, this may lead to an increase of the interval of anthesis since the pollen responds to temperature. It is surprising for pollen of *N. sativa* to continue for five days. Another reason for this long period of anthesis is the large number of anthers in staminate. Climatic factors affect the anthesis intervals in *N. sativa*, there is evidence that high temperature had a direct effect on pollen performance since the pollen responds to temperature. However, at the same time they are advantageous for the pollen by hastening its tube growth rate. On the other hand, low temperatures may act against the pollen by reducing its germination and growth rate, which could limit the fertilization success (Thompson and Liu, 1973; Jakobsen and Martens, 1994)

The duration of stigmatic receptivity in Nigella sativa *was approximately hours*

In angiosperms, the stigma is the first female structure, the pollen grains and pollen tubes have to face on their way to the female gametophyte. The stigma provides an adequate environment for pollen grain germination (Knox, 1984; Helsop-Harrison and Shivanna, 1997). One of the most important features of stigmas is stigmatic receptivity, defined as the ability of the stigma to support pollen germination, which is a decisive stage in fertilization success and has a large variability among plant species (Helsop-Harrison, 2000). At the end of the fifth day on the male stage, the female stage started to be active during 8:00 A.M. to 13:00 P.M. and then ended up. It is interesting to point out that the stigma of *N. sativa* is receptive throughout anthesis. Inspite of the flowering period in April when we don't have high temperature which may hurt the plant; the stigma receptive only for hours. The explanation for that is that the stigma is exposed in direct way to the sun which may increase the exposed area. In addition, the receptivity of stigma occurred after the stigma lost most of the anthers that surrounded the stigma so that the whole stigma is exposed to the sun which may also increase the exposed area to sun. That means high temperature affects stigma receptivity and reduces receptivity interval. There is evidence that ensures stigma responds to high temperature. High temperatures are detrimental for the female part by reducing the length of stigmatic receptivity and accelerating ovule degeneration (Postweiler *et al*., 1985).

It is well documented that the reproductive phase, especially from pollination to fertilization, is highly vulnerable to the prevailing environmental conditions including temperature (Hall, 1992; Stephenson *et al.,* 1992). The duration of stigmatic receptivity is variable depending on the species, and it is also variable within genus. There is evidence that indicates duration of stigmatic receptivity is variable, that the duration of stigmatic receptivity is variable depending on the species and is usually greater in wind-pollinated than in insect-pollinated species (Khadari *et al*., 1995). Thus, the stigma can be receptive for not much more than an hour or so, as in *Avena* or *Dactylis*, to as long as several days, as in other grass species (*Pennisetum* or *Zea*) or *Eucalyptus* in which it can remain receptive for more than a week, particularly in hostile environments (Helsop-Harrison, 2000).

From an agricultural perspective, stigmatic receptivity has also a clear practical implication as it limits floral receptivity, the effective pollination period (Guerrero-Prieto *et al.*, 1985) and hence fruit set (reviewed in Sanzol and Herrero, 2001). Moreover, in an ecologist context, by altering stigmatic receptivity, flowering plants may influence the likelihood of fertilization by indirectly controlling the number and the quality of mating through the control of the number of pollen grains deposited and the time of germination (Cruden *et al*., 1984; Primack, 1985; Galen *et al*., 1986).

Autonomous pollination

First of all, I would like to define the Autonomous phrase for the reader to understand. As Lloyd, 1992 defines it: Prior self pollination within-flower: self-pollination that occurs before the opportunity for outcross-pollen receipt for that flower has occurred, competing self pollination within-flower; self-pollination that occurs during the opportunity for outcross-pollen receipt for that flower has occurred, and delayed selfing pollination within-flower; self-pollination that occurs after the opportunity for outcross-pollen receipt for that flower has occurred. One of these three types of self pollination occurred in our research in *N. sativa*, which is delayed selfing pollination. Automonous delayed selfing late in *N. sativa* flower's life

is favored when honey bees service and thus outcross-pollen receipt is unpredictable. *N. sativa* flowers attract honey bees but they can also autonomously perform delayed self pollination, which provides reproductive assurance if pollinators fail to visit. The delayed self pollination occurred in our research because the synchronization between male and female occurred in the end of flowering period. I agreed with Darwin (1877), Muller (1883), Baker (1955, 1965) and Lloyd (1979, 1992) that pollinator absence or low pollinator abundance during some periods within or among flowering seasons favor shifts from outcrossing to autonomous self-fertilization because self-pollinated seeds provide reproductive assurance. Some authors support the research result that absence of pollinators can shift to delayed self pollination; the extinction of pollinators or range expansion in a plant lineage can favor shifts to biotic modes of pollination, including wind pollination and autonomous self fertilization (Baker, 1955; Stebbins, 1957; Regal, 1982; Cox, 1991; Weller *et al*., 1998).

The results agreed with Barrett and Harder (1996), and Ramsey and Vaughton (1996) that pollinator scarcity and reduced pollinator services may result in high selfing rates. Cross pollination and bagged self pollinations occur; approved by seed set achieved by all treatments applied on the research where bagged selfing and outcrossing boosted seed production means of 45% and 77% respectively. The results agreed with Zohary (1983) as he found that *N. sativa* are capable of setting seed without being cross-pollinated, but he didn't mention the mechanism for such a result. The results also agreed with Faegri and Van Der Pijl (1971) who reported: There are a few flowers that can self-pollinate by their own, but this limits them to in breeding. The results agreed with Goodwillie (1999) in believing the ability of self pollination to provide some insurance against pollination failure.

In addition to the reproductive assurance benefits, prior selfing could be favored since it reduces the costs associated with the longer floral maintenance time required for outcrossing, and sets the stage for the evolution of reduced investment in cues for pollinators and the amount of pollen per flower. In contrast with early selfing, later-selfing species will retain floral traits and costs associated with outcrossing (i.e., cues to attract pollinators, pollinator rewards, and prolonging floral maintenance relative to prior selfing species. At one extreme, selfing early in a flower's life (prior) is favored when a population requires pollinators are chronically absent (Lloyd, 1992), or when population size is so low as to be undetectable by pollinators (Lloyd, 1992; Fausto *et al*., 2001; Goodwillie, 2001), or when a population experiences high levels of interspecific pollen flow (Fishman and Wyatt, 1999). Many authors are interested in common type of pollination as cross, open and self pollination, but through our research I have been devoted all our efforts to point out some thing out of traditional efforts such as delayed self pollination. Thus, delayed selfing may be achieved by either a partial overlap in timing of male phase with female phase or changes in the relative position of anther and stigma during development. For example, delayed selfing in *Hibiscus laevis* (Klips and Snow, 1979) and Campanula species (Faegri and Van der Pijl, 1979) is characterized by a progressive downward curling of the stigmatic area towards the style where anthers or pollen are located. Conversely, in the protogynous *Aquilegia canadensis* (Eckhert and Schaeffer, 1998) the stamens progressively elongate towards the exerted stigma. In *Kalmia latifolia* (Lyon, 1992), anthers collapse into the stigma on the final day of floral development; thereby achieving self pollination. Others have found in self pollination late in floral life without changes in morphology. The breakdown of self incompatibility as the flower ages in both Lilium and Longifolium (Ascher and Peloquin, 1966) is attributed to degradation of the proteins that control self incompatibility and can be viewed as another form of delayed selfing. Faegri and Van der Pijl (1971) used the term ''self pollination'' or "autogamy" when pollination takes place within one flower (idiogamy), and "allogamy" or "cross pollination" when pollen from one flower is carried out to the stigma of another one. Allogamy may further be divided into "geitonogamy" if the flowers are on the same plant and "xenogamy" if they are from different plants. However, it is that geitonogamy that has the ecological properties of cross-fertilizer but the genetic properties of self fertilization. Thus, geitonogamy appears to be equivalent to autogamy (Lloyd and Schoen, 1992).

Style movement acts towards promoting self-pollination and leads Nigella sativa *to delayed self-pollination*

Weber (1995) has produced a presentation film showing the pollination mechanism for *Nigella arvensis.* He concisely presented the mechanism in written steps. The mechanism was demonstrating style movement in *N. arvensis* which exactly resembled our observations on style movement of *N. sativa*; through pictures shown above. I used his written description has quotation for its meaningful. I have measured the style and anther length and style twisting angle. Hence the equal length of anther and stamen demonstrate the style twisting, whereas Weber (1995) did not mention the length. Weber (1995) mentioned that insects bear pollen on their thorax after touching the horizontal anthers. Our observations showed that honey bees are landing on the horizontal anthers and twisting point of style and anther to bear pollen grains on their legs. So, how could insect carry pollen to another flower if the pollen is on their thorax. The beginning of receptivity caused a strong twist for stamens and style that leads to self pollination. This was observed as the end of male stage and the beginning of the receptivity stage. Style movement acts towards promoting self-pollination as in *N. sativa*. In another plant, style movement leads to avoiding self-pollination and promoting cross-pollination as Verma and Magotra (2004) reported for *Eremurus himalaicus* where they observed the mechanism of the stigma movement away from the dehiscing anthers, hence, it avoided receiving any left over pollen, and so self pollination is impossible. It is interesting to point out that *N. sativa* plant relies solely on animal vectors to move pollen among individuals, and if

pollinators are absent or in low numbers at certain times or years, individuals of *N. sativa,* that can self pollinate if not previously out crossed, will be at a selective advantage. This reproductive assurance process has been termed delayed selfing.

N. sativa *mixed mating is a better strategy than selfing alone*

Mixed mating is a better strategy; that means open pollination system is better to seed setting than other pollination treatment. This open system leaves the plant exposed to biotic and abiotic factor. The plant will be without any restriction which may cause any reduction in seed setting. The open system includes the role of honey bees and role of plant to pollinate itself by delayed self pollinated flowers. The manual pollination, which included: hand cross, hand geitongamy and hand forced self, ranked second after open pollination, and this significant difference is attributed to human performance which is not like natural performance. Excluding the biotic and abiotic factor from the plant by bagged self, that means plant will be restricted without honey bees, and the plant depends on itself to develop its style to reach the maximum length to catch the anthers in order to twist. In spite of the style movement towards the anthers, it gained half of the seed setting from open pollination, and this attributed to the fact that the style's movement occurred once the stigma was receptive and at the final stage of anthesis when there is small number of anthers, and then sink down, this may not be enough to get high percent of seed setting as there isn't enough quantity of pollen.

Honey bees are pollinator to N. sativa *which is considered unattractive to wild bees*

The only diurnal visitor and pollinator were honey bees. Honey bees frequently visited *N. sativa* in the Jordan Valley. The honey bee had similar behavior in the two locations. In the evening no pollinators were found in the flowers in both sites and seasons. Flower visitors can only be considered pollinators if four pollination conditions have been met: pollen transfer to the vector is observed; pollen transport by the vector is observed, pollen transfer from vector to stigma is observed; and pollen deposited by the vector is shown to result in fertilization of the value (Cox and Knox, 1988). The flowers of *N. sativa* were unattractive to wild bees' visitors. An important aspect used in many pollination studies is the number of visits made by a pollinator (Proctor *et al*., 1996). *Apis mellifera* engaged in pollen and nectar collection as a pollinator of *N. sativa* flowers with low frequency. The unattractively of *N. sativa* flowers to wilds bees may be attributed to several factors such as the presence of other floral resources. During our research, *N. sativa* flowering coincided with that of other species such as *Centurea syriaca* and *S. arevensis* which are important for apiculture in Jordan due to their abundant nectar and the large floral patches through out the area. The attractiveness of any species is a function such as favor, color, nectar volume, sugar concentration (Frisch, 1967), and the bees fly to plant species that yield the greatest nectar and pollen (Gary, 1979).

The role of honey bees in the pollination of N. sativa *is too small*

Honey bees' role as pollinator in fertilizing *N. sativa* flower buds was very small compared to the role of plant itself and the role of open natural conditions in pollination. The emasculated buds were let exposed to the pollinators in order to fulfill the pollination where it sets up 12% of seed formation percent, while the natural conditions and self pollination conditions gave 87% and 45%, respectively. It is necessary to ask whether the removal of stamens affected subsequent flower development, e.g. the growth of the perianths, a factor that would make it difficult to distinguish between the costs of stamens or pistils and the costs of structures associated with display and reward (Andersson, 2003). Such effects seem likely considering the work of Andersson (2000), who detected a cost of producing and maintaining sepals and petals in a related species (*N. degenii*), and Plack (1957), who found a negative effect of emasculation on corolla size in hermaphroditic plants of the gynodioecious *Glechoma hederacea* (Lamiaceae). In the present study of *N. sativa*, stamen removal caused significant reduction in the mean of seed set. The results agreed with Andersson's study (2003) where he observed the stamen removal produced reduction in total seed number. As a furthermore for *N. sativa,* Andersson (2003) carried out removal of styles from *N. sativa* flowers and he found that; style-less plants initiated almost three times more flowers and invested 57% more biomass in stamens, than plants whose flowers were permitted to set fruit.

He found also stamen-less plants produced significantly heavier seeds after hand-pollination. These observations indicate that stamens draw upon the same pool of resources as the other floral organs and that the removal of immature stamens therefore influences patterns of resource allocation. Furthermore, Andersson and Jorgensen (2005) carried out removal of perianth from *N. sativa* flowers and found that; perianth removal produced 12.5% heavier seeds and allocated 15.8% more biomass to seed production than plants on which all perianths were left intact, whereas differences in flower production and total seed number were not significant. Perianth removal did not significantly affect the proportion of seeds that germinated, but caused a shift toward earlier germination dates.

The ultimate visitation rates for *N. sativa* flower in both Locations was diurnal visitation type especially at early morning

The ultimate visitation rates for flower in both locations were during 9:30 A.M. to 12:30 P.M., because the bees' activity is limited by environmental factors; the radiation rate and the daily temperature. Visitation rate was estimated by counting the number of visiting tours, those with anther or stigma contact. Counts were made for one hour periods during (8) hours a day, while plant species flowers were open. Pollinators may accidentally take place without any relationship existing between blossom and agent. Even

with concept of definite relationship in mind, it is not always easy to draw the line between pollinators and accidental visitors. The quantity of pollen transferred from anthers to stigmas, visit frequency to flower, pollinator forage pattern during anthesis, and floral rewards availability are parameters that can adequately explain the pollination efficiency of floral visitors (Primack and Silander, 1975; Herrera, 1987; 1989). It is generally thought the more visits made, the more efficient is the pollinator, though this also depends on the per visit pollen contribution to the pistillate flower part (Primack and Silander, 1975; Herrera, 1989).

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Disbudding effects on growth analysis of Celosia (*Celosia cristata*)

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Key words: Celosia cristata, disbudding, growth analysis.

Abstract: Experiments to investigate the effects of disbudding on growth analysis of two celosia cultivars, 'Carmine' and 'Chief Gold', were carried out on the field in 2009 and 2010 at the Sinna Garden of Department of Crop Science, University of Ghana, Legon, Accra, Ghana. The treatments consisted of disbudding once, disbudding twice, and no disbudding, as control, and were arranged in a 3x2 split plot in a randomized complete block design with four replications in 2009 (experiment 1) and three in 2010 (experiment 2). The two cultivars were harvested weekly during the growing period and separated into the various plant parts and oven-dried for dry weights, using appropriate formulae to calculate the various growth parameters. Analysis of variance (ANOVA) was used to analyse the data and a correlation coefficient matrix showed relationships among growth parameters. Disbudding resulted in increased leaf area index, leaf area ratio, leaf area duration, relative growth rate, and harvest index, but reduced crop growth rate and net assimilation rate. 'Chief Gold' had a higher harvest index than 'Carmine'. Disbudding plants once gave the best flower head size and weight result. 'Carmine' gave the best flower yield and quality results in experiment 1 and 'Chief Gold' in experiment 2.

1. Introduction

Celosia, a C_3 plant, belongs to the Amaranthaceae family and is of tropical origin. In Ghana, Celosias are not only grown as a cut flower crop, but also as bedding plants, pot plants, and vegetable crops (Norman, 2004).

In this study, disbudding entailed the removal of axillary buds or bud breaks on a single stem, leaving the terminal flower bud intact and able to develop into a large flower head. This practice is reported to be a standard operation in the cultivation of roses, carnations, chrysanthemums and celosias (Machin and Scopes, 1978; Janick, 1986; Dole and Wilkins, 1999; Norman, 2004). Celosia requires one stem as a cut flower; here developing flower buds on a flowering shoot must be disbudded in order to improve the quality of the terminal flower (Norman, 2004). Reports indicate that disbudding increases plant height in dahlias (Parshall, 2007) and in Celosias reduces the number and size of undesirable side (or axillary) shoots on the flower stem and thus resulting in increased plant height, flower stem length and flower head size (Norman *et al.,* 2009). Also, disbudding induces early harvesting and a more concentrated harvesting period (Norman, 2004).

Growth analysis has been widely used to study yield-influencing factors and plant development as net photosynthates accumulation over time (Gardner *et al*., 1985). This approach uses simple primary data in the form of weights, areas, volumes and contents of plant components to investigate processes within and involving the whole plant (Hunt, 1990).

The leaf area index (LAI) of a crop at a particular growth stage indicates its photosynthetic potential or the level of its dry matter accumulation. The greater the LAI, the higher the dry matter accumulation potential of the crop and vice versa (Rasheed *et al.,* 2003). Its value can vary with environmental and cultivation conditions (Board and Harville, 1992). The leaf area and its duration (LAD) are measurements of growth of plants and plant physiological processes (Miralles *et al*., 1997). LAI and LAD control the total production of dry matter and subsequently yield and yield attributes (Jirali *et al*., 1994). Crop growth rate (CGR) is a prime factor in determining crop yield because it reflects the capacity of assimilates production and affects dry matter accumulation. There is a close association between maximum dry matter production and maximum CGR (Ball *et al*., 2000). The analysis of CGR has been shown to be important in evaluating treatment differences among crop species or cultivars with species in relation to yield (Fageria *et al*., 2006).

Relative growth rate (RGR) is described as the rate of increase of total dry weight per plant (Hunt, 2003). Relative growth rate curves of crops are in opposition to DM accumulation during the life cycle of crops (Fageria *et al*.,

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2006). Results from the studies of Medhet *et al*. (2000) on growth analysis of sunflower, *Helianthus annuus*, under drought conditions indicated a reduction of RGR value from early growth stages to final stages. However, recent reports by Fageria *et al*. (2006) indicate that values of RGR are generally higher during early growth stages of the crop and decrease with age.

Measurement of net assimilation rate (NAR) is important to detertmine the efficiency of plant leaves for DM production. NAR values decrease with crop growth due to both the shedding of leaves and reduced photosynthetic efficiency of older leaves (Fageria *et al*., 2006). Similarly, Law-Ogbomo and Egharevba (2008) reported that abscission with plant growth of the lower leaves in tomato causes a decrease in NAR.

However, there is no detailed information on the quantitative growth aspects and growth analysis of *Celosia cristata* grown for cut flower production. The only previous reference to growth characteristics is that of *Celosia argentea* (grown as a leafy vegetable crop) by Ojo (2001) who reported a positive relationship between yield and leaf area, which was enhanced by increasing population density and cutting height. The present experiment was therefore undertaken to investigate the effects of disbudding on the growth indices of two cultivars (Carmine and Chief Gold) of *Celosia cristata* and to identify relationships between these indices (parameters) and flower yield.

2. Materials and Methods

Experimental site

The study was conducted at the Sinna Garden, Department of Crop Science, University of Ghana, Legon, between July and September 2009 for experiment 1 and December 2009 to February 2010 for experiment 2. The soil at the experimental site is of the Adenta series (Brammer, 1960) and classified as Ferric Acrisol (FAO/UNESCO, 1990). The soil is sandy loam and moderately well drained with moderate levels of organic matter. Climatological data during the experimental period are shown in Table 1.

Experimental design

A randomized complete block design with split plot arrangement and cultivars as the main plots and disbudding as the subplots were used for the experiment. There were four replications in experiment 1 and three in experiment 2. The disbudding treatments were: disbudding once; disbudding twice; and no disbudding (as control). The cultivars used were 'Carmine' and 'Chief Gold'. The plants were established at a spacing of 15 x 9 cm. There were 90 plants (experiment 1) and 96 plants (experiment 2) per sub-plot in which five plants were sampled weekly for dry weights and 10 plants were tagged for field data collection.

Cultivation practices

In experiment 1, seeds were first sown in seed boxes using sandy soil on 17 July 2009 and the germinated seedlings were planted in the field on 7 August 2009. Before planting, each sub-plot (0.9 x 1.5 m) received an application of 15-15-15 NPK fertilizer at the rate of 674 kg/ha on 6 August 2009. In experiment 2, cow dung was incorporated into the plots at 25 t/ha on 8 December 2009. Seeds were sown in plastic seed trays on 16 December 2009 using peat as the soil mix and the germinated seedlings were pricked out into plastic seed trays on 30 December 2009. The seedlings were planted in the field on 13 January 2010. A day before planting, each sub-plot (0.99 x 1.65 m) received an application of 15-15-15 NPK fertilizer at the rate of 600 kg/ha.

In each experiment, hand watering was done twice a day. Routine weed control was carried out either by handpicking of weeds or by hoeing when necessary. Diseases and insect pests were controlled by spraying of insecticide and fungicide. Dithane M45 was sprayed on 19 August 2009 and 11 February 2010, in both experiments respectively, to control leaf spot diseases. On 2 September 2009, 28 January and 3 February 2010, in both experiments respectively, Cydim Super was also sprayed to control grasshoppers and whiteflies. In experiment 1, plants were sidedressed four weeks after planting with potassium nitrate at

Table 1 - Climatological data during experimental period

Month	Mean maximum temperature (°C)	Mean minimum temperature (°C)	Total rainfall (mm)	Mean maximum relative humidity (%)	Mean minimum relative humidity (%)
Experiment 1 - 2009					
July	28.3	23.3	91.5	94	77
August	28.3	23.0	11.5	92	74
September	30.5	23.2	6.3	91	69
Experiment 2 - 2009					
December 2010	33.4	24.8	10.3	92	64
January	33.3	25.0	49.6	94	65
February	33.7	25.4	57.2	94	66

Source: Meteorological Services of Ghana, Mempeasem, Accra, Ghana.

a rate of 100 kg/ha while in experiment 2, side-dressing was done at three weeks after planting at the same rate.

Disbudding

Disbudding was carried out as follows:

1. Disbudding once: Axillary flower heads and side shoots were removed on all the plants in the field except the control plants at 22 days after planting (DAP) on 29 August 2009 and at 18 DAP on 1 February 2010.
2. Disbudding twice: The removal of axillary flower heads and side shoots was undertaken on only the plants designated for this treatment at 27 DAP on 3 September 2009 and at 25 DAP on 8 February 2010.

Sampling

Sampling started two weeks after planting and every week thereafter until the sixth week in experiment 1; in experiment 2 it started a week after planting and every week thereafter until the fifth week. Five plants were randomly sampled from each sub-plot, carefully dug up and the roots washed of soil particles. The leaf area was calculated using a leaf area meter (Model AM 100 by Analytical Development Company Limited, England). The plant parts (leaves, flower heads, flower stems, side shoots, axillary flowers and roots) were separated and chopped into pieces and put in different sampling envelopes and oven-dried to a constant weight of 80°C for 48 hr to determine their dry matter.

Two types of measurements are needed for growth analysis: the plant weight, usually the oven dry weight (g); and the size of the assimilating system, usually in terms of leaf area (cm^2). The crop growth rate, net assimilation rate (NAR), relative growth rate (RGR), leaf area index, leaf area ratio (LAR), leaf area duration and harvest index were calculated as follows.

Leaf area index (LAI)

Leaf area index is defined as leaf area per unit area of land. It is a dimensionless ratio (Watson, 1947) and calculated with the formula:

$$\text{Leaf area index} = \frac{\textit{Total Leaf Area}}{\textit{Land Area}}$$

Crop growth rate (CGR)

Crop growth rate is defined as the increase in plant dry matter per unit of time per land area unit (Radford, 1967) with the formula:

$$\text{CGR } (gm^{-2}\ day^{-1}) = \frac{W_2 - W_1}{t_2 - t_1}$$

Relative growth rate (RGR)

Relative growth rate is the increase of plant material per time unit. It was calculated for each interval between sampling with the formula given by Radford (1967). The RGR of the first harvest could not be calculated because there was no dry weight before the first harvest.

$$\text{RGR } (mgg^{-1}\ day^{-1}). = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

Net assimilation rate (NAR)

The net assimilation (NAR) is the increase of plant material per unit of the assimilating material per unit of time. It was calculated for each interval between two samplings with the formula described by Watson (1947) and Radford (1967). The NAR of the first harvest could not be calculated because there was no leaf area value before the first harvest.

$$\text{NAR } (gm^{-2}\ day^{-1}) = \frac{(W_1)(W_2)}{LAD}$$

Leaf area duration (LAD)

Leaf area duration is the photosynthetic potential of a plant, i.e. a measurement of the entire opportunity for assimilation a plant possesses during a growth period (Watson, 1947). This was calculated using the formula:

$$\text{LAD} = \frac{(LAI_1 + LAI_2) \times (t_2 - t_1)}{2}$$

Leaf area ratio (LAR)

Leaf area ratio of a plant at an instant in time (t) is the ratio of the assimilatory material per unit of plant material present. The LAR was calculated with the following formula:

$$\text{LAR } (cm^2/g) = \frac{(LAI_1) - (LAI_2)}{(W_1)(W_2)}$$

Harvest index (HI)

The harvest index is the ratio of economic yield (flower head and stem) to biological yield (Donald and Hamblin, 1976). Its computation uses the following formula:

$$\text{Harvest index (HI)} = \frac{\textit{Economic yield (Flower head and stem)}}{\textit{Biological yield (Total dry weight)}}\ x\ 100$$

Where W_2 and W_1 = dry weight at second and first harvest, t_2 and t_1= time corresponding to second and first harvest.

Leaf chlorophyll content

Leaf chlorophyll content was measured using a chlorophyll meter (model SPAD, Minolta, Japan).

Flower head size index

This was calculated as the product of the vertical and horizontal lengths of the flower head divided by 2.

Number of side shoots

This was obtained by stripping off side shoots on the flower stem and counting.

Harvesting

Harvesting of the 10 tagged plants of each plot started 60 days after sowing for experiment 1 and 63 days after sowing for experiment 2. 'Carmine' was harvested two days earlier than 'Chief Gold'.

Statistical analysis

The data collected were analysed using analysis of variance (ANOVA) (GenStat, ver. 9). Significant differences among treatment means were determined using the least significant difference (LSD) test at P = 0.05.

Correlation analysis

Correlation analysis for flower quality parameters and other measured growth variables was also determined using Spearman's rank correlation coefficient.

3. Results and Discussion

Flower head dry weight and size

Tables 2 and 3 show the effects of disbudding and cultivar on flower head dry weight and size. Disbudding significantly influenced flower head size production. In experiment 1, disbudding twice produced the heaviest flower heads with the control producing the lightest. Plants subjected to disbudding once produced the heaviest flower heads with the control producing the lightest in experiment 2. Larger flower heads were also produced by disbudding-once plants followed by disbudding-twice, with the control producing the smallest flower heads. 'Carmine' produced significantly larger flower heads than 'Chief Gold' in experiment 1. However, the opposite was true in experiment 2.

Flower head size has the potential to increase when the sink-source ratio is reduced, i.e. when the number of competing sinks for assimilates is reduced or the source activity is increased (Cockshull, 1982; Lee *et al*., 2001). In the present study, disbudding increased flower head size significantly. Similar observations were made by Carvalho *et al*. (2006) in chrysanthemums and Norman *et al*. (2009) in celosia. In a celosia plant, the axillary flower heads, roots, leaves and side shoots compete with the flower stem and head for assimilates. As the number of flower heads per plant increases, the flower head size tends to decrease. Reducing the number of flower heads on a flower stem allows the plant to distribute assimilates to the terminal flower that then attains a larger size. Competition among the terminal flowers as well as between the flower and the vegetative plant parts for available assimilates explains the smaller and lighter flower heads produced by the control plants. These experienced a high intra-plant competition for photosynthetic radiation, thus influencing the assimilate allocation to the terminal flower. An increased in the number of small flowers has also been reported in Chrysanthemum by Carvalho *et al*. (2006) as a result of removal of the terminal flower bud.

Crop growth rate (CGR)

Figure 1 shows the effects of disbudding and cultivar on CGR of celosia plants. Significant interactions were observed but showed no differences among treatments at 3 WAP in experiment 1. However, in experiment 2, disbudding significantly affected CGR with the control plants recording the highest CGR and this was significantly different from the other treatments. Significant

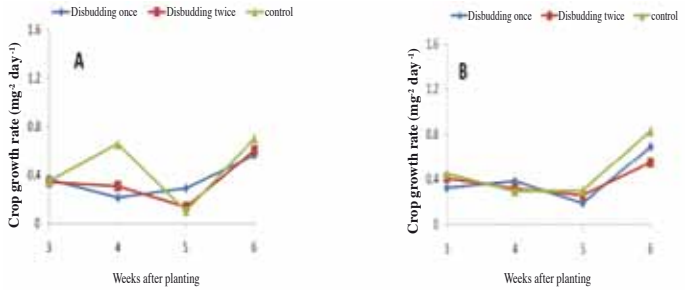
Table 2 - Effects of disbudding on flower head dry weight and size of two celosia varieties at harvest. Experiment 1

	Flower head dry weight (g)			Flower head size index (cm)		
Treatment	'Carmine'	'Chief gold'	Mean	'Carmine'	'Chief Gold'	Mean
Disbudding once	2.08	2.20	2.34	21.7	19.7	20.7
Disbudding twice	2.48	1.81	1.94	18.9	15.0	17.0
Control	1.09	0.84	0.97	12.2	9.5	10.9
Mean	1.89	1.62		17.6	14.7	
$LSD_{(5\%):CULTIVAR}$	NS			NS		
$LSD_{(5\%):DISB}$	0.56			7.20		
$LSD_{(5\%):CULTIVAR \times DISB}$	1.22			9.65		

Table 3 - Effects of disbudding on flower head dry weight and size of two celosia varieties at harvest. Experiment 2

	Flower head dry weight (g)			Flower head size index (cm)		
Treatment	'Carmine'	'Chief gold'	Mean	'Carmine'	'Chief Gold'	Mean
Disbudding once	2.21	2.47	2.34	16.9	23.9	20.4
Disbudding twice	2.11	2.13	2.12	18.1	20.2	19.1
Control	1.43	1.02	1.08	11.9	10.2	11.1
Mean	1.82	1.87		15.7	18.1	
$LSD_{(5\%):CULTIVAR}$	NS			NS		
$LSD_{(5\%):DISB}$	0.36			3.50		
$LSD_{(5\%):CULTIVAR \times DISB}$	0.41			4.30		

Experiment One



Experiment Two

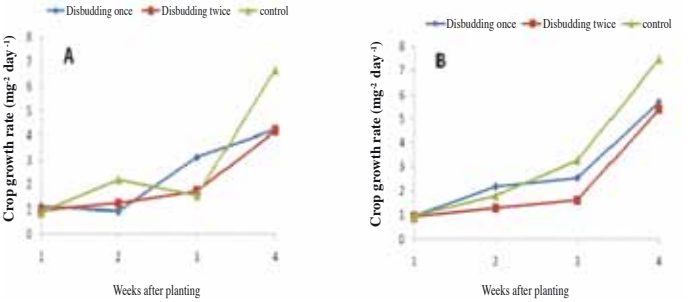


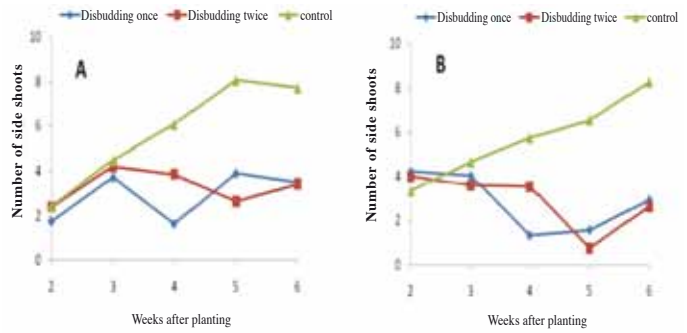
Fig. 1 - Crop growth rate as affected by disbudding and cultivar: 'Carmine' (A) and 'Chief' Gold (B).

interactions were observed with 'Chief Gold' having significantly higher CGR than 'Carmine' at the 3 WAP. Crop growth rate was observed to increase with plant development (Fig. 1A and B). An increase in CGR was recorded for both experiments at the final sampling (5-6 WAP). Crop growth rate is a prime factor in determining crop yield because it reflects the capacity of assimilates production and affects dry matter accumulation. There is a close association between maximum dry matter production and maximum CGR (Ball *et al*., 2000). The observed significant and positive correlation between total aboveground biomass and CGR (r = 0.241*) and (r = 0.245*) in both experiments, respectively, supports this hypothesis. Celosia plants produced a lot of side shoots and axillary flower heads during growth. Therefore, it can be speculated that the DM accumulated in these organs, in addition to the other plant organs, accounted for the higher CGR and also enhanced NAR in the control plants (Fig. 2A and B). Crop growth rate was significantly and positively correlated with flower stem dry weight (r = 0.3*).

Leaf area index (LAI)

Disbudding did not significantly affect LAI at the initial growth stages. Leaf area index was significantly different among the various treatments (Fig. 3A and B) and at harvesting (5-6 WAP), disbudding significantly affected LAI in both experiments. In experiment 1, plants disbudded twice had the highest LAI (2.47) followed by those disbudded once (2.43); the control plants produced the lowest (1.55). In experiment 2, plants disbudded once had the highest LAI (2.58) followed by those disbudded twice (2.52), while the control plants had the lowest LAI (1.83). All disbudded treatments had a significantly higher LAI than the control treatments. 'Carmine' produced a lot of leaves in experiment 1, and they were broader than the ones of 'Chief Gold', hence 'Carmine' had a higher LAI. 'Chief Gold' responded earlier to disbudding than 'Carmine' in LAI as the control plants recorded lower values right from the initial stages.

Experiment One



Experiment Two

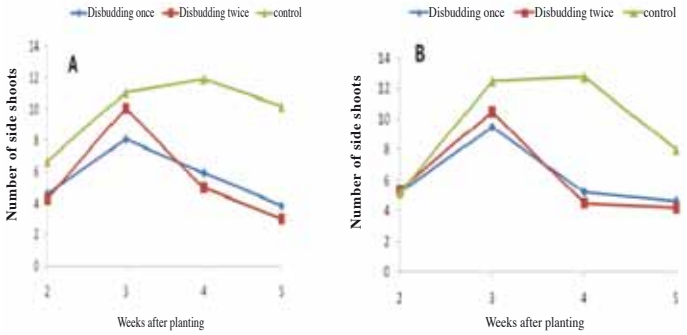
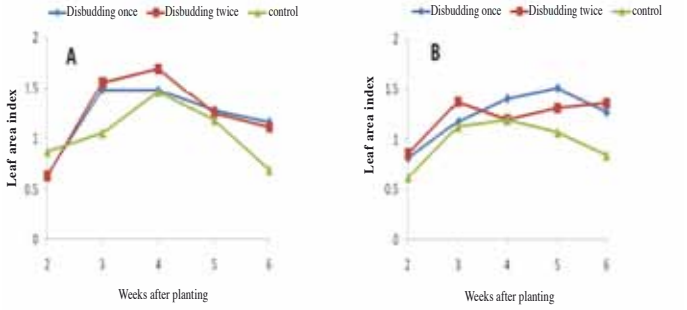


Fig. 2 - Effects of disbudding and cultivar on side shoot production: 'Carmine' (A) and Chief Gold' (B) cultivars of celosia over the growing period in both experiments.

Experiment One



Experiment Two

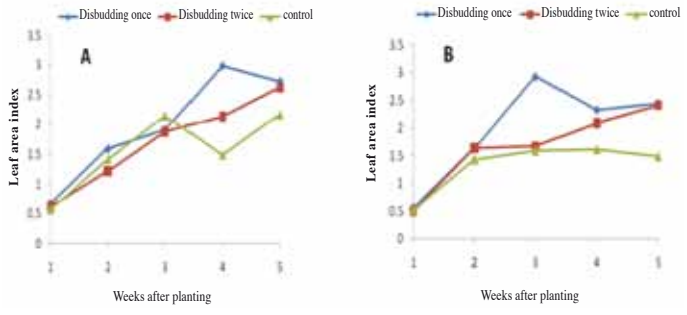


Fig. 3 - Leaf area index as affected by disbudding and cultivar: 'Carmine' (A) and 'Chief Gold' (B).

The LAI determines the photosynthetic capacity of a crop. The higher LAI of the disbudded treatments means that there were more (expanded) leaves per plant for higher radiant energy interception for photosynthesis and, therefore, more dry matter partitioning into the economic yield. This assertion is supported by the significant positive correlation between chlorophyll content and LAI in experiment 1 (r = 0.040*). A significantly higher number of leaves per plant was produced by 'Carmine' (17.44) than 'Chief Gold' (12.82) (Fig. 4). The lower LAI induced by the control treatments might be due to a lower leaf number and area which might have resulted from the competition among the various plant parts for assimilate partitioning. Maximum DM production is achieved at an optimal LAI. The optimal LAI obtained for disbudded plants in celosia is between 2 and 2.5. Although the control plants had a lower LAI, they had the highest CGR. Previous reference to growth characteristics of *Celosia argentia* was made by Ojo (2001) who reported a positive relationship between yield and leaf area. The results of experiment 2 confirm what reported above this. A linear relationship was observed between total aboveground biomass and LAI (r = 0.040*). However, total aboveground biomass had a significantly negative relationship with LAI (r = -0.343*) in experiment 1. LAI had a positive and significant association with flower stem dry weight (r = 0.04*) and flower head dry weight (r = 0.07*).

Relative growth rate (RGR)

In both experiments, RGR decreased linearly during the early growth and increased towards maturity (Fig. 5A and B). In experiment 1, plants disbudded twice exhibited a higher RGR than control and disbudded-once plants. However, in experiment 2, plants disbudded once had a higher RGR than the other treatments. 'Carmine' exhibited a higher RGR in experiment 1 than 'Chief Gold' in experiment 2. The observed decrease in RGR may be attributed to the decreasing trend in leaf area ratio (LAR) with plant growth as indicated by the linear relationship between LAR and RGR (r = 0.343*), (r = 0.168*) in both experiments, respectively. Relative growth rate had a positive and significant association with flower stem dry weight (r = 0.12*) and flower head dry weight (r = 0.39*). Increased RGR due to disbudding also resulted in increased flower yield. Relative growth rate also had a significant negative relationship with CGR (r = -0.04*).

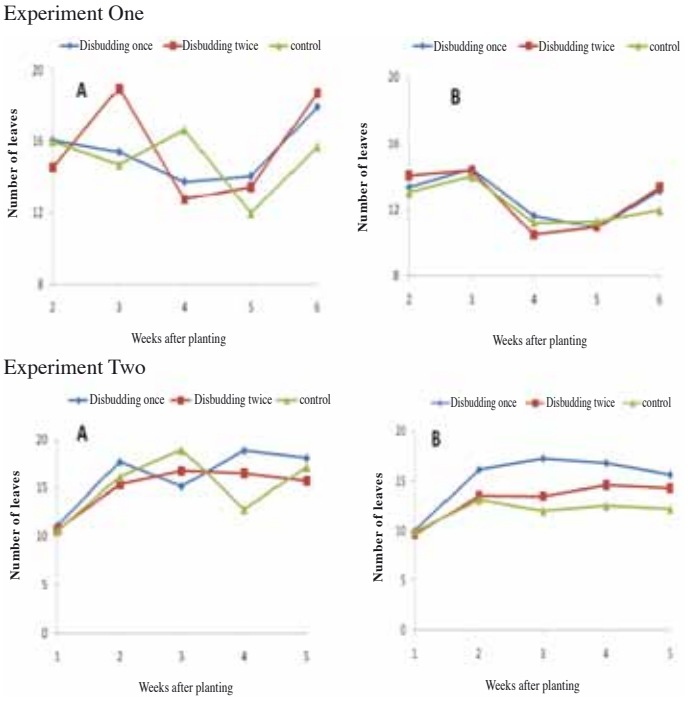


Fig. 4 - Leaf growth as influenced by disbudding and cultivar: 'Carmine' (A) and 'Chief Gold' (B) cultivars of celosia.

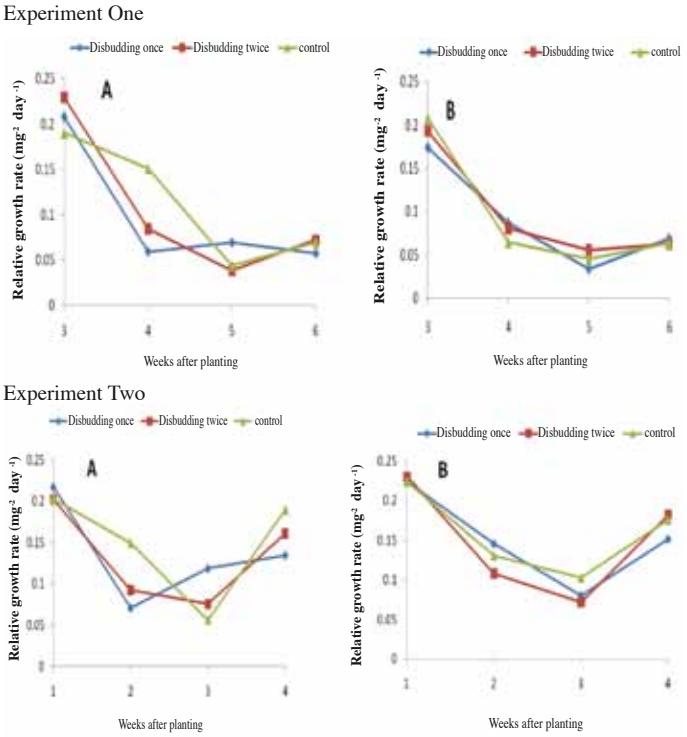


Fig. 5 - Relative growth rate as affected by disbudding and cultivar: 'Carmine' (A) and 'Chief Gold' (B).

Net assimilation rate (NAR)

Net assimilation rate showed no significant differences among treatments at 3 WAP in experiment 2 (Fig. 6A and B). In experiment 1, disbudding did not affect NAR significantly; in experiment 2 disbudding lowered NAR significantly. Correlation analysis shows that NAR had a negative and significant correlation with flower head dry weight (r = -0.05*) and flower stem dry weight (r = -0.02*) in experiment 1 but correlated positively and significantly with flower head dry weight (r = 0.13*) in experiment 2. Thus, the lower NAR observed in experiment 2 was compensated for bigger flower head production. Generally, 'Chief Gold' had a higher NAR than 'Carmine'. The decline in NAR with plant growth observed in experiment 2 after disbudding might be due to both the shedding of leaves and reduced photosynthetic efficiency of older leaves (Fa-

geria *et al*., 2006). Similarly, Law-Ogbomo and Egharevba (2008) reported that the abscission of the lower leaves with plant growth in tomato causes a decline in NAR.

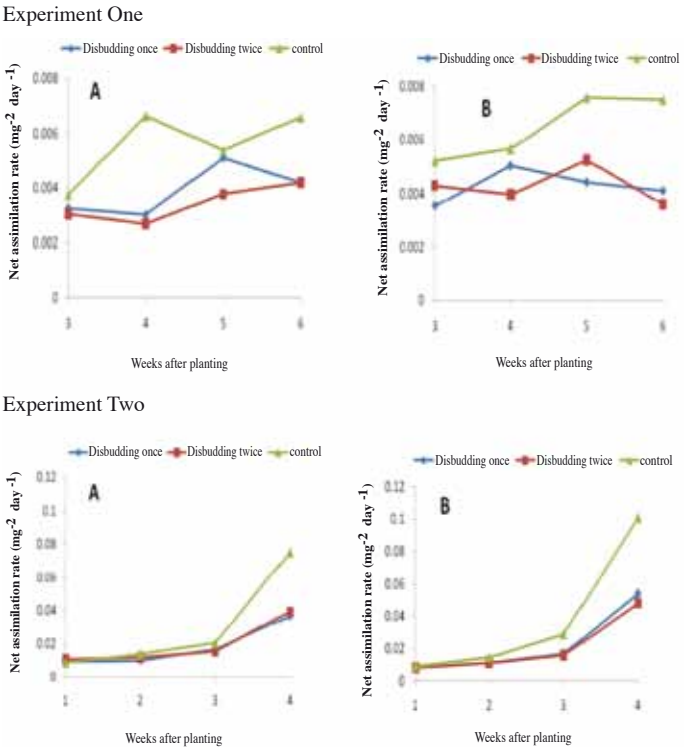


Fig. 6 - Net assimilation rate as affected by disbudding and cultivar: 'Carmine' (A) and 'Chief Gold' (B).

Leaf area ratio (LAR)

Leaf area ratio decreased for both cultivars in both experiments with plant age. From 3-6 WAP, disbudding significantly affected LAR (Fig. 7A and B). The disbudded plants had a significantly higher LAR than the control plants. Since LAR indicates how much leaf area a plant produces per gram of dry matter, a high LAR indicates that a plant is efficient at producing leaf area. Since leaf area determines light interception, which is also an important parameter affecting plant growth, a high LAR would be expected to result in a high growth rate (Kang and Van Iersel, 2004). This further explains the linear relationship between LAR and RGR (r = 0.343*), (r = 0.168*) in both experiments, respectively. Leaf area ratio correlated negatively and significantly with flower stem dry weight (r = -0.05*) and flower head dry weight (r = -0.02*).

Leaf area duration (LAD)

The effect of disbudding and cultivar on LAD is shown in figure 8A and B. Disbudding affected LAD but this was significant. However at harvesting (5-6 WAP), significant differences were observed among disbudded treatments. Significant interactions were also observed with all disbudded plants of 'Carmine' producing a higher LAD than that of 'Chief Gold' (Fig. 8A and B). According to Gifford and Evans (1981),

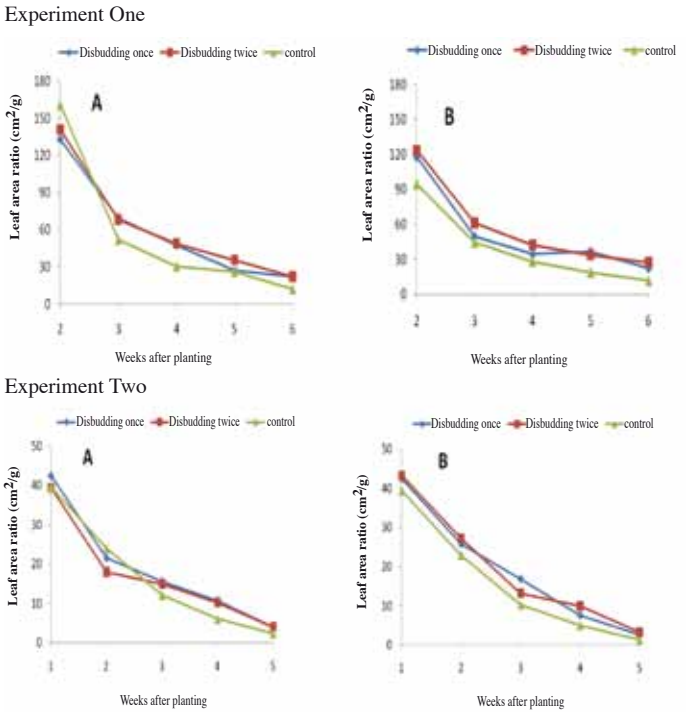


Fig. 7 - Leaf area ratio as affected by disbudding and cultivar: 'Carmine' (A) and 'Chief Gold' (B).

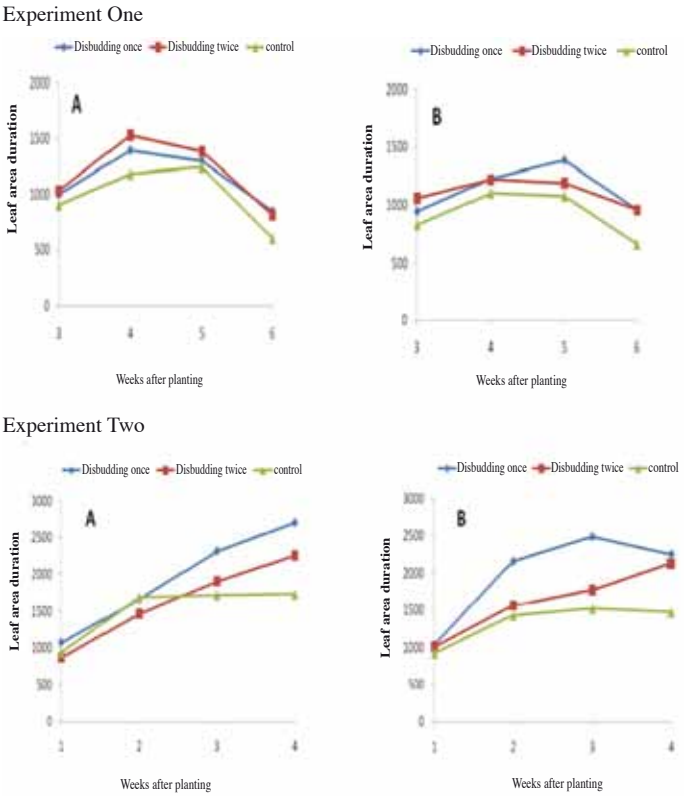


Fig. 8 - Leaf area duration as affected by disbudding and cultivar: 'Carmine' (A) and 'Chief Gold' (B).

LAD is more important for determining the final yield. However, in the current study, the higher LAD of 'Carmine' in both experiments did not lead to a yield advantage since 'Chief Gold' had a higher yield in terms of both the flower head and flower stem (economic sinks).

The most likely explanation for this disagreement is the inefficiency of 'Carmine' to use its entire LAD for DM production even though flower heads were harvested before they were fully matured (market requirement) for both cultivars. A positive linear association was observed between LAD and flower head dry weight (r = 0.02*) and flower stem dry weight (r = 0.04*).

Harvest index (HI)

The effects of cultivar and disbudding on mean harvest index are presented in Table 4. Disbudding significantly influenced HI. All disbudded plants had a higher HI than the control plants (Table 4). Cultivars did not differ significantly in mean HI. Significant disbudding and cultivar interactions were also observed. The HI for the disbudded 'Chief Gold' plants was relatively higher than that of 'Carmine', indicating that 'Chief Gold' had a more efficient translocation system compared to 'Carmine'. Differences in HI may be related to differences in the pattern of allocation of photosynthate (Gent and Kiyomoto, 1989). 'Chief Gold' had higher HI than 'Carmine', which indicates that 'Carmine' is less efficient in converting DM to flower stem and head yield (flower yield). HI showed linear associations with LAI (r = 0.131*), LAR (r = 0.154), NAR (r = 0.019*) and RGR (r = 0.010*).

4. Conclusions

The overall result of the present study shows the effect of variations in disbudding on growth and development of the two considered cultivars. Disbudding increased leaf area index, leaf area ratio, leaf area duration, relative growth rate, and harvest index, but reduced crop growth rate and net assimilation rate. 'Chief Gold' had a higher harvest index than 'Carmine'. Disbudding plants once gave the best flower yield and quality in terms of flower head size and weight. In addition, 'Carmine' gave the best flower yield and quality results in experiment 1 and 'Chief Gold' in experiment 2. Disbudding once is therefore a highly recommended technique for celosia cut flower growers.

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Table 4 - The effects of cultivar and disbudding on mean harvest index of two celosia cultivars in both experiments

Treatment	Mean harvest index					
	'Carmine'		'Chief gold'		Mean	
	Epx 1	Exp 2	Epx 1	Exp 2	Epx 1	Exp 2
Disbudding once	36.99	29.34	39.26	29.34	38.12	29.34
Disbudding twice	37.41	37.03	42.90	28.49	40.15	27.76
Control	21.35	15.58	20.10	15.47	20.73	15.53
Mean	31.92	23.99	34.09	24.44		
$LSD_{(5\%):CULTIVAR}$	NS	NS				
$LSD_{(5\%):DISB}$	4.35	4.04				
$LSD_{(5\%):CULTIVAR\ x\ DISB}$	5.48	4.87				

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Effects of mulching with compost on growth and physiology of *Acer campestre* L. and *Carpinus betulus* L.

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Key words: chlorophyll fluorescence, leaf gas exchange, mulching, soil respiration, soil temperature, SPAD.

Abstract: The aim of this work was to evaluate the effects of mulching with compost on growth and leaf gas exchange of two widely-used ornamental trees in comparison to local nursery management standards. In addition, effects on soil respiration, soil temperature and water evaporation from soil were determined. An equal number (180 each) of uniform hedge maple (*Acer campestre* L.) and 180 hornbeam (*Carpinus betulus* L.) were planted in an experimental plot located in Pistoia. Treatments compared were: 1) chemical weeding by herbicides; 2) natural grass cover, mowed twice per year; 3) harrowing once a year; and 4) mulching with mixed compost (50% green+50% from household waste, 5 to 10 cm thick). Over a two-year period, stem diameter, shoot extension and leaf gas exchange were measured. In the second year leaf chlorophyll content, chlorophyll fluorescence, soil respiration, soil evaporation content and soil temperature were also recorded. Mulching with compost influenced shoot extension and stem diameter growth of field maple and white hornbeam. Plants grown with natural grass cover had, generally, smaller stem diameters and shoot growth than the other treatments. Leaf gas exchange, chlorophyll content and chlorophyll fluorescence were influenced by the different treatments. Soil respiration was unaffected by the different treatments while soil temperature was significantly lower in mulched plots.

1. Introduction

A key to success for the planting of new trees, both in open-field nursery and in the urban environment, is the protection of young plants from non-crop plant species (including some hardwoods, shrubs, grasses, and forbs). These fast-growing plants often kill or greatly suppress desired trees by competing with them for light, water, and nutrients. As a result, nurserymen, arborists and urban forest managers generally use herbicides to suppress non-crop vegetation.

However, the use of herbicides in the urban environment may be limited or even banned in certain countries and/or municipalities. As a consequence, to protect young trees, environmentally sound, effective, cost-efficient, and socially acceptable techniques for managing non-crop vegetation are needed.

In this scenario, we focused on the need for the establishment of environmentally friendly and low cost management methods for nurseries and urban green areas. Mulching and its skilled use can contribute to such a development by improving organic matter content in the soils and by affecting other soil characteristics (Harris *et al.*, 2004).

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For tree management in the urban landscape, especially in the first years after planting, mulching with organic materials can be advantageous. Organic mulching with different materials (mainly shredded wood, chipped wood, pine bark and composted materials), when skillfully applied, is an environmentally friendly way of establishing, protecting and managing young trees at a low cost in a new plantation. A recent review compared the costs and benefits of landscape mulches as reported in the technical and scientific literature, underlining how plants and soil can both benefit in terms of weed suppression, evaporation reduction and other environmental modifications (Chalker-Scott, 2007).

Even if mulching is a world-wide practise in urban green areas and different materials (as noted above) can be used for this purpose (Rakow, 1989), little research has been done in Italy to determine its effectiveness.

Positive effects following organic mulch application have been obtained in previous research, demonstrating beneficial effects on soil physical and chemical properties (Fraedrich and Ham, 1982; Litzow and Pellett, 1983; Watson, 1988; Appleton *et al.*, 1990; Himelick and Watson, 1990; Smith and Rakow, 1992; Iles and Dosmann, 1999; Dahiya *et al.*, 2007; Tiquina *et al.*, 2007) and on plant growth and physiology (Watson, 1988; Green and Watson, 1989; Appleton *et al.*, 1990; Himelick and Wat-

son, 1990). However, sometimes the results from mulching are variable as they are affected by different environmental conditions and different tree species (Whitcomb, 1979; Iles and Dosmann, 1999). Moreover, if the quality of the mulching materials supplied by the producers is not satisfactory, tree performances can be affected in a negative way. This fact can be related either to quality or misuse, i.e adding too much material which can negatively affect soil oxygen content (Gilman and Grabosky, 2004; Hanslin *et al*., 2005), although Watson and Kupkowski (1991) found no detrimental effect from the application of 0.45 m of wood chip mulch over soil in which the roots of trees were growing. The application of bark mulch can sometimes decrease growth in the first year, but the effects on plant growth are positive when examined in the long term (Samyn and de Vos, 2002). This can be due to a temporary nitrogen depression until the microorganisms are able to decompose a sufficient amount of organic material to provide the needed nitrogen (Craul, 1992). In fact, although this temporary depression mainly affects the interface between mulch and soil, it has been shown that fine roots tend to grow into the organic mulch layer (Watson, 1988; Watson and Kupkowski, 1991), where N-concentration is temporary depressed.

As far the composted material as concerned, it has to be remarked that it needs to be well characterised for nutrient values, stability and other properties for the support of tree growth and effect against weeds. In a review of the use of composts for mulching and soil amendments, Sæbø and Ferrini (2006) suggest designing the composts to fit the specific effects that are wanted. For example composts for mulching should consist of layers of compost of different particle sizes, so that nutrients can be supplied and weeds are not given good germination conditions.

The purpose of the present study was to investigate the use of mulching materials and their possible influence on growth and physiology of two shade tree species widely grown in the urban environment. In addition, effects on soil respiration, soil temperature and water evaporation from soil were assessed in the second year of the experiments.

2. Materials and Methods

One hundred eighty uniform *Acer campestre* L. and 180 *Carpinus betulus* L. two-year-old seedlings 1.20 m in height from container were planted in an experimental plot located in Pistoia (43°56' N; 10°54' E). Planting density was 1.80 m in-row and 2.50 m between rows. Mean temperature and rainfall over the last 50 years are 14.3°C and 1257 mm/year respectively. However, during the years of the current experiment, a decrease in rainfall and an increase in mean temperatures were recorded: rainfall in the first and second year was 971 and 903 mm, respectively; while in both the first and second year, an extended dry period with no rainfall was recorded from early June to the end of July. Plants were irrigated the first year after planting to help establishment. From the second year no irrigation or fertilization were applied to plants.

After two years from planting, when trees had overcome transplant phase, the experiment was started and the following thesis were compared: 1) weeding by herbicides using glyphosate twice a year (W); 2) natural grass cover, which was mechanically cut twice per year (G); 3) tillage by harrowing at a depth of 15 cm, once per year (T); and 4) in-row mulching with green compost (layer 5-10 cm thick and 1.5 m wide) and natural grass cover soil between the rows (M). The experimental design was a randomized complete block with six blocks and 30 plants per block.

The following year (three years after planting and one year after treatments), biometric and physiological analyses were performed. Stem diameter was measured 5 cm above the root flare on all plants at the end of the growing season. At the same time shoot extension was determined on 10 shoots per plant on three plants per block (18 plants per treatment). During the growing season, leaf gas exchange was measured with an infrared gas analyzer (Ciras-2, PP-System, New Hertfordshire, UK) on six leaves per block (36 leaves per treatment x species) twice during the first growing season and four times during the second. Measured parameters were net assimilation (A, $\mu mol*m^{-2}*s^{-1}$) and transpiration (E, $mmol*m^{-2}*s^{-1}$). Measurements were taken between the hours 8.00 and 13.00 on the first fully expanded leaf from the shoot apex. Measurements were taken under fixed CO_2 concentration (360 ppm) and saturating irradiance (1300 $\mu mol*m^{-2}*s^{-1}$) provided with a built-in red light emitting diode radiation source. Water Use Efficiency (WUE) was calculated as the ratio between A and E as described in a previous work (Ferrini *et al*., 2008). Leaf greenness index was determined in the second growing season with a Minolta SPAD-meter (Spectrum Technologies, Plainfield, IL, USA) on six leaves per block (36 leaves per treatment x species). For each leaf, the SPAD value was obtained averaging three different measurements made in different points of the leaf blade. This parameter is a good indicator of leaf chlorophyll, N and carotenoid content (Percival *et al.*, 2008). Chlorophyll fluorescence was measured three times in the second year with a portable plant efficiency analyzer HandyPea (Hansatech Ins., King's Lynn, UK) on six leaves per block (36 leaves per treatment x species). Fv/Fm values were obtained by placing leaves in darkness for 30 min by attaching light-exclusion clips to the leaf surface. Fv/Fm is the maximum quantum yield of the PSII and it is a reliable indicator of the occurrence of environmental stress (Maxwell and Johnson, 2000). Soil respiration and evaporation were measured with a soil respiration chamber (SRC, PP-System, New Hertfordshire, UK) at a depth of 5 cm below soil surface. Measurements were taken twice in July around midday on soil exposed to full sunlight (not shaded by the trees). At least 30 days had passed since the last remarkable rainfall

(Fig. 1). Four measurements per treatment per species and per replicate were made. Before measuring respiration and evaporation, the mulch was removed from an area of about 25 cm^2 and the chamber was placed on the soil beneath. After taking the measurements, mulch was spread again. Soil temperature was measured with a temperature probe at a depth of 10 cm below soil surface four times per treatment per species and per replicate.

All data were analyzed with GLM using the SPSS statistical package for Windows (SPSS Inc., Chicago, IL, USA). Effects of soil management technique and species were analyzed with a random model two-way ANOVA. When no significant interaction between factors was found, differences among soil management techniques were tested with Duncan's multiple range test ($P \leq 0.05$ and $P \leq 0.01$). Parameters which showed significant interaction between factors were plotted separately in order to compare each level of factor A (soil management) for each level of factor B (species) (Chew, 1976). Data on leaf gas exchange, Fv/Fm and soil parameters were analyzed per single sampling date, merged together and processed again to obtain an average value on annual basis.

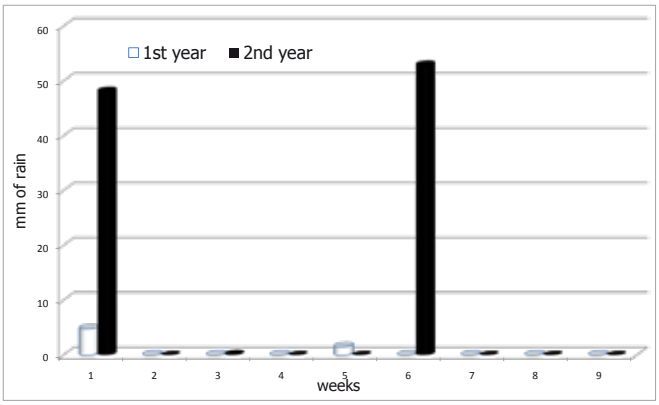

Fig. 1 - Rainfall from 1 June (day 1) to 31 July (day 61) in 2006 and 2007 measured in the experimental centre where the research was carried out.

3. Results and Discussion

Mulching with compost (M) affected shoot growth of *Acer campestre* and *Carpinus betulus* (Table 1). Significant interaction between species and soil management technique was found for shoot growth (Table 1). In the first year of measurements, mulched plants of both species had higher shoot growth than the other treatments (Table 2). In maple, no difference was found among treatments W (herbicide), T (tilling) and G (ground cover). In hornbeam, shoot growth was lower in G than in T and W. In the second year, M *Carpinus* plants had greater shoot growth than W and T which, in turn, had longer shoots than G. In the second year, M and T *Acer* had greater shoot growth than W. Again, the shortest shoots were found in G maples. In the first year and second years, G plants had lower stem di-

Table 2 - Effect of soil management technique on shoot extension in *Acer campestre* and *Carpinus betulus* in the first and second years

Shoot growth (cm)	Acer campestre		Carpinus betulus	
Treatment	1 year	2 year	1 year	2 year
Chem. weeding	70.2 b	58.3 b	70.0 b	44.7 b
Tillage	70.0 b	57.5 b	67.0 b	53.4 a
Grass cover	69.6 b	45.5 c	57.1 c	35.6 c
Mulching	86.0 a	72.0 a	74.0 a	54.4 a

Different letters within the same column indicate significant differences at $P \leq 0.01$.

Table 1 - Summary table of Two-way ANOVA for the investigated parameters

Parameter	Measurement unit	Management technique	Species	Management x Species
Shoot growth (1 year)	cm	**	**	**
Shoot growth (2 year)	(g)	**	**	**
Stem diameter (1 year)	(g)	**	NS	NS
Stem diameter (2 year)	(g)	**	NS	NS
Leaf greenness index	SPAD unit	**	**	NS
Fv/Fm		**	NS	NS
A (1 year)	$\mu mol\ m^{-2}\ s^{-1}$	**	**	NS
A (2 year)	$\mu mol\ m^{-2}\ s^{-1}$	**	**	NS
E (1 year)	$mmol\ m^{-2}\ s^{-1}$	NS	**	NS
E (2 year)	$mmol\ m^{-2}\ s^{-1}$	**	**	NS
WUE (1 year)	$\mu mol\ CO_2/mmol\ H_2O$	NS	NS	NS
WUE (2 year)	$\mu mol\ CO_2/mmol\ H_2O$	**	**	NS
Soil temperature (2nd year)	° C	**	NS	NS
Soil respiration (2nd year)	$\mu mol\ m^{-2}\ s^{-1}$	NS	NS	NS
Soil evaporation (2nd year)	$mmol\ m^{-2}\ s^{-1}$	**	NS	NS

* and ** indicate significant differences between treatments at $P<0.05$ and $P<0.01$, respectively.

ameter than the other treatments (Table 3); no differences in stem diameter were found between species (Table 3). There are several reports on how turf or natural grass cover decrease plant growth (Garrity and Mercado, 1994; Stork and Jerie, 2003; Yao *et al.,* 2005; Chalker-Scott, 2007). In the present experiment, natural grass cover decreased shoot growth and stem diameter in both the species studied. Also, *Carpinus betulus,* whose growth was affected both in the first year and second year, is probably a worse competitor with turf than *Acer campestre.* Mulch increased growth of both species, and mulched plants had shoot growth and stem diameter similar or higher than plants grown in tilled soil. Greater plant growth in response to mulching has been observed by many authors (Sæbø and Ferrini, 2006; Chalker-Scott, 2007; Ferrini *et al*., 2008). Some authors found that mulching decreased growth in the first year after application and most of the authors attributed this reduced growth to temporary immobilization of soil N due to the high C/N ratio of the mulch (Ferrini *et al*., 2009). The mulch applied in this experiment had relatively low C/N ratio (about 30), so no nitrogen immobilization occurred and growth was enhanced even in the first year after application, in agreement with that reported in previous works (Tilander and Bonzi, 1997; Erhart and Hartl, 2003; Sonsteby *et al.*, 2004; Granatstein and Mullinix, 2008).

Table 3 - Effects of soil management technique and species on stem diameter growth in the first and second years

	Stem diameter (cm)	
	1 year	2 year
Effect of different treatments		
Chem. weeding	6.5 a	7.5 a
Tillage	6.1 a	7.6 a
Grass cover	5.6 b	6.7 b
Mulching	6.2 a	7.9 a
Effect of species		
A. campestre	6.1	7.4
C. betulus	6.1	7. 4

Different letters within the same column indicate significant differences at P≤0.01.

Leaf greenness index was affected by the different treatments and species (Table 4). Regardless of the species, mulched plants showed higher values than the other treatments. The lowest readings were found in W and G plants, whereas T plants performed intermediately. Higher SPAD readings following low C/N mulch application were also found by Granatstein and Mullinix (2008), who found a soil N-enrichment due to mulch mineralization. We did not consider the effect of management technique on soil N, however according to Percival *et al*. (2008), the higher SPAD-reading of M plants may reflect a better nutritional status generated by mulch application and its decomposition. Leaf greenness index was also affected by species, with field maple having higher values than hornbeam (Table 4). Fv/Fm was affected by soil management technique but not by species. Fv/Fm measurements of healthy, unstressed plants are associated with values ranging from

Table 4 - Effects of soil management technique and species on leaf greenness index and Fv/Fm in the second year

	Greenness Index (SPAD)	Fv/Fm
Effect of different treatments		
Chem. weeding	38.7 c	0.721 ab
Tillage	40.2 b	0.731 a
Grass cover	38.8 c	0.701 b
Mulching	42.3 a	0.743 a
Effect of species		
A. campestre	43.0 a	0.728
C. betulus	40.0 b	0.719

Data are the average of two measurement campaigns made in the second year. Different letters within the same column indicate significant differences at P≤ 0.01.

0.76 to 0.85 (Percival, 2004; Percival *et al*., 2006). Regardless of the treatment, all plants in this experiment were subjected to some degree of stress because of the very low rainfall during the summer (Fig. 1), since Fv/Fm values were lower than 0.75. In any case, M and T plants had a significantly higher Fv/Fm than G plants (Table 4). Fv/Fm is very sensitive to oxidative stresses, and to drought stress in particular (Angelopulos *et al.*, 1996; Maxwell and Johnson, 2000; Rong-Hua *et al.*, 2006). Thus, the lower SPAD and Fv/Fm observed in G plants must be attributed to grass competition for nutrients and water.

Leaf gas exchange was affected by soil management technique and species (Table 5). In the first year, net assimilation was higher in M plants than in the other treatments. In the second year, M and T had greater assimilation than G and W. Transpiration was not affected by management technique in the first year, while in the second year T plants had greater transpiration than the other treatments. Water Use Efficiency was not affected by soil management technique in the first year. In the second year, M and W had greater WUE when compared to T and G. Leaf gas exchange was also affected by species: hedge maple always showed higher values than hornbeam (Table 5). A significant quadratic relation was found for *Acer* ($P<0.01$; $R^2=0.855$) and *Carpinus* ($P<0.01$; $R^2=0.298$) between leaf greenness index and net assimilation (Fig. 2). Leaf greenness index has shown to be accurate in predicting leaf N content (Follett *et al.*, 1992; Wood *et al*., 1992). In our experiment, the relationship between SPAD and A was much stronger in hedge maple than in hornbeam, suggesting a N limitation to photosynthesis in the case of maple and a less N-dependant limitation in hornbeam.

Soil temperature was affected by management technique: in July (second growing season) plots mulched with compost were 13°C, 10.8°C and 7.2°C cooler than bare soil, tilled and turf plots respectively (Table 6). This is consistent with previous works, which found that mulching is more effective than cover crops and tillage to reduce extreme summer temperatures and that, in dry climates, mulching can lead to a reduction in soil temperature of up to 10°C (Martin and Poultney, 1992; Zhang *et al*, 2009).

Table 5 - Effect of soil management technique and species on net assimilation (A, μmol*m^{-2}*s^{-1}), transpiration (E, mmol*m^{-2}*s^{-1}) and Water Use Efficiency (WUE, μmol CO_2/mmol H_2O). Data are the average of two (the first year) and four (the second year) measurement campaigns

	A (μmol m^{-2} s^{-1})		E (mmol m^{-2} s^{-1})		WUE (μmol CO_2/ mmol H_2O)	
	1 year	2 year	1 year	2 year	1 year	2 year
Effect of different treatments						
Chem. weeding	8.2 b	6.3 b	2.5	2.0 b	3.6	3.5 a
Tillage	8.4 b	7.5 a	2.5	2.6 a	3.8	2.9 b
Grass cover	7.7 b	6.3 b	2.2	2.1 b	3.8	3.1 b
Mulching	9.3 a	7.5 a	2.5	2.0 b	4.0	3.7 a
Effect of species						
A. campestre	10.3 a	8.3 a	2.9 a	2.4 a	3.8	3.6 a
C. betulus	6.5 b	5.5 b	1.9 b	1.9 b	3.7	2.9 b

Different letters within the same column and factor indicate significant differences at P<0.01.

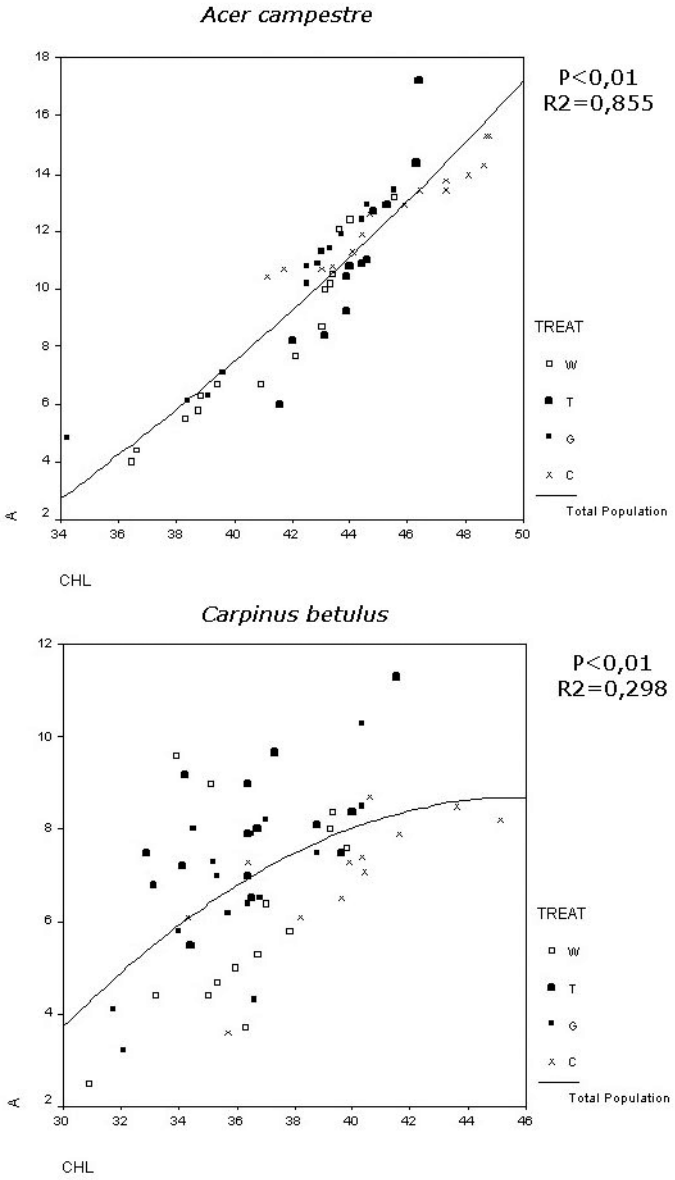

Fig. 2 - Relationship between leaf greenness index (chl, SPAD-units) and net assimilation (A, mmol*m^{-2}*s^{-1}) in *Acer campestre* and *Carpinus betulus.*

The significant reduction in soil temperature contributed to create a more favourable environment for root growth: soil temperature in mulched plots did not exceed 35°C, which is considered the threshold temperature above which root growth is hampered and root mortality increases (Coder, 1996; Fini and Ferrini, 2007). The reduction of soil temperature under mulch is due to the low albedo and thermal conductivity of woody mulches (Montague and Kjelgren, 2004). This finding means that the radiation reaching the mulch was not reflected, but the mulch acted as insulation and prevented energy from being conducted to the soil. Soil temperature under natural grass cover was lower than tilled and bare soil, but higher than mulch. The cover crop acted as a barrier which absorbed solar energy and shaded soil surface, but it also transpired soil water, reducing soil water content and, by consequence, its buffering capacity (Zhang *et al*., 2007, 2009). This is confirmed by water evaporation from soil (Table 6). Evaporation was measured 30 days after the last rainfall. In the mulched plot, the mulch layer was temporary removed and the measurement taken on the soil below. The lowest evaporation was found in G plots and the highest value was found in M plots. In absence of irrigation and natural rainfall, the higher value measured in mulched soil provides further confirmation of the effectiveness of mulching to reduce evaporation: after 30 days of drought, soil was very dry

Table 6 - Effect of the different treatments on soil temperature (measured 10 cm below soil level), soil evaporation (measured 5 cm below soil level), and soil respiration (measured 5 cm below soil level). Data are the average of two measurement campaigns made in the second year

Treatment	Soil temperature (°C)	Soil evaporation (mmol m^{-2} s^{-1})	Soil respiration (μmol m^{-2} s^{-1})
Chem. weeding	43.4 a	28.0 b	0.3
Tillage	41.2 a	29.7 b	0.4
Grass cover	37.6 b	18.3 c	0.3
Mulching	30.4 c	94.2 a	0.5

Different letters within the same column indicate significant differences at P≤ 0.01.

in treatments G, W and T and no further evaporation was possible. On the contrary, moisture was still available under the mulch so that, after mulch removal, evaporation was higher than in the other treatments. Soil respiration was somewhat higher in M and T plots, but differences were not significant (P=0.172).

Most of the parameters measured showed that plants performed better when mulching was used and there are probably multiple causes which determined these results. The effect of mulching in reducing soil temperature which can allow a higher root growth is probably the most important under the conditions of this study, also because it might have prevented dehydration. Compost mineralization might also have increased soil nutrient content. These effects could have allowed a greater root growth and, as a consequence, greater water and nutrients absorption. Unfortunately we did not analyze the soil and this might be a potential shortcoming of this research.

4. Conclusions

The use of compost as mulching material had great impact on plant growth and physiology. In agreement with the results obtained in a previous work (Ferrini *et al.*, 2008), the present study confirms that mulching with compost is a useful practice to improve plant growth, leaf gas exchange and leaf chlorophyll content. Positive effects of organic mulching on soil organic matter, soil water holding capacity and weed suppression have already been revealed in previous works (Sæbø and Ferrini, 2006; Chalker-Scott, 2007; Granatstein and Mullinix, 2008; Mulumba and Lal, 2008). The present investigation provides the evidence that mulching also has positive effects on plant growth and physiology, comparable or even superior to tillage. Despite being inexpensive and very effective in weed control, the use of chemical herbicides reduced shoot growth and gas exchange when compared to mulching. An even greater reduction in growth and carbon assimilation and a significant increase of oxidative stress on PSII was found in plants growing with natural grass cover, mechanically cut twice per year. Considering that mulching is cheaper than tillage and mechanical weeding (Zhang *et al*., 2009), it can be considered an environmentally-friendly and sustainable alternative for managing plants in the forest, in the nursery and in the urban environment.

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Selecting parents for developing superior hybrids in cucumber (*Cucumis sativus* L.)

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Key words: Cucumber, *Cucumis sativus*, gca, hybrids, sca.

Abstract: Estimates of general combining ability of parents and specific combining ability of the crosses help to select desidered parents for hybridization and development of superior hybrids. Crosses among eight parents were attempted in a half-diallel fashion. The material comprising eight parents, 28 F_1s and one check (Pusa Sanyog) was sown at two locations in Randomized Block Design with three replications. The highest estimates of general combining ability (gca) were exhibited by G_2 and Gyn_1 for most of the characters at both the locations. In general, there was close agreement between gca effects and per se performance, but in some cases it did not hold good, which may be due to a higher degree of gene action involved. The superior cross combinations which recorded high specific combining ability (sca) estimates and per se performance for yield and number of fruits were K-90 x G_2 and K-90 x Gyn_1 and hence may be exploited for the development of F_1 hybrid (s) after testing their performance at multi-locations for two to three years.

1. Introduction

Cucumber (*Cucumis sativus* L.), a member of the Cucurbitaceae family, is grown as a summer and rainy season crop in the low and mid hills of the northwestern Himalaya from April to August and fruits are available from June to October to the plains of northern India. The crop raised in the hills, being of high quality and off-season, brings good returns to the growers.

F_1 hybrids in cucumber, as in many vegetable crops, have several well known advantages over open-pollinated varieties (Dogra and Kanwar, 2011) and hence provide a scope for the breeder to find more appropriate combinations to develop superior hybrids. F_1 hybrids are early, vigorous, high yielding, tolerant to diseases and insect-pests and more efficient in the use of water and fertilizers. Currently, farmers are purchasing hybrid seeds from private firms who charge exorbitant prices for seed. To tide over the situation, there is a need to develop F_1 hybrids and make their seed available to farmers at a reasonable price. For the development of superior hybrids, estimates of general combining ability of parents and specific combining ability of the crosses help to properly select parents for hybridization. Moreover, use of gynoecious lines for developing cucumber hybrids makes the production of F_1 seed more cost effective. Furthermore, there is urgent need to develop stable hybrids adapted to a wide range of climatic conditions.

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2. Materials and Methods

The present investigations were carried out at two locations: Experimental Farm Nauni (L1) and Experimental Farm Chambaghat (L2) of the Department of Vegetable Crops, Dr Y S Parmar University of Horticulture and Forestry, Solan (Himachal Pradesh), India, which are 1276 m a.m.s.l. and 1300 m a.m.s.l., respectively. Both locations fall in the mid-hill sub-temperate zone of the state of Himachal Pradesh; Nauni lies at latitude and longitude of 30^0 52'N and 77° 11' and Chambaghat, 30° 55' N and 77° 06'. All the parents except two gynoecious lines were of monoecious type. Crosses among eight parents were attempted in a half-diallel fashion. The material comprising eight parents, 28 F_1s and one check (Pusa Sanyog) was sown in Randomized Block Design with three replications. Spacing was 1.25x1.00 m. Data were recorded on randomly selected plants for yield and horticultural characters at both the locations. Griffing's (1956) method II model I was used to derive general and specific combining ability estimates. The analysis of variance for combining ability was based on following mathematical model:

$$P_{ijk} = m + g_{ii} + g_{jj} + s_{ij} + b_k + e_{ijk}$$

where,

P_{ijk} = phenotypes of the hybrids between i^{th} and j^{th} parents in k^{th} plots

m = population mean

g_{ii} = GCA effects of i^{th} parent

g_{jj} = GCA effects of j^{th} parent

s_{ij} = SCA of the crosses between i^{th} and j^{th} parents

b_k = block effects

e_{ijk} = environmental effect associated with ijk^{th} observation

3. Results and Discussion

Analysis of variance (Table 1) for combining ability revealed that the importance of gca (σ^2g) was more than sca (σ^2s), indicating the preponderance of additive gene action for days to first female flower appearance (DFFFA) at location 1 and days to marketable maturity (DMM) at both locations. However, in all the other traits, the sca component was higher in magnitude than gca's, indicating the preponderance of non-additive gene effects. However, mean sum of squares for gca and sca were highly significant for all the characters except TSS, suggesting the importance of both additive and non-additive genetic variance in agreement with the findings of Om *et al*. (1978). Similar trends at both the locations proved that the conclusions on gene actions are authentic.

The parents G_2, Gyn_1 and Poinsette had negative estimates for DFFFA and node at which first female flower appears (NFFF) at both the locations (Table 2) showing earliness in fruit bearing and were good general combiners for these characters. Among F_1's, the sca effects were significantly negative in 12 and 15 crosses, respectively, for these two traits at L1 (Table 3) whereas significantly negative in 15 crosses for each of these two traits at L2 (Table 4). The crosses LC-11 x Gyn_1 (poor x high) and EC 173934 x LC-40 (poor x poor), respectively, had the highest sca effect at L1 and the crosses LC-11 x LC-40 (poor x poor) and EC 173934 x LC-40 (poor x poor), respectively, had the highest sca effects at L2 for these traits. The parents G_2 and Gyn_1 (L1) and G_2, Gyn_1 and Poinsette (L2) with significantly high gca estimates (with negative value) were good general combiners for DMM. Crosses LC-11 x Gyn_1, EC 173934 x LC-40, K-90 x G_2 and K-90 x EC 173934 had high sca estimates at both the locations for DMM. El-Shawaf and Baker (1978), Om *et al.* (1978), and Wang and Wang (1980) also reported greater additive genetic variance for DMM. The parents G_2 and Gyn_1 may be used in the hybridisation programme for developing early hybrids adapted to a wide range of climate. LC-11 x Gyn_1 and EC 173934 x LC-40 may be exploited as early hybrids after further multi-locational testing. These crosses may also be exploited to produce transgressive segregants in advanced generations.

With regard to fruit length, the parents Gyn_1, LC-11 and K-90 were good general combiners as is evident from their high gca estimates at both locations. Fourteen crosses exhibited significant sca effects. The sca effects were high in crosses Poinsette x LC-40 and G_2 x Poinstte involving poor x poor general combiners. K-90, K-75 and EC 173934 had the highest gca with respect to fruit width and hence were good general combiners. The sca effect was maximum in G_2 x Gyn_1 involving poor x poor general combining parental lines (at L1) and in G_2 x K-75 involving poor x high general combining parental lines (at L2). In India, slicing cucumbers are preferred, therefore lengthy fruits are desirable. Kupper and Staub (1988) and Hormuzdi and More (1989) reported contrasting results for fruit length and width due to different experimental material and environment.

Table 1 - Analysis of variance for combining ability for different characters in F_1 cucumber

Source of variation	Df	Character										
		Days to first female flower appearance	Node of first female flower	Days to marketable maturity	Fruit length	Fruit width	TSS	Flesh to seed cavity ratio	Fruit weight	No. of fruits per plant	Yield per plant	Internodal length
Location 1 - Nauni												
Gca	7	678.818 *	27.997 *	705.436 *	6.425 *	1.087 *	0.005	0.001 *	3787.657 *	9.898 *	0.735 *	9.512 *
Sca	28	42.264 *	3.049 *	45.029 *	3.237 *	0.243 *	0.021	0.0015 *	693.149 *	1.159 *	0.193 *	2.183 *
Error	70	0.557	0.228	0.562	0.004	0.002	0.0013	0.00004	62.357	0.112	0.0013	0.272
σ2g		67.826	2.777	70.487	0.642	0.108	0.0004	0.0001	372.53	0.979	0.073	0.924
σ2s		41.707	2.821	44.467	3.0233	0.240	0.020	0.002	630.79	1.047	0.191	1.911
σ2g/ σ2s		1.626	0.984	1.585	0.199	0.451	0.021	0.068	0.591	0.934	0.383	0.483
Location 2 - Chambaghat												
Gca	7	390.457 *	35.726 *	577.811 *	7.820 *	0.993 *	0.012 *	0.0016 *	3515.486 *	14.247 *	0.786 *	7.800 *
Sca	28	67.477 *	4.551 *	37.300 *	3.895 *	0.268 *	0.028 *	0.0009 *	612.551 *	1.582 *	0.181 *	1.510 *
Error	70	0.431	0.205	0.442	0.089	0.023	0.006	0.000035	49.232	0.148	0.0096	0.358
σ2g		39.003	3.552	57.737	0.773	0.097	0.0006	0.000159	346.630	1.409	0.078	0.744
σ2s		67.046	4.346	36.859	3.806	0.245	0.022	0.00088	563.320	1.434	0.171	1.153
σ2g/ σ2s		0.582	0.817	1.566	0.203	0.395	0.029	0.081	0.615	0.983	0.452	0.646

* Significant at 5% level of significance.

The best general combiners for TSS at both locations in order of merit were EC 173934 and LC-40. Among 28 specific combinations, 16 (at L1) and 14 (at L2) crosses exhibited positive sca effects being maximum in K-90 x Poinsette and Poinsette x K-75 at L1 and LC-40 x Gyn_1, K-90 x Poinsette and K-75 x LC-40 at L2. For flesh to seed cavity ratio (FSR), the best general combiners were Poinsette, EC 173934 and Gyn_1, irrespective of locations. Cross combination K-90 x K-75 at L1 and Poinsette x EC 173934 at L2 had maximum sca among seven significant and positive specific combinations. In contradiction to the present results, importance of additive gene action for FSR has been reported (Dogra, 1995).

The parents LC-11, K-90 and K-75 depicted high *per se* performance with respect to fruit weight at both locations as is evident from their high gca effect (Table 2). These parents had maximum concentration of favourable genes for increasing fruit weight. Eleven (at L1) and 12 (at L2) specific cross combinations had significantly positive sca effects (Tables 3 and 4), being maximum in K-90 x LC-11 (high x high) and K-90 x EC 173934 (high x poor). Non-additive gene action for fruit weight was also obtained by Ghaderi and Lower (1979) in consonance with the present findings. However, Gyn_1 and G_2 were identified as good general combiners for number of fruits per plant. The top specific combinations in order of merit were K-90 x G_2, K-90 x Gyn_1 and K-75 x Gyn_1 involving medium high, medium x high and poor x high general combiners, respectively. The situation holds good for both the locations with respect to number of fruits. Importance of non additive gene action for number of fruits per plant was also reported (Om *et al*., 1978; Ghaderi and Lower, 1979; Dogra, 1995). However, the present results with regard to fruit weight and number of fruits are in disagreement with El Hafeez *et al.* (1997). This may be due to differences in the parental material used for making diallel crosses.

For yield per plant, K-90 was the best general combiner in addition to Gyn_1 and G_2 irrespective of location (Table 2). The sca effects (Tables 3 and 4) were high for K-90 x G_2 (high x high), K-90 x Gyn_1 (high x high) and LC-11 x Gyn_1 (poor x high). The present results on yield per plant were similar to earlier findings of Om *et al*. (1978), Ghaderi and Lower (1979), Wang and Wang (1980) and Doligibh and Sidorova (1983) but in contradiction to the work of Gu *et al.* (2004). Parents such as G_2, Gyn_1 and LC-40 had negative gca effects and were considered good general combiners for internodal length. Nine (at L1) and 10 (at L2) specific combinations had significant negative values with the maximum in K-90 x Poinsette and Poinsette x EC 173934, poor x poor general combiners at each location.

As is evident from the data in Tables 2, 3 and 4, environmental effect was observed as non-significant on geno-

Table 2 - Estimates of general combining ability of parents for different characters in cucumber

Source of variation	Character										
	Days to first female flower appearance	Node of first female flower	Days to market-able maturity	Fruit length	Fruit width	TSS	Flesh to seed cavity ratio	Fruit weight	No. of fruits per plant	Yield per plant	Internodal length
Location 1											
K-90	0.000	0.367*	-0.550*	0.361*	0.364*	-0.016*	-0.0002	20.083*	0.017	0.276*	0.021
G2	-12.133*	-2.567*	-12.217*	-1.404*	-0.041*	0.004	0.004*	-25.250*	1.317*	0.302*	-1.856*
Poinsette	-2.433*	-0.767*	-2.0183*	-0.105	-0.531*	-0.031	0.014*	-4.917*	-0.217*	-0.055*	1.048*
EC173934	8.167*	1.633*	8.517*	-0.390*	0.191*	0.037*	0.011*	7.417*	-0.617*	-0.346*	0.144
K-75	0.733*	0.733*	1.017*	-0.050*	0.320*	-0.004	0.017*	10.083*	-0.017	0.024*	1.084*
LC-11	6.600*	0.633*	6.583*	0.388*	0.136*	-0.022	0.007*	32.750*	-0.783*	-0.089*	0.604*
LC-40	9.800*	2.067*	9.950*	-0.225*	-0.008*	0.029*	0.005*	-8.417*	-1.283*	-0.379*	-0.593*
Gyn1	-10.733*	-2.100*	-11.117*	1.425*	-0.433*	0.002	0.008*	-16.917*	1.583*	0.268*	-0.453
SE (gi)	0.221	0.141	0.222	0.019	0.013	0.011	0.0019	2.336	0.099	0.011	0.154
CD0.05 (gi)	0.441	0.281	0.443	0.037	0.026	0.021	0.0038	4.658	0.197	0.022	0.307
Location 2											
K-90	0.075	0.258*	-0.267*	0.208*	0.269*	-0.021	-0.013*	20.492*	0.508*	0.301*	0.116
G2	-10.092*	-2.908*	-11.600*	-1.355*	0.016	-0.015	-0.016*	-23.341*	1.842*	0.285*	-1.828*
Poinsette	-1.158*	-0.375*	-1.133*	-0.285*	-0.574*	-0.008	0.018*	-7.141*	-0.325*	-0.053*	0.693*
EC173934	7.642*	1.192*	7.867*	-0.592*	0.196*	0.065*	0.014*	-5.342*	-0.858*	-0.384*	0.489*
K-75	2.908*	0.792*	1.100*	-0.025	0.309*	-0.013	-0.010*	8.825*	-0.258*	0.058*	0.869*
LC-11	2.875*	1.325*	6.300*	0.495*	0.083*	-0.028*	-0.0001	30.825*	-1.092*	-0.125*	0.513
LC-40	5.675*	2.358*	7.900*	-0.148*	0.083*	0.045*	-0.0007	-5.342*	-1.358*	-0.363*	-0.364*
Gyn1	-7.925*	-2.642*	-10.167*	1.702*	-0.381*	-0.026*	0.009*	-18.375*	1.542*	0.279*	-0.488*
SE (gi)	0.194	0.134	0.197	0.088	0.044	0.023	0.0018	2.076	0.114	0.029	0.177
CD0.05 (gi)	0.387	0.267	0.393	0.175	0.088	0.046	0.0036	4.139	0.227	0.058	0.353

* Significant at 5% level of significance.

Table 3 - Estimates of specific combining ability of F_1 for different characters in cucumber at Nauni (L1)

Crosses	Characters										
	Days to first female flower appearance	Node of first female flower	Days to marketable maturity	Fruit length	Fruit width	TSS	Flesh to seed cavity ratio	Fruit weight	No. of fruits per plant	Yield per plant	Inter-nodal length
K-90x G2	-7.422*	-1.059*	-7.252*	-2.481*	-0.411*	-0.082*	0.122*	-13.259*	2.685*	1.023*	-0.627*
K-90x Poinsette	-4.789*	-0.526*	-4.618*	-0.440*	0.086*	0.275*	-0.046*	-38.593*	0.848*	-0.324*	-2.530*
K-90x EC173934	-7.056*	-1.259*	-7.612*	-0.445*	-0.573*	-0.073*	-0.040*	40.574*	-0.752*	-0.059*	-1.894*
K-90x K-75	10.378*	1.974*	9.484*	1.382*	0.451*	0.011	0.012*	-35.259*	-0.352*	-0.543*	0.466
K-90x LC-11	9.178*	-0.259	9.948*	-0.406*	0.516*	-0.061*	-0.031*	55.407*	-0.252	-0.309*	2.246*
K-90x LC-40	-5.356*	-1.693*	-5.085*	1.324*	-0.794*	0.006	0.006*	-1.759	-0.085	-0.303*	2.043
K-90x Gyn1	-4.489*	-0.526*	-5.352*	-1.343*	-0.182*	-0.051*	-0.013*	-23.259*	2.382*	0.509*	-0.030
G2 xPoinsette	2.011*	1.074*	3.715*	2.334*	-0.393*	0.002*	-0.045*	0.074	-0.119	-0.316*	0.680*
G2 x EC173934	3.411*	0.007	3.348*	1.819*	0.482*	-0.032	-0.029	-4.093	-1.052*	-0.398*	0.883*
G2x K-75	0.178	-1.093*	0.515	2.113*	0.306*	0.112*	-0.009*	21.074*	1.348*	0.494*	1.143*
G2 x LC-11	-3.356*	0.674*	-4.052*	-1.142*	-0.367*	0.043	-0.014*	24.074*	0.115	0.268*	-0.044
G2 x LC-40	9.444*	0.574*	9.248*	0.638*	-0.240*	0.160*	0.003	-26.426*	-1.385*	-0.659*	0.453
G2 x Gyn1	-0.356*	0.741*	0.982*	1.421*	0.756*	0.093*	-0.023*	-14.593*	0.082	-0.140*	1.013*
Poinsette x EC173934	1.044*	-1.126*	1.315*	2.070*	0.192*	0.085*	0.080*	5.574	0.482*	0.065*	-2.387*
Poinsettex K-75	-0.522*	0.107	-1.185*	2.002*	0.343*	0.223*	-0.039*	16.407*	-0.118	0.201*	2.006*
Poinsettex LC-11	-5.056*	-1.126*	-5.418*	-0.325*	-0.527*	-0.023*	-0.035*	10.407*	0.648*	0.478*	1.419*
Poinsettex LC-40	-4.922*	-1.226*	-5.452*	2.622*	0.654*	-0.256*	0.032*	-28.426*	0.481*	0.088*	-1.517*
Poinsettex Gyn1	14.944*	0.941*	15.615*	-1.995*	0.126*	0.120*	0.032*	18.407*	-0.385*	0.260*	1.109*
EC173934x K-75	-1.456*	1.041*	-2.885*	0.033	-0.276*	-0.205*	0.0006	-7.759*	-0.718*	-0.314*	1.009*
EC173934x LC-11	-0.322*	-2.526*	-0.785*	1.161	0.139*	-0.074*	0.044*	9.574*	0.048	0.189*	0.489*
EC173934x LC-40	-9.189*	-3.293*	-8.818*	1.174*	-0.525*	-0.107*	-0.002	-5.926	1.548*	0.446*	0.353
EC173934x Gyn1	9.678*	3.541*	8.915*	-0.092*	-0.059*	0.036*	-0.018*	-14.093*	-1.985*	-0.602*	0.179
K-75x LC-11	3.778*	0.374	3.715*	1.238*	0.436*	0.020	-0.014	-36.259*	0.448*	-0.258*	-2.084*
K-75x LC-40	4.244*	-2.059*	4.682*	0.901*	-0.677*	0.134*	-0.013*	-0.093	-0.052	-0.028*	-1.954*
K-75x Gyn1	-6.556*	-0.893*	-6.585*	-1.116	-0.379*	-0.213*	-0.020*	13.407*	1.415*	0.538*	0.006
LC-11x LC-40	4.044*	4.041*	4.115*	0.496*	-0.593*	-0.005	-0.003	22.241*	-0.285	0.049*	1.259*
LC-11x Gyn1	-9.422*	-1.459*	-9.818*	-0.454*	-0.158*	0.128*	-0.030*	25.741*	0.181	0.518*	-0.614*
LC-40x Gyn1	3.378*	-0.226	2.482*	-1.707*	0.173*	0.211	-0.019*	-16.426*	-1.318*	-0.352*	-2.084*
SE (ij)±	0.676	0.433	0.680	0.058	0.044	0.032	0.0057	7.160	0.303	0.033	0.472
CD0.05	1.994	0.883	1.356	0.116	0.088	0.064	0.011	14.280	0604	0.066	0.941

*Significant at 5% level of significance.

Table 4 - Estimates of specific combining ability of F_1 for different characters in cucumber at Chambaghat (L2)

Crosses	Characters										
	Days to first female flower appearance	Node of first female flower	Days to marketable maturity	Fruit length	Fruit width	TSS	Flesh to seed cavity ratio	Fruit weight	No. of fruits per plant	Yield per plant	Inter-nodal length
K-90x G2	-7.826*	-0.915*	-7.207*	-2.255*	-0.745*	-0.095*	0.009*	-8.641*	3.696*	1.058*	-1.097*
K-90x Poinsette	-3.759*	0.885*	-3.674*	-0.025	0.012	0.265*	-0.390*	-28.174*	-0.137	-0.361*	-0.917*
K-90x EC173934	-6.893*	-2.015*	-6.674*	-0.152*	-0.625*	-0.142*	-0.028*	43.359*	-1.270*	-0.140*	-1.580*
K-90x K-75	9.174*	1.719*	11.426*	1.081	0.295*	0.070	0.042*	-27.474*	-0.537*	-0.565*	1.140*
K-90x LC-11	11.207*	-0.148	7.893*	-0.172	0.088	-0.049	-0.014*	33.859*	-0.370*	-0.252*	2.263*
K-90x LC-40	-1.593*	-1.515*	-3.374*	1.705*	-0.412*	-0.022	-0.004	-1.641	-0.437*	-0.288*	-0.193
K-90x Gyn1	-7.659*	-1.182*	-5.974*	-1.578*	-0.082*	-0.050	-0.007*	-19.674*	1.663*	0.431*	-0.737*
G2 xPoinsette	3.741*	1.385*	4.659*	2.838*	-0.502*	-0.009	-0.036*	-17.674*	-0.470*	-0.255*	0.027
G2 x EC173934	0.607*	0.819*	2.326*	2.845*	0.428*	0.018	-0.022*	-14.474*	-1.937*	-0.434*	1.363*
G2x K-75	-5.659*	-1.448*	-2.907*	2.278*	0.782*	0.196*	-0.001	23.026*	0.796*	0.334*	-0.583*
G2 x LC-11	-1.293*	0.352	-3.774*	-1.275*	-0.392*	0.045	-0.008*	24.359*	-0.704*	0.184*	-0.327
G2 x LC-40	18.574*	1.319*	7.293*	0.702*	0.008	-0.095*	0.023*	-12.807*	-1.437*	-0.515*	0.717*
G2 x Gyn1	-0.826*	0.652*	2.026*	1.018*	0.738*	0.143*	-0.014*	-7.507*	-0.670*	-0.087	0.807*
Poinsette x EC173934	2.674*	-1.048*	2.859*	0.908*	0.318*	0.145*	0.077*	14.326*	0.896*	0.147*	-1.957*
Poinsettex K-75	-3.593*	-0.315	-1.374*	-1.792*	0.072	0.190*	-0.042*	30.159*	0.296	0.255*	1.330*
Poinsettex LC-11	-2.893*	0.485*	-6.574*	-0.478*	-0.302*	0.005	-0.012*	11.493*	0.796*	0.492*	1.587*
Poinsettex LC-40	-0.693*	-1.548*	-2.841*	2.665*	1.065*	-0.170*	0.029*	-29.007*	0.729*	0.066	-1.903*
Poinsettex Gyn1	11.574*	1.118*	13.893*	-2.252*	-0.372*	0.069	0.015*	9.959*	-0.170	0.344*	0.753*
EC173934x K-75	-1.726*	3.452*	-0.041	-0.085	-0.165*	-0.250*	0.002	-3.307	-0.170	-0.247*	-0.200
EC173934x LC-11	4.307*	-2.082	0.759*	1.095*	0.195*	-0.069	0.042*	19.693*	0.330*	0.236*	-0.343
EC173934x LC-40	-6.159*	-4.115*	-8.507*	1.105*	-0.205*	-0.175*	-0.004	-19.141*	1.263*	0.340*	1.600*
EC173934x Gyn1	2.774*	0.219	4.893*	-0.412*	-0.109*	-0.070*	-0.014*	-18.841*	-1.970*	-0.618*	1.423*
K-75x LC-11	5.707*	-1.015*	4.193*	1.062*	0.582*	-0.157*	-0.014*	-41.141*	0.396*	-0.309*	-0.857*
K-75x LC-40	4.907*	-3.715*	4.259*	0.938*	-0.885*	0.203*	-0.017*	-6.041	-0.004	-0.131*	-1.613*
K-75x Gyn1	8.507*	-1.048*	-5.674*	-1.578*	-0.255*	-0.242*	-0.020*	10.326*	1.429*	0.307*	0.077
LC-11x LC-40	-22.726*	3.085*	6.393*	0.618*	-0.825*	-0.015	-0.014*	23.026*	0.163	0.025*	0.077
LC-11x Gyn1	-5.793*	-2.248*	-7.541*	-0.865*	0.238*	0.123*	-0.030*	28.326*	-0.404*	0.574*	-1.467*
LC-40x Gyn1	2.074*	5.052*	1.859*	-2.322*	0.105	0.317*	-0.023*	-12.174*	-1.137*	-0.302*	-1.322*
SE (ij)±	0.595	0.411	0.603	0.271	0.134	0.070	0.0054	6.362	0.349	0.089	0.542
CD0.05	1.186	0.819	1.202	0.540	0.267	0139	0.011	12.685	0.696	0.177	1.081

*Significant at 5% level of significance.

types and hybrid combinations for most of the characters. The results are similar at both locations with developed hybrid combinations and hence hybrids K-90 x G_2 and K-90 x Gyn_1 can be exploited in similar types of climates.

K-90, G_2 and Gyn_1 may be used in hybridisation for developing high yielding hybrids with higher number of fruits per vine, long fruits and high TSS on the basis of results from location 1, whereas G_2 and Gyn_1 are promising for developing high yielding hybrids with higher number of fruits per vine and short inter-nodal length on the basis of results from location 2. It can be concluded that G_2 and Gyn_1 may be used in hybridisation for developing high yielding hybrids with more fruits per vine and wider adaptability. The crosses K-90 x G_2 and K-90 x Gyn_1 can be released as hybrids after further testing.

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Short- and long-term effect of sulphite on sucrose transport in grapevine (*Vitis vinifera* L.) leaves. An electrophysiological study

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Key words: membrane potential, sucrose transport, sulphite, sulphur dioxide, *Vitis vinifera* L.

Abstract: Sucrose is the main carbohydrate translocated in grapevine and its transport may be restricted or inhibited by a number of factors such as the pollutant sulphur dioxide. The present study investigated, for the first time in grapevine, the effects of sulphite on membrane electrical response of sucrose transport in the mesophyll cells. Co-transport of sucrose across the membrane is linked to the free energy in a electrochemical proton gradient. Without the pollutant, electrophysiological traces displayed a metabolic-dependent sucrose electrical response in which an initial depolarization was followed by complete repolarization. In the presence of sulphite, instead, there were different trends depending on time of contact with the tissue of the pollutant. In the short-term, a slower repolarization was observed and in the long-term (after 6 and 12 h) the extent of depolarization (Δ mV) was also reduced. Transmembrane electrical potentials, measured in the presence of sulphite, became significantly ($P < 0.01$) more positive with increasing time of incubation of the tissue. On the whole, electrophysiological results highlight a direct or indirect effect of the pollutant on the activity of proton pump H^+/ATP ase. Since carbohydrate translocation has a central role in the balance between source and sinks in the plant, the results of the research suggest that sulphite can modify the above balance with negative implications for the export of carbohydrate from the leaves.

1. Introduction

The potential impact of atmospheric deposition of pollutants such as sulphur dioxide on vegetation has been the subject of many papers (Tingey and Olszyk, 1985; Darral, 1989; Mesanza *et al*., 1996; Johnson *et al.*, 1999). Plant injury caused by air pollution is most common near large cities and industrial enterprises, and damage in isolated areas occurs when pollutants are spread over long distances by wind currents (Cicek, 2003). Some studies have also begun to surface from developing countries (Hassan *et al*., 1995; Wahid *et al*., 1995), where yield losses of cereals and legumes have been attributed to both SO_2 and ozone. Exposure to SO_2 has been reported to decrease or inhibit photosynthesis (Silvius *et al*., 1975; Ziegler, 1975; Carlson, 1983; Rao *et al*., 1983; Katainen *et al*., 1987; Kropff, 1987; Sheu, 1994; Lorenc-Plucińska, 1998; Ranieri *et al*., 1999) and pollen tube growth (Karnosky and Stairs, 1974; Varshney and Varshney, 1981).

Even if the SO_2 sensitivity of grapevine is poorly documented (Ishikawa, 1972; Weinstein, 1984; Garcia-Huidobro *et al*., 2001), the species is considered sensitive to chronic SO_2 exposure, and adverse effects on growth or yield were presumed possible in the field (Daines, 1968; Fujiwara, 1970). Development of black and brown lesions was observed especially on leaves (Cicek, 2003); shoot growth reduction and, in some cases, leaf abscission were reported (Shertz *et al*., 1980). The interaction of SO_2 with ozone is evident. The combination of the two gases led to an increase in oxidant stipple in leaves (Forsline *et al*., 1983). In other species this interaction has not occurred (Kreess *et al*., 1986), suggesting the role of the genotype in resistance to the pollutant. Interaction SO_2/carbon black was also suggested by Ionescu *et al*. (1971) in the industrialized Copsa Mica zone of Romania, where the damaging effects of sulphur-containing effluents were enhanced by the presence of carbon black. Fujiwara (1970) found, with increasing SO_2 concentration, that leaf abscission, in cv. Fredonia, began earlier and progressed at a greater rate. Effects of sulphur dioxide were also found on the stomatal apparate of *Vitis labrusca* L. cv. Ives, where SO_2 induced both stomatal closure and a higher stomatal resistance (Rosen *et al.,* 1978).

Unlike other species (Lorenc-Plucińska and Ziegler, 1989; Maurousset and Bonnemain, 1990), there is not, for the vine, specific knowledge on the effects of SO_2 on

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sugar transport in leaves. In *Phaseolus vulgaris* and *Ricinus communis* SO_2 inhibits assimilate translocation and this inhibition seems to depend on damage to the sucrose transport system or proton pump (Noyes, 1980; Teh and Swanson, 1982; Lorenc-Plucińska and Ziegler, 1987 a, b, 1988). According to a more recent and detailed study on purified plasma membrane vesicles of *Ricinus communis*, the decreased uptake of sucrose may be attributed to a dissipation in the transmembrane pH gradient (Russell *et al.,* 1999).

Sucrose transport in leaves is a secondary active transport (co-transport) in which solute (sucrose) translocation across the membrane is linked to the free energy available in a proton electrochemical potential difference (Bush, 1993). It is widely known in the leaves of many plants (Giaquinta, 1979; Delrot, 1981; Delrot and Bonnemain, 1981; Huber and Moreland, 1981), and also in the vine is assumed to be so (Mullins *et al.,* 1992). Since sucrose transport involves the entry of protons into the cell, it gives rise to changes in transmembrane potential. Thus electrophysiological techniques are very useful for following, in real time, the transport of solutes. Regarding the effect of sulphite on sucrose transport, the problem has been studied mainly on a biochemical level, while there is little information on the electrophysiological aspects. To our knowledge, only one paper (Maurousset and Bonnemain, 1990) reports some information about these aspects, however it does not include combined electrophysiological tests with sucrose and sulphite. Therefore we felt it was interesting to investigate, for the first time in grapevine, how sucrose membrane electrical response was influenced by SO_2 supplied as sulphite.

2. Materials and Methods

Plant material

Electrophysiological tests were carried out on leaves of *Vitis vinifera* L., cv. Sangiovese clone SS-F9-A5-48, removed from two-year-old plants grown in a container and grafted on rootstock 420 A. Preliminary tests were performed on whole leaves, while the subsequent tests were on leaf segments (3 x 7 mm), cut with a scalpel under B.S. solution.

Chemicals

For electrophysiological experiments, the substance to be tested has to be dissolved in the solution bathing the tissue (treatment solution). Sulphur dioxide is highly soluble in water but, being gaseous, it is not easy to dose the exact amount to be dissolved. Therefore, in this research, Na_2SO_3 was employed, which at pH 5.5, is found mainly in the form of bisulfite ion (HSO^{3-}). Previous research conducted on isolated chloroplasts of *Spinacia oleracea* L. showed that SO_2 and bisulphite ions, at the same equimolecular concentration, have a parallel mode of action in the inhibition of photosynthetic oxygen evolution (Silvius *et al*., 1975). Moreover the effects of SO_2 on chloroplast enzyme systems were studied by means of hydration products of bisulphite and sulphite (Ziegler, 1975).

The components of the various treatments were added to the basal solution (B.S.) for the electrophysiological experiments (Table 1). In all treatments in which Na_2SO_3 alone was employed, even the B.S. contained the same amount of sodium supplied as Na_2SO_4. Carbonyl cyanide m-chloro phenyl hydrazone (CCCP), a protonophore and uncoupler of oxidative phosphorilation (Marrè *et al*., 1973) was used in 0.05% ethanol, starting from a stock solution of 0.5 mol m^{-3}. Controls, in the different trials, also contained the same percentage of ethanol in the treatments. The salts present in the basal solution were only in sulphate form. The presence of Cl^- anions was avoided since their entry by symport with H^+ can influence cytoplasmic pH (Bellando *et al*., 1995).

Table 1 - Composition of the basal solution (B.S.) and concentration of the components in the treatments

Basal solution		Treatment components*	
K_2SO_4	2.5	sucrose	20.0
$CaSO_4$	0.5	Na_2SO_3	1.0
MES	5.0	CCCP	10^{-2}

All solutions were adjusted to pH 5.5 by way of TRIS or dilute H_2SO_4 when Na_2SO_3 was present. MES: 2-N-morpholinoethane-sulphonic acid (Sigma); CCCP: Carbonyl cyanide m-chlorophenylhydrazone (Sigma); TRIS: 2-amino-2-hydroxymethyl-1,3-propanediol (Fluka). *Concentrations are in mol m^{-3}.

Electrophysiology

Before beginning the electrophysiological experiments, whole leaves and/or leaf segments were incubated in basal solution in the light for 2 h (short-term). During this period, the solution was renewed twice and constantly aerated. Subsequent tests were performed after incubation of leaf segments in sulphite for 6 and 12 h (long-term). Even in this case the solution was constantly aerated and renewed every hour. Membrane potential (Em) was measured according to standard electrophysiological technique as previously adopted (Rinaldelli and Bandinelli, 1999; Rinaldelli, 2005) with some modification. In brief, whole leaves and/or leaf segments were mounted on a Poly(methyl methacrylate) chamber secured to a microscope stage. The chamber for whole leaves was slightly inclined and wide enough to accommodate a grapevine leaf, while the one for the segments was small (3 ml) and placed horizontally. Continuously aerated B.S. or treatments were permitted to perfuse through the chamber at a flow rate of 10 ml min^{-1}. They reached the tissue by gravity, each through its own adductor channel controlled by a manual valve. In the case of whole leaf, only the stomatal area around the insertion point and the petiole were perfused. A small glass thermometer placed inside the chamber allowed verification of the temperature of the solution. Heating or cooling of the solution was obtained by way of a Peltier-effect heat pump located along the solution conduit before the Poly(methyl methacrylate) chamber; it was electronically controlled.

The measuring electrodes used were micropipettes (tip diameter <1 μm) obtained from single-barrelled borosilicate capillaries (W.P.I., USA) by way of a vertical homebuilt puller. The micropipettes and the reference electrode were filled with 500 mol m^{-3} KCl. The electrodes were connected, by Ag/AgCl wires, to a high input impedence electrometer (AD 549, 10^{15} Ω). The output signals from the electrometer, before being transmitted to a chart recorder, were passed through a low-pass filter (10 Hz) in order to eliminate possible noise. The insertion of microelectrodes into the cells of the central part of the mesophyll took place under two different magnifications. In the case of whole leaves the electrode passed through a stoma and a magnification of 500x was necessary, obtained with a 50x long working distance objective (Mitutoyo, Japan). In the case of leaf segments, instead, a magnification of 250x, obtained with a 25 x long working distance objective (WPI, USA), was sufficient. The insertion of the microelectrodes was, in both cases, at an angle of about 45°, and took place by way of a very stable, manual, three-axis, homebuilt micromanipulator.

Treatments started after Em stabilized for 5 min. All experiments were performed under Faraday cage. The complete electrophysiological set-up is presented in figure 1. Except where indicated otherwise, preincubation and electrophysiological tests were carried out at +22°C (± 0.5) in the light (30 watt/m^2).

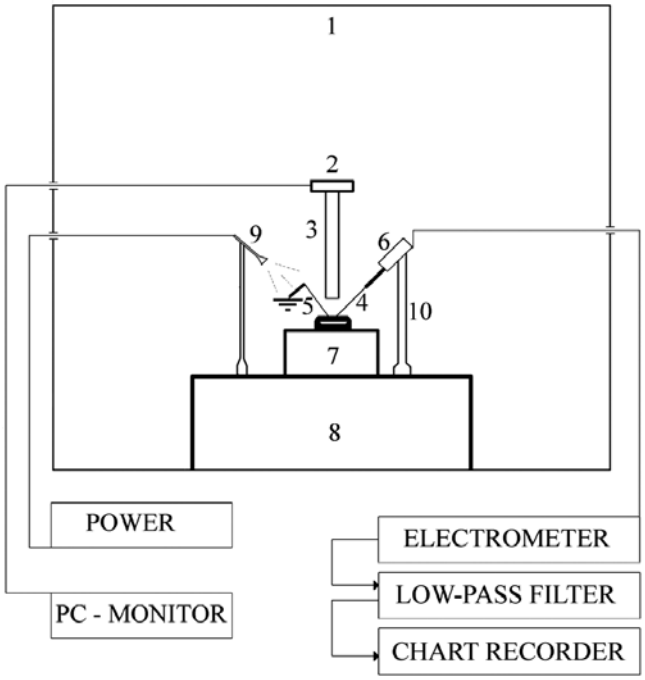

Fig. 1 Schematic electrophysiological set-up for membrane potential measurements. 1. Faraday cage. 2. Digital cameras. 3. Microscope tube. 4. Measuring microelectrode. 5. Reference electrode. 6. Probe. 7. Microscope stage. 8. Vibration-free table. 9. Lamp. 10. Micromanipulator.

Statistical analysis

The data relating to transmembrane potentials, measured at different times, in BS and sulphite, were subjected to statistical analysis of variance (ANOVA). Comparisons were carried out using Duncan's test. Statistical significance of differences were accepted when P <0.01. In the electrophysiological traces, depolarizations (Δ mV) are presented as mean ± SE.

3. Results

Preliminary experiments on whole leaves and leaf segments

Since the cuticle is often a barrier to organic molecules (Rinaldelli and Bandinelli, 1999; Rinaldelli, 2005), before working on a tissue it is necessary to verify its permeability to the solutions that will be employed. Thus, preliminary tests were performed on whole leaves and leaf segments. They showed that both were permeable to sulphite (Fig. 2, a, c) but not to sucrose. In whole leaves, in fact, sucrose depolarization was almost one-quarter that in leaf segments (Fig. 2, b, d). Based on this evaluation, all subsequent tests were performed on leaf segments.

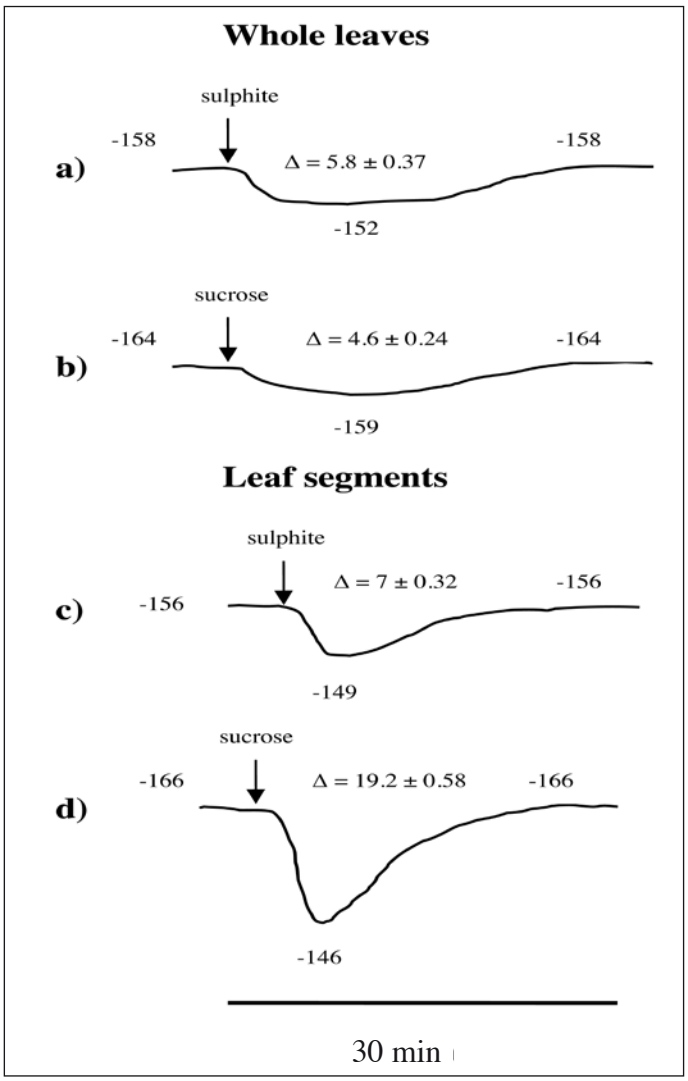

Fig. 2 - Effects of treatments with sucrose and sulphite on whole leaves and leaf segments. All measurements were carried out in light. Numbers preceded by - are negative mV. Electrophysiological traces are representative of five equivalent measurements. Δ is the depolarization (mV) as the mean of five measurements ± SE.

Sucrose transport and its metabolic support

As co-transport of sucrose in grapevine leaves was assumed (Mullins *et al*., 1992) but not electrophysiologically investigated, initial tests were carried out to verify this assumption. In the light, in the presence of the uncoupler CCCP, sucrose depolarization was less than half, compared to the same test without the inhibitor (Fig. 3 a, b). Subsequent repolarization was also very slow. In the dark, instead, sucrose depolarization was lower than in the light, and it was completely abolished by the uncoupler (Fig. 3 c, d). The different extent of depolarization, in light and dark, reflects metabolic support to the operation sucrose/H^+ symport. As discussed later, CCCP only inhibited oxidative phosphorylation and had incomplete or no effect on photophosphorylation.

At 5°C sucrose depolarization was nearly annulled (Fig. 3 e) and a similar response occurred when the pH of the treatment solution was brought to 8.0 (Fig. 3 f).

Short- and long-term experiments under sulphite

In the short-term tests, sucrose depolarization, in the light, was about 20 mV (Fig. 3 b) whereas in the dark it was much less (Fig. 3 c). Also, in the presence of sulphite, differences in extent of depolarization, in light and dark, remained approximately the same as previous tests without pollutant, but a slower repolarization was observed (Fig. 4 a, b). Based on this evidence, in the short-term sulphite affected only in part the membrane electrical response of sucrose transport.

Figure 5 shows the electrical responses to sucrose after leaf segments were preincubated in B.S. (control) or sulphite, for 6 and 12 h. Since short- and long-term treatments in the dark showed the same trends, only the first are reported.

Sulphite already reduced the sucrose depolarization after 6 h of incubation (Fig. 5, b), but the result was more evident after 12 h where the extent of depolarization de-

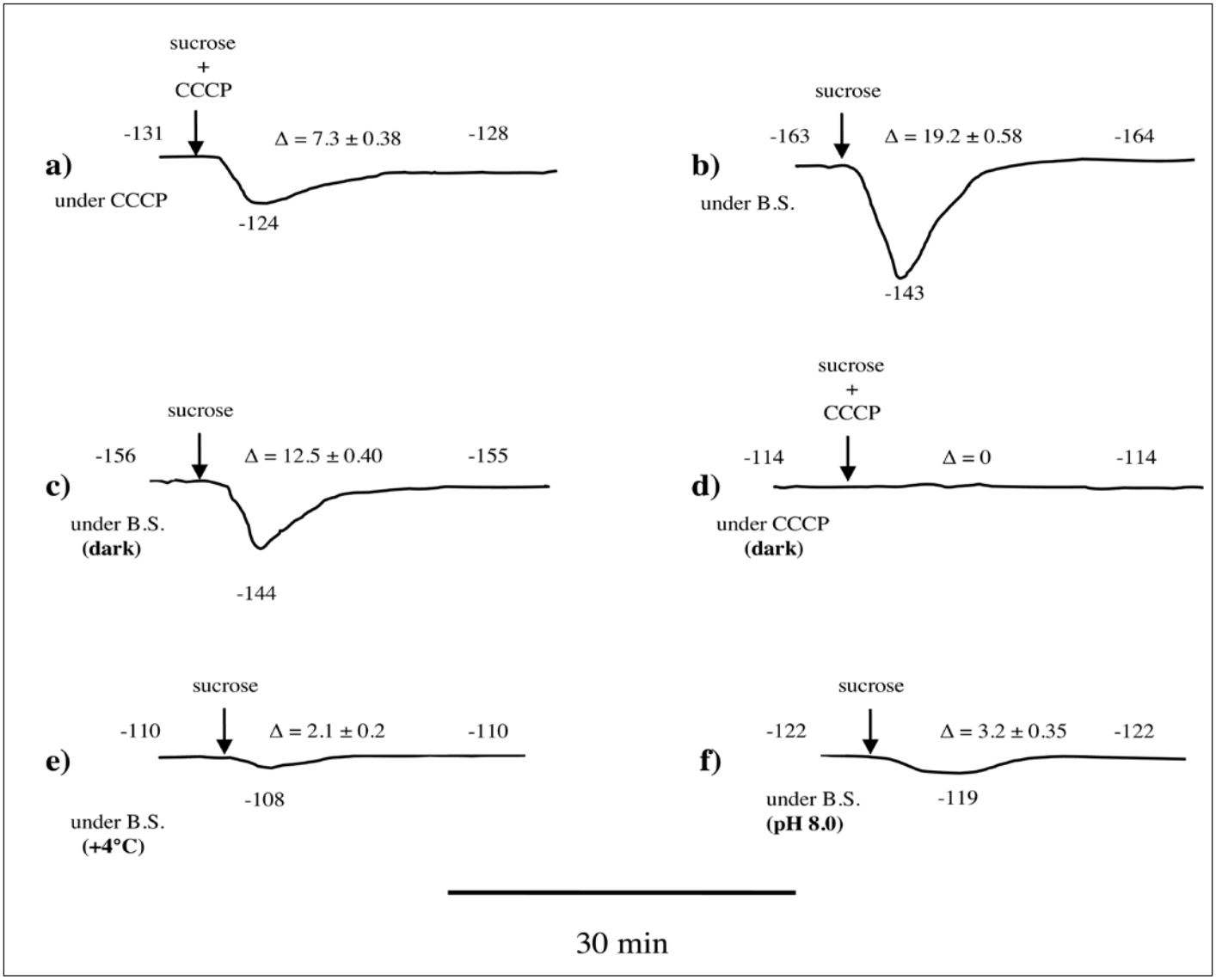

Fig. 3 - Effects of chemical and physical treatments to verify energetic support of sucrose transport. Except where indicated otherwise, measurements were carried out in light. Solution at pH 8.0 was buffered with 2 mol m^{-3} HEPES [N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid] -TRIS. Numbers preceded by - are negative mV. Electrophysiological traces are representative of five equivalent measurements. Δ is the depolarization (mV) as the mean of five measurements ± SE

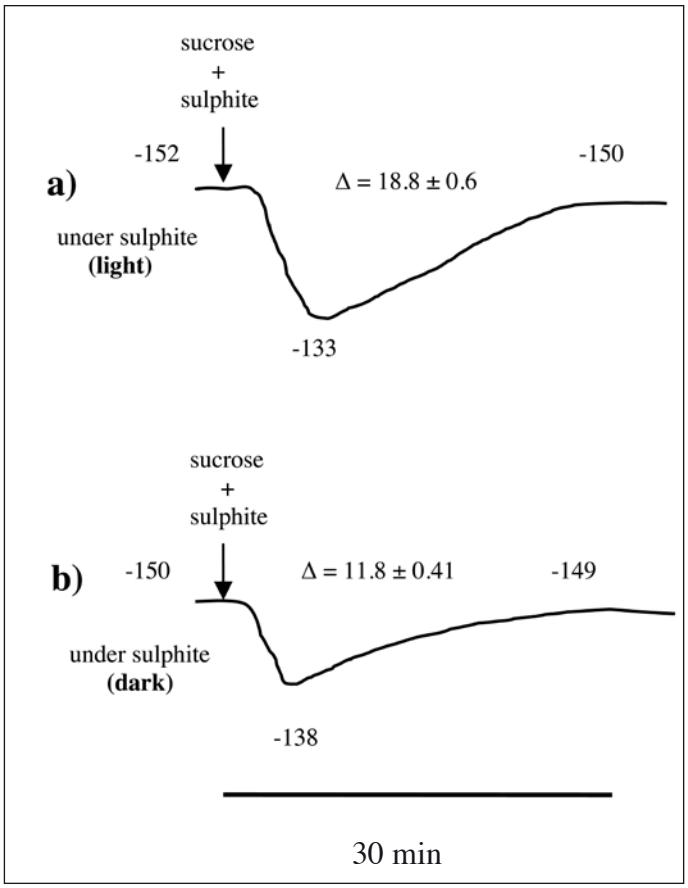

Fig. 4 - Short-term treatments in light and dark. Numbers preceded by - are negative mV. Electrophysiological traces are representative of five equivalent measurements. Δ is the depolarization (mV) as the mean of five measurements ± SE.

creased by almost half as compared to the control (Fig. 5d). Also the repolarization, after 6 h, and even more after 12 h, was strongly slowed. It should be noted that even with increasing incubation time there is a progressive decrease in the extent of depolarization. Also the transmembrane potentials decreased (they became more positive) with increasing incubation time (Table 2). These effects were evident under both, B.S. and sulphite. Under sulphite, however, they were more marked.

Table 2 - Membrane potentials (mV) recorded at different times of incubation, in B.S. and sulphite

	Membrane potentials	
Incubation time (h)	B.S. (no sulphite)	1 mol m^{-3} Na_2SO_3
0	-160.2 ± 2.61 a	-153.0 ± 2.05 b
6	-152.2 ± 2.04 b	-139.7 ± 2.45 d
12	-149.0 ± 2.44 c	-125.1 ± 2.33 e

Each value is the mean ± SD of ten experiments.

Different letters denote statistically significant differences between membrane potentials (P<0.01).

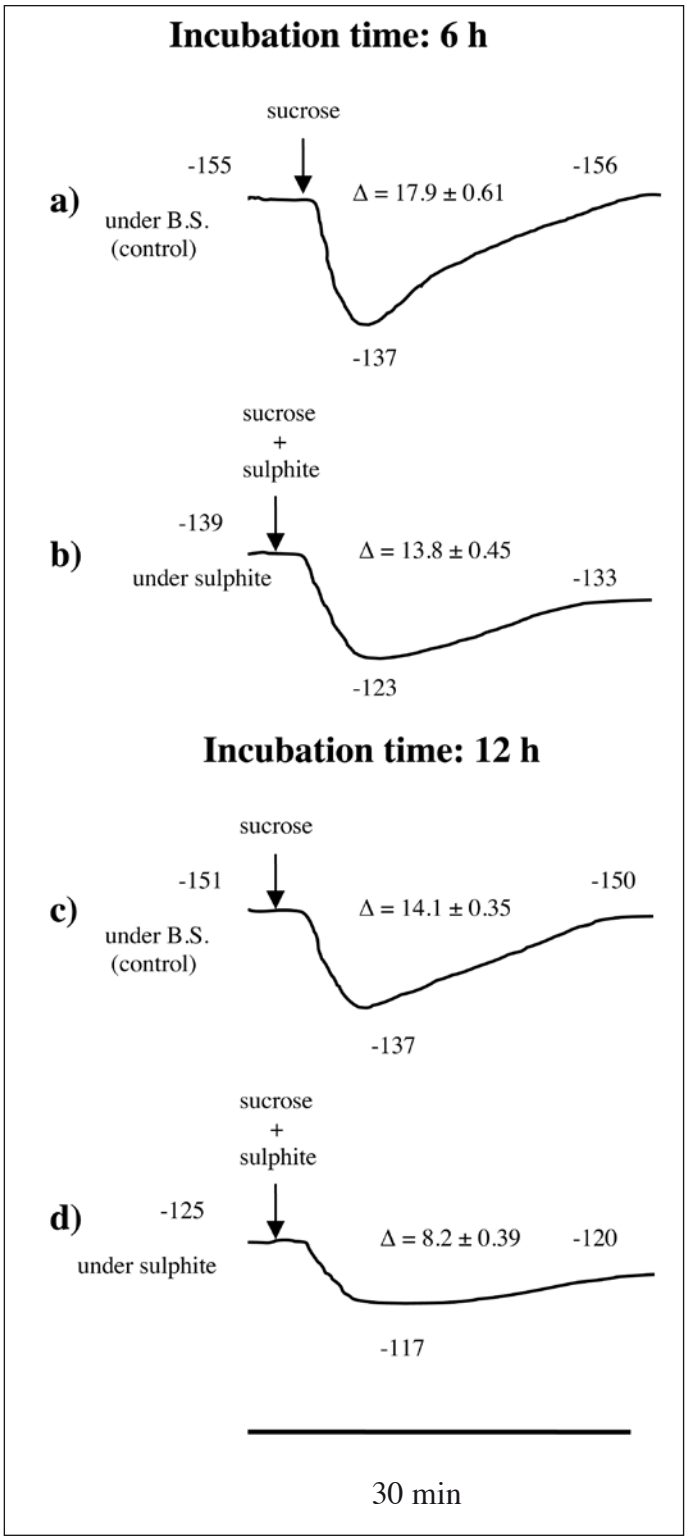

Fig. 5 - Long-term treatments, after 6 h and 12 h of incubation in B.S. and/or sulphite. All measurements were carried out in light. Numbers preceded by - are negative mV. Electrophysiological traces are representative of five equivalent measurements. Δ is the depolarization (mV) as the mean of five measurements ± SE.

4. Discussion and Conclusions

Energetic support to sucrose transport

CCCP is an uncoupler of oxidative phosphorilation which, in agreement with the chemiosmotic hypothesis (Mitchell, 1961), dissipates the electrochemical gradient

of H^+ ions preventing ATP synthesis. Consequently, active transport systems (primary and also, indirectly, secondary), lacking energy, are deactived.

Under CCCP, membrane electrical response of sucrose showed, in light and dark, two different results (Fig. 3 a, d). This finding suggests that the metabolic support of the operation of sucrose/H^+ symport comes from both respiration and photosynthesis. In dark, since there is no photosynthesis, ATP production is only of respiratory origin and it is blocked by the uncoupler CCCP. In light, instead, some energetic support appears because CCCP does not inhibit, or only partially inhibits, the photophosphorylation. This may be the case, taking into account the variable effects of CCCP on photophosphorylation as a function of leaf greening (Oelze-Karow and Butler, 1971; Butler *et al*., 1972) or light intensity (Saha *et al*., 1970; Prins *et al*., 1980).

Since chemical inhibitors may simultaneously affect many different processes of cells (Khalilov *et al*., 2002), we also tested the effect of low temperature. Treatment with sucrose at 5°C nearly led to the annulment of the depolarization. This suggests a metabolic dependence of sucrose transport on the activity of a plasmalemma ATP-driven proton pump. At 5°C, since the metabolic energetic support is highly limited (Mengel and Shubert, 1985; Rinaldelli and Bandinelli, 1999; Rinaldelli, 2000; 2004), the activity of the pump is inhibited.

The use of sucrose solution buffered at pH 8.0 strongly reduced depolarization. Under these conditions, the lack of protons in the apoplast would explain the annulment of H^+ co-transport. This response has also been observed for nitrate (Ullrich and Novacky, 1981; McClure *et al*., 1990) and urea co-transports (Rinaldelli, 2004).

All these results are considered sufficient to support a metabolic and pH-dependent sucrose co-transport.

Membrane electrical responses under sulphite

Sucrose is the main carbohydrate translocated in grapevine. From the leaves, where it is produced, it moves to the various sinks according to the specific needs of the plant (Coombe and McCarthy, 2000; Hunter and Ruffner, 2001). During the annual vegetative and reproductive cycle, it plays a preeminent role in regulation of the carbon/nitrogen ratio (Rodriguez-Lovelle and Gaudillère, 2002).

Sucrose, being a non-polar molecule, does not have direct effects on membrane potential when it enters the cell, but only indirect, because its transport is related to the flow of protons that move along an electrochemical gradient. This co-transport results in an initial depolarization observed under both B.S. and sulphite (Figs. 2 b, d; 3, 4, 5). Under sulphite, it has to be excluded that the depolarization could be due to Na^+ or the accompanying anion, since sucrose and perfusing solutions contained an equal amount of Na_2SO_3.

Repolarization of the membrane, which sometimes exceeded the starting level (overshoot), is proposed to depend on stimulation of the H^+-ATPase, caused by either changes in the cytoplasmic pH or the membrane potential itself. The H^+-ATPase has a pH optimum at 6.6, i.e. well below the physiological pH of the cell cytoplasm (usually around 7.2-7.5). Thus, whenever protons start accumulating in the cytoplasm, the activity of the pump increases to remove excess protons from the cell (Michelet and Boutry, 1995). This response is comparable, as cause and effect, to the one caused by permeant weak acids (Marrè *et al*., 1983; Rinaldelli and Bandinelli, 1999).

Sulphite alone, at the concentration used, induced a slight depolarization followed by partial or complete recovery. This is visible in both whole leaves (Fig. 2 a) and leaf segments (Fig. 2 c). Maurosset and Bonnemain (1990) found that the depolarization by sulphite, does not depend on the concentration used. Effect of the pollutant on the membrane would be, initially, that of "exciting agent". Instead, the repolarization would depend, according to the same authors, on the concentration of sulphite in the treatment. In our study we tested sulphite concentrations below and above 1 mol m^{-3} (data not shown) finding, however, less marked differences compared to the aforementioned authors. However the comparison is difficult because the study of Maurosset and Bonnemain (1990) did not include our same electrophysiological tests. Their tests, in addition, were conducted on a different species (*Vicia faba* L.) and this may also affect the reported results. When the treatment with sucrose was under sulphite, however, repolarization slowed and decreased gradually moving from short- to long-term. This is the first and most important effect of sulphite on sucrose transport. Since repolarization is dependent on proton pump activity, the direct or indirect effect of sulphite should be placed at this level.

In the long-term, under B.S. and sulphite, sucrose depolarization was lower (Fig. 5) and the transmembrane electropotentials became significantly more positive (Table 2). Since these effects are evident even in B.S., they should be attributed, at least in part, to phenomena of senescence which start when leaves are detached or segments are cut (Thimann *et al*., 1977; Gepstein, 1982; Malik, 1982). However, since the variations under sulphite were greater, there is clear evidence, in addition to senescence, of a direct or indirect effect of pollutant on proton pump activity. Proton pumps, in fact, are considered the primary motors that build up transmembrane electrochemical proton gradients (Felle, 2001).

The complex of electrophysiological tests performed in this research highlights a clear membrane electrical response of sucrose transport to sulphite. Two points stand out clearly enough: a) in grapevine leaves sucrose membrane electrical response is supported by metabolic energy; b) sulphite alters the above response by acting on the proton pump and/or energetic metabolism, from which the pump draws energy to extrude H^+ ions outside the cell. An effect of sulphite on respiration and photosynthesis cannot be excluded, especially in the long-term. The link may be there because both slowed repolarization and more positive transmembrane potentials under sulphite reflect a partial inhibition of the proton pump, whose activity is supported by respiratory and photosynthetic ATP.

The results obtained constitute an interesting acquisition in grapevine physiology, but it should be taken in account that conditions for the SO_2 effect, in the vineyard ecosystem, may differ from those adopted in the laboratory due to the high number of cultural, climatic and pedological variables.

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Starch accumulation in the leaves of root-restricted pepper affects plant growth by a feedback-inhibition of the photosynthesis

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Key words: *Capsicum annuum* L., carbohydrates, leaf gas exchange, root restriction.

Abstract: The mechanism behind the reduced growth occurring in plants subjected to root restriction is still not fully understood. Therefore, this investigation was planned to determine the morphological and physiological changes induced in response to root volume reduction and to determine the time frame within which these changes occurred. In particular, this research focused on the effect of root restriction on growth, leaf gas exchange parameters, carbohydrate production and water relations in pepper (*Capsicum annuum* L.). Our results show that the reduced growth is mainly linked to a feedback inhibition of the photosynthesis, caused by a concurrent limited stomatal conductance (probably driven by both stomatal factors and hormonal substances) together with a strong accumulation of starch in the leaves.

1. Introduction

Root-restricted cultivation is an effective technique for saving resources, to control root environment, to anticipate yield and to regulate the quality of vegetables. For all these reasons, its use has significantly increased during the last decades in vegetable nurseries (Shi *et al*., 2008). Root restriction (RR) may occur when container size and/or rooting volume is physically constrained (Tschaplinski and Blake, 1985; Ismail and Noor 1996; Saito *et al*., 2008; Mugnai *et al.,* 2009), especially in greenhouse-grown horticultural crops (Thomas, 1993). A reduced container volume stimulates the formation of a denser root mass, with decreased root growth (Ismail and Noor, 1996). Together with a strong limitation of the soil available to the root system for water and nutrients uptake, RR also reduces canopy growth (Ismail and Noor, 1996; Shi *et al*., 2008) by affecting many plant physiological and biochemical processes. The mechanism behind the reduced shoot growth is not yet fully understood. Several hypotheses have been proposed: water and nutrient stresses (Hameed *et al*., 1987), decrease in root respiration (Shi *et al*., 2007), reduction in photosynthesis (Shi *et al.,* 2008), and synthesis and translocation of plant hormones (Liu and Latimer, 1995). However, contradictory results are reported as to which of these factors plays a significant role in the response of aerial plant parts to restricted root growth, with strong differences between species. Leaf photosynthesis strongly depends on environmental conditions such as radiation, CO_2 concentration and temperature. In addition to these environmental conditions, photosynthesis is subjected to internal regulation associated with sink demand for assimilates (Marcelis, 1991). In the presence of a physical restriction to root growth, a major metabolic sink for photosynthetically fixed carbon at seedling stage (Thomas and Strain, 1991) may result in feedback inhibition mechanisms (Shi *et al*., 2008). This investigation was therefore planned to determine the morphological and physiological changes induced in response to RR conditions and to determine the time frame within which these changes occurred. In particular, this research aims to study the link between leaf gas exchange parameters and carbohydrate production in regulating growth in pepper (*Capsicum annuum* L.) plants.

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2. Materials and Methods

Plant material

Experiments were carried out at the Department of Plant Biology, University of Pisa (Italy). Seeds of pepper (*Capsicum annuum* L.) cv. Sienor were sown in seedling flats filled with vermiculite and placed in a germinating room at constant temperature (25°C) and light intensity (300 mol m^{-2} s^{-1} PPFD). After germination, seedlings with the first true leaves were selected for uniformity and single plants were transplanted into 7 ml (root restricted, RR)

and 230 ml (control) speeding flats filled with vermiculite. Flats were placed in a greenhouse and suspended 15 cm above the benches to facilitate air pruning of roots and to induce RR treatment throughout the experiment period. In each flat 24 seedlings were planted regardless of the original number of cells per flat to minimize the effect of mutual shading, to avoid light competition between plants and to allow for uniform plant density. In order to avoid any water or nutrient stress, a closed fertirrigation system controlled by a timer was established to supply water and nutrients at frequent and regular intervals. The nutrient solution was composed as follows: 10 mM NO_3^-, 1 mM $H_2PO_4^-$, 8 mM K^+, 4 mM Ca^{2+}, 1.5 mM Mg^{2+}, 1 mM SO_4^{2-}, 0.04 mM Fe^{2+} and microelements (pH 6.0, EC=1.2 mS/cm). The nutrient solution was renewed every week.

Growth measurements

Five plants per treatment were sampled at weekly intervals. Roots were carefully washed, and then plants were separated into leaves, stems and roots. Leaf area was measured with an area meter (Delta T-Devices Ltd., Cambridge, UK), plant height was estimated using a ruler, and dry weight for each organ was obtained after oven drying (48 h at 70°C).

Leaf gas exchange measurements

Net CO_2 assimilation (A), stomatal conductance (g) and transpiration (E) measurements were performed weekly (n=5) on the central sector of the youngest fully expanded leaf by using an open system (CMS 400, Heinz Walz, Effeltrich, Germany) connected to an assimilation chamber and equipped with a high sensitivity IRGA (BINOS, Leybold Haeraeus, Germany) under temperature (24°C) and growing light (400 µmol/m*s PAR) conditions provided by a mercury vapour lamp (Osram HQI-TS 250 W/NDL). Calculation of all the parameters was performed following Von Caemmerer and Farquhar (1981) using a specific software (Diagas 2.02, Walz, Effettrich, Germany). Water use efficiency (WUE) was calculated as the ratio between A_{max} and E_{max}. For each crop species, E, A and g were also measured under different light intensities (0, 20, 50, 100, 200, 400, 600, 800 and 1000 µmol/m*s) as described above. A piece of black cloth was used to provide complete darkness, whereas different layers of wire mesh with very small holes were used to provide the required light intensity.

Chlorophyll content

Five leaf disks (10 mm diameter) were randomly taken from the uppermost fully expanded leaves at weekly intervals, and extracted in 2 ml of N,N-dimethylformamide for 24 h in the dark. Absorbance was then determined for each sample using a spectrophotometer at 647 and 663 nm. Chlorophyll *a* and *b* contents, and a/b ratio were calculated according to Moran (1982).

Determination of total, osmotic and turgor potentials

Leaf water potential measurements were taken on the same leaf immediately after measuring gas exchange (n=5). Total water potential (ψ_w) was determined using a pressure chamber (Pardossi *et al*., 1991). Osmotic potential (ψ_s) of the leaf xylem sap was determined using an osmometer (Precision System, USA) by determining the freezing point depression of the sample. Leaf turgor potential (ψ_p) was calculated using the following equation (Eq. 1):

$$\psi_p = \psi_w - \psi_s \quad \text{(Eq. 1)}$$

Measurement of sugar content

Leaf, stem, and root samples (approx. 50 mg each) were taken at weekly intervals (n=5) and directly freeze-dried in liquid nitrogen. Samples were homogenized and extracted with 1 ml hot 80% ethanol, boiled for 5 min, centrifuged at 12000 rpm for 15 min and then the supernatant was collected. The pellet was extracted again as described above, and the supernatant was collected again. At the end, the pellet was evaporated to remove any excess of ethanol. Particulates including starch were suspended in 1 ml of KOH 20 mM, boiled and centrifuged at 8000 rpm for 15 min and the supernatant was collected. The extract from ethanol was used for sucrose, glucose and fructose determinations, and the extract from KOH was used for starch determination. For sugar determination, two 200 µl aliquots from the ethanol extract were taken, one incubated for 30 min at 37°C with 100 µl solution containing invertase (1 mg invertase/ml Na-acetate 50 mM at pH 4.6), and the other one with 100 µl solution containing Na-acetate 50 mM at pH 4.6, they were then both brought to the final volume (1 ml) with a solution containing 100 mM Tris-HCl, pH 7.6, 3 mM $MgCl_2$, 2 mM ATP, 0.6 mM NADP, 1 unit hexokinase and 1 unit glucose-6-P-dehydrogenase (incubated at 37°C for 30 min). Absorbance at 340 nm was then measured by a spectrophotometer. The concentration of glucose in each solution was determined from glucose standard curves according to Guglielminetti *et al.* (1995). The solution without invertase was used to calculate the amount of free glucose in the sample and the difference between the two gave the amount of sucrose (as glucose equivalent). For each of them, 10 µl of solution containing 15 µl of phosphoglucoisomerase in 150 µl of tris-HCl 300 mM at pH 7.6 were incubated at 37°C for 15 min, then absorbance at 340 nm was determined. The difference between the one without invertase and treated with phosphoglucoisomerase and the other without invertase at the first determination gave the amount of free fructose (as glucose equivalent). For starch determination, 100 µl of extract was incubated at 37°C for 1 h with 100 µl solution of Na-acetate 100 mM pH 5.2/10 u α-amylase. This solution was then incubated with 100 µl of Na-acetate 100 mM pH 4.6/10 u amyloglucosidase at 55°C for 1 h. Finally, the solution was boiled and centrifuged to eliminate denaturated protein from α-amylase and amyloglucosidase. 100 µl of this solution was taken and brought to 300 µl with distilled water; starch analysis (as glucose equivalent) was then carried out as mentioned above for glucose.

Statistical analysis

Data were analyzed by one-way ANOVA, and means (n=5) were separated using Duncan's Multiple Range Test (P≤0.05). Statistical analysis was performed using GraphPad Prism 4.0 (GraphPad software).

3. Results and Discussion

Root volume reduction greatly affected growth parameters, confirming several previous results concerning growth depression induced by RR in many horticultural crops (see for example Kharkina *et al.,* 1999; Saito *et al.,* 2008; Shi *et al.,* 2008), but scarcely in pepper (Ismail and Davies, 1998). Total dry weight significantly decreased starting from day 30 after emergence with reducing container size (Fig. 1A). In detail, RR pepper plants showed a 3.85-fold lower total dry weight compared to control at the end of the experiment. Leaf area was also greatly affected by volume reduction: RR plants showed a 4.15-fold reduction (Fig. 1B) at the end of the experiment. RR plants appeared to be smaller (reduced height values) (Fig. 1C), denoting a slackened development compared to control plants, with a preferred allocation of dry matter in the root system than in the aerial system, as demonstrated by a slight increase in the root:shoot ratio (Fig. 1D). RR generally caused an increase in root:shoot ratio (Mugnai *et al.,* 2000), with roots growing in smaller volume forming a highly branched mat. The increased root:shoot ratio reported by some researchers for many crop species subjected to RR might be attributed to an increased substrate temperature in smaller containers in conjunction with a possible temperature dependence of root elongation, as suggested by Hurley *et al.* (1998).

During the first month, no significant differences were noticed in leaf gas exchange parameters. Stomatal conductance (*g*) significantly decreased in RR plants (Fig. 2A)

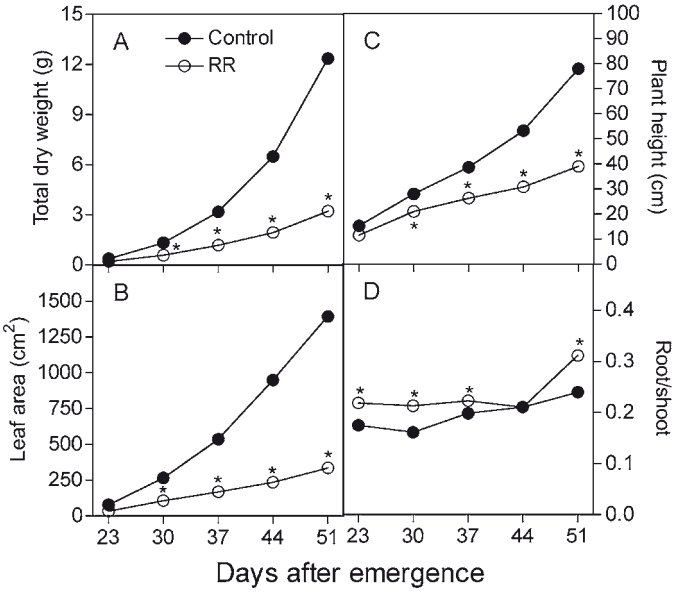

Fig. 1 - Growth parameters measured at weekly intervals from day 23 to the end of the experiment in both control and root-restricted (RR) plants:total dry weight (A), leaf area (B), plant height (C) and root:shoot ratio (D). * indicates significantly different values for P≤0.05 (n=5), when means were separated by Duncan's test.

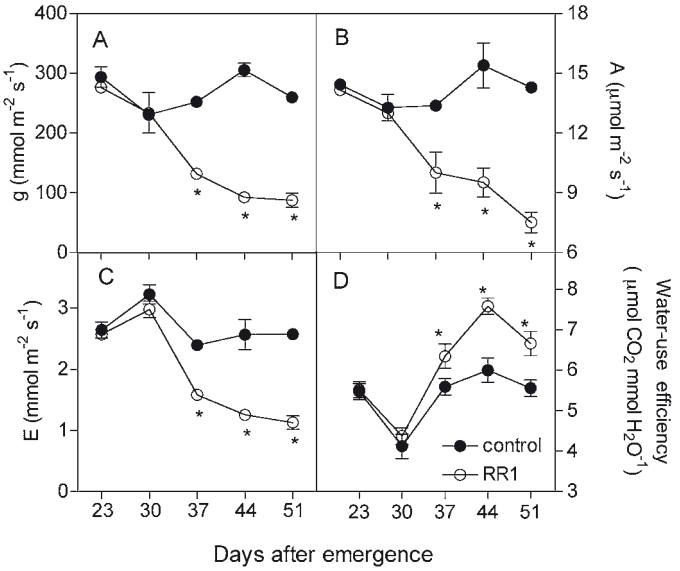

Fig. 2 - Leaf gas exchange parameters measured at weekly intervals from day 23 to the end of the experiment in both control and root-restricted (RR) plants: stomatal conductance (A), net CO_2 assimilation (B) and transpiration (C). * indicates significantly different values for P≤0.05 (n=5), when means were separated by Duncan's test.

Table 1 - Chlorophyll content (*a*, *b* and *a/b* ratio) measured at weekly intervals from day 22 to the end of the experiment in leaves collected from control and root-restricted (RR) plants

Day	Control plants			Root-restricted plants (RR)		
	Chl a (mg cm^{-2})	Chl b (mg cm^{-2})	a/b	Chl a (mg cm^{-2})	Chl b (mg cm^{-2})	a/b
23	12.469	4.485	2.780	11.453	4.170	2.746
30	13.387	4.756	2.814	13.107	4.642	2.823
37	14.671	5.639	2.601	13.845	5.259	2.632
44	16.274*	6.347*	2.564	14.058*	5.589*	2.515
51	17.784*	7.450*	2.387	11.493*	4.768*	2.410

* indicates significantly different values between the two treatments for the same parameter and date for P≤0.05 (n=5), when means were separated by Duncan's test.

from day 36, leading to a significant reduction in both net CO_2 assimilation (*A*, Fig. 2B) and transpiration (E, Fig. 2C) until the end of the experiment. The reduction in *A* was not related to a decrease in the chlorophyll content of RR plants (Table 1), as significant differences between the two treatments were noticed for chlorophyll *a*, *b,* and *a*/*b* ratio only after 43 days from the beginning of the experiment. Also, RR plants showed increased instantaneous water-use efficiency values (Wue) (Fig. 2D), as reported for several species under stress (Blum, 2009). RR treatment also affected leaf gas exchange parameters' response to light (Fig. 3). All the parameters (*g*, *A* and *E*) strongly decreased their values, leading to less pronounced response curves. In details, photosynthetic parameters, such as dark respiration, light compensation point and maximum CO_2 assimilation, started to significantly decrease after 37 days (Table 2).

Leaf water status did not seem to be the cause of the stomatal closure in RR plants, as total water potential (Fig. 4A) and turgor potential (Fig. 4C) did not show any significant difference in either of the treatments, even if slight reductions in total water potential and osmotic potential were measured at the end of the experiment in RR plants.

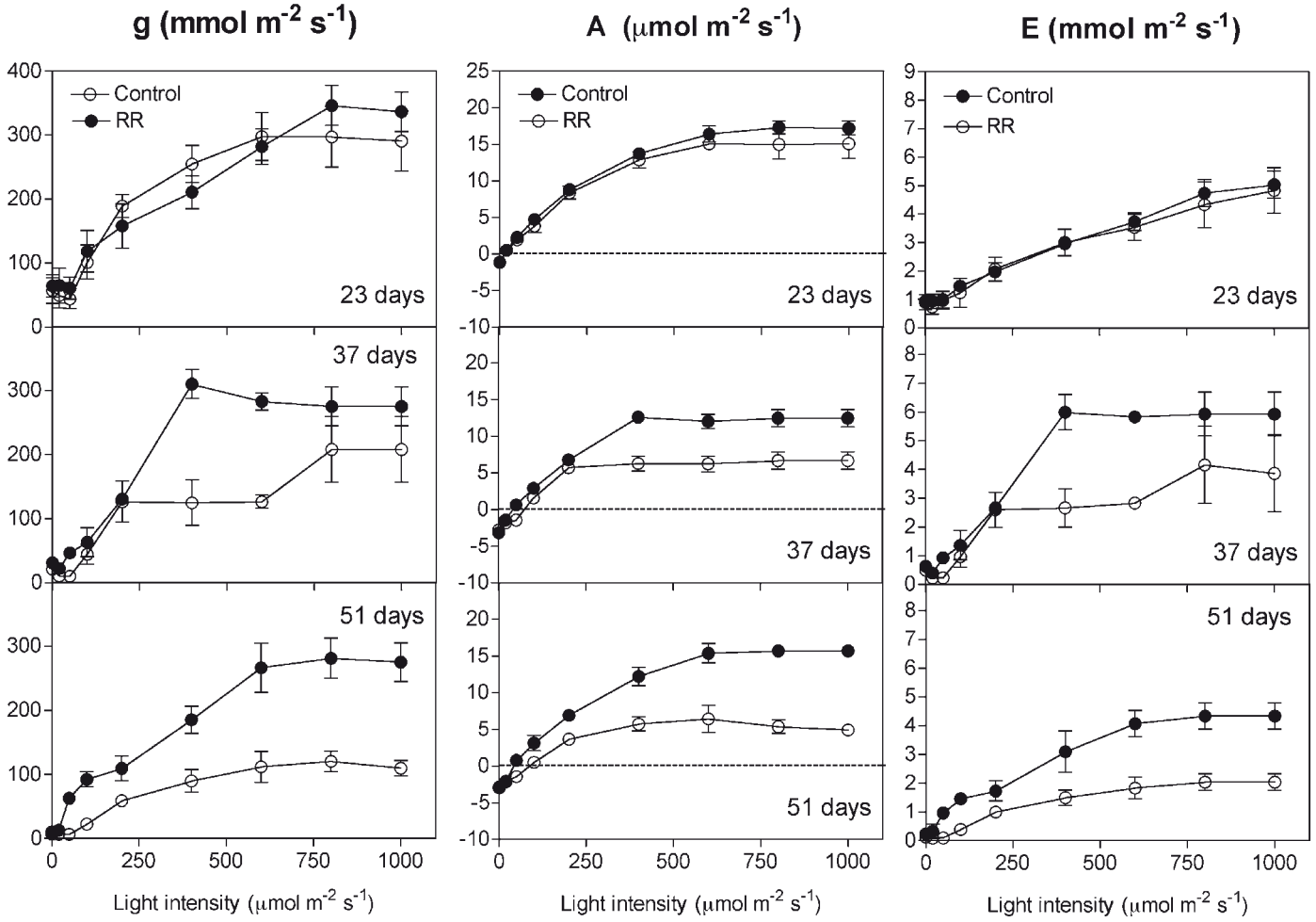

Fig. 3 - Light saturation curves for the three leaf gas exchange parameters (*g*, *A and* E) measured every two weeks from day 23 to the end of the experiment in both control and root-restricted (RR) plants.

Table 2 - Dark respiration (DR, μmol CO_2 m^{-2} s^{-1}), light compensation point (LCP, μmol m^{-2} s^{-1} PAR), maximum CO_2 assimilation (Amax, μmol CO_2 m-2 s^{-1}), water-use efficiency (WUE, μmol CO_2 mmol H_2O^{-1}) measured every two weeks from day 22 to the end of the experiment in leaves collected from control and root-restricted (RR) plants

Day	Control plants				Root-restricted plants (RR)			
	DR	LCP	A_{max}	WUE	DR	CP	A_{max}	WUE
23	-1.07	14.06	17.31	5.47	-0.9	12.83	15.11	5.51
37	-3.31*	41.56*	24.87*	5.59*	-2.75*	71.50*	6.67*	6.34*
51	-2.93	42.17*	15.71*	5.55*	-2.87	85.56*	6.46*	6.65*

* indicates significantly different values between the two treatments for the same parameter and date for P≤0.05 (n=5), when means were separated by Duncan's test.

This result also confirmed that no symptom of water stress ever occurred during the experimental period, leading to a positive feedback about our experimental system.

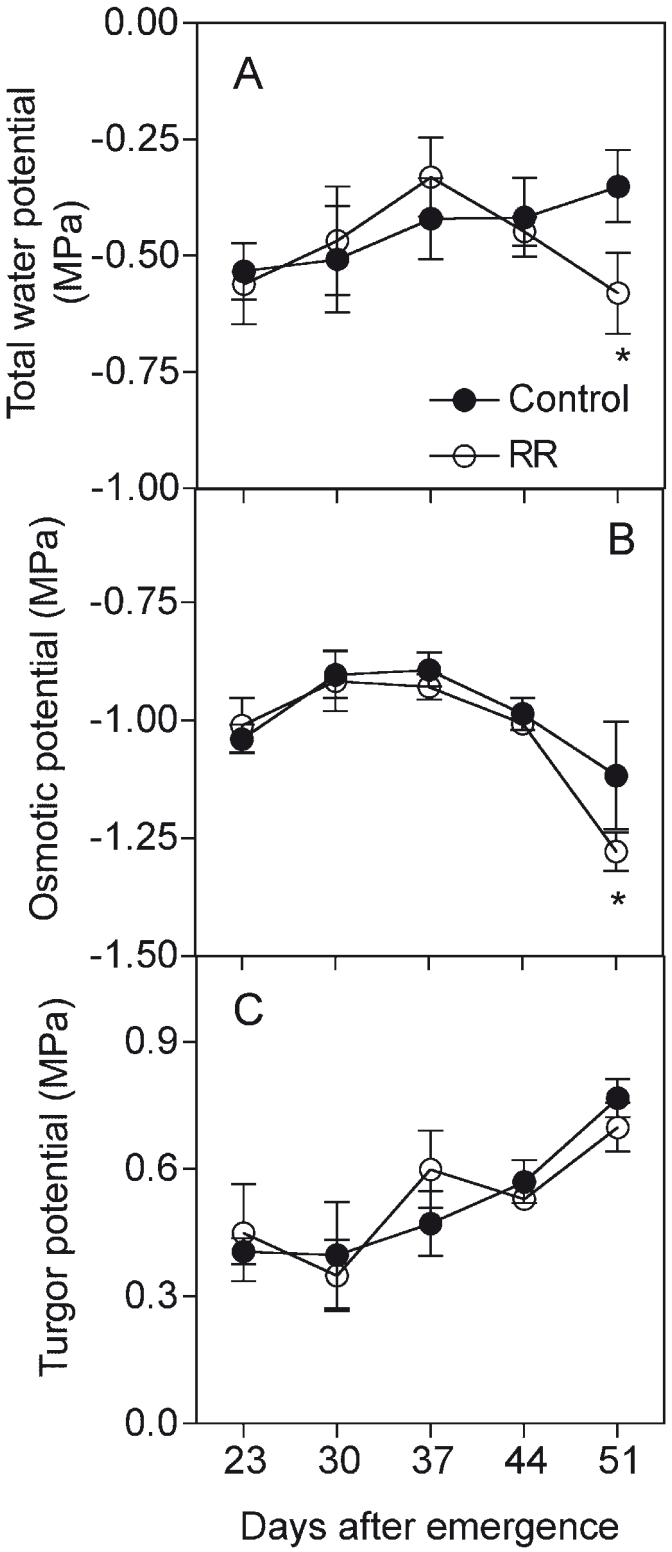

Fig. 4 - Leaf water status determined at weekly intervals from day 23 to the end of the experiment in both control and root-restricted (RR) plants:total water potential (A), osmotic potential (B) and turgor (C). * indicates significantly different values for P≤0.05 (n=5), when means were separated by Duncan's test.

Our results reveal that RR significantly reduces *g*, as previously noticed by other authors on different species (Ismail and Noor, 1996; Kharkina *et al.,* 1999), and that *g* is the primary cause of the reduction in *A* in RR plants, suggesting a stomatal factor limiting the photosynthetic rate under RR conditions (Shi *et al.,* 2008). However, the decline in *g* was not correlated to a concurrent decline in total water potential, as leaf tissues were able to maintain a high level of turgor during the whole experiment. Therefore, other factors should be involved in the stomatal closure. It has been suggested that RR induces a reduction in *g* through a decrease in the supply of growth substances from roots to shoots and/or an imbalance in root and shoot hormones. For example, Shi *et al*. (2008) reported that shoot growth suppression might be caused by the influence of ABA originating from the restricted roots. Ismail and Davies (1998) found that the slight increase in xylem sap [ABA] measured in pepper plants could not account for the reduction in leaf growth and *g*. They suggested that insufficient ABA synthesis occurred to trigger the processes that cause reductions in leaf growth and *g*.

Sugar content determination led to interesting results. Sucrose content significantly increased in RR plants starting from day 37 (Fig. 5A). Also, RR treatment led to a clear increase in glucose content (Fig. 5B) and a concurrent decrease in fructose content (Fig. 5C) together with a great accumulation of starch (Fig. 5D). In particular, starch accumulation in the tissues began early in the developmental process (day 29). Starch was mainly compartmentalised in the leaves (Fig. 6A) of RR plants, whereas no significant differences were noticed both in stems (Fig. 6B) and in roots (Fig. 6C) between control and RR plants, except for day 33. The decline in *A* observed in RR conditions was often interpreted as a feedback inhibition by carbohydrate accumulation (Pezeshki and Santos, 1998). Plant

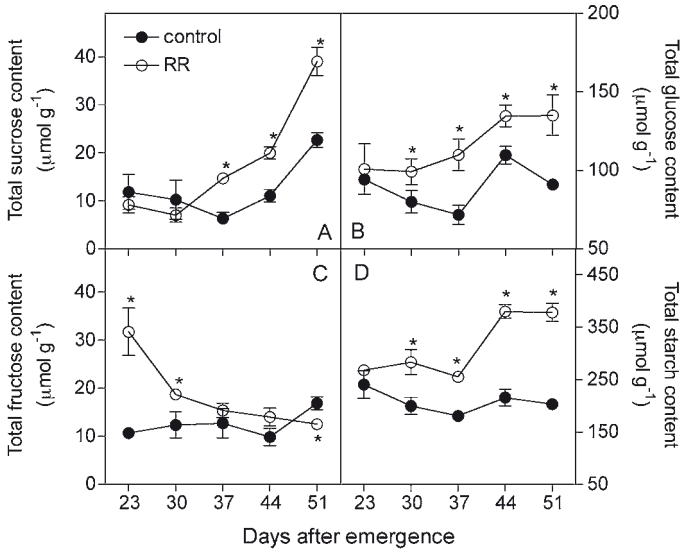

Fig. 5 - Sugar content measured at weekly intervals from day 23 to the end of the experiment in both control and root-restricted (RR) plants:total sucrose (A), total glucose (B), total fructose (C) and total starch (D). * indicates significantly different values for P≤0.05 (n=5), when means were separated by Duncan's test.

growth is strongly affected by leaf photosynthetic activity, since photosynthates are essential either as the source of carbon used for the build-up of organic compounds or as the source of energy needed for biochemical reactions involved in growth and maintenance processes. Growth rate may regulate photosynthesis either through effects on the supply of growth substances translocated into leaves or through effect on the translocation rate of photosynthates from leaves to the growing organs (Carmi *et al.,* 1983). The accumulation of photosynthates is influenced by the rate of their translocation to the sink organs (Sonnewald and Willmitzer, 1992), and sink demand for photosynthates has a marked influence on source leaf photosynthesis, which is greatly dependent on SINK strength, considered as a product of sink size and sink activity (Sonnewald and Willmitzer, 1992). However, sink size is determined by different parameters. Roots are recognized as a metabolic sink that influences the partitioning of photosynthetically fixed carbon (Gifford and Evans, 1981; Robbins and Pharr, 1988). Sink limitation caused by RR can greatly reduce leaf photosynthetic rate in many crop species (Hameed *et al*., 1987; Ismail and Noor, 1996; Whiley *et al*., 1999; Shi *et al*., 2008), and reduced translocation of assimilates from leaves (Robbins and Pharr, 1988; Kharkina *et al*., 1999). RR often promotes an accumulation of non-structural carbohydrates in the stem and leaves in response to the lack of the active sinks (Nishizawa and Saito, 1998), meaning that the difference in the growth rate between RR and control treatments was not due to a decrease in assimilates' supply to the organs whose growth was restricted (Mandre *et al*., 1995). Our results suggest that the role of the leaves as sink organs may increase when root growth is extremely limited by volume restriction and a relatively larger amount of carbohydrate may accumulate in the canopy. A new shoot to root equilibrium may be established for an increased function of leaves and stem, together with a concurrent diminished function of the roots. Therefore, it can be concluded that as a result of reduced vegetative growth an excess of assimilates was produced which could not be used for growth, and thus accumulated in the form of starch, as also indicated by Shi *et al.* (2008). Accumulation of non-structural carbohydrates in the leaves in response to RR could provide a feedback mechanism that reduces carbon metabolism (Thomas and Strain, 1991). Starch accumulation may reduce net photosynthetic rate by avoiding intracellular CO_2 transport (Shi *et al*., 2008). However, contradictory results were obtained by Rieger and Marra (1994), suggesting that reduced CO_2 assimilation cannot always be explained by a feedback inhibition of carbohydrates. The relatively low maximum assimilation (A_{max}) rates for container-grown plants compared to field-grown plants may be attributed to containers restricting the root sink, thus causing the photo assimilate supply to exceed the capacity of demand (i.e. end-product inhibition of photosynthesis) as indicated by Whiley *et al.* (1999).

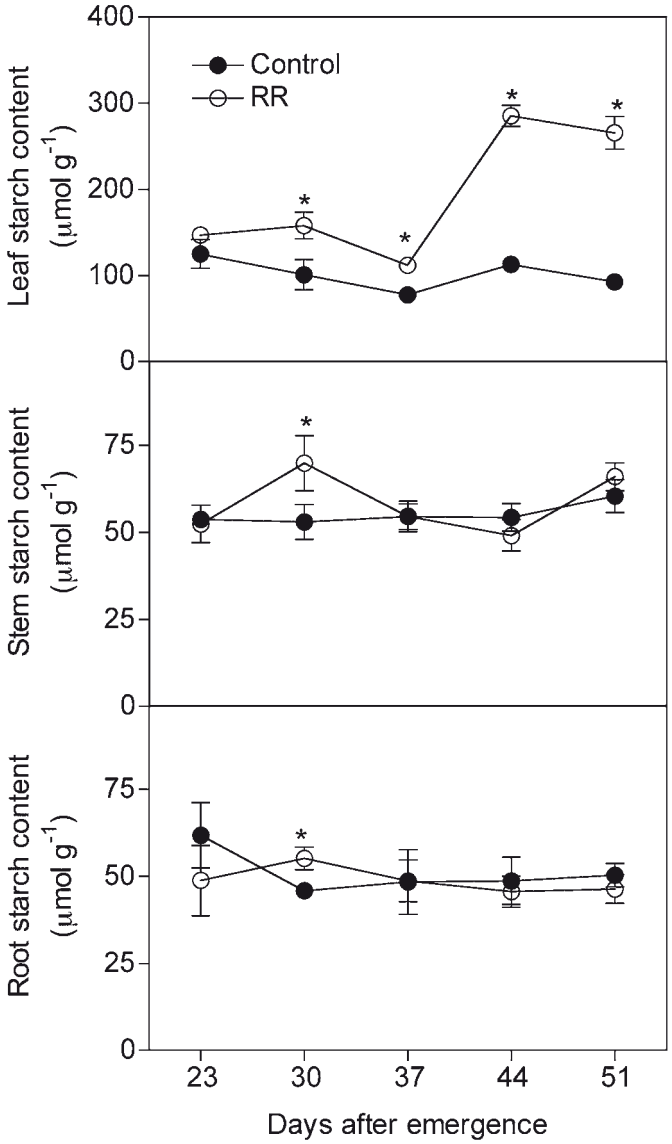

Fig. 6 - Starch content in the different plant organs measured at weekly intervals from day 23 to the end of the experiment in both control and root-restricted (RR) plants:leaf (A), stem (B) and roots (C). * indicates significantly different values for P≤0.05 (n=5), when means were separated by Duncan's test.

In conclusion, our results show that growth reduction by RR is mainly linked to a photosynthetic limitation, caused by a reduced stomatal conductance (probably driven by both stomatal factors and hormonal substances) and a strong accumulation of starch in the leaf tissues, which led to a feedback inhibition of the photosynthetic process.

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Towards *in vitro* selection studies for salinity tolerance in Canino apricot cultivar. Effect of gamma irradiation on *in vitro* mutation and selection for salt-tolerance

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Key words: IBA, BAP, M3 and MS3 medium, mutation gamma ray, propagation, salt tolerance.

Abstract: *In vitro* mutation method was used to obtain salt-tolerant clone in apricot. Small propagules of Canino apricot cultivar were irradiated with gamma ray at doses of 0, 10, 25, 35, 50, 75 and 100 Gy. After 30 days from treatment, both the radio sensitivity and post-irradiation recovery were assessed as the number of proliferated shoots per explants, fresh weight of cultures, shoot length and productivity of irradiated explants. A sudden and sharp decrease in the survival percentage occurred with the dose 75 Gy, while the highest dose (100 Gy) was lethal for all propagules. A marked decline in the number of regenerated shoots per explant and fresh weight of produced cultures was associated with an increase of irradiation doses. Doses in the range of 10-75 Gy, which preserved high survival percentage of irradiated explants, seemed to be more suitable for *in vitro* mutation in Canino apricot cultivar. Irradiated shoots were exposed to different concentrations of NaCl which were added to the multiplication medium at the rates of 25, 50, 75, 100, 125 mM and after 30 days, vigorous shoots were selected from salinity treatments. In conclusion, apricot tissues exposed to different doses of gamma irradiation in the range of 10-75 Gy, followed by culturing the plantlets produced in a medium containing additional salts (ranging from 25 to 125 mM) can be considered a good method to identify the most tolerant mutants to salts in apricot cultivars.

1. Introduction

Salinity is a widespread problem around the world, especially in arid and semi-arid regions. Each year more and more land becomes non-productive due to salt accumulation. At least 25% of currently cultivated land throughout the world suffers from excess salinity (Bohnert and Jensen, 1996) and all major crop species are intolerant to salt (Fairbairn *et al*., 2000). The most economic and sustained way to overcome the problem of salt-stress is to develop salt-tolerant varieties (Frommer *et al*., 1999). *In vitro* culture has been widely used for the propagation and conservation of crop genetic resources in both agriculture and horticulture crops (Barakat and El-Lakany, 1992).

Mutation breeding programs using gamma irradiation on apricot buds were carried out by several investigators (Legave and Garcia, 1988; Ageeva, 1989; Gulcan and Aksoy, 1995). Legave and Garcia (1988) reported that bud sticks of five apricot cultivars were exposed to up to 70 Gy gamma rays and scored for bud survival and growth. The effect of gamma irradiation in the range 10-70 Gy on variation in the characters of apricot was also investigated (Ageeva, 1989). The varieties reacted in different ways to treatment. Induced mutations in apricot breeding were also investigated (Gulcan and Aksoy, 1995). Apricot (*Prunus armeniaca*) was treated with 0.3 KR gamma irradiation (source 60Co). Mutagenesis affected vigour, dry matter and vitamin C content, and the level of carotene in fruit. *In vitro* cultures of Japanese plum (*Prunus salicina*) cv. Shiro were also gamma-irradiated by Predieri and Gatti (2000). *In vitro* culture may offer potential for quick evaluation of germplasm against salt stress (Cano *et al*., 1998). Recently, Jain (2001) reported that tissue culture generates a wide range of genetic variation in plant species which can be incorporated into plant breeding programs. The effect of NaCl and $CaCl_2$ in *Prunus cerasifera* was investigated by Lucchesini and Vitagliano (1993). The *in vitro* response of peach cv. Redhaven and of the peach/almond hybrid rootstock GF677 to increasing concentrations of NaCl in the medium was reported (Biricolti and Pucci, 1995). The response to increasing rates of NaCl or $CaCl_2$ and proline on 'Mr.S 2/5' (*Prunus cerasifera*) peach rootstock cultured *in vitro* has also been reported (Dimassi-Theriou, 1998).

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Recently, increased sodium chloride (NaCl) salinity effects on bitter almond (*Amygdalus communis*) (*Prunus dulcis*) growth, cell osmolarity and nutrient acquisition were studied *in vitro* (Shibli *et al*., 2003) and it was found that elevating salinity from 0.0 (control) to 50, 75, 100 mM NaCl resulted in reductions in shoot growth (shoot height, shoot dry weight) and rooting (rooting percentage, root number, root length).

The objective of the present work was to obtain salt-tolerant clone (s) in apricot using an *in vitro* mutation method.

2. Materials and Methods

The present work was carried out in the Biotechnology Laboratory, Crop Science Department, Faculty of Agriculture, Alexandria University from 2001 to 2005. Small propagules of Canino apricot cultivar initiated from *in vitro* culture by the follwing protocol: two explants shoot tips and single node cutting; of 0.5-1 cm in length was used. Shoot tips and single node were soaked in 100 mg/l ascorbic acid +150 mg/l citric acid for 30 min. Explants were dried for 30 min. before they were immersed in fungicide Ridomil (1 g/l solution) for 30 min. and then washed with distilled water, then soaked in Clorox (7% Sodium hypochlorite) for 7 min and washed with sterile distilled water three times. The explants were aseptically excised and placed in Jar containing 50-60 ml of culture medium. Each Jar contained one explant, considered as one replication. Cultures were incubated at 25±2°C under 16 hour's illumination (2000 lux, day light fluorescent tubes). In order to check the phenol oxidation and to establish the explants with free from phenol, the explants were cultured on two medium (MS and M_3,) supplemented with four PVP concentrations (0.0, 40.0, 80.0, 160.0 mg/l). The effect of five medium protocols was examined: modified woody plant medium (M_3) (Perez-Tornero *et al*., 2000), MS medium (MS_1) and MS medium (Murashige and Skoog, 1962) modified by reducing KNO_3 and NH_4NO_3 by 25%, 50% and 75% which were designated MS_2 , MS_3 and MS_4, respectively. All media were supplemented with 3.0% sucrose, 4 mg/l adenine sulfate, 160 mg/l PVP, 0.4 mg/l BAP and 0.01 mg/l IBA. The pH of the media was adjusted to 5.7 by using 1.0 N HCl or 1.0 N NaOH and agar was added after adjusting the pH. The best two media (M_3 and MS_4) for proliferation were used to test the optimum effect of three types of cytokinins benzyladenine (BAP), Kiniten and isopentenyladenine (2iP) and their concentration on shoot tip proliferation, four different concentration of cytokinin 0.2, 0.4, 0.6 and 0.8 mg/l for each type using M_3. Also, four different concentration of cytokinin 0.5, 1.0, 2.0 and 4.0 mg/l for each type using MS_4. The proliferation was evaluated six weeks after the beginning of the experiment and the number of shoots, (longer than 5 mm) per explant, their length and productivity (number of shoots x the average shoot length) were recorded. Shoots of apricot derived from the shoot tips multiplication were cultured on M_3 medium Perez-Tornero et *al*. (2000). The medium was supplement with either NAA (0.0, 0.5, 1.0, 2.0 mg/l) or IBA (0.0, 2.0, 4.0, 6.0 mg/l) were employed.

In vitro *mutation and selection for salt-tolerance*

Effect of gamma irradiation on in vitro *shoot culture*. Small propagules of Canino cultivar initiated from *in vitro* culture (Fig. 1) were irradiated in a gamma cell with a cobalt^{60} source at the Middle-East Regional Radioisotopes centre for Arab Countries, El-dokki, Giza with 10, 25, 35, 50, 75 and 100 Gy doses. The irradiated propagules were removed from the jar and recultured on a fresh proliferation medium. After six weeks incubation, impact of the irradiation was assessed by determinating the number of shoots, the fresh weight of shoot multiplication, shoot length and productivity.

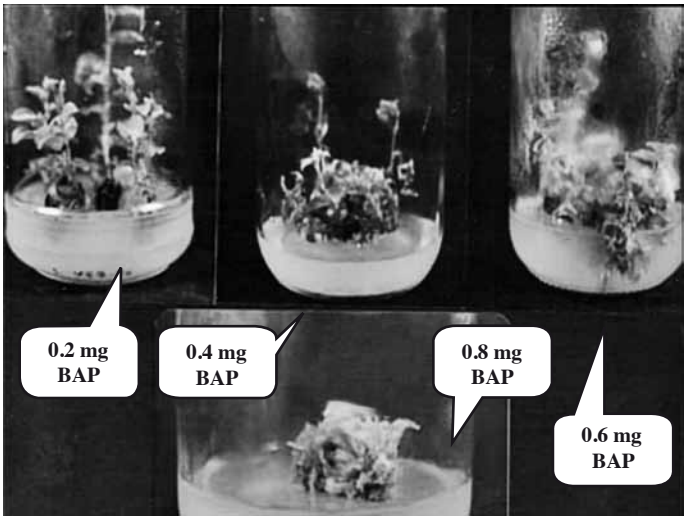

Fig. 1 - *In vitro* micro propagation of apricot cv. Canino on M_3 medium derived from shoot tip explants.

In vitro *selection*. Small propagules of the cultivar (Canino) (Fig. 1) were irradiated in a gamma cell with cobalt ^{60} source Gy at a dose of 10, 25, 35, 50 and 75 Gy. The propagules were subcultured five times before *in vitro* selection for salt-tolerance. Individual shoots from irradiated cultures were grown on the proliferation 0.6 mg/l BAP M_3 medium supplemented with different concentrations of NaCl (25, 50, 75, 100 and 125 mM). After six weeks of incubation, the vigorous shoots were selected and transferred to fresh medium free from salt. Analysis of variance with SAS software (SAS Institute, 1988) was carried out. Treatment means were compared using the LSD test at 5% level probability. Data were analyzed as a factorial arrangement in Randomized Complete block design according to Steel and Torrie (1980).

3. Results and Discussion

Effect of gamma irradiation on in vitro *apricot culture*

The basic requirement for effective use of mutation induction in plant breeding programs is the analysis of radio sensitivity of the explant material (Walther and Sauer,

1986). Predieri (2001) reported that one of the first steps in mutagenic treatment is the estimation of the most appropriate dose to apply. The aim of the present work was to determine the radio-sensitivity of *in vitro* apricot culture, as assessed by the number of regenerated shoots, the fresh weight of shoot multiplication, the shoot length and productivity in order to select the suitable dose of gamma irradiation to conduct *in vitro* mutation for improvement.

The collected data, reported in Table 1, indicate that a clear decrease in *in vitro* traits occurred with increasing irradiation dose. Complete lethality (100% death) was observed with an irradiation dose higher than 75 GY (Fig. 2). Several other studies have been conducted on the radio-sensitivity of *in vitro* cultures of fruits, such as *Prunus avium* (Walther and Sauer, 1985), kiwifruits (Shen *et al*., 1990), grapevine (Lima da Silva and Doazan, 1995; Charbaji and Nabulsi, 1999) and *Prunus salicina* (Predieri and Gatti, 2000). Previously, Laneri *et al*. (1990), working with *Gerbera jamesonii*, stated that in a mutation breeding experiment, the dose chosen for the main experiment should result in the highest survival of irradiated explants and that a low inhibition of the rate of production of new shoots gives the highest efficiency in recovering useful mutants. In light of these studies, the results obtained in the present investigation suggest that doses of 10 Gy to 75 Gy seem to be the most suitable for inducing mutation for apricot improvement.

Table 1 - Effect of gamma irradiation six weeks after the treatment on *in vitro* apricot shoot

Gamma irradiation (gy) doses	Weight	Number of shoots	Shoot length	Productivity
Control	5.85 a	23.40 a	6.15 a	147.36 a
10	3.58 b	14.30 b	2.99 b	42.27 b
25	3.45 b	13.80 b	3.14 b	42.52 b
35	2.40 bc	9.60 bc	3.63 b	41.68 b
50	1.50 cd	6.00 cd	0.77 c	11.05 c
75	0.68 d	2.70 d	0.48 c	4.18 c

Means within a column or a row followed by the same letter(s) are not significantly different at the 0.05 level of probability.

Fig. 2 - Effect of gamma irradiation 100 Gy, six weeks after the treatment on *in vitro* apricot shoot.

In vitro *mutation and selection for salt-tolerance in Canino apricot cultivar*

Mutation breeding can be employed as a promising technique that allows diversification of apricot. Induced mutations change only one or a few specific traits of an elite cultivar without undesired additional variations (Predieri, 2001). In fact Predieri concluded that the most suitable method may be mutation treatment and propagation of *in vitro* axillary shoots without passage through undifferentiated growth, and it can contribute to fruit improvements without upsetting the requirements of the fruit industry nor the consumers. Through *in vitro* selection, mutation with a useful agronomic trait, e.g. salt or drought tolerance or disease resistance, can be isolated in a short time (Jain, 2001).

The present work was conducted to obtain salt-tolerant clone(s) in apricot cv. Canino using *in vitro* shoot mutation. Small propagules were irradiated with 0, 10, 25, 35, 50, or 75 Gy and explants were multiplied for five subcultures. The generated irradiated shoots were subjected to a salt (NaCl) which was added to the medium with the concentrations 25, 50, 75, 100, or 125 mM. The number of vigorous shoots of cv. Canino showed marked differences in their *in vitro* salinity tolerance (Table 2). It is clear that the number of vigorous shoots decreased rapidly with increasing salinity. The highest number of vigorous shoots was obtained in medium supplemented with 25 mM selective agent of salinity when the propagules were exposed to 25 and 50 Gy, respectively (Table 2). From these results, it can be concluded that apricot cv. Canino tissues exposed to different doses of gamma irradiation in the range 10-75 Gy, followed by culturing in medium containing a higher concentration of additional salts (ranging from 25 to 100 mM) can be considered a good method to identify mutants in apricot cv. Canino which are the most tolerant to salts. FAO/IAEA (1997) reported that plant biotechnology in combination with mutation induction and conventional breeding might open new frontiers for obtaining salt-tolerance rice varieties. The application of mutation techniques in breeding has increased constantly over the past years. These techniques must be rapid to keep pace with the large quantity of breeding materials generated after mutagenesis. Screening under field conditions is difficult due

Table 2 - Effect of irradiation doses on the number of vigorous shoots of apricot cv. Canino, after six weeks from culturing in media containing different salt concentrations

Salt concentration mM	Irradiation dose (Gy)					
	0	10	25	50	75	Total
25	0	2	5	4	3	14
50	0	2	3	1	2	8
75	0	0	0	2	2	4
100	0	0	0	1	2	3
125	0	0	0	0	0	0
Total	0	4	8	8	9	29

to stress heterogeneity, presence of salt-related stress and the significant influence of environmental factors such as temperature, relative humidity and solar radiation. Genetic modification of crop plants to improve their salt-tolerance is a possible way of increasing production, especially for regions of the world where arable lands must be extended to marginal areas, and sometimes irrigated with saline water (Dorion *et al*., 1999).

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Short note

Potato response to potassium application rates and timing under semi-arid conditions

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Key words: aggregate tuber yield, potassium application rates, potassium application timing, *Solanum tuberosum* L., specific gravity, tuber dry matter.

Abstract: A two-year experiment (2004-2005) was conducted at Tal Amara Research Station in the Bekaa Valley of Lebanon to evaluate the influence of progressive application of K rates and application timing on yield, yield components and tuber quality of potato (*Solanum tuberosum* L. cv. Agria). Four levels of potassium (0 (K_0), 75 (K_{75}), 150 (K_{150}), and 225 (K_{225}) kg K_2O ha^{-1}) and two application timings (tuber initiation and tuber bulking stages) were used in a split-plot design. The progressive application of potassium fertilizer from 0 to 225 kg K_2O ha^{-1} significantly affected the yield and yield components of potato. In both years, small grade tubers and aggregate tuber yield increased quadratically with increasing K application rates up to 150 kg K_2O ha^{-1}, reaching a plateau thereafter, showing luxury consumption of the nutrient at 225 kg K_2O ha^{-1}. In 2004 when averaged over K application rates, large and medium grade tubers and aggregated tuber yield were 120%, 22%, and 12% greater, respectively, with K application at tuber bulking than at tuber initiation. A similar trend was also observed in 2005, when the small grade tubers and aggregate tuber yield were 20% and 12% higher, respectively, with K application at tuber bulking than at tuber initiation stage. Finally, no significant difference among treatments was observed for tuber dry matter (avg. 19.8%) and specific gravity (1.08 g cm^{-3}).

1. Introduction

Potato (*Solanum tuberosum* L.) is one of the major crops contributing to the world's food requirement because it is a rich source of starch, having protein of high biological value (Eppendorfer and Eggum, 1994). In Lebanon, potato occupies an area of 19,700 ha with a total production of 460,000 t, but average yield is only 23 t ha^{-1}, which is much below the crop's potential productivity. Such a low yield seems to be due to the imbalance in nutrients applied for the agricultural production of this crop.

Owing to the current trend of intensive cropping in Lebanon, soils have developed multi-nutrient deficiencies. Farmers usually diagnose and correct the deficiencies of nitrogen (N) and phosphorus (P), but often neglect the effect of deficiencies of other essential macronutrients such as potassium (K). In addition to N and P, potato is a heavy remover of soil potassium and its response to potassium varies with variety, source and method of potassium fertilizer application (Sharma and Sud, 2001; Kumar *et al*., 2007; Abd El-Latif *et al*., 2011).

Since biomass and bulking rate of potato tubers are positively affected by synthesis and accumulation of starch, K plays a key role in this regard as it is the most efficient monovalant cation that stimulates the activity of the starch synthase enzyme, catalyzing the incorporation of simple glucose molecules into complex molecules of starch (Moinuddin *et al.*, 2004). Starch accumulation is coupled with cell and tissue growth of the tubers as K enhances the overall growth of the plants (Singh and Singh, 1996), and facilitates the translocation of assimilates to the sinks/tubers (Moinuddin *et al.*, 2005), which could ultimately increase the tuber bulking capacity and, thereby, its biomass and yield. Thus, potato removes large quantities of K and other soil nutrients, particularly N and P, in a short period coupled with a high rate of dry matter production (Perrenoud, 1993; Singh and Trehan, 1998). An optimum K level, along with optimum levels of N and P would, therefore, be required to exploit the full genetic potential of the crop and achieve an improved level of tuber yield and quality.

In spite the efforts aimed at optimizing potato response to K fertilization, little has been done on the time of K application for potato farming. This study, conducted in the Central Bekaa Valley of Lebanon, aims at assessing potato response to increasing in-season potassium rates (four progressive rates of K) applied at different times of tuber

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growth (tuber initiation and tuber bulking stages) and to depict the optimal rate to achieve target yield.

2. Materials and Methods

Experimental site

Field experiments were conducted from April to August during the 2004 and 2005 growing years at Tal Amara Research Station in the Central Bekaa Valley of Lebanon (33º 51' 44'' N lat., 35º 59' 32'' N long., 905 m a.s.l). The details of the experimental site have been described elsewhere (Karam *et al*., 2003, 2005, 2006, 2007, 2009 a,b, 2011). Tal Amara has a well-defined hot and dry season from May to October and very cold conditions for the remainder of the year. Average seasonal rainfall is 592 mm, with 95% of the rain occurring between November and March, and a maximum of 145 mm in January. Historical data indicate no rain occurrence at Tal Amara from June to September. Rainfall amounts during the growing period were 35 and 25 mm during 2004 and 2005, respectively. Soils of the experimental site were deep, non-calcareous, clay Eutric Cambisols with an average bulk density of 1.2 g cm^{-3}. Soil chemical and physical properties were: available N content 45.5 g kg^{-1}, available P content 17.0 g kg^{-1}, and available K content 11.5 g kg^{-1}, organic matter content 1.2% and pH 7.9.

Crop management, K-treatments and experimental design

Potato (*Solanum tuberosum, L.*) seeds of cultivar Agria were sown under field conditions on 5 April 2004 and 11 April 2005. The soil was plowed and disked each year in anticipation for bed preparation. In both years, seeds were planted in a conventional "hill" system, where single soil beds were separated by relatively deep furrows spaced 70 cm apart, giving a theoretical plant density of 70000 plants ha^{-1}. The experiments were conducted under optimum irrigation conditions in both years. At planting, the soil surface was thoroughly moistened using a sprinkler irrigation system at the application rate of 4.5 mm h^{-1}. When plants reached 8 to 10 cm in height (two weeks after emergence), a drip irrigation system was installed along the furrows. The drip system consisted in polyethylene (PE) distribution lines, 16 mm in diameter, 40 cm spaced drippers, delivering each 4 L h^{-1} at 1 bar of head pressure. Experiments were set up in a split plot design (main plot: potassium application rate; sub-plot: application timing). The trial covered four levels of potassium (0 (K_0), 75 (K_{75}), 150 (K_{150}), and 225 (K_{225}) kg K_2O ha^{-1}) and two application times (tuber initiation and tuber bulking stages), with five replications. Each experimental unit consisted of six rows, 5 m in length. In both years, preplant fertilizer was broadcast (150 kg·ha^{-1}; 17N - 17P -17K) and incorporated into the soil. Moreover, a fertilizer dose of 144 kg N and 96 kg P_2O_5 ha^{-1} was applied in two splits after planting (35 days after planting, DAP) and at tuber initiation (60 DAP) uniformly to all the plots to assure rigorous shoot development. Potassium was applied as K_2O (0-0-46) in one split at tuber initiation (60 DAP) and tuber bulking (80 DAP) stages in four application rates with irrigation water. The experiments were concluded on 3 August 2004 (120 days after planting) and on 8 August 2005 (119 days after planting).

Data collection

After harvest, the tuber yield was grouped into three grades, grade 1 (200-400 g), grade 2 (85-200 g) and grade 3 < 85 g. The grade-wise and aggregate tuber yields were recorded. Tubers were dried in a forced-air oven at 80°C for 72 h and weighed to determine the tuber dry matter (DM). Tuber specific gravity (tuber weight in air/tuber weight in water) (Dunn and Nylund, 1945) was determined on subsamples of acceptable tubers.

Statistical analysis

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 10 for Windows, 2001). Duncan's multiple range test was performed at p=0.05 on each of the significant variables measured.

3. Results and Discussion

In experiment 1 (2004), small grade tubers and aggregate tuber yield were significantly affected by K application rates, whereas large and medium grade tubers and aggregate tuber yield were highly influenced by K application timing, with no 'K rates x K timing interaction' (Table 1). While in experiment 2 (2005), small grade tubers and aggregated tuber yield were significantly influenced by K application rates, K application timing, with no significant 'K rates x K timing interaction' (Table1). In both years, no significant difference among treatments was observed for tuber dry matter (avg. 19.8%) and specific gravity (1.08 g cm^{-3}) (Table 1). These results on tuber quality (i.e. dry matter and specific gravity) are consistent with the findings of Davenport and Bentley (2001) who observed no response in tuber quality, mainly specific gravity, in response to increasing K rates. In contrast, others have reported that excess K fertilizer reduces dry matter content and specific gravity of tubers (Westermann *et al.,* 1994 a, b). Explanations for this disagreement could be the different environments in which the plants were grown, and variations between potato genotypes in response to potassium application rates.

In 2004 when averaged over K application rates, large and medium grade tubers and aggregated tuber yield were 120%, 22%, and 12% greater, respectively, with K application at tuber bulking than at tuber initiation. A similar trend was also observed in 2005, when the small grade tubers and aggregate tuber yield were 20% and 12% higher, respectively, with K application at tuber bulking than at tuber initiation stage (Table 1). In both years, irrespective of K application timing, the highest small grade tubers yield was recorded with K application rates of 150 kg K_2O ha^{-1} (avg. 30 and 33 t ha^{-1}, in 2004 and 2005 respectively),

whereas the highest aggregate tuber yield was observed at both K_{150} and K_{225} with no significant difference observed between the two K application rates followed by K_{75} and finally K_0 treatment.

In both experiments, aggregate tuber yield increased quadratically with increasing K application rates up to 150 kg K_2O ha^{-1}, reaching a plateau thereafter, indicating the luxury consumption of the nutrient at 225 kg K_2O ha^{-1} (Table 1). Significant increase in tuber yield of potato as a result of K application is well documented (Cordova and Valverede, 2001; Singh *et al*., 2001; Tawfik, 2001; Umar and Moinuddin, 2001; Moinuddin *et al*., 2004, 2005). In fact, potato has a higher potassium requirement for optimum production compared to cereals, pulses, oilseeds, and other commercial crops and produces much more dry matter in short growth duration. It produces large amounts of starch due to K-mediated carbohydrate metabolism (Perrenoud, 1993; Singh and Trehan, 1998). In addition, it helps in efficient translocation of photoassimilates to the developing sinks/tubers (Beringer, 1978) and enabling the plants to fully utilize applied N and P fertilizers (Mengel and Kirkby, 1987). Thus, K helps the potato tubers to attaine large size and heavier weight. This was evident in the current study, as we observed a progressive increase in aggregate tuber yield. These results are consistent with the findings of Moinuddin *et al*. (2004, 2005) and Abd El-Latif *et al*. (2011) who showed an increase in tuber yield with a progressive application of K fertilizer from 0 to 225 kg K_2O ha^{-1} (Moinuddin *et al*., 2004, 2005) and from 72 to 120 kg K_2O $fed.^{-1}$ (Abd El-Latif *et al*. 2011). Moreover, in line with our results, Singh *et al*. (1997) reported that an increase in K application rates resulted in an increase in the yield of small-grade tubers.

To summarize, we can conclude that the progressive application of potassium fertilizer from 0 to 225 kg K_2O ha^{-1} significantly affected the yield and yield components of potato. In both experimentation years, small grade tubers and aggregate tuber yield increased quadratically with in-

Table 1 - Effects of potassium application rates and K application timing on grade-wise and aggregate tuber yield, tuber dry matter and specific gravity of potato plants grown in 2004 and 2005

Year	K timing	K rate	Grade-wise tuber yield (t ha^{-1})			Aggregate yield	Tuber dry matter	Specific gravity
		Kg K_2O ha^{-1}	Grade 1	Grade 2	Grade 3	t ha^{-1}	%	g cm^{-3}
2004	Tuber initiation Stage	0	0.6	30.7	19.9	51.2	20.0	1.078
		75	0.3	23.4	28.1	51.8	19.8	1.079
		150	0.9	24.2	28.9	54.0	19.5	1.077
		225	0.2	26.0	29.4	55.6	20.2	1.081
	Tuber bulking Stage	0	1.0	27.4	25.5	53.9	19.5	1.077
		75	1.7	30.8	24.3	56.8	19.2	1.075
		150	0.4	33.0	31.1 a	64.5	20.0	1.079
		225	1.3	36.5	25.2	63.0	20.0	1.080
Significance [z]	K rate		NS	NS	**	**	NS	NS
	K timing		*	*	NS	*	NS	NS
	K rate x K timing		NS	NS	NS	NS	NS	NS
2005	Tuber initiation Stage	0	0.8	29.1	22.7	52.6	19.8	1.078
		75	1	27.1	26.2	54.3	19.5	1.077
		150	0.6	28.0	30	58.6	19.8	1.078
		225	0.8	31.2	27.3	59.3	20.1	1.081
	Tuber bulking Stage	0	0.9	28.2	27.3	56.4	19.7	1.077
		75	1.3	29	31.4	61.7	19.3	1.076
		150	0.5	31.0	36	66.5	19.9	1.079
		225	1	33.9	32.8	67.7	20.0	1.080
Significance [z]	K rate		NS	NS	*	**	NS	NS
	K timing		NS	NS	*	*	NS	NS
	K rate x K timing		NS	NS	NS	NS	NS	NS

[z] NS * ** ***Non significant or significant at $P < 0.05$, or 0.01 respectively.

creasing K application rates up to 150 kg K_2O ha^{-1}, reaching a plateau thereafter, showing luxury consumption of the nutrient at 225 kg K_2O ha^{-1}, indicating the detrimental effect of over fertilization. The results also demonstrated that K application during tuber bulking stage was more effective in terms of yield and yield components, and not in terms of quality, than at tuber initiation stage.

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