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Antioxidant capacity and total phenolic content of hydrothermally-treated 'Fuerte' avocado

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Key words: antioxidant capacity, bioactive compounds, *Persea americana Mill*., refrigeration.

Abstract: Avocados possess high nutritional value with proven effectiveness in preventing cardiovascular diseases, attributed primarily to their unsaturated fatty acids content. This fruit is also rich in carotenoids and vitamins, particularly vitamin E. This work evaluates the antioxidant capacity and total phenolic content of hydrothermally-treated Fuerte avocado. Fruits were selected and hydrothermally treated at 45ºC for 5, 10, 15 and 20 min. They were then stored in a refrigerator (10 ± 1ºC and 90±5% relative humidity) and evaluated over a 15-day period. The total phenolic content increased up to the sixth day of storage, and decreased thereafter, without differences between the treatments. The percentage of antioxidant capacity of the control and the hydrothermally-treated samples for 5 and 10 min increased during storage. Untreated fruits showed the highest percentage of antioxidant capacity. However, the antioxidant capacity of avocado fruits subjected to these treatments declined starting on the twelfth day of storage, possibly due to the fruits' senescence. Hydrothermal treatments for 15 and 20 min delayed fruit senescence while the antioxidant capacity continued to increase up to the fifteenth day of storage. No significant correlation was found between antioxidant capacity and total phenolic content. The antioxidant capacity of ripe Fuerte avocado was higher than that of unripe or overripe avocado.

1. Introduction

Avocado (*Persea americana* Mill.) has considerable nutritional quality, with a high content of fibers, proteins and mineral salts, particularly potassium and vitamins, especially vitamin E (USDA, 2007). It also contains significant amounts of unsaturated fatty acids, which are beneficial for the prevention of cardiovascular diseases (Tango *et al*., 2004). Previous studies have also shown that this fruit contains anticarcinogenic lipophilic compounds such as carotenoids (Ding *et al*., 2007).

Wang *et al*. (2010) pointed out the scantiness of studies on the phytochemical composition of avocados and the lack of knowledge about the total phenolic content and antioxidant capacity of different avocado varieties, or cultivars. The aforementioned authors conducted studies to determine the antioxidant capacity of the pulp, seed and peel of different avocado varieties, but not the Fuerte variety. 'Fuerte' avocados are small and are highly valued in European and American markets.

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Antioxidants, which are compounds that inhibit and/or reduce the effects of free radicals (Soares *et al*., 2005), can be defined as compounds that protect the cells against the harmful effects of oxygen and nitrogen free radicals that are formed in oxidative processes. High free radical levels generate an imbalance, triggering oxidative stress, the metabolic process responsible for the onset of several types of chronic degenerative diseases. Antioxidants can be obtained by eating food containing vitamins E and C, carotenoids, phenolic compounds, and other compounds (Ali *et al*., 2008).

Phenolic compounds are responsible for most of the antioxidant activity in fruits, making them a natural source of antioxidants (Heim *et al*., 2002). The phenolic content in food and plants depends on a number of intrinsic factors such as the genus, species and cultivar, and on extrinsic factors such as agronomic and environmental factors, handling and storage (Thomas-Barberán and Espín, 2001).

Avocado is a climacteric fruit which ripens a few days after harvest (Hardenburg *et al*., 1986; Seymour and Tucker, 1993) and whose postharvest behavior can be influenced by temperature and storage time. The literature contains several studies about the increase in

the conservation period of avocado, involving the evaluation of storage temperature, the use of modified atmosphere with the application of wax, gamma irradiation and thermal treatment to prevent chilling injury (Zauberman *et al*., 1973; Castro and Bleinroth, 1982; Seymour and Tucker, 1993; Germano *et al*., 1996; De Oliveira *et al*., 2000; Sanches, 2006; Morgado, 2007; Donadon, 2009).

Thermal treatment has been applied postharvest to solve the problem of contamination by fungal diseases and insect infestation in fruit (Fawcett, 1922 *apud* Couey, 1989) or to reduce problems caused by low storage temperatures (Kluge *et al*., 2006). To this end, thermal treatments are performed prior to refrigeration, in the form of conditioning, or during refrigerated storage, in the form of intermittent warming. Thermal conditioning consists of exposing fruits briefly to moderate (15 to 25ºC) or high temperatures (37 to 53ºC) before putting them in refrigerated storage (Kluge *et al*., 2006).

Daiuto and Vieites (2008) conducted a study on Hass avocado to evaluate the polyphenol oxidase (PPO) and peroxidase (POD) content in unripe and ripe fruits hydrothermally treated at 45ºC for 10 min and stored at 9ºC (±1). The enzyme inactivation in ripe fruits subjected to the treatment was 78 to 94% compared to untreated fruits. Daiuto *et al*. (2010) evaluated the weight loss and respiratory rate of 'Hass' avocado by subjecting it to different physical treatments (thermal, UV and gamma radiation) and reported a decrease in the intensity of the fruit's respiratory peaks.

The evaluation of antioxidant capacity has become increasingly important to determine the effectiveness of natural antioxidants in protecting vegetable products against oxidative damage and loss of their commercial and nutritional value. Therefore, the present research focused on an evaluation of the antioxidant capacity and total phenolic content of 'Fuerte' avocado subjected to hydrothermal treatment.

2. Materials and Methods

'Fuerte' avocados were harvested carefully at the point of physiological maturation and according to their oil content. The fruits, which were selected with a view to uniform size, color and absence of injuries and defects, were hydrothermally treated in a water bath at 45°C for 5, 10, 15 and 20 min (four treatments), after which they were stored under refrigeration (10±1°C and 90±5% relative humidity). Fruits not subjected to the hydrothermal treatment were used as control. The fruits of these five treatments were evaluated at three-day intervals for two weeks.

Fruit extraction

The extraction process was performed with a solvent mixture of ethanol:water (80:20 v/v). Fruit extracts were obtained in triplicate. Aliquots of 3.0 g of pulp were weighed and placed in Falcon tubes, to which were added 30 ml of an ethanol:water mixture (80:20 v/v). The tubes containing pulp and solvent were then processed at room temperature in a Turrax crushing disperser for several minutes, and then centrifuged at 5000 X G for 15 min. The extracts were filtered and stored in dark vials at 8ºC for no longer than a week prior to analysis.

Total polyphenol analysis

The total phenolic content was determined by the Folin-Ciocalteu spectrophotometric method, as described by Singleton *et al*. (1999), using gallic acid as standard. An aliquot of 0.5 ml of the resulting extracts was then transferred to a test tube and 2.5 ml Folin-Ciocalteu reagent diluted in water 1:10 was added. The mixture was allowed to rest for 5 min, after which 2 ml of sodium carbonate 4% was added and the tubes were left to stand for 2 hr in the dark. The absorbance was measured in a spectrophotometer operating at a wavelength of 740 nm. A blank sample was subjected to the same procedure and conditions. The results are expressed in μg GAE/100 g^{-1} of dry weight.

DPPH radical scavenging activity

The radical scavenging activity was determined by DPPH method (Mensor *et al*., 2001). Tocopherol and BHT at a concentration of 90 μg ml^{-1} were used as standards. The reaction mixture consisted of 500 μl of fruit extract, 3.0 ml of ethanol 99%, and 300 μl of the DPPH radical in a solution of ethanol 0.5 mM, which was incubated for 45 min at room temperature in the dark. The negative control was prepared by replacing the volume of extract for an equal volume of the extraction solvent. A processing time of 45 min was defined after determining the half maximal effective concentration, Ec50. To determine the stabilization time, readings of the antioxidant in five concentrations (1, 2, 3, 4 and 5 g) were taken at 15-min intervals (Sanches-Moreno *et al*., 1998). According to Do Rufino *et al*. (2007), in subsequent experiments with the same fruit, readings can be limited to the previously established time (Ec50 time), accompanied by the initial reading of the control. The blank was prepared by substituting the volume of the DPPH solution for an equal volume of solvent.

The free radical scavenging activity was determined in the form of Antioxidant Activity (AA), using the equation: AA (%) = 100- [(Aa – Ab) x 100] / Ac, where: Aa = absorbance of the sample; Ab = absorbance of the blank; and Ac = absorbance of the negative control. All the analyses were performed in triplicate and accompanied by a control.

A variance analysis was performed using Tukey's test for multiple comparisons of the averages, at a significance level of 5%. The data were then subjected to a regression analysis and to Pearson's correlation for the two parameters evaluated, using the SAS version

9.2 software program.

3. Results and Discussion

Table 1 presents the average and standard deviation of total polyphenols identified in 'Fuerte' avocado. Although the four treatments produced similar results, a difference was detected as a function of storage time (p=0.007).

Total polyphenol content was higher in the control treatment. Mean values of 45.7, 47.0, 47.1 and 47.2 μg GAE/100 g^{-1}, respectively, were obtained in 5, 10, 15 and 20 min hydrothermal treatment, while the control showed 49.8 GAE/100g^{-1}. The lowest value obtained was 42.7 μg on the first day of analysis and the highest was 62.1 μg for the control treatment on the sixth day of analysis. The composition of phenolic compounds in fruit may be modified as a function of the environment and postharvest factors, including processing and storage. Processing and storage can induce prolonged enzymatic and chemical oxidation of phenolic compounds, contributing to their reduction (Kaur and Kapoor, 2002). Many studies have shown that phenolic compounds generally decrease in climacteric fruit such as tomatoes, bananas, mangos and guavas during ripening (Haard and Chism, 1996; Lakshimnarayana *et al*., 1970; Mitra and Baldwin, 1997; Selvaraj and Kumar, 1989).

The total phenolic content increased up to the sixth day of storage, decreasing thereafter due to the onset of senescence (Fig. 1). Daiuto *et al*. (2010) found that the average respiratory peak of 'Hass' avocado subjected to different physical treatments occurred on the ninth day of storage, after which senescence set in. This decrease can be attributed to a series of chemical and enzyme amendments that occur during the accelerated process of maturation of this fruit. These changes may include glycoside hydrolysis by glycosidases, phenol oxidation by phenoloxidases, and polymerization of free phenolic content (Robards *et al*., 1999).

The average antioxidant capacity measured in the treatments varied from 21.1% (20 min treatment) to 28.5% (control). The lowest value obtained was 17.6% on the first day of analysis and the highest was 67.6% on the twelfth day of analysis in the control treatment (Table 2). The overall average, taking into account the days of storage time, indicated an increase in antioxidant capacity.

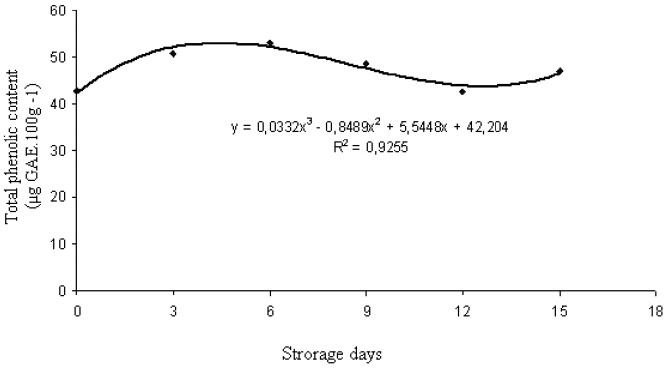


Fig. 1 - Total phenolic content (μg GAE/100g^{-1}) of hydrothermally-treated 'Fuerte' avocado (overall average of storage days).

Table 1 - Average and standard deviation of the total polyphenol content in hydrothermally-treated 'Fuerte' avocado as a function of treatment and storage day

	Storage days						
Treatments	0	3	6	9	12	15	Overall average per treatment
Control	42.7±2.7	41.8±2.7	62.1±20.5	54.2±1.3	36.5±9.3	61.7±3.5	49.85±13.2
5 min	42.7±2.7	51.8±12.8	42.3±13.5	52.9±15.4	42.7±3.7	42.2±6.0	45.75±9.9
10 min	42.7±2.7	58.5±2.7	48.0±4.6	48.8±6.5	44.1±7.5	39.8±15.1	47.05±9.5
15 min	42.7±2.7	51.6±5.1	58.4±9.1	41.4±15.8	42.8±4.5	45.6±6.0	47.15±9.4
20 min	42.7±2.7	49.3±1.7	54.6±10.1	45.3±6.8	45.6±2.9	45.4±7.9	47.25±6.5
Overall average per storage day	42.7B±2.7	50.6AB±8.8	53.1A±13.0	48.5AB±10.3	42.4B±6.1	46.9AB±10.8	

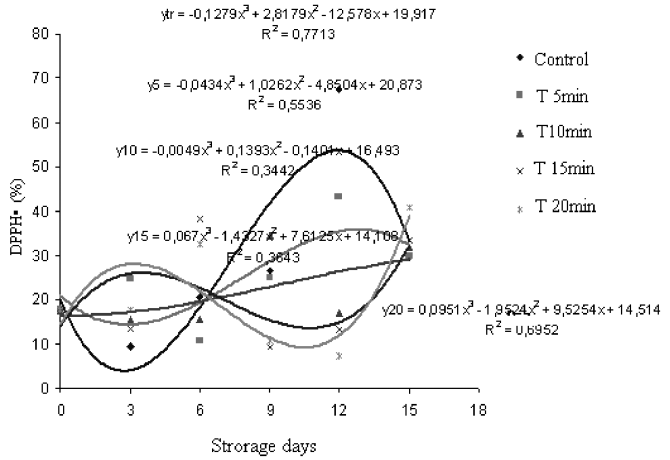
Upper case letters compare overall averages on each storage day

Table 2 - Average and standard deviation of antioxidant capacity of hydrothermally-treated 'Fuerte' avocado as a function of hydrothermal treatment and storage day

	Storage days						
Treatments	0	3	6	9	12	15	Overall average per treatment
Control	17.6aB±1.1	9.3aB±2.0	20.6abB±7.2	26.6aB±6.8	67.6aA±1.4	29.4aAB±3.6	28.5±19.5
5 min	17.6aB±1.1	24.7aAB±1.0	10.8bB±6.4	25.1aAB±9.7	43.2aA±33.6	29.6aAB±12.6	25.1±16.6
10 min	17.6aB±1.1	15.4abA±4.0	15.7abA±7.4	34.3aA±16.9	16.9bA±0.7	31.7aA±12.2	21.9±11.2
15 min	17.6aB±1.1	13.2aAB±4.3	38.4aA±5.4	9.3aB±4.5	13.2bAB±0.7	33.4aAB±8.4	20.9±13.4
20 min	17.6aB±1.1	17.7aAB±2.5	32.6AB±13.3	10.81B±3.8	7.2bB±4.8	40.9aA±34.6	21.1±17.8
Overall average per storage day	17.6±1.1	16.1±5.9	23.6±12.9	21.2±12.9	29.6±17.5	33.0±15.7	

Lower case letters compare averages per treatment per day.
Upper case letters compare averages of each treatment on each storage day.

Considering all the treatments, the highest antioxidant capacity was found in the control treatment, followed by the 5-min thermal treatment. Figure 2 shows increasing values of antioxidant capacity over storage time in the control and the 5- and 10-min hydrothermal treatments.



Legend: T = thermal treatment

Fig. 2 - DPPH antioxidant activity of hydrothermally-treated Fuerte avocado.

The highest percentages of antioxidant capacity were obtained in the fruit without hydrothermal treatment. The percentage of antioxidant capacity declined in thermally-treated fruits starting on the twelfth day of storage, possibly due to senescence.

The fruits thermally treated for 15 and 20 min showed values of 33.4 and 40.9%, respectively, after 15 days of storage. However, the values declined between the sixth and twelfth day of storage. This tendency for the percentage of antioxidant capacity to decrease may be a result of the thermal treatment, but the profile presented here with low values at nine and 12 days may be a consequence of the heterogeneity of fruit samples. This may explain the results of this research, which indicated that the antioxidant capacity of pulp thermally treated for 15 and 20 min increased up to the fifteenth day of storage. With a less intense respiratory peak, the degradation reactions were also diminished.

Arancibia-Avila *et al*. (2008) reported that total polyphenols, flavonoids and anthocyanins were significantly higher ($p < 0.05$) in ripe durian fruit than in unripe or overripe fruit (*Durio zibethinus* Murr., cv. Mon Thong).

The overall average antioxidant capacity during the storage period was 17.6, 16.1, 23.6, 21.2, 29.6, and 33.0% for the different treatments, indicating the tendency for antioxidant capacity to increase as the fruits ripened. The total phenolic content is not necessarily involved in the quantification of antioxidant activity (Jacóbo-Velasquéz and Cisneros-Zevallos, 2009). The correlation analysis of antioxidant capacity and phenolic compounds in Fuerte avocado did not reveal significant results ($p=0.992$ and $r=0.001$). Arancibia-Avila *et al*. (2008) found a correlation of 0.98 between the total phenolic content and antioxidant capacity of durian fruit. These authors concluded that the high polyphenol content was the main factor responsible for the fruit's antioxidant capacity. Wang *et al*. (2010) found a significant correlation between the total phenolic content and antioxidant capacity ($\geq = 0.79$) of avocados of different cultivars. The two parameters evaluated by these authors showed no correlation with the chlorophyll and carotenoids content ($r<0.1$). Furthermore, for these authors the high correlation found between procyanidins and the polyphenol content and antioxidant capacity suggests that this compound is the main polyphenol contributing to the antioxidant capacity of avocado. In the present research, the low correlation found for the evaluated parameters may indicate that another food metabolite is responsible for the antioxidant activity of avocado. It should be noted that vitamin E is a powerful antioxidant which may also contribute to the antioxidant capacity of avocado fruits.

In a study of the effect of heat treatment on the antioxidant capacity of vegetables, Melo *et al*. (2009) found that several events that occur during this treatment explain changes in the antioxidant activity of foods, which may be increased, reduced or unaltered. In situations in which the antioxidant activity of food increases, heat treatments favor the partial oxidation of the bioactive compound with the highest ability to donate a hydrogen atom to a radical starting from the hydroxyl group, and/or the aromatic structure of the polyphenol is more able to withstand the displacement of the unpaired electron around the ring. Moreover, heat treatments may favor the formation of new compounds such as Maillard reaction products (reductones), which exhibit antioxidant activity (Nicoli *et al*., 1999). Because refrigeration is the most efficient method for controlling fruit maturation, it may have contributed to maintaining the antioxidant capacity of the fruits during the storage period. The heat treatment had a negative effect on the maintenance of the fruit's antioxidant capacity compared to that of the control. The longer the fruit is exposed to a hydrothermal treatment, the higher the loss of its antioxidant capacity.

4. Conclusions

The total phenolic content increased up to the sixth day of storage, decreasing thereafter, without differences between the treatments. The antioxidant capacity of the control fruit and the fruit hydrothermally treated for 5 and 10 min increased throughout the storage period. The highest percentages of antioxidant capacity were obtained for the fruit without heat treatment. The percentage of antioxidant capacity of these treatments declined starting on day 12 of storage, possibly due to senescence. Hydrothermal treatments of 15 and 20 min delayed senescence, with antioxidant capacity continu-

ing to increase up to the fifth day of storage. No significant correlation was found between the antioxidant capacity and the content of phenolic compounds. The antioxidant capacity of ripe 'Fuerte' avocado was higher than that of unripe or senescent fruit.

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Influence of crop cycle and nitrogen fertilizer form on yield and nitrate content in different species of vegetables

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Abstract: Research was carried out in Latina province (Italy) on rocket, radish and zucchini grown under tunnel. Ten treatments, obtained by the factorial combination of two crop cycles (autumn-winter and winter-spring) and six nitrogen fertilizer forms (organic, organic-mineral, mineral in three modes, control with no nitrogen fertilization) were compared. The effects of these treatments were evaluated in terms of yield and nitrate content in the edible organs. In rocket, no significant difference in yield was detected between the autumn-winter and winter-spring crop cycles, although the former cycle resulted in a higher leaf nitrate content. The organic fertilizer treatment and the N-unfertilized control gave the lowest yields, but the mineral fertilizers caused the highest leaf nitrate accumulation. Radish yield did not vary between the two crop cycles, but the hypocotyl nitrate content was higher in the autumn-winter cycle. The crops fertilized with the two highest mineral supplies produced the highest yields, compared with the organic or organic-mineral treatments. In the autumn-winter crop, the mineral N fertilization resulted in the highest hypocotyl nitrate content, whereas in the winter-spring crop only the highest mineral N dose caused a higher nitrate content compared with the organic fertilizer. The highest zucchini yield was obtained from the winter-spring cycle at the two highest mineral fertilizer supplies. In the autumn-winter crop the highest mineral nitrogen dose resulted in the highest fruit nitrate content, while in winter-spring the two highest supplies caused this effect.

1. Introduction

An adequate supply of nitrogen fertilizers is generally needed to achieve high yield and quality performance of vegetable crops (Hochmuth, 1992), although a positive correlation between nitrogen availability and production has not always been shown (McCall and Willumsen, 1998). The ratio between nitric and ammonium nitrogen in N fertilizers also affects yield: in a study on zucchini, Chance *et al*. (1999) reported that a ratio between 1:0 and 1:3 is more effective than 3:1 in terms of production. On the other hand, an excessive supply of N fertilizer may have negative effects both on the quality of vegetables and on the environment (Beretta *et al*., 1990).

Excessive nitrogen fertilization may also result in nitrate accumulation, especially in leafy vegetables and a high food nitrate content is considered to be potentially dangerous to human health. This occurs because 5-10% of the ingested nitrate is reduced by bacterial enzymes in saliva and the gastrointestinal tract into the more toxic nitrite ion (Walters and Smith, 1981) which can, in turn, react with amines and amides giving rise to carcinogenic N-nitrose compounds (Hill, 1999). A positive correlation between drinking water nitrate levels and diabetes mellitus incidence in northern England was also reported (Parslow *et al*., 1997). However, some beneficial effects of nitrate on human health were also recognized (Duncan *et al*., 1997; Addiscott and Benjamin, 2000), while the high content in antioxidant compounds of some vegetables, like wild rocket (Martinez-Sanchez *et al*., 2008), can inhibit the formation of carcinogenic compounds (Steinmetz and Potter, 1991). In this respect, a contrasting report was published by Vermeer *et al*. (1998) who found that despite the low

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nitrate content of cauliflower, peas, beans and carrots, the presence of vitamin C and other antioxidants in the same vegetables could not prevent nitrosamine formation. Therefore, the nitrate content alone of vegetable foods cannot be considered as a quality indicator.

Genetic, environmental and cultural factors affect nitrate uptake and accumulation in plants (Bonasia *et al*., 2002). Nitrate in the soil is readily taken up by the plant where it is reduced to nitrite and ultimately converted into organic compounds by the nitrate-reductase enzyme complex, whose presence in the leaves increases in response to light and to nitrate availability in the soil (Guerrero *et al*., 1981). When the rate of nitrate uptake is faster than its assimilation rate, it is mainly accumulated in the plant cell vacuoles where is not toxic, differently from the ammonium ion (Crawford and Glass, 1998).

Nitrate accumulation rate depends on the vegetable species and on the plant organs, the highest concentrations being found in leafy/petiole species while lower nitrate contents are found in root/hypocotyl (Meah *et al*., 1994, Santamaria, 1999 b) or in fruit crops (Quince and Dvorak, 1980). The nitrate content also varies among cultivars within the same species: large differences were found in spinach (Cantliffe, 1972), celeriac (Delorez and Vulsteke, 1985), lettuce (Reinink *et al*., 1987), endive (Reinink *et al*., 1994) and carrot (Gutezeil and Fink, 1999).

In rocket, the nitrate content was found to increase under low solar radiation, as did the flavonoid content and antioxidant activity (Jin *et al*., 2009). However, when this crop is grown under high light intensity, more rapid plant growth requires high organic nitrogen availability, which consequently prevents nitrate accumulation. In fact, according to Padgett and Leonard (1993), organic nitrogen compounds can replace nitrate as osmolyte and adjust the plant nitrate absorption. Temperature also affects the uptake, translocation and assimilation of nitrate: nutrient solution heating stimulates nitrate absorption (Malorgio *et al*., 1995) but excessive air temperature favours its accumulation in the plant tissues (Behr and Wiebe, 1992).

As regards fertilization, ammonium and nitrate ions are the main nitrogen sources for plants. Even though nitrate assimilation is energetically rather expensive, it is usually the plant-preferred form (Salsac *et al*., 1987). In fact, a rapid ammonium uptake rate, exceeding its assimilation rate, would result in ammonium accumulation and toxicity (Maynard and Barker, 1969), while nitrate accumulation in plant tissues does not cause negative consequences. However, the preference between the two nitrogen forms depends on several factors: plant species, plant age, cultural method and the ratios between the concentrations of nitrogen/ammonium and other nutrients in the growth medium. In fact, while celery and fennel prove indifferent to the inorganic nitrogen form, chard is inhibited by ammonium nutrition (Santamaria *et al*., 1999 b). In lettuce, nitrate content can be reduced by distributing a part of the whole nitrogen supply as ammonia form, without changing the overall yield results (van der Boon *et al*., 1990) while in endive the exclusively ammonia form allows for no nitrate heads (Elia and Santamaria, 1997). Moreover, Rouphael and Colla (2005) reported that a greater nitrate content in zucchini was found in soil-grown plants compared to a soilless cultural system. In the latter context, it was found that interrupting the nitrogen supply at an advanced stage of the crop cycle, the fruit nitrate content was significantly reduced (Santamaria *et al*., 1998). This reduction appears to be related to the plant's ability to use the nitrate previously accumulated in vacuoles for protein synthesis (Blom-Zandstra and Lampe, 1983), in order to ensure growth when the substrate resources decrease (Koch *et al*., 1988). Finally, nitrapirine, a nitrification inhibitor, did not improve productive results in radish (Mills *et al*., 1976).

The present study was carried out to evaluate the effects of different nitrogen fertilization forms on yield and nitrate content of wild rocket, radish and zucchini in the Pontina plain (Latina, Italy), grown under tunnel in two cultural cycles, autumn-winter and winter-spring.

2. Materials and Methods

Wild rocket, radish and zucchini were grown on sandy soil (Table 1) in Fondi (Pontina plain, Latina province, Italy) in 2003-2004. The crops were grown under a thermal PE tunnel equipped with anti-freeze irrigation, activated at a temperature of 5°C. The structural unit was 7.20 m wide, 40.00 m long and 2.00 to 3.50 m tall, respectively, from wall to roof. Temperature and solar radiation values of the trial environment are reported in figure 1.

The experimental protocol was planned in order to compare 10 treatments, which originated from the factorial combination of two crop cycles (autumn-winter and winter-spring) with six nitrogen fertilizer forms (organic, organic-mineral, mineral in three modes, control not fertilized with nitrogen). For the treatment distribution within each crop cycle, a randomized block

Table 1 - Soil characteristics Fondi (Latina, Italy), 2003-2004

Soil characteristics		
Sand	%	86.00
Silt	%	4.00
Clay	%	10.00
Organic matter	%	2.06
Total nitrogen - Kjeldhal method	%	0.14
Available phosphorus - Olsen method	ppm	69.00
Available potassium - ammonium acetate method	ppm	245.00
Total lime	%	not detected
pH		6.40
Electrical conductivity (1:5) at 25°C	dS·m-1	0.74

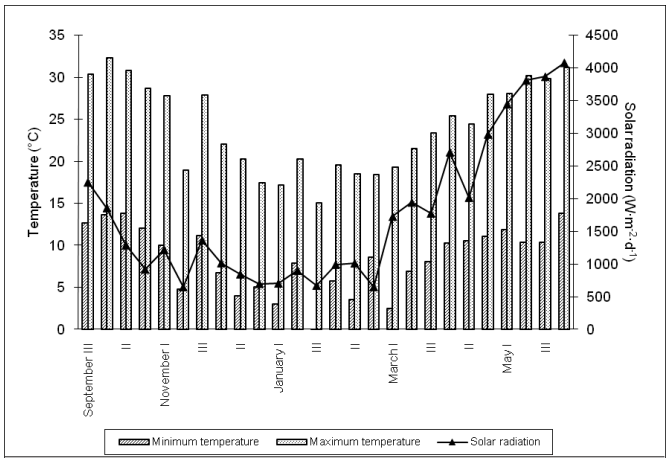

Fig. 1 - Trend of temperature and solar radiation. Fondi (Latina), 2003-2004.

design with three replicates was followed.

For each crop, the local standard agricultural practices were respected: plowing, hoeing, ridging, pre-planting chemical weed control, drip irrigation and parasite control. The fertilization plans were scheduled according to the average nutrient requirements of the crops (Tesi and Lenzi, 2005) and to the expected production levels.

Crops were harvested at midday in all of the plots and random representative samples of the edible plant parts were collected. The samples were transferred to the laboratory, where nitrogen content was determined by high-performance liquid chromatography (HPLC) using a Waters 600E chromatographic system, managed through the Millennium software version 3.05.01. The analytical column was a Dionex anion exchange column (model AS11, 4×250 mm, code P/N 044076) with a Dionex pre-column (4×50 mm, code P/N 044078). The eluent was 21 mmol l^{-1} NaOH at a flow rate of 1 ml min^{-1}. The detector was a Dionex pulsed electrochemical detector together with an anion self-regenerating suppressor.

Statistical data processing was performed by ANOVA, using the Duncan test for mean separation.

Wild rocket (Diplotaxis tenuifolia (L.) D.C.)

Pre-planting fertilization was applied with 50 kg·ha^{-1} of P_2O_5 from mineral superphosphate 18/20 and 150 of K_2O from potassium sulphate 48/50. With the exception of the N-unfertilized control, in all plots 90 kg·ha^{-1} of N were supplied as follows: organic N fertilization was applied at pre-planting as roasted leather; mixed organic-mineral N fertilization was supplied with 45 kg·ha^{-1} at planting from roasted leather and 45 kg·ha^{-1} at dressing from ammonium nitrate 26/27; mineral N fertilization was supplied as 25 kg·ha^{-1} at planting from ammonium sulphate 20/21 plus 65 kg·ha^{-1} from ammonium nitrate during the crop cycle. Organic-mineral N fertilization was applied in two phases, at six and three weeks before the expected second harvest, while the three mineral N treatments differed with respect to the application time at one, two or three weeks before the expected second harvest date.

Alveoli containing 10 plants each were transplanted on 25 September 2003 and on 15 January 2004 in the first and the second crop cycle, respectively. In plots of 12.60 m^2 (3.00 x 4.20 m) three ridges were made 0.40 m apart from each other, which included six rows with spacing 0.20 m, with a density of 215 plants per m^2.

Rocket was harvested in two phases, between 13 November and 9 January (2003-2004) in the first cycle and from 14 March to 28 April 2004 in the second cycle, according to the fertilization treatment. This crop was hand harvested by cutting the plants at about 2 cm above the soil surface when they reached 18 cm in height; crop weight was measured. At the second harvest, a random sample of 100 plants was collected in each plot and transferred to the laboratory for nitrate determination.

Radish (Raphanus sativus L. Subsp. parvus)

Radish cv. Suprella, a commonly grown variety in the experimental area, was sown on 24 November 2003 in the first crop cycle and on 2 March 2004 in the second. The plots had a 5.20 m^2 (2.60 x 2.00 m) surface area including 0.80 m wide beds, with 0.10 x 0.05 m spacing between plants.

Pre-planting fertilization was applied with 80 kg·ha^{-1} of P_2O_5 from mineral superphosphate 18/20 and 100 kg·ha^{-1} of K_2O from potassium sulphate 48/50. In addition, 100 kg·ha^{-1} N, corresponding to the crop requirement, was supplied to all plots with the exception of the N-unfertilized control: organic N fertilization was applied at pre-planting as roasted leather; mixed organic-mineral N fertilization was applied in two phases as 50 kg·ha^{-1} N as roasted leather at pre-planting plus 50 kg·ha^{-1} N as ammonium nitrate at 20 days after sowing. The mineral N fertilization treatments supplied 70, 100 or 130 kg·ha^{-1} N: one-half of the total N supply was given at pre-planting as ammonium sulphate while the remaining 50% was given as ammonium nitrate in two applications, at 12 and 24 days before the expected harvest date. Therefore, the range of mineral N fertilization treatments supplied the intermediate amount (100 kg·ha^{-1}) +/- a 30% increase or reduction.

Radish crops were harvested 16-22 January and 6-11 April in 2004, depending on the crop cycle and fertilization treatment. Radish plants were manually harvested when the hypocotyls reached the 30-40 mm caliber: weight, number and average weight data were recorded. Moreover, a sample of 100 units was collected in each plot and transferred to the laboratory for nitrate assessment.

Zucchini (Cucurbita pepo L.)

Zucchini cv. Velvia, a commonly grown variety in the experimental area, was used for this study. The plants were transplanted on 23 September 2003 in the

first cultural cycle and on 20 January 2004 in the second. Each plot was 35.10 m^2 (5.40 x 6.50 m) and plant density was 1.71 plants per m^2 (1.80 m between double rows and 0.65 m between plants along the row).

The fertilization doses were adjusted to the expected yields, which changed with the crop cycle: 60 kg·ha^{-1} of P_2O_5 from mineral superphosphate 18/20 and 240 of K_2O from potassium sulphate 48/50 were supplied at planting for the autumn-winter cycle; while 120 kg·ha^{-1} of P_2O_5 and 480 of K_2O were supplied at planting for the winter-spring cycle. In addition, nitrogen fertilization supplied 150 or 300 kg·ha^{-1} N for the autumn-winter or the winter-spring cycle, respectively. N fertilization was supplied as follows: organic N fertilization was applied at pre-planting as roasted leather; organic-mineral N fertilization was applied in two phases (50% at planting as roasted leather and 50% at dressing as ammonium nitrate). Mineral N fertilization treatments supplied 105, 150 and 195 kg·ha^{-1} or 210, 300 and 390 kg·ha^{-1} to the autumn-winter or winter-spring crop, respectively. One-third of the total N dose was applied at planting (35, 50, 65 kg·ha^{-1} as ammonium sulphate in the autumn-winter cycle or 70, 100, 130 kg·ha^{-1} in the winter-spring crop) while the remaining two-thirds of the total N dose was applied during the crop cycle (70, 100, 130 or 140, 200, 260 kg·ha^{-1} as ammonium nitrate). Therefore, the range of mineral N fertilization treatments supplied an intermediate rate (150 or 300 kg·ha^{-1}) +/- a 30% increase or reduction. The N fertilization at dressing was evenly distributed in three applications with a 21-day interval.

Fruits were hand harvested when the corolla was open, from 23 October to 22 December 2003, and from 29 March to 27 May 2004, depending on the crop cycle and fertilization treatment. Fruit weight, fruit number and average fruit weight were recorded. In addition, 10 days after the last fertilizer application a sample of 20 marketable fruits was collected from each plot and transferred to the laboratory for nitrate assessment.

3. Results

No significant difference in rocket yield was found between the autumn-winter and winter-spring crop cycles (data not shown). In the first cycle, however, the leaf nitrate content was as much as 64.3% higher than in the winter-spring.

Table 2 shows that in the autumn-winter cycle there was no yield difference between the mineral and mixed organic-mineral N fertilization forms. However, the mineral and organic-mineral treatments produced better results compared with the organic N fertilization and with the N-unfertilized control. In rocket, mineral N fertilization resulted in the highest leaf nitrate content compared with the other treatments, while the N-unfertilized control resulted in the lowest.

In the winter-spring cycle (Table 3), the production trend was similar to that observed in the autumn-winter cycle. However, organic-mineral fertilization did not produce significantly different results from the organic N fertilization treatment. The mineral and organic-min-

Table 2 - Wild rocket under tunnel: yield results and leaf nitrate content in the autumn-winter cycle, as influenced by nitrogen fertilizer form. Fondi (Latina, Italy), 2003-2004

Treatment	Marketable yield (t·ha^{-1})	Leaf nitrate content (mg·kg^{-1} of fresh weight)
Nitrogen fertilizer form		
Non-fertilized control	10.3 c	2706.7 c
Organic	13.9 b	3866.7 b
Organic-mineral	15.4 a	4240.3 b
Mineral 1: one week before the second harvest	16.0 a	5032.0 a
Mineral 2: two weeks before the second harvest	16.3 a	5150.0 a
Mineral 3: three weeks before the second harvest	16.4 a	5317.7 a

Means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

Table 3 - Wild rocket under tunnel: yield results and leaf nitrate content in the winter-spring cycle as influenced by nitrogen fertilizer form. Fondi (Latina, Italy), 2003-2004

Treatment	Marketable yield (t·ha^{-1})	Leaf nitrate content (mg·kg^{-1} of fresh weight)
Nitrogen fertilizer form		
Non-fertilized control	11.0 c	1626.0 c
Organic	15.1 b	2322.8 b
Organic-mineral	16.8 ab	2838.3 a
Mineral 1: one week before the second harvest	17.3 a	2842.2 a
Mineral 2: two weeks before the second harvest	17.4 a	3083.3 a
Mineral 3: three weeks before the second harvest	17.6 a	3300.0 a

Means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

eral N fertilization treatments resulted in higher leaf nitrate content compared with the organic N fertilizer application or with the N-unfertilized control.

Radish yield did not vary between the two crop cycles: the hypocotyl number per unit surface area and their average weight were unaffected by the crop cycle factor (Table 4). In contrast, N fertilizer form significantly affected the edible organ production: mineral fertilization at the two highest doses was more effective than the organic and the organic-mineral N fertilizer forms. The organic N supply had better effects on yield, compared to N-unfertilized control, but it was less effective than the other treatments. These results were mainly affected by the average hypocotyl weight, which changed significantly in response to the different N fertilization forms while only the unfertilized control resulted in a reduced hypocotyl number.

Table 5 - Radish under tunnel: hypocotyl nitrate content as a function of crop cycle and nitrogen fertilizer form. Fondi (Latina, Italy), 2003-2004

Treatment	Nitrate content (mg·kg^{-1} of fresh weight)	
	Autumn-winter	Winter-spring
Nitrogen fertilizer form		
Non-fertilized control	1020.3 d	478.3 c
Organic	1229.7 c	1038.3 b
Organic-mineral	1431.7 b	1258.3 ab
Mineral 1: 70 kg·ha^{-1}	1670.0 a	1311.7 ab
Mineral 2: 100 kg·ha^{-1}	1697.0 a	1357.3 ab
Mineral 3: 130 kg·ha^{-1}	1743.0 a	1571.7 a

Means followed by different letters are significantly different according to the Duncan test at p≤0.05.

ized control, whereas in the autumn-winter cycle a 71% increase was recorded between the same two treatments.

Table 4 - Radish under tunnel: hypocotyl yield as a function of crop cycle and nitrogen fertilizer form. Fondi (Latina, Italy), 2003-2004

Treatment	Marketable hypocotyl yield		
	Weight (t·ha^{-1})	No. per m^2	Mean weight (g)
Crop cycle			
Autumn-winter	26.9	139.9	19.2
Winter-spring	27.4	137.0	20.0
	NS	NS	NS
Nitrogen fertilizer form			
Non-fertilized control	20.8 D	128.2 B	16.2 E
Organic	25.4 C	138.0 A	18.4 D
Organic-mineral	27.9 B	140.0 A	20.0 C
Mineral 1: 70 kg·ha^{-1}	28.5 AB	140.2 A	20.4 BC
Mineral 2: 100 kg·ha^{-1}	30.0 A	142.2 A	21.1 AB
Mineral 3: 130 kg·ha^{-1}	30.5 A	142.3 A	21.5 A

Means followed by different letters are significantly different according to the Duncan test at p≤0.05.

Similarly to rocket, also in radish (Table 5) nitrate content was higher in the autumn-winter cycle, on average as much as 25.3% compared to the winter-spring cycle. In the autumn-winter cycle, leaf nitrate content was higher in response to organic-mineral and mineral N fertilization. Instead, during the winter-spring cycle the highest mineral nitrogen supply caused a nitrate concentration increase only in comparison with the organic form. Moreover, in the winter-spring cycle, the maximum mineral fertilizer dose (130 kg·ha^{-1} equally distributed before and after planting) resulted in a three-times higher leaf nitrate content compared with the unfertil-

In both crop cycles, the highest zucchini production was obtained with the two highest mineral fertilizer supplies (Tables 6 and 7). However, in the autumn-winter cycle (Table 6) the intermediate dose was not different from the lowest one and the latter was as effective as the organic-mineral treatment in both crop cycles. The control treatment resulted in lower fruit number and average weight while the mineral N fertilization produced the highest values.

The winter-spring yield (Table 6 and 7) was more than double the autumn-winter yield, as a consequence of the increased fruit number (+100%), while their

Table 6 - Zucchini under tunnel: yield results and fruit nitrate content in the autumn-winter cycle as a function of nitrogen fertilizer form. Fondi (Latina, Italy), 2003-2004

Treatment	Marketable fruit yield			Fruit nitrate content (mg·kg^{-1} of fresh weight)
	Weight (t·ha^{-1})	No. per plant	Mean weight (g)	
Nitrogen fertilizer form				
Non-fertilized control	13.8 e	9.3 c	87.1 c	496.7 e
Organic	19.9 d	12.0 b	97.5 b	638.7 d
Organic-mineral	23.1 c	13.0 ab	103.8 ab	723.7 d
Mineral 1: 105 kg·ha^{-1}	23.9 bc	13.3 ab	105.7 ab	850.0 c
Mineral 2: 150 kg·ha^{-1}	25.8 ab	14.0 a	107.5 a	1022.0 b
Mineral 3: 195 kg·ha^{-1}	26.9 a	14.3 a	110.6 a	1186.0 a

Means followed by different letters are significantly different according to the Duncan test at p≤0.05.

Table 7 - Zucchini under tunnel: yield results and fruit nitrate content in the winter-spring cycle as a function of nitrogen fertilizer form. Fondi (Latina, Italy), 2003-2004

Treatment	Marketable fruit yield			Fruit nitrate content ($mg \cdot kg^{-1}$ of fresh weight)
	Weight ($t \cdot ha^{-1}$)	No. per plant	Mean weight (g)	
Nitrogen fertilizer form				
Non-fertilized control	30.3 d	19.3 c	91.3 d	331.7 c
Organic	39.9 c	23.3 b	100.3 c	426.7 bc
Organic-mineral	47.0 b	25.7 ab	107.6 b	443.0 bc
Mineral 1: 210 $kg \cdot ha^{-1}$	48.3 b	25.7 ab	109.7 ab	536.3 ab
Mineral 2: 300 $kg \cdot ha^{-1}$	54.5 a	28.0 a	113.6 ab	606.0 a
Mineral 3: 390 $kg \cdot ha^{-1}$	55.0 a	28.0 a	115.5 a	678.3 a

Means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

average weight was not significantly different.

In the autumn-winter cycle (Table 6), the maximum mineral N rate and the unfertilized control resulted in the highest and lowest fruit nitrate concentrations (1186 vs 497 $mg \cdot kg^{-1}$), respectively. Furthermore, no difference was detected between the organic and organic-mineral N fertilization treatments. In the winter-spring cycle (Table 7), the mineral N treatments led to a greater fruit nitrate accumulation compared with the control and the organic N fertilization treatment.

4. Discussion and Conclusions

In the present investigation rocket and radish did not show yield differences between the autumn-winter and winter-spring cycles. This is presumably due to the light and temperature requirements of these crops which allow for an equally good production in both seasons. In fact, the two crop periods are quite similar in terms of duration and day-length, though the latter decreases from the beginning to the end of the autumn-winter cycle while the opposite trend occurs in winter-spring. Our results are in accordance with those reported by Inada and Yabumoto (1989) who found that growth of a radish crop was promoted by increasing day-length, but it was less sensitive to variations in temperature regime. Differently from rocket and radish, zucchini yield was affected by the cultural cycle, as the winter-spring cycle resulted in a higher fruit production compared with the autumn-winter cycle. Presumably, the increasing day-length, light intensity and temperature of the second part of the winter-spring cycle played a crucial role in improving crop productivity. Similar results were reported by Rouphael and Colla (2005).

As regards N fertilization, the wild rocket yield was favourably affected by the mineral fertilizer treatment as well as the organic-mineral treatment. A contrasting report was published by Cavarianni *et al*. (2008) who found that an increasing mineral N supply caused the rocket yield to decrease.

Differently from rocket, radish showed a positive response to nitrogen increase, both in organic-mineral and in mineral form. Guven (2002) reported that radish yield benefited from an increase of inorganic nitrogen supply, while Fuke *et al*. (2000) reported a better effect of the mixed organic-mineral fertilization compared with the exclusively mineral treatment. In the present work, the organic N fertilization was less effective on radish production than the other examined treatments, although it produced better results than the unfertilized control. Yield was influenced almost exclusively by the hypocotyl average weight, which gradually decreased from the highest nitrogen rate to the unfertilized control. The number of marketable edible organs was conditioned instead only by the absence of N in the control treatment, which resulted in 9% deformed or undersized hypocotyls. The zucchini yield in both crop cycles was better affected by mineral nitrogen fertilization achieved with the intermediate or 30% increased rate, compared to the organic-mineral or organic fertilization forms. Nevertheless, in the autumn-winter cycle the intermediate mineral supply did not produce better yield results than the 30% reduced treatment. Zotarelli *et al*. (2008) reported that, in the same cultural cycle, a 50% increased N supply, compared to the crop requirement, did not modify zucchini yield but a 50% decrease was less effective. Moreover, the reduced mineral nitrogen application did not result in a better production than the organic-mineral supply while, as recorded also for wild rocket and radish, the organic N treatment accelerated zucchini plant development only compared to the control. However, it should be stressed that the lower yield in the organic N fertilization treatments resulted from a combination of the production factors. In fact, these treatments did not affect the fruit emission rate nor their size. Termine *et al*. (1987) reported that organic N fertilization did not condition the production level of leek and turnip.

In this study the tested species showed a different attitude toward accumulation of nitrate in their edible organs. In particular, rocket was the only crop that in the autumn-winter cycle displayed nitrate levels above the highest EC regulation limits (4500 mg of $NO_3^- \cdot kg^{-1}$ of fresh weight), confirming also the influence of cultivation time on vegetable nitrate content. A clear tendency to accumulate more nitrates in the autumn than in the spring was shown, the latter being characterized by low cloud cover and increasing photoperiod. This is in agreement with the reports of Rouphael and Colla

(2005) on zucchini and Elia *et al*. (1997) on broccoli rab grown in growth chamber and subjected to additional lighting. In the latter case, the leaf blade nitrate reduction was a result of nitrate-decreased absorption rather than its assimilation increase. In fact, in spinach (Steingröver *et al*., 1986 b), parallel to the leaf blade nitrate reduction, the net nitric ion adsorption was reduced while the nitrate-reductase activity did not change except at the end of the night period. This effect would not result from direct light inhibition, but from an adsorption feedback regulation achieved by the amino acids formed in the leaf blades and then transported to the roots. Moreover, it was found that under low solar radiation, typical of the winter season, the nitrate content is high even with a low nitrogen supply, whereas with high solar radiation the ion concentration increases only by supplying nitrogen (Roorda van Eysinga and van der Meijs, 1985; Santamaria *et al*., 1999 a). This indicates that solar radiation intensity and photoperiod length regulate the nitrate-reductase activity, the enzyme which reduces nitrate to nitrous ion (Steingröver *et al*., 1986 a). In particular, the solar radiation effect is multiple, as it gives input to the nitrate-reductase synthesis and induction, supplying also the reducing power (NADH) through photosynthesis (Behr and Wiebe, 1992). It is believed that in the vacuole, nitrate acts as the cell turgor osmotic regulation, as an alternative to sugars and organic acids, poorly synthesized under low radiation conditions (Blom-Zandstra and Lampe, 1985).

Plant nitrate concentration is known to be also subject to the available nitrogen amount and quality (Citak and Sonmez, 2010), and this explains why the unfertilized control plants always exhibited the lowest tissue nitrate levels. Only in the zucchini fruits harvested in spring, values were not different between the control and organic or mixed treatments, confirming this species' lower attitude. On the contrary, wild rocket displayed a remarkable tendency to accumulate nitrates in the aerial apparatus: in the unfertilized plants, a nearly triple (2.9) and more than five-fold (5.2) content was detected, compared with the corresponding control of radish and zucchini, respectively. According to Quinche and Dvorak (1980), the latter accumulate less nitrate because they receive organic nitrogen mainly through the phloem, which does not carry inorganic nitrogen forms. In other studies, rocket was reported to accumulate high nitrate levels both under reduced nitrogen availability (Bianco *et al*., 1998; Santamaria *et al*., l999 a) or increased supply (Santamaria *et al*., 2002).

Organic fertilization, compared to the other supply forms, caused the lowest nitrate content in the edible organs; it was only higher than the unfertilized control (as much as 29% in zucchini and 51% in radish on average). Similar results were reported for lettuce (Stopes *et al*., 1989) and radish (Ebid *et al*., 2008) crops fertilized with composted manure. The organic-mineral treatment resulted in nitrate content increases between 41% in zucchini and 80% in radish compared with unfertilized plants. Therefore, even in such circumstance zucchini demonstrated to be a refractory species to nitrate accumulation. The mineral nitrogen fertilization caused the highest nitrate content in the edible organs: compared to the non-fertilized control, the increase was +90.2, +108.0 and +96.3% on average, respectively in rocket, radish and zucchini. Santamaria *et al*. (1993) reported that mineral nitrogen caused a higher nitrate accumulation than the organic-mineral form in spinach. Nevertheless, in the autumn-winter cycle a 30% reduction of the mineral nitrogen dose resulted in a lower nitrate accumulation, compared to the maximum and to the intermediate supply, respectively in radish and in zucchini. Previously, other researchers found a direct relationship between the nitrogen rate supplied and the vegetable nitrate content (Maynard *et al*., 1976; Tesi *et al*., 1995; Cavarianni *et al*., 2008).

With regard to the time of fertilizer supply, in rocket, in both crop cycles, the latest N dressing application (one week before harvest) did not cause a higher leaf nitrate accumulation than the earliest one. Opposite findings were reported by Graifenberg *et al*. (1990) for lettuce: leaf nitrate content increased with the reduction of the interval between nitrogen fertilizer application and harvest.

In conclusion, the studies carried out in the Pontina plain (Latina, Italy) under tunnel, in 2003-2004, showed that the cultivation time produced a significant effect only on zucchini yield, which was better affected by the winter-spring cycle. Moreover, the autumn-winter crops exhibited higher nitrate content in the edible organs. As for the fertilization treatments, in rocket N fertilization in organic-mineral form was the most appropriate, as it gave yield values as high as the exclusively inorganic supply. In addition, the organic-mineral fertilizer resulted in a lower leaf nitrate accumulation in the autumn-winter cycle, which did not exceed the EC threshold. In contrast, in radish and zucchini the two mineral supplies corresponding to nitrogen crop requirement or to its 30% increase produced the best yield results. Moreover, they also caused the highest radish hypocotyl nitrate content, which was however much lower than the limits set by some European countries for vegetable trade (Santamaria, 2006).

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The status of Chestnut cultivation and utilization in the Canary Islands

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Key words: *Castanea sativa*, food consumption, timber.

Abstract: Chestnut was introduced to the Canary Islands at the beginning of the 16th century during the time of the Spanish Conquest. It was utilised by the conquerors as a means of claiming property for communal lands. From that time until today, chestnut has been an important crop in the Canary Islands. It is important both as a source of food and timber and has contributed to the subsistence of the population, particularly at times when both resources were scarce. Nowadays it is mainly cultivated on the Islands of Tenerife (around 1300 ha) and La Palma. On other islands, such as Gran Canaria, La Gomera and El Hierro, semi-wild chestnut forests and small plots where farmers collect fruit still exist. Morphological and molecular marker (SSRs) studies have shown a great variability within the local population of chestnut trees in the Canaries. The main use for chestnut is fruit consumption, but it was also utilised in the past as an exchange commodity to obtain fish and other food. The fruit is consumed in many different ways, mainly toasted or roasted, but also cooked in soups, fish or meat dishes and even as an ingredient for the typical Canary Islands' sweet 'morcillas' (a type of sausage). The wood of the tree has been used for furniture, with some shoots being utilised for basket making, and also as cattle food. The trunk of the chestnut has also been used to obtain cork or as a bee hive. Recent efforts to add value to chestnut cultivation in the Canary Islands have included the creation of a Chestnut Farmers Association in Tenerife that commercializes their products under a brand name.

1. Historical background

According to recent studies, chestnut was first introduced to the Canary Islands on El Hierro and Gomera and then on Gran Canaria, La Palma and finally the Island of Tenerife, shortly after the process of conquest and colonization of the islands. In the case of La Palma, it was most likely introduced around 1493 and quickly expanded and used both for its fruit and wood (Ríos Mesa, 2004). In fact, there is evidence that 5 'arrobas' (a measure of weight, 1 arroba = 11.502 kg) of fruits were sent from La Palma to the island of El Hierro in 1546 (Hernández-Martín, 1999), which seems to indicate the presence of numerous chestnut trees to allow for surplus production. By 1590 Frutuoso (2004) had mentioned the existence of chestnuts in the region of Puntallana, and there are also many other reports about the presence of chestnut early after the conquest of the islands (Hernández-Rodríguez, 1983; Browne, 2005).

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Many ancient trees are still alive on La Palma at present. Two are especially important: one located in Puntallana with circumference of more than 7 m, and another even larger one with a perimeter of 10 m in the Municipality of Breña Alta. According to the information gathered from the local population, both of them seem to be of the denomination known as "*Temprano*", one of the few varietal denominations identical to those of Spain's mainland cultivars, which might be a clear indication of its early introduction.

The great relevance of the chestnut tree on La Palma is reflected in the information given by Bandini (1816) in 1813, indicating that 46% of the total production of chestnuts in the Canary Islands (CI) came from that island. Information provided by Von Fritsch (2006) on exports of chestnut fruits in 1862 from different areas of La Palma also confirms the importance of this crop for the island. The role of this tree as part of the landscape of La Palma was also highlighted in school texts used at the end of the 19th century (de Las Casas Pestana, 1894). It is also important to mention that the first reference to grafting of chestnuts on La Palma was

made during the last quarter of the 18th century (Rodríguez-Benítez, 2004).

There are also historical references to the presence of chestnut trees on Tenerife as early as 1517 (Pereira-Lorenzo *et al*., 2007), soon after the Conquest. Many historical chestnut trees reported previously still survive on this island, such as the 'Castaño de Las Siete Pernadas', in the area of Aguamansa (Fig. 1), La Orotava county (Mendez Pérez, 2002), which may be around 500 years old. There is an interesting description of this tree given by Rodríguez (2001) in which he indicates that the trunk had a circumference of more than 12 m and that the seven branches of its trunk (siete pernadas) had been reduced to five by wind action. According to Ríos-Mesa (2004) it is possible that this tree was one of the chestnut trees planted by El Adelantado (name given to the first Governor of the island), D. Alonso Fernández de Lugo, in the Valley of La Orotava in the first decades of the 16th century.

Many well known visitors and naturalists, among them Mac-Gregor (2005), Verneau (2003), Von Humboldt (2005), Du Cane (1993), Glass (1982), Von Fritsch (2006) and Berthelot (2005), mentioned the presence of chestnut forests as part of the natural landscape of Tenerife in their Canary Island trip reports during 18th and 19th centuries.

Fig. 1 - Ancient chestnut tree with 7 main branches named castaño del las 7 pernada.

2. Economic importance, cultivated area and geographical distribution

Chestnut seems to have been an important crop in the middle of the 19th century according to the great number of localities in the Canary Islands producing chestnuts by 1852 (De Leon y Falcón, 2005).

On Tenerife: Arafo, Candelaria, La Matanza, La Orotava, El Rosario, Santa Ursula.

On Gran Canaria: Galdar, Moya, San Mateo, Telde, Teror.

On La Palma: Barlovento, Punta Gorda, Punta Llana, San Andres y Sauces, Santa Cruz de la Palma.

On Lanzarote: Tinajo.

On La Gomera: Vallehermoso.

There are no updated figures on the economic importance of chestnut cultivation at a regional level at present. A thorough sampling done by Pereira-Lorenzo *et al*. (2001) indicated that chestnut could be introduced in the Archipelago as a crop with a double use (fruit and wood) following the example of the Spanish mainland from where it was introduced. This study also revealed the presence of isolated, testimonial chestnut trees on the drier eastern islands of Lanzarote and Fuerteventura.

Chestnut fruit is nowadays almost exclusively commercialized in a few areas on Tenerife and La Palma. Recent efforts were made to increase the value of chestnut in the Canary Islands by the creation of a Chestnut Farmers Association on Tenerife to market all their products through a brand name. They even differentiate chestnuts harvested in the south one month earlier than those in the north due to microclimate differences, and which usually obtain the highest prices of the year.

Elorrieta (1949) indicated the presence of chestnut on all the western islands of the Canaries but he did not report it as a crop. A recent detailed study carried out in 2006 and 2007 by Hernández *et al*. (2010) localised chestnut trees in 28 counties on the Island of Tenerife, 23 of them with chestnut orchards. According to this study, the cultivated area of chestnut on Tenerife is 1374 ha, with the biggest cultivated area (280 ha) located in La Orotava County. In addition 2,567 isolated trees were also found on the island of Tenerife. Chestnut trees on Tenerife are found between 400 and 1,800 m altitude in the southern part of the island (Granadilla County). Most of the area (647 ha) is located between 400 and 1000 m above sea level, and diminishes progressively after this altitude. Only some isolated chestnut trees can be found below 400 m above sea level in the northern county of La Laguna.

Chestnut distribution is different in north and south portions of Tenerife. The higher rainfall and humidity on the north slopes due to trade winds, in contrast with a lack of the beneficial influence of them on the south side, explains why chestnut cultivation has been developed at higher altitudes in this latter area than on the north side (Ríos-Mesa, 2004; Hernández *et al*., 2010).

In northern Tenerife the area covered by chestnut totals 1,121.72 ha (81.6% of total) and increases constantly from 400 m to 1,000 m above sea level, with a minimum area between 1,000 and 1,200 m. Chestnut orchards are located mainly in the Orotava Valley and Tacoronte-Acentejo County. Most of the 112 ha cultivated in the south are located in the Guimar Valley, more favoured by trade winds than other southern

counties, between 800 and 1,000 m and only 5.6 ha are cultivated between 600 and 800 m. Another 118 ha are located between 1,000 and 1,400 m altitude, most of them also in the Guimar Valley, being a merely testimonial presence of chestnuts at higher altitude (Fig. 2).

Fig. 2 - Chestnut trees in The south of Tenerife.

Three different systems of chestnut cultivation can be found on Tenerife. There is a first band at lower altitudes with low density plantings where chestnuts are associated with mixed plantings of vegetables and other fruit trees; a second band with more dense plantings where the lack of light penetration does not allow association with other crops; and the last band occurs at higher altitudes where chestnuts are largely associated with endemic fayal-brezal (*Myrica faya* and *Erica arborea*) or even Pinus (*Pinus canariensis*) populations that, in many instances, constitute a part of the native forest and cannot be considered cultivated trees (Ríos-Mesa, 2004).

Chestnut is more uniformly distributed on the Island of La Palma with the maximum concentration of chestnut trees being on the west side of the Cumbre Nueva area in Santa Cruz de La Palma and Breña Alta counties, where there is more humidity, also in this case, due to an influence of trade wind; on the north and east sides of the island chestnut is more important at middle altitudes. On the drier south and west sides of the island, chestnut as well as other temperate fruit crops is less present and located at higher altitudes. As in the case of Tenerife, chestnut is associated with the native vegetation at higher elevations, with laurisilva and fayal-brezal in the north and east and pine forest in the west. Frequent fires, the most serious of which occurred in August 2009, have caused the death of many chestnut trees or, on occasion, destruction of the grafted portion of the tree and regrowth of the rootstock (Pereira-Lorenzo *et al*., 2007).

Cultivation of chestnut on Gran Canaria was important years ago but is now only testimonial with isolated plots in the counties of Arucas, Firgas, Valleseco and Teror (Naranjo-Rodríguez and Escobio-García, 2002). Most chestnut trees on the island of El Hierro are located in the most humid and cooler areas of El Golfo, Tiñor, Asofa and Honduras. Chestnut trees are also found on La Gomera in the higher parts of the valleys of the north where they are cultivated on mountain slopes. Scarce, isolated trees on Lanzarote are found in the area affected by the Timanfaya volcanic eruptions (1730-1736) which may suggest a later introduction on this island and, as in the case of the other dry island of Fuerteventura, a lack of appropriate climatic conditions for chestnut development (Pereira-Lorenzo *et al*., 2007).

3. Horticultural aspects

Around 1852, De León y Falcón (2005) indicated that chestnuts showed excellent growth on the CI at middle and higher altitudes in soils with a predominant clay, propagated either by seeds or from shoots emerging from their roots, with grafting seldom being practiced.

In the case of Tenerife, chestnuts are located in highly fertile acid soils, in most cases andisoles and alfisoles, and cultivated, whenever orography and planting density allow, together with other crops such as potato, rye, corn or others (Ríos-Mesa, 2004).

Only around 430 ha (31%) of the total surface covered by chestnut on the island of Tenerife can be considered to be under good conditions of cultivation and 138 of these, distributed in different counties of the island, can be considered well managed. About 670 ha (48.7% of the total), concentrated mostly on the south side, are in a clear state of abandonment (Hernández *et al*., 2010).

In Tenerife, extensive areas previously devoted to chestnut cultivation have been abandoned in recent years, although in many cases new trees have grown from seeds among the old grafted trees. These new plants are called chestnut machos or ladrones (males or thieves). The only cultivation practice carried out in these places consists of possible cleaning of the soil surface to facilitate harvesting of early chestnut fruits, perhaps being the only regular horticultural practice undertaken for chestnut cultivation in the different cultivated areas of the Islands.

Plant spacing differs greatly from place to place but it is possible to consider three main systems (Ríos-Mesa, 2004; Hernandez *et al*., 2010).

1 - Trees planted at high density (30-50 trees/ha) in places where chestnuts were planted for fruit and that correspond with the areas now in a state of abandonment.

2 - Planted at lower density (15-30 trees/ha) allow-

ing intercropping with associated crops.

3 - Chestnut trees (10-15 trees/ha) planted only along the edges of plots used for cultivation of other crops such as potatoes, grapes, corn, cabbage, lupines or other vegetables (Figs. 3 and. 4).

All chestnuts are cultivated without irrigation and no fertilisers are applied, but in cases 2 and 3 above they benefit from the fertilisers applied to the main associated crop. Grafting on Tenerife (Fig. 5) was done in a similar way to that on the Spanish mainland, where seedling rootstocks were grafted with two scions placed at 0.5 m height. However peculiarly shaped shrubby chestnuts found in the volcanic south of the island with large pendulous branches touching the soil indicate that this practice was not always carried out.

Maintenance pruning is seldom practiced except in the case of planting system 3 indicated above. Even in this case pruning is scarce and rudimentary, mostly limited to the removal of dead wood or wood that

Fig. 3 - Chestnut-corn association.

Fig. 4 - Chestnut-grape Association.

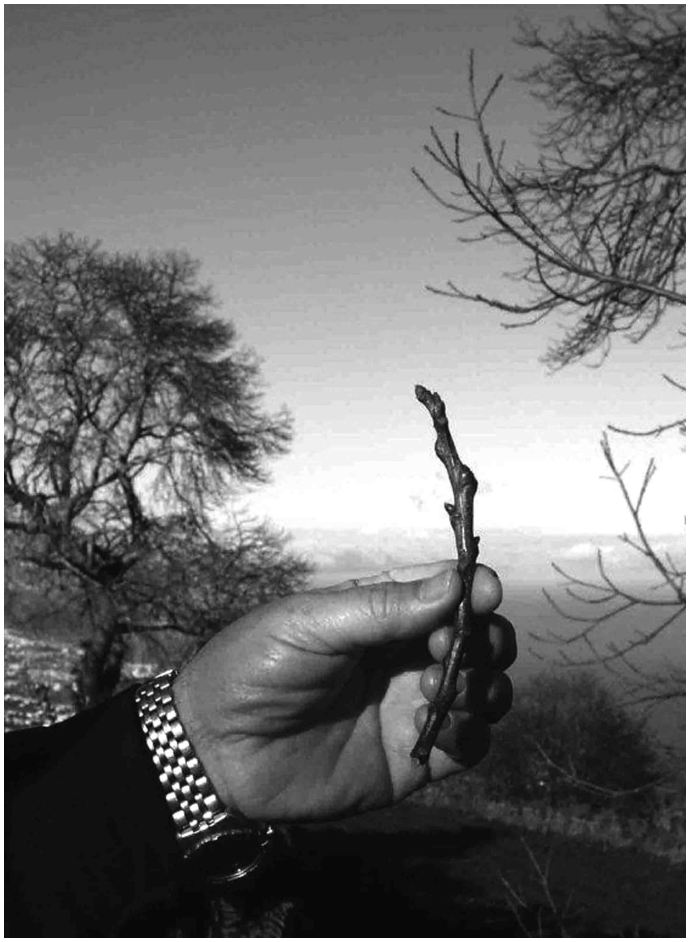

Fig. 5 - Scionwood for grafting.

makes the cultivation of the rest of the plot more difficult. There is, however, an important exception in the case of the trees cultivated on volcanic lapilli soils in the counties of Arafo (Tenerife) and El Paso (La Palma). In both places, grafting is practiced closer to the soil with a training process orientated at keeping branches as close to the soil as possible to facilitate harvest and to obtain wind protection. Trees are also protected against wind on the Island of El Hierro where a circular wall of rocks, locally called gorona, is built to protect the tree from wind and animals. Chestnuts on the CI are in good phytosanitary condition, with no reports of the serious diseases which usually affect this plant on the Spanish mainland, such as *Phytophthora cambivora* and *Phytophthora cinnamomi*. This is most likely due to the isolation of the islands from other chestnut areas and no introduction of new plant material for new plantings. Several arthropods, such as *Cydia splendana* and *Balaninus elephas* that attack chestnut fruits in the Archipelago, are being studied (Pereira-Lorenzo *et al*., 2007).

4. Plant material

Efforts made to localise and identify the plant material of chestnuts on the Canary Islands have only

recently been made through extensive samplings and collecting of material during field visits to interview farmers in the producing areas: on Tenerife this was carried out between 2000 and 2004; on La Palma between 2001 and 2004; on El Hierro in 2001; and on La Gomera and Gran Canaria in 2003. The main objectives of these surveys were to finalise the Chestnut Cultivar Spanish Inventory, realise the morphological and molecular characterization of CI chestnut plant material, to propose the material to be kept in a germplasm bank and to define the most interesting cultivars for commercial cultivation (Pereira-Lorenzo *et al*., 2007). A total of 47 different varietal nominations, according to the names given by farmers, with one or several accessions, were found on the CI (21 on Tenerife, 17 on La Palma, six on Gran Canaria, two on El Hierro and one on La Gomera) coinciding on many occasions with the same names on different islands like '*Blanco*' (White) on La Palma and El Hierro, '*Mollar*' (freestone) on Tenerife, La Palma, Gran Canaria, Gomera and El Hierro, and '*Negro*' (white), '*Picudo*' (Pointed), '*Manso*' (Tame) and *'Temprano'* (early) on Tenerife and La Palma.

Some new plant materials have been found in recent investigations carried out by the same authors (unpublished data): '*Piñuda*' (with the shape of a pine-cone), '*Menuda*' (small) and '*Merina*' (as the sheep race) on Tenerife; '*Colorado*' (Red) and '*Arrancado*' (pulled out) on La Palma; and '*Chabetudo*' (unknown meaning) on Gran Canaria.

The 39 accessions found on Tenerife correspond to 21 varietal nominations (Fig. 6), '*Mulato*' (Mulatto) with seven accessions being the most common. The 34 accessions of La Palma correspond to 17 varietal nominations, '*Jabudo*' (unknown meaning) with six accessions being the most common. From all the varietal nominations of these two islands, only '*Redondo*' (Round) and '*Temprano*' coincided with names of cultivars from the Spanish mainland, the former in Galicia and the latter in Andalucía and Extremadura.

Fig. 6 - Fruits of Castagrande cultivar.

Only the plant material from Tenerife and La Palma has been characterised morphologically and phonologically, using the same methodology as for previous studies of the cultivars of Galicia on mainland Spain and allowing a comparison among them (Pereira-Lorenzo *et al*., 1996 a, b, c; Pereira-Lorenzo and Fernández-López, 1997 b, c; Pereira-Lorenzo *et al*., 2006).

Analogous molecular characterizations of plant material from Tenerife and La Palma have also been made following the same methodology as for the cultivars of Galicia, comprising the results obtained from 10 microsatellites being utilised in the research Project "Evaluation, analyses and biodiversity management of *Castanea sativa* Mill. (European chestnut) in the Atlantic regions (CASTANEAREG)", INTERREG IIIB, ESPACIO ATLANTICO, FEDER, 2004-2006. To further allow comparison among cultivars, the four most discriminating morphological characteristics previously studied for the cultivars of Galicia (Pereira-Lorenzo, 1994 and Pereira-Lorenzo *et al*., 1996 a), favourably tested later for cultivars of different origin (Pereira-Lorenzo *et al*., 2006 and 2007) and also utilised under the framework of this same project, have been employed: i) fruit size; ii) fruit shape; iii) type of male flowering; and iv) length of burr spines.

From the results obtained through this molecular characterization, it has been possible to group the 74 accessions of Tenerife and La Palma into 57 different genotypes. It is worth mentioning that, as in the case of the cultivars of Galicia (Pereira-Lorenzo *et al*., 1996 b), Andalucia (Pereira-Lorenzo and Ramos-Cabrer, 2003), León (Ramos-Cabrer *et al*., 2003) and Asturias (Pereira-Lorenzo *et al*., 2005; 2006), the cultivars from the CI are all polyclones. The few cases in which more that one genotype was found under the same name seem to indicate that more than one clone of a cultivar might have been propagated. Most of the genotypes found were singulars and only 10 groups of coincidence by microsatellites have been detected. When the CI genotypes were compared with those of the Spanish mainland (data unpublished), no synonyms were found. All the cases of varietal denominations with only one accession found in the CI have been found to be of a single genotype different from all the others (Pereira-Lorenzo *et al*., 2007).

5. Tradition and uses

Chestnuts were traditionally used as an important source of food in times of scarcity and also for wood of important value (Pereira-Lorenzo *et al*., 2007). Among the different uses of this plant on Tenerife, Ríos-Mesa (2004) specifies the following:

The fruits

Larger ones used for fresh food, for family consumption, exchange or sale, the smaller ones as cattle

feed. Chestnut fruits are important for traditional cooking on the islands. They are consumed either boiled or roasted, or even fried in oil alone or accompanying salty fish (a very traditional dish on the islands), in purees or, more recently, in desserts.

The wood

Used for house building, furniture, wine presses, staves for wine barrels and for the bottom of boats, it is also used for making boxes to store various local units of weight, cuartillas, and sowing quantities, almudes (Fig. 7). The wood has also served to build small tools such as those used to blow cereals or to fan the grain. Ungrafted trees have been preferred, generally, for wood purposes as they are straighter and present fewer nodes than grafted plants. It should be mentioned that the wood has always been cut when the moon is in "its last quarter".

Despite early introduction of the tree to the CI chestnut wood was not used, at least on Tenerife, for building wine barrels until the middle of the 17th century, as there are reports of imports of wood for that purpose from Galicia and Portugal (Méndez Pérez, 2002).

Chestnut suckers were also utilised to make baskets (Fig. 8) of different sizes, such as hand baskets or bigger ones for transporting rocks and even those known as raposas placed on the sides of donkeys to carry various items. Suckers also served to make horquetas, long wood sticks ending in a 'v' shape which first served to raise and hold the grapevines away from the soil and later in the 20th century for banana cultivation, both to keep the bunch separated from the pseudostem (small horquetas) or to give support to the whole plant in case of wind (bigger horquetas called horquetones).

- The dry leaves have been used as cattle beds and the green ones as cattle food.
  Ríos-Mesa (2004) indicates that chestnut is closely related to cultural aspects of CI life. Chestnuts are an important element of the gastronomy linked to popular celebrations. Special mention should be given to:
- the "All the Saints Feast" when the early chestnut fruits are roasted and consumed together with the "aguapié" (the first liquid extracted from the grapes after the men performed the traditional stomping of the grapes).
- the "Saint Andrews Feast", when chestnuts are eaten together with the new wine of the year when the cellars are first opened.
- at Christmas time when chestnuts are considered important Christmas symbols, like the Spanish turrones (hard almond cakes). It is tradition in some villages to give a small basket full of chestnuts to children as a present.
- during Carnival in some localities in the north of Tenerife dry chestnuts are also consumed accompanying wine.
- for the "Saint Anthony Feast" in the county of Acentejo, in the north of Tenerife, it is customary to use chestnuts as rosaries to be placed over domestic animals.

Fig. 7 - Box for sowing the grain made of chestnut.

Fig. 8 - Traditional harvesting basket made with chestnut wood.

Chestnut wood has been used on the island of La Palma for wine barrels and also for making receptacles to export fruits preserved in syrup, made with the sugar coming from the sugar cane industry, or to keep salty meat. They were also used as support poles for grape cultivation (Pereira-Lorenzo *et al*., 2007). Peeled chestnut wood skin bands were used for building the structure and handles of baskets. Several types of baskets, including bread baskets are also made, like those on Tenerife (Santos-Cabrera, 2002). The wood is also appreciated for smoking cheese, a very traditional and delicious dish of the gastronomy of this island. The tender young shoots coming from the main trunks have been traditionally used as cattle feed.

Unifloral chestnut honey is produced in Tenerife and La Palma where sometimes bee hives are made of chestnut wood. In the past chestnuts were preserved

buried in volcanic lapilli, but it was more common to cover them with sand or to dry them in the sun (Pereira-Lorenzo *et al*., 2007).

It is worth mentioning the historical use of chestnut trees as a means of claiming property for communal lands. It is indicated by Rodríguez-Brito (1982) that the planting of a chestnut tree in a deforested plot constituted the first step toward claiming it.

The "feast of Saint Martín" (11 November) is the day chosen to drink the new wine and on Tenerife it is accompanied by the consumption of chestnut fruits. The night before this feast, children dragged empty oil tins, "cacharros", in the air which are open at the top and filled with chestnuts and coals.

With an evident sense of humour, the Old People's Association in the village of San Juan de Puntallana organise a contest a few days after "Saint Martín", coinciding with the time of the chestnut harvest, to nominate the Queen of Chestnuts, the Big Chestnut, the Small Chestnut and the Nice Chestnut. Women participate in the contest by bringing chestnuts collected by themselves or by their husbands. The woman that presents the biggest one is nominated as the Queen of Chestnuts, and similarly for the smaller or nicer fruits. In addition, the chestnut has erotic relevance on this island as it is used to refer to the female sexual organ (Pereira-Lorenzo *et al*., 2007).

On the Island of El Hierro chestnuts have lost their traditional importance as food during autumn and winter except at the "Feast of All Saints", when chestnut fruits are used as tafeñas (local chestnut grill) and consumed with new wine. In the past chestnuts were dried in the sun and used as ingredients for the preparation of "morcillas" (blood sausages), a local product still prepared on other islands. These morcillas were used by the inhabitants of the "El Golfo" county as exchange for dry figs or potatoes coming from other counties of the island (Gil-González 1998).

The use of chestnuts on the island of La Gomera must have been similar to that of other islands, but in addition chestnut wood is used for making chácaras, a musical percussion instrument similar to the Spanish castañuelas (Perera-López, 2005) (Note that chestnut in Spanish is castaño.)

As indicated above, the consumption of chestnut fruits is very common on the CI where they have been an important source of calories since soon after the Spanish conquest of the islands. They are not normally consumed raw, except occasionally when they are very soft, but generally boiled or roasted (Fig. 9). Boiling is generally done after making a cut in the external shell or after eliminating it. They are cooked for half an hour in plenty of water to which anise, vanilla sticks or cinnamon is added, particularly when used for desserts (Iglesias García, 2004). They are usually roasted in homes by putting them (after making a cut in the external shell) in a perforated biscuit tin which is then put directly on the kitchen fire. Chestnuts are traditionally cooked in small metal boilers placed above charcoal fires on many street corners. In both cases, coarse salt is added during cooking.

While roasted chestnuts are directly consumed, boiled chestnuts may be incorporated into typical dishes of the Canary Island gastronomy, such as roasted sardines, meat compositions or salty fish dressed with oil and vinegar or with the typical "mojo" (a sauce made with *Capsicum annun*) to which they give a sweeter taste. They are also smashed into a puree, either to accompany a dish or as a filling for dishes prepared in the oven mixed with dry fruits (crushed pine nuts or almonds), spices and herbs or even previously fried giblets, adding a special flavour. In addition, they are a typical side dish for wild game (Iglesias García, 2004), or as an ingredient in sweet CI bloody sausages.

Chestnuts may be consumed in a broth in the typical "caldo de castañas" (chestnut soup), cooked with potatoes or sweet potatoes, offering consistency and nutritive value which is much appreciated on cold winter days (Ríos-Mesa, 2004; Pereira-Lorenzo *et al*., 2007).

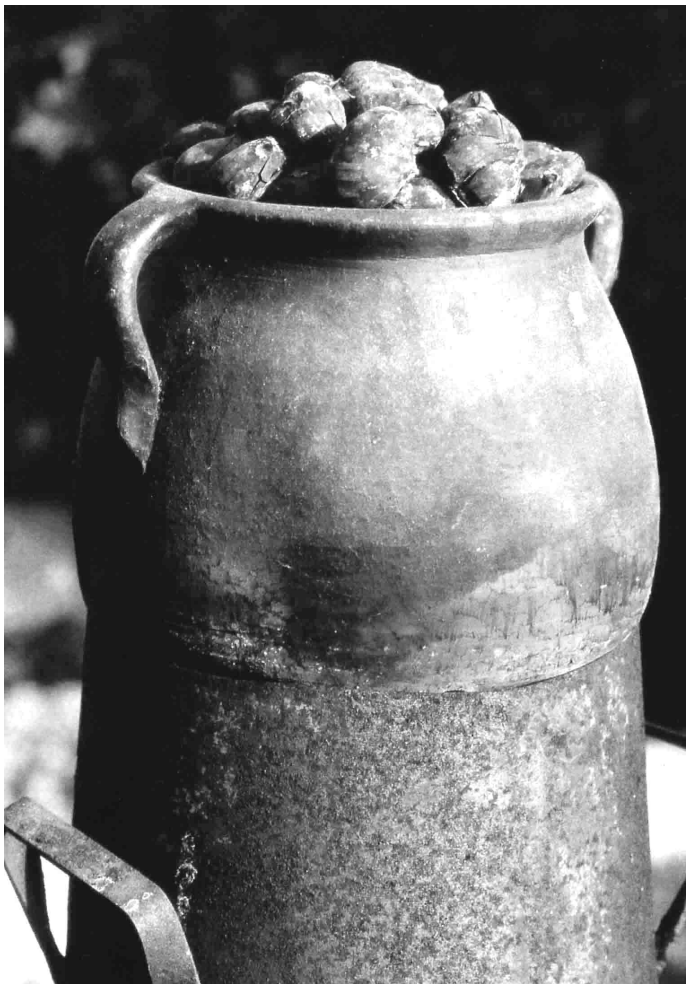

Fig. 9 - Traditional chestnut roaster.

Chestnuts boiled with vanilla sticks are mixed, after removing all the skin, with syrup and eaten or ground as a filling for various sweets and cakes. One of the older recipes for these fillings includes ground chestnuts in addition to sugar, milk and beaten egg yolks. The mixture is cooked on a very slow fire for a few minutes, without allowing it boil. Chestnuts are also used as ingredients in stewed fruit preserves. For this use chestnuts are boiled in abundant water, well cleaned to eliminate any remaining skin and then plenty of sugar is added along with anise, mint or hortelan (*Menta spitaca*) and a glass of orange liquor or brandy. When the chestnuts become soft and thick, a syrup is formed; they are kept well covered in the syrup in sealed jars in a dry place (Iglesias, 2004).

6. Future prospects

Technical meetings have been organised since 2003 at La Matanza Municipality on the Island of Tenerife with the assistance of the Centre for Conservation of Crop Biodiversity of Tenerife (CCBAT) and the Extension Service. Lectures by national and international experts have been given to include horticultural aspects such as pruning and training, marketing and preparation of chestnut products as well as economical and structural aspects and many other subjects dealing with chestnut cultivation on Tenerife.

Special mention should be made of the process of recovery and adding of value to chestnut cultivation initiated in 2004 by the Cabildo (local government) of the Island of Tenerife. This project was developed by the Extension Service and the CCBAT have initiated "ex situ" and "in situ" conservation programmes and aims to create, in connection with the Chestnut Farmers Association of Tenerife, a quality label for chestnuts produced on Tenerife. This initiative, if continued and extended to other islands, may make a great contribution toward the recovery of commercial cultivation of chestnuts on the Canary Islands.

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Growth reduction in root-restricted tomato plants is linked to photosynthetic impairment and starch accumulation in the leaves

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Key words: chlorophyll, leaf gas exchange, *Solanum lycopersicum* L., sugar content, total water potential.

Abstract: The mechanisms responsible for reduced shoot growth due to restricted root growth is still not fully understood. Therefore, this investigation was planned to determine the morphological and physiological changes induced in response to root restriction conditions and to determine the time frame within which these changes occurred. In particular, this research aims to evaluate the effect of root restriction on growth, leaf gas exchange parameters, carbohydrate production and water relations in tomato (*Solanum lycopersicum* L.). Our results show that growth reduction by root restriction is mainly linked to a photosynthetic impairment, caused by a concurrent limited stomatal conductance (probably driven by stomatal factors and hormonal substances) together with a strong accumulation of starch in the tissues, which led to a feedback inhibition of the photosynthetic process.

1. Introduction

The use of root-restricted cultivation for vegetable production has significantly grown in the last decades (Shi *et al*., 2008), as it appears an effective technique for saving resources, controlling root environment, and regulating early yield and quality (Marsh and Paul, 1988; Shi *et al*., 2008). Root restriction may occur wherever pot size or rooting volume is physically limited (Tschaplinski and Blake, 1985; Ismail and Noor, 1996; Saito *et al*., 2008; Mugnai *et al*., 2009), mostly with greenhouse-grown horticultural crops (Thomas, 1993). Root restriction leads to a denser root mass and a reduced root growth (Ismail and Noor, 1996). Besides limiting the volume of the soil available to the root system for water and nutrient uptake, it also suppresses canopy growth (Ismail and Noor, 1996; Shi *et al*., 2008) via many plant physiological and biochemical processes. The mechanisms responsible for reduced shoot growth due to restricted root growth is still not fully understood. Several hypotheses were investigated including water and nutrient stresses (Hameed *et al*., 1987), decrease in root respiration (Shi *et al*., 2007) and photosynthesis (Shi *et al*., 2008), and production of plant hormones (Liu and Latimer, 1995), but reports indicated that there are contradictory results as to which of these factors play a significant role in the response of aerial plant parts to restricted root growth and indicated 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). The presence of a physical restriction to root growth, a major metabolic sink for photsynthetically fixed carbon at seedling stage (Thomas and Strain, 1991) resulted in feedback inhibition mechanisms, with lower rates of carbon metabolism and photosynthesis as a result of carbohydrate accumulation (Schaffer *et al*., 1996; Shi *et al*., 2008). Therefore, this investigation was planned to determine the morphological and physiological changes induced in response to root restriction conditions and to determine the time frame within which these changes occurred. In particular, this research aims to study the effect of root restriction on growth, leaf gas exchange parameters, carbohydrate production and water relations in tomato (*Solanum lycopersicum* L.).

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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 tomato (*Solanum lycopersicum* L.) cv. 'Cal J' 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} of PAR). 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 root restriction treatment through out 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 thus: 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^{-1}). The nutrient solution was renewed every week.

Growth measurements

Five plants per treatment were sampled at weekly intervals. Roots were carefully washed, 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 hr 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 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 mmol m^{-2} s^{-1} PAR) conditions provided by a mercury vapour lamp (OSRAM HQI-TS 250 W/NDL). Calculation of all the parameters was performed following von Cammerer and Farquhar (1981) using a specific software (Diagas 2.02, Walz, Effettrich, Germany).

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 hr 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):

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

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, and centrifuged at 12000 rpm for 15 min; the supernatant was then collected. The pellet was extracted again as described above, and the supernatant was collected again. At the end of the procedure, the pellet was evaporated to remove any excess 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^{-1} Na-acetate 50 mM at pH 4.6), the other with 100 µl solution containing Na-acetate 50 mM at pH 4.6, 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 using 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 hr with 100 µl solution of Na-acetate 100 mM pH 5.2/10 α-amylase. This solution was incubated with 100 µl of Na-acetate 100 mM pH 4.6/10 u amyloglucosidase at 55°C for 1 hr. Finally, the solution was boiled and centrifuged to eliminate denaturated protein from α-amylase and amylogluco-sidase. 100 µl from this solution was taken and brought to 300 µl with distilled water, then starch analysis (as glucose equivalent) was 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

Growth parameters were greatly affected by root restriction treatment (RR), with significant reductions in total dry weight, leaf area and plant height (Fig. 1A, B and C) starting from an early stage of seedling development. RR plants also showed a significantly higher root:shoot ratio (Fig. 1D), due to a higher allocation of biomass in the root system compared to canopy (stem and leaves).

During the first month, no significant differences were noticed in leaf gas exchange parameters. From day 29, however, stomatal conductance (g) started to significantly decrease in RR plants (Fig. 2A), leading to a significant reduction from day 36 in both net CO_2 assimilation (Fig. 2B) and transpiration (Fig. 2C) until the end of the experiment. The reduction in net CO_2 assimilation was not related to a decrease in the chloro-

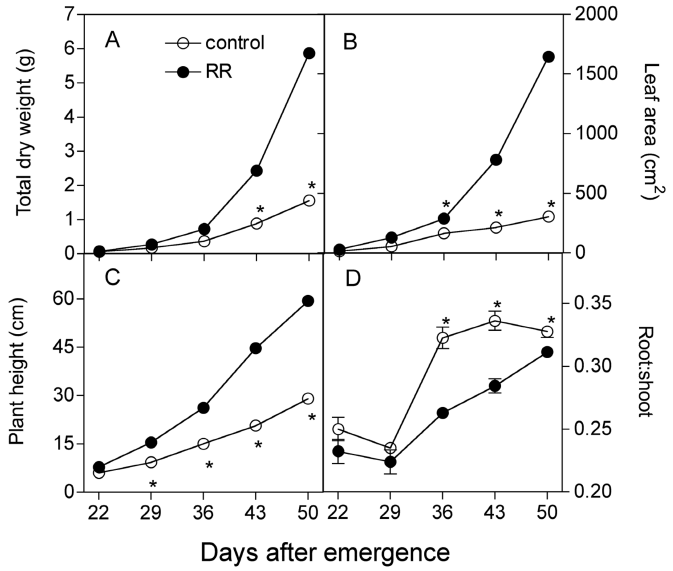

Fig. 1 - Growth parameters measured at weekly intervals from day 22 to the end of the experiment in both control and root-restricted (RR) plants: total dry weight (A), leaf area (B), plant height (C) and root:shoot ratio (D). * indicates significantly different values for P≤0.05 (n=5), when means were separated by Duncan's test.

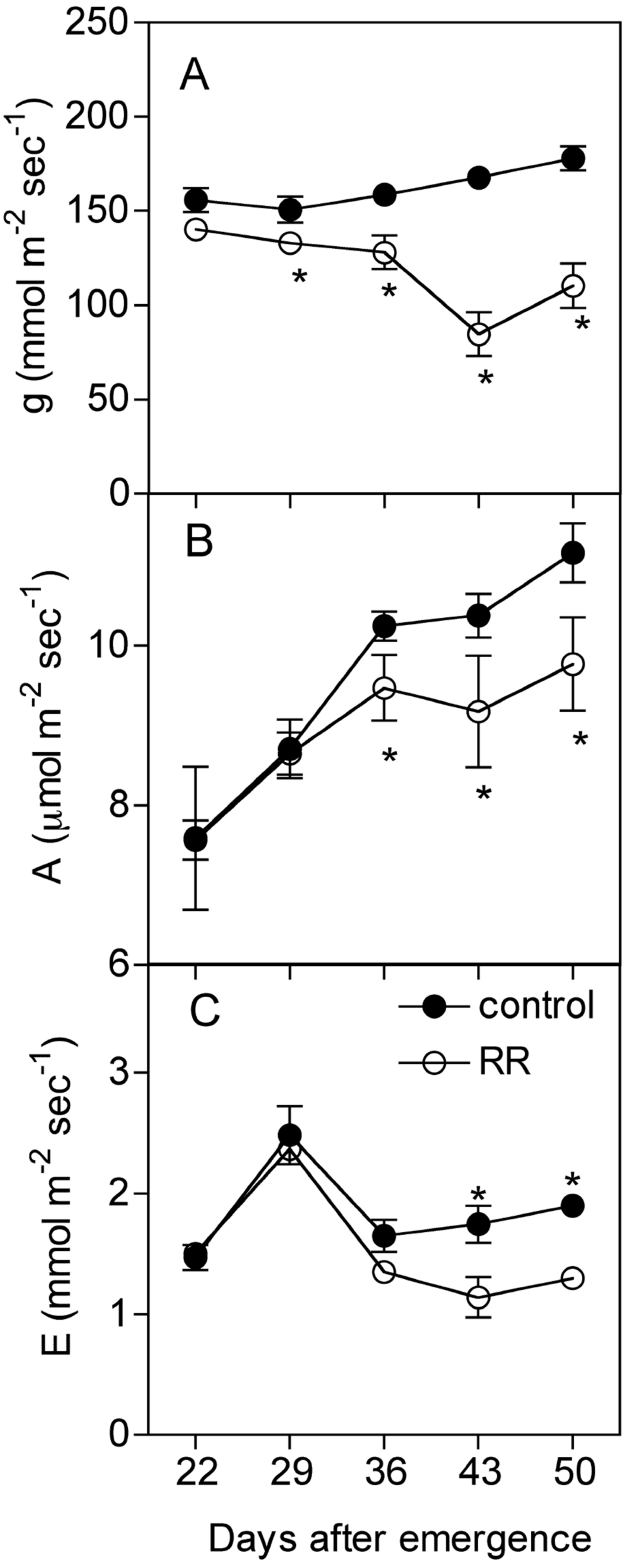

Fig. 2 - Leaf gas exchange parameters measured at weekly intervals from day 22 to the end of the experiment in both control and root-restricted (RR) plants: stomatal conductance (A), net CO_2 assimilation (B) and transpiration (C). * indicates significantly different values for P≤0.05 (n=5), when means were separated by Duncan's test.

phyll content of RR plants (Table 1), as no significant differences were noticed for chlorophyll *a*, *b,* and *a*/*b* ratio between the two treatments. Leaf water status did not affect stomatal closure, as total water potential (Fig. 3A) and turgor potential (Fig. 3C) did not show any significant difference throughout the entire experiment in both the treatments, even if a slight, but not significant, reduction in total water potential was measured on day 43 in RR plants. This behaviour also confirmed the fact that no water stress symptoms occurred during the experimental period, giving a positive feedback of our experimental system.

On the contrary, sugar content determination led to interesting results. While sucrose content trend was not uniform during the experiment, leading to contradictory results (Fig. 4A), RR treatment led to a clear increase in glucose content (Fig. 4B) and a concurrent decrease in fructose content (Fig. 4C) together with a great accumulation of starch (Fig. 4D). In particular, starch accumulation in the tissues began early in the developmental process (day 29). Starch was mainly compartimentalized in the leaves (Fig. 5A) and stems (Fig. 5B) of RR plants, whereas no significant differences were noticed in roots between control and RR plants (Fig. 5C).

4. Discussion and Conclusions

Our growth data are in line with several previous results concerning growth depression induced by root restriction in other horticultural crops (Carmi and Heuer, 1981; Tschaplinski and Blake, 1985; Thomas and Strain, 1991; Rieger and Marra, 1994; Liu and Latimer, 1995; van Iersel, 1997; Kharkina *et al*., 1999; Saito *et al*., 2008; Shi *et al*., 2008). Root restriction generally caused an increase in root:shoot ratio (Carmi *et al*., 1983; Mugnai *et al*., 2000); roots in smaller volume formed a highly branched mat, whereas plants in large volume had long tap roots and showed little branching. The increased root:shoot ratio reported by some researchers for many crop species subjected to root restriction 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).

Our results reveal that root restriction significantly reduces stomatal conductance, as previously noted by other authors for different species (Carmi *et al*., 1983; Thomas and Strain, 1991; Ismail and Noor, 1996;

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*
22	8.075	3.221	0.484	8.423	3.333	0.483
29	7.403	3.274	0.703	9.261*	3.708	0.566
36	7.414	3.276	0.702	9.949*	3.500	0.216*
43	9.076	3.686	0.596	9.809	3.863	0.547
50	10.924	4.320	0.624	10.873	4.054	0.422*

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

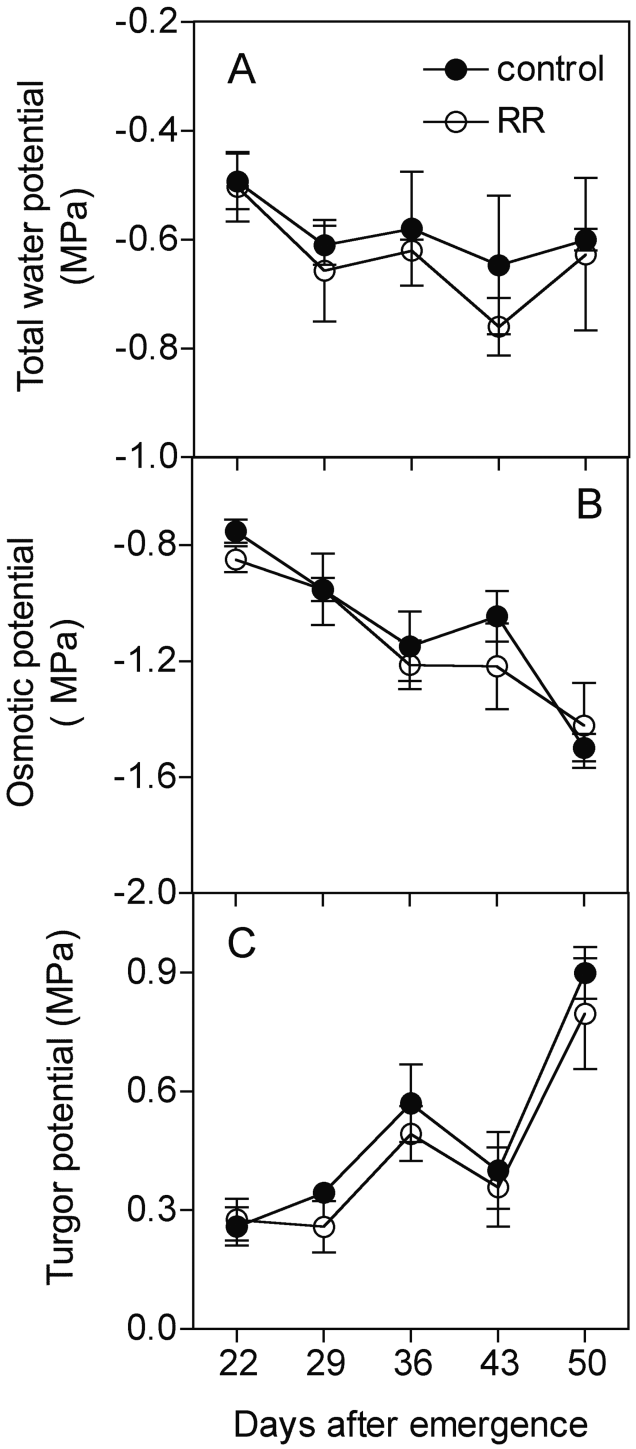

Fig. 3 - Leaf water status determined at weekly intervals from day 22 to the end of the experiment in both control and root-restricted (RR) plants: total water potential (A), osmotic potential (B) and turgor (C). * indicates significantly different values for P≤0.05 (n=5), when means were separated by Duncan's test.

Kharkina *et al*., 1999), and that stomatal conductance was the primary cause of decrease in CO_2 assimilation in root-restricted plants suggesting a stomatal factor limiting the photosynthetic rate under root-restriction conditions (Shi *et al*., 2008). The decline in stomatal conductance 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. This means that other factors are largely involved in the stomatal closure. It has been suggested that root volume restriction induces a reduction in the stomatal conductance *via* 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 may be caused by the influence of ABA originating from the restricted roots. Carmi (1995) found that the higher level of ABA in the leaves of root-restricted plants was not a consequence of an enhanced transport from the restricted roots, concluding that root-zone restriction might promote ABA accumulation in the root and the shoot, with a possible influence of such accumulation on other processes in root-restricted plants, such as leaf gas exchange.

The decline in net CO_2 assimilation observed in root-restricted conditions was also interpreted as a feedback inhibition by carbohydrate accumulation (Pezeshki and Santos, 1998). Plant 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 the 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

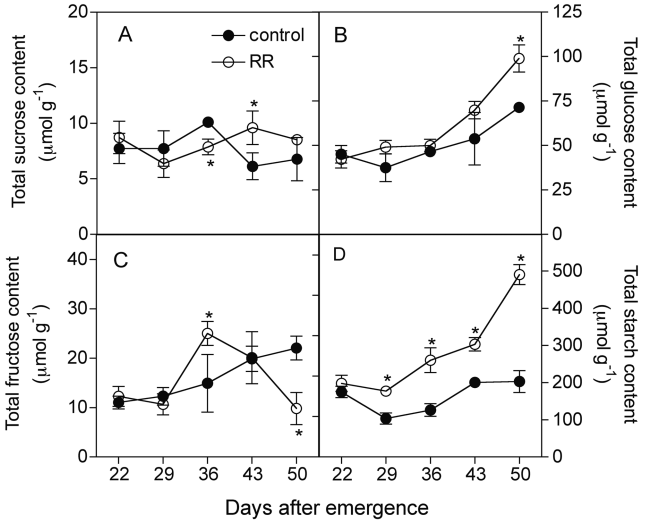

Fig. 4 - Sugar content measured at weekly intervals from day 22 to the end of the experiment in both control and root-restricted (RR) plants: total sucrose (A), total glucose (B), total fructose (C) and total starch (D). * indicates significantly different values for P≤0.05 (n=5), when means were separated by Duncan's test.

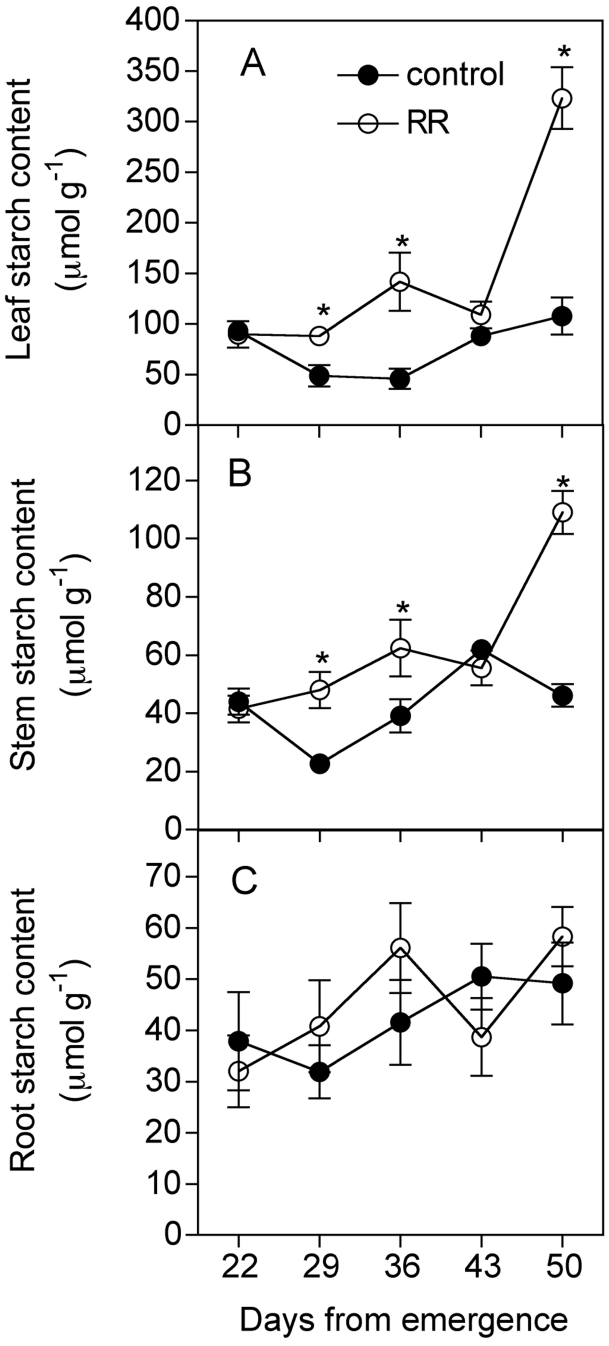

Fig. 5 - Starch content in the different plant organs measured at weekly intervals from day 22 to the end of the experiment in both control and root-restricted (RR) plants: leaf (A), stem (B) and roots (C). * indicates significantly different values for P≤0.05 (n=5), when means were separated by Duncan's test.

a metabolic sink that influences the partitioning of photosynthetically fixed carbon (Gifford and Evans, 1981; Robbins and Pharr, 1988). Sink limitation caused by root restriction can greatly reduce leaf photosynthetic rate in many crop species (Hameed *et al*., 1987; Ismail and Noor, 1996; Schaffer *et al*., 1996; Whiley *et al*., 1999; Shi *et al*., 2008), and reduced translocation of assimilates from leaves (Robbins and Pharr, 1988; Kharkina *et al*., 1999). Root volume restriction 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 root-restricted 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 and stem as sink organs may increase when root growth is extremely limited by volume restriction and a relatively larger amount of carbohydrates 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 Carmi and Heuer (1981), Robbins and Pharr (1988) and Shi *et al*. (2008).

Accumulation of non-structural carbohydrates in the leaves in response to root restriction 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), who suggested 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 photoassimilate supply to exceed the capacity of demand (i.e. end-product inhibition of photosynthesis) as indicated by Arp and Drake (1991) and Whiley *et al*. (1999).

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

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In vitro mutagenesis and detection of variability among radiomutants of chrysanthemum using RAPD

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Key words: Dendranthema grandiflora, *in vitro* mutagenesis, radiomutants, somaclones.

Abstract: The present study was undertaken to induce mutations in *Dendranthema grandiflora* cv. Snow Ball through *in vitro* mutagenesis by exposing the *in vitro* shoots to 5, 10, 20 and 30 Gy gamma radiation. RAPD analysis was used to detect genetic polymorphism among the variants and the control. Morphological variations were not observed with the 5 Gy gamma dose during the first season. Shoot regeneration, rooting and survival of shoots were affected by gamma ray doses. 10 Gy of gamma irradiation was the most effective in inducing mutations in flower colour through direct *in vitro* mutagenesis. The shoots irradiated with 20 and 30 Gy gamma radiation did not root and died. Twenty RAPD primers were used to amplify DNA segments from the genomic DNA of the control and its 10 variants, and the genetic similarity among them ranged from 0.06 to 0.79 revealing high genetic diversity.

1. Introduction

Chrysanthemum is a major horticultural crop and it is the second largest in terms of cut flowers after rose, among the ornamental plants traded in the global flower market (Kumar *et al*., 2006). The common garden chrysanthemum is hexaploid with 54 chromosomes (Wolff, 1996). It is propagated vegetatively and has a strong self incompatibility system (Richard, 1986), hence new cultivars are difficult to obtain by crossing. Traditionally, new cultivars have been obtained from spontaneous mutations in vegetative reproduction, sports, being some variations more stable than others (Miñano *et al*., 2009). In the last few years, induced mutations and somaclonal variations derived from the tissue culture process have been employed as a new source of variability (Schum, 2003; Datta *et al*., 2005; Jain and Spencer, 2006; Zalewska *et al*., 2007; Jain, 2010; Barakat *et al*., 2010).

Although extensive work has been carried out to develop novelties in chrysanthemum through induced mutations using physical and chemical mutagens (Broetjes and Van Harten, 1978), there is always a need to explore the possibility of new variety for floriculture trade. Mutation breeding by radiation has been widely used to upgrade well-adapted plant varieties and also to develop new variations within improved agricultural characteristics. Since most cultivated chrysanthemum cultivars are polyploids with high genetic heterogeneity, mutants with allied flower colour, shape, floral size and shape are often recovered. Allied flower colours with chimeric tissue can be easily induced by radiation and can be isolated using *in vitro* tools (Kumar *et al*., 2006). Identification and characterization of cultivars is extremely important in horticultural crops in order to protect the plant breeder's rights. Traditionally, identification has been based on morphological characters; however the development of new technologies has made it possible to base this analysis on DNA information. One approach is a PCR-based technique - RAPD (William *et al*., 1990) - that has been widely used for plant germplasm characterization (Wolff *et al*., 1994; Huang *et al*., 2000; Martin *et al*., 2002; Martin and Gonzalez-Benito, 2005). The aim of the present study was to induce mutation in chrysanthemum cv. Snow Ball through *in vitro* mutagenesis by treating the *in vitro* shoots with gamma radiation and to apply RAPD analysis for the detection of genetic polymorphism among the mutants and control.

2. Materials and Methods

Plant material and in vitro mutagenesis

Nodal segments (2-3 cm) of *Chrysanthemum grandiflora* (Tzelev) cv. Snow Ball, collected from one-

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year-old mother plants maintained in the glasshouse of the Department of Biotechnology, University of Horticulture and Forestry Solan (Himachal Pradesh), India, were used as explants. The explants were surface sterilized with 5% sodium hypochlorite ($NaOCl_2$) for 10 min, washed thoroughly three to four times with sterilized water and cultured on MS (Murashige and Skoog, 1962) medium supplemented with 2 mg/l benzyladenine (BA), 0.5 mg/l α-naphthalene acetic acid (NAA), 30 g/l sucrose (w/v) and 8 g/l agar (w/v; Sigma-Aldrich, Bangalore). The culture without growth regulators served as control. The pH of the medium was adjusted to 5.8 before autoclaving at 121ºC at a pressure of 1.1 kg cm^{-2} for 15 min. The cultures were maintained under 16-hr photoperiod with a light intensity of 50-60 μmol cm^{-2} s^{-1} provided by cool, white fluorescent lamps at 24±2ºC. After four weeks of culture, the shoots were subcultured on shoot multiplication medium consisting of MS salts supplemented with 0.5 mg/l BA, 0.1 mg/l indole acetic acid (IAA) and 1 mg/l gibberellic acid (GA_3). Shoots about 2.5-3.0 cm long were irradiated in a gamma cell (60 cobalt source) with 5, 10, 20 and 30 Gy doses. After four weeks, the shoots were multiplied and 3-4 cm long shoots were transferred to $^1/_2$ strength MS medium supplemented with 0.3 mg/l indolebutyric acid (IBA) and 0.2% activated charcoal for rooting. The shoots with well developed roots were removed from the culture vessel, washed with running tap water to remove the adhering agar and transferred to plastic pots (25 cm diameter) filled with soil:sand:FYM in 1:1:1 ratio and kept in the glasshouse with 80-90% relative humidity. After acclimatization for four weeks, the plants were observed for any variations in morphological characters from the control and PCR analysis was carried out using the genomic DNA.

Statistical analysis

The data recorded for different parameters were subjected to completely randomized design (Gomez and Gomez, 1984). The statistical analysis based on mean value per treatment was made using analysis of variance (ANOVA). The LSD multiple range test ($p \leq 0.05$) was used to determine differences between treatments.

Polymerase chain reaction

Genomic DNA was isolated from fresh, young green leaves of the control and gamma ray-irradiated plants following the method of Doyle and Doyle (1987) with some modifications. For amplification, reaction mixtures (21.8 μl) contained 1 μl oligonucleotide, 2.5 μl 10X Taq polymerase buffer, 1.5 nM $MgCl_2$, 2 μl each of dNTPs, 3 μl genomic DNA and 3 μl Taq polymerase (3 units/μl). Random oligonucleotide primers were used for RAPD amplification (Bangalore Genei, Bangalore, India). Amplification was performed in a thermocycler (MJ Research, USA) programmed for a first denaturation step of 3 min at 94ºC followed by 36 cycles of 94ºC for 30 s for denaturation, 50ºC for 30 s for annealing, and 72ºC for 2 min (extension). Twenty Operon random primers (Operon Technologies, Inc., USA), ten of the OPA series (1-10) and ten of the OPB series (1-10), were employed for amplification using the cycling condition mentioned above.

All samples were given 10 min at 73ºC for post-amplification. PCR products were separated on a 1.4% agarose gel using 1 x TAE buffer and were stained with ethidium bromide. In all amplification reactions, a reaction mixture with water instead of genomic DNA was used as negative control. 1 kb DNA ladder (Fermantas, Lithuania) was used as the size marker. The amplified products were visualized using a UV transilluminator and photographed using gel documentation system (Alpha Imager, USA). Amplified DNA was scored as either presence (1) or absence (0) of band. Pairwise comparison between the control and variants was performed to calculate similarity (J) between the samples (Jaccard, 1908) using SIMQUAL programme of NTSYS-PC (version 2). A dendrogram was produced from the resulting similarity using UPGMA method.

3. Results

Effect of gamma radiation on in vitro *cultures*

The data presented in Table 1 reveal that the survival of shoots was affected by gamma ray doses. A decrease in survival was observed with an increase of irradiation dose; a significantly higher survival percentage was observed in the control shoots. The variation in survival percentage between 5 and 10 Gy was statistically insignificant. A decrease was also observed on shoots forming roots. A lethal effect of higher doses (20 and 30 Gy) was noticed on rooting of shoots. The shoots did not root, hence they turned yellowish brown and died. No variation was observed in shoots forming roots with 5 and 10 Gy irradiation. A significant difference in percent root initiation was observed between the control and irradiated shoots. The rooted plants were acclimatized as explained above and allowed to grow in the glasshouse till flowering.

Table 1 - Effect of radiation doses on survival of *in vitro* shoots in *D. grandiflora*

Dose strength	Survival (%)	Number of shoots producing roots (%)
0	90.47 (79.93)	100.00 (90.90)
5	66.60 (59.25)	82.60 (65.48)
10	52.38 (46.39)	82.35 (65.40)
20	42.85 (40.83)	0(0)
30	19.05 (20.15)	0(0)
$LSD_{0.05}$	25.11 (21.89)	6.25 (4.88)

Figures within parentheses are arc sine transformed values.

Effect of gamma radiation on morphological characters

Morphological variations were not observed in the acclimatized plants exposed to 5 Gy gamma radiation. All the plants flowered true to the mother floret colour/shape. Therefore, selections were made among the plants exposed to 10 Gy gamma radiation for agronomic and molecular characterization of mutants.

At flowering stage, plants in the glasshouse were observed for any variations in morphological characters. Results revealed that there were 10 variants with different morphological characters compared to the control. The data presented in Table 2 reveal that morphological characters of *D. grandiflorum* cv. Snow Ball and its mutant were statistically, highly significantly affected by gamma ray doses.

Plant height

Plant height was significantly reduced in the variants compared to control (Table 2). A significant variation in plant height was also observed among the variants (Table 3). Variant V10 gave the significantly highest value for plant height (69.00 cm), compared with the control (62.10 cm). Variant V8 showed the lowest value for plant height, followed by variant V2. The plant height was significantly lower in most of the variants as compared to the control.

Leaf number and leaf area (cm^2)

The number of leaves was significantly reduced in the variants compared to control (Table 2). There was a significant increase in the number of leaves in variant V4. In general, the number of leaves in the variants was lower compared to the control. Variant V2 had the fewest number of leaves, followed by variants V7 and V8. Statistically leaf area did not differ between the variants and control (Table 2), however leaf area differed among the variants (Table 3). Variant V7 had significantly higher mean values of leaf area, followed by variants V10, V2 and V9 in comparison to the control.

Number of flower buds and flowers

Table 2 reports that the number of flower buds was significantly different between the variants, whereas the difference in the number of flowers was not significant. The number of flower buds and flowers varied among the variants (Table 3) with variants V3 and V8 having a significantly lower number of flower buds and flowers, followed by variant V7. It was observed that in most of the variants about 82-83% of buds opened into flowers while in variant V3 only 50% of the buds opened into flowers.

Flower diameter and flower colour

The variation in flower diameter between the control and the variants was statistically not significant (Tables 2 and 3). The original flower colour of the cv. Snow Ball was white with incurve ray florets (Fig. 1A), whereas one branch of variant V9 produced yellow-coloured flowers with incurve ray florets (Fig. 1B). The results indicate that the irradiation dose of 10 Gy was an effective dose in inducing mutations in flower colour, but no changes were observed in flower shape and size.

Table 2 - Analysis of variance of the morphological characters of control and variants of chrysanthemum

Treatment	Plant height (cm)	Number of leaves	Leaf area (cm^2)	Number of buds	Number of flower	Flowers diameter (cm^2)
Control	62.10	38.70	8.60	15.10	11.40	6.60
Variants	54.80	31.60	10.93	11.70	8.10	6.50
$LSD_{0.05}$	2.97	4.18	2.35	4.05	3.44	1.58

Table 3 - Effect of gamma radiation (10 Gy) on morphological characters in *D. grandiflora*

Treatment	Plant height (cm)	Number of leaves	Leaf area (cm^2)	Number of buds	Number of flower	Diameter flowers (cm^2)
Control	62.10	38.70	8.61	15.10	11.40	6.60
V1	56.00	23.00	7.39	15.00	10.00	5.50
V2	40.00	16.00	13.00	5.00	4.00	6.00
V3	60.00	40.00	8.48	14.00	7.00	8.00
V4	58.00	49.00	7.87	16.00	9.00	5.50
V5	60.00	38.00	10.85	14.00	11.00	6.60
V6	55.00	35.00	10.48	10.00	7.00	7.30
V7	58.00	19.00	15.16	7.00	5.00	6.00
V8	37.00	19.00	9.32	5.00	3.00	7.50
V9	55.00	40.00	12.56	13.00	10.00	6.80
V10	69.00	37.00	14.15	18.00	15.00	6.60

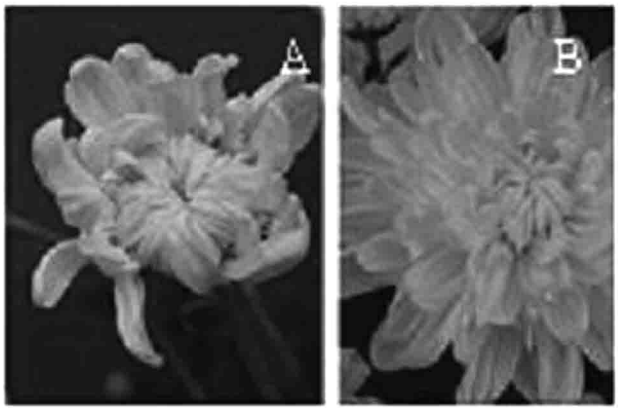

Fig. 1 - Induced somatic mutations in chrysanthemum cv. Snow Ball after 10 Gy gamma radiation treatment. (A) control, white-coloured flowers; (B) mutated, yellow-coloured flower.

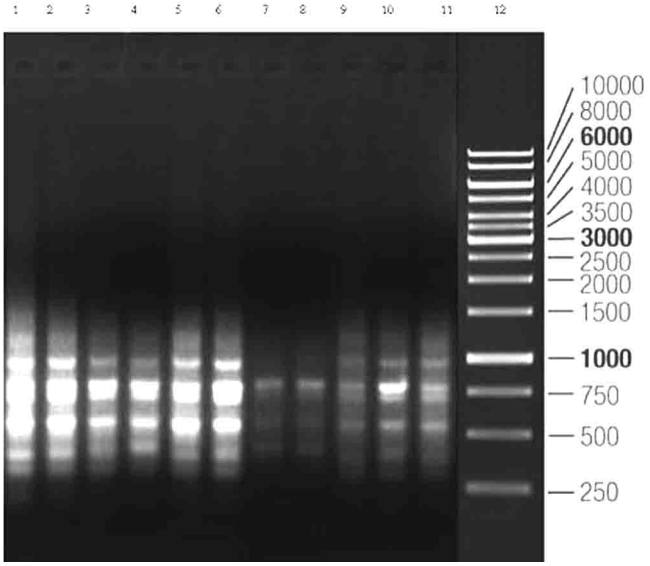

Fig. 2 - RAPD profile with primer OPB-4 showing polymorphism in control and its variants in chrysanthemum cv. Snow Ball. Lanes 1- 10: variants (V1 to V10); Lane 11: control; Lane 12: DNA ladder (1kb).

Characterization

Twenty decamer primers (Table 4) were used for the amplification of genomic DNA of control and mutants of *Dendranthema grandiflora* Snow Ball. The number of DNA fragments amplified ranged from one to five depending upon the primer and the DNA sample with a mean value of 2.90 bands per primer (Table 4). The amplification product ranged from 250 to 3000 bp. A total number of 58 markers were produced by the 20 primers. A total of 100% of the 58 scored bands were polymorphic in 11 genotypes (one control and its ten variants). Figure 2 shows the amplification profiles, generated by primer OPB-4 across the chrysanthemum genotypes. The RAPD markers produced by the 20 primers were used to construct a similarity matrix (Table 5). Five clusters can be observed. The first cluster includes only

Table 4 - Nucleotide sequences and RAPD amplification results of the primer used in the PCR amplification

Primer	Sequence (5'-3')	Scored bands	Polymorphic bands	Polymorphism (%)
OPA-1	GTTTCGCTCC	3	3	100
OPA-2	TGATCCCTGG	4	4	100
OPA-3	CATCCCCCTG	3	3	100
OPA-4	GGACTGGAGT	1	1	100
OPA-5	TGCGCCCTTC	4	4	100
OPA-6	TGCTCTGCCC	5	5	100
OPA-7	GGTGACGCAG	4	4	100
OPA-8	GTCCACACGG	4	4	100
OPA-9	TGGGGGACTC	0	0	0
OPA-10	CTGCTGGGAC	2	2	100
OPB-1	CAGGCCCTTC	4	4	100
OPB-2	TGCCGAGCTG	1	1	100
OPB-3	AGTCAGCCAC	1	1	100
OPB-4	AATCGGGCTG	3	3	100
OPB-5	AGGGGTCTTG	2	2	100
OPB-6	GGTCCCTGAC	2	2	100
OPB-7	GAAACGGGTG	4	4	100
OPB-8	GTGACGTAGG	3	3	100
OPB-9	GGGTAACGCC	3	3	100
OPB-10	GTGATCGCAG	5	5	100
Total		58		58

Table 5 - Jaccard's similarity matrix in control (C) and mutated (V) plants based on RAPD analysis

	C	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
C	1.00										
V1	0.32	1.00									
V2	0.31	0.79	1.00								
V3	0.31	0.67	0.54	1.00							
V4	0.28	0.76	0.65	0.67	1.00						
V5	0.37	0.37	0.37	0.37	0.42	1.00					
V6	0.20	0.30	0.26	0.32	0.32	0.37	1.00				
V7	0.29	0.65	0.68	0.53	0.72	0.40	0.45	1.00			
V8	0.20	0.50	0.54	0.37	0.50	0.26	0.37	0.62	1.00		
V9	0.06	0.27	0.26	0.28	0.29	0.24	0.26	0.33	0.25	1.00	
V10	0.28	0.62	0.61	0.44	0.51	0.31	0.32	0.62	0.59	0.35	1.00

variant 9 (V9); the second includes the control (C); the third includes variants 5 and 6 (V5 and V6); the fourth includes variants 8 and 10 (V8 and V10), and the last cluster includes variants 3, 4, 7, 2 and 1 (V3, V4, V7, V2, V1). It can be seen from figure 3 that the shortest genetic distance was found between variant 1 (V1) and variant 2 (V2), whereas the greatest distance was observed between the control and variant 9 (V9).

4. Discussion and Conclusions

Analysis of explant sensitivity is one of the basic requirements for an effective use of mutation induction in plant breeding programmes (Walther and Sauer, 1986). In the present study, the radio sensitivity of shoots was assessed by survival and rooting of shoots after irradiation in order to select a suitable dose of gamma irradiation. Predieri (2001) also reported the necessity to identify an appropriate dose to apply in mutagenic treatments. The effect of gamma radiation on *in vitro* cultures in crop breeding has been studied by many workers (Shen *et al*., 1990; Charbaj and Nabul, 1999; Predieri and Gatti, 2000; Datta *et al*., 2005; Barakat *et al*., 2010).

Reductions in survival, plant height, number of leaves and flowers were recorded after gamma irradiation. Mutation in flower colour was detected as chimera in one branch of the plant, which produced yellow-coloured flowers (Group 5C, Fan 1) (British Colour Council, 1938). In order to check the stability of flower colour, the mutated branch was propagated vegetatively. The plants were grown in a glasshouse, where they expressed the same colour/shape.

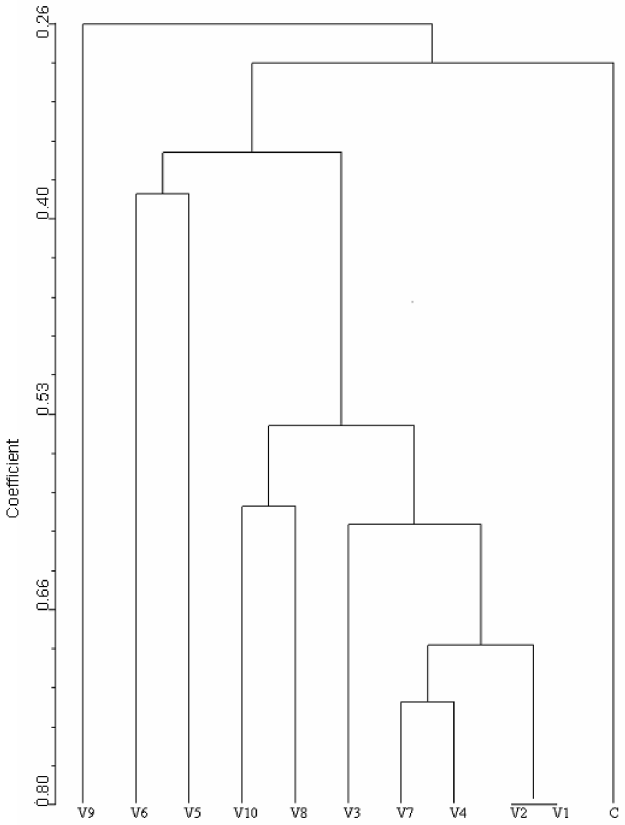

Fig. 3 - Dendrogram of control and mutants of chrysanthemum cv. Snow Ball

Over recent years, RAPD analysis has been used in ornamental breeding to characterize genotypes and to identify genes controlling important traits (Huang *et al*., 2000; Rumińska *et al*., 2004; Kumar *et al*., 2006; Miñano *et al*., 2009). In the present study, a high level of polymorphism was observed in 10 radiomutants of chrysanthemum. Wolff and Van Rijn (1993) also noticed a high degree of polymorphism in chrysanthemum cultivars using RAPD markers. This high level of polymorphism may be due to the strict out-crossing, resulting in a higher level of heterozygosity in chrysanthemum (Wolff *et al*., 1994). RAPD markers were used to construct a similarity matrix and the results of characterization analysis revealed a high diversity between the control and its somaclones. The greatest genetic distance between control and variant 9 (V9) may be due to the fact that V9 is a somaclone where one branch was mutated and produced yellow-coloured flower heads. Although the plants differed in flower colour, bands specific for flower colour could not be distinguished due to the resolution capacity of the tested primers. Wolff (1996) reported that the choice of the primers may be an important factor in obtaining a rapid discrimination between samples. Barakat *et al*. (2010) reported that mutants with different flower colour could be identified at the molecular level using RAPD technique, holding promise to identify unique genes as SCAR markers. Bhattacharya and Teixeira da Silva (2006) attempted to understand the molecular systematic and genetic difference between 10 original chrysanthemum cultivars and 11 mutants and reported that similarity ranged from 0.17 to 0.90 using rapid analysis. Kumar *et al*. (2006) reported genetic distances from 0.43 to 0.96 between 13 chrysanthemum cultivars. A great genetic distance among the different cultivars showed the existence of introgressing new and novel genes from the chrysanthemum gene pool. It may be suggested, with regard to the present study, that by using RAPD markers, it is possible to differentiate newly evolved chrysanthemum cultivars from their parents which can be a useful tool to supplement the distinctness, uniformity and stability analysis for plant variety protection in future. Our results are in accordance with those of other workers who reported RAPD as a powerful tool in the assessment of genetic variability as well as for genetic characterization, allowing differentiation of chrysanthemum mutants/cultivars (Huang *et al*., 2000; Rumińska *et al*., 2004; Kumar *et al*., 2006; Chatterjee *et al*., 2006; Miñano *et. al*., 2009).

Therefore, it may be concluded that 10 Gy gamma irradiation was most effective in inducing variations in flower colour and other morphological characters. Morphological variations were not observed with the 5

Gy gamma dose and a lethal effect of 20 and 30 Gy doses was observed on rooting and survival of *in vitro* shoots. One hundred percent polymorphism was observed among the radiomutants using PCR technique. The present results also indicate the applicability of gamma radiation in crop breeding, and the assessment of genetic variability and characterization of radiomutants at genomic level by RAPD can be a useful tool in breeding programs aimed to improve ornamental characters of chrysanthemum cultivars.

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Root distribution in young Chétoui olive trees (*Olea europaea* L.) and agronomic applications

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Key words: irrigation, overall root length, root-canopy ratio, root density, root volume, water requirement.

Abbreviations: ETc (mm)= Crop evapotranspiration as determined by the FAO method (Allen *et al*., 1998).
ET* (m^3)= Evapotranspiration volume of an individual tree relative to its root area.
K_c =Crop coefficient.
K_r = Minorative coefficient introduced in the formulae of ET_c - FAO to take into account the soil coverage.
K_{supply} = A supply ratio determined in order to link the water supplied to trees to the evaporative demand, it takes into account only the tree-related quantities.
ET_o (mm)= Reference evapotranspiration determined following to Penman-Monteith equation.
P_e (mm)= Effective rainfall determined following the USDA-SCS method (FAO, 1976).
I (mm)= Irrigation amount supplied during the irrigation period.
I*(m^3)= Irrigation amount supplied by localized system or in small basins around the trunk.
P (mm)= Total rainfall.
P* (m^3)= Effective rainfall for a single tree received around the trunk.
Sc (m^2)= The maximum projected canopy area determined for each of the six trees assuming a circular shape.
Sr (m^2) = Area concerned by tree transpiration i.e. where roots are active.
(t)= The number of years from planting.
Lo, Lx= Dimension of interest respectively at planting and at maximum growth.
α, β = Adjustment parameters within the logistic root and canopy growth curve.

Abstract: The study was carried out to have a comprehensive view of the root system behavior of young olive trees cultivated under field conditions. The experiment involved irrigated trees (*Olea europaea* L., cv., Chétoui) cultivated at 6x6 m^2 spacing in Mornag (36.5°N, 10.2°E), northern Tunisia. The way in which roots explore the soil volume during the first years after planting was explored through 'in situ' root system drawings and estimation of root densities. The relationship between canopy and root growth parameters was also investigated. The last section of this paper proposes a methodological approach for determining irrigation requirements of young olive trees and how water supply could be linked to the development of canopy and root system during the first years of cultivation when ground cover and the root system are not completely developed. Some agronomic applications were then deduced concerning water and fertilizers for such orchards. Results show that the main development of the olive root system occurs during the two to four first years of cultivation confining most roots (70%) to the top soil layers (20-40 cm). Maximum root densities were observed at this depth at a distance of 0.4 m from trunks. For young trees, water and fertilizers should be supplied at these depths and distances from trunk to allow easy and efficient root absorption. Obtained results also show a significant relationship between canopy and root areas which can be approximated by a linear model (r = 0.94). The root-canopy ratio estimated from their areas decreased rapidly beginning from the second year after planting, resulting from the establishment of competition between vegetative growth and fruiting. The optimum ratio root length/leaf canopy area of 2.3 km m^{-2} was found for the six-year-old tree indicating good equilibrium between the above and underground parts. The mathematical model developed on the basis of canopy cover and root extension allows precise estimation of water needs taking into account the actual root surface. However, while the canopy cover measurement was relatively easy to carry out, it was much more difficult to determine the surface covered by the root system. Results obtained in the present work also show an over-estimation of water needs when the FAO method is adopted to estimate the evapotranspiration of young trees.

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1. Introduction

The primary function of the root system, i.e. water absorption and acquisition of soil nutrients, has a great influence on many of the physiological processes in the tree (Doussan *et al*., 2003). However, despite of this importance, the root system is possibly the least explored area in crop physiology because of the difficulty involved in reaching it, in addition to the highly spatial-temporal variability which can generate many constraints to root extension. Amongst the first papers dealing with this area, are those of Yankovictch and Berthelot (1947), Vernet and Mousset (1963) and Abd-El-Rahman *et al*. (1966) which were carried out in North Africa, mainly on cultivars Chemlali and Picholine Marocaine. Research conducted a few years later in Spain and Italy investigated the relationship between water and root extension (Pisanu and Corrias, 1971; Bohm, 1979; Nunuez-Aguilar *et al*., 1980; Martin-Aranda *et al*., 1982; Michelakis and Vougioucalou 1988; Pastor *et al*., 1998; Smit *et al*., 1999; Palease *et al*., 2000). It was shown that apart from genetics and the origin of the plant (Ayachi-Mezghani, 2009) root distribution and extension can be markedly influenced by neighboring trees and soil texture and depth (Ben Rouina *et al*., 1996). Also, roots proliferate within the potential root zone regardless of irrigation application and method (Fernandez *et al*., 1991, 1992, 2003; Fernandez and Moreno, 1999; Connor and Fereres, 2005). These last authors noted that localized irrigation increased root length density of Manzanilla olive trees but it decreased their spread, largely confining them within the wetted volume and nearby trunks. They reported also that except under the canopy, roots were less frequent in the top layers than in deeper strata.

Root extension is also dependent on the available carbohydrate resources (Dichio *et al*., 2002) and growth stage (Michelakis, 2000). Rapid growth is observed in spring and autumn; it depends on water supply. Root growth precedes shoot growth and may be drastically limited by the previous year's fruit load. In fact, when no competition for carbohydrates occurred with other organs, for example for young olive trees or/and for vigorous canopy growth trees, important root extension and greater root densities were reported (Palease *et al*., 2000). In contrast, limited carbohydrate resources led plants to reduce their canopy growth and root length and even could deteriorate the root-canopy ratio as a result of competition between shoots, flowers, fruits and roots (Dichio *et al*., 2002). This relationship between root growth and the above-ground development is complex because it integrates many other factors and physiological processes like temperature, radiation, hormones, variety and alternate bearing.

Reduction of the root-canopy ratio implies systematic reduction of the capacity of the rooting system to absorb water. In terms of root balance, the importance of the water collecting system resides in its capacity to obtain water to support the transpiring leaf area (Connor and Fereres, 2005; Connor, 2006) and it can be determined via an estimation of total root length through monitoring of root density. These techniques are reported by Tennant (1975) and Fernandez and Moreno (1999). Such measurements could provide reliable estimates of comparative activities. For olive trees, Connor and Fereres (2005) reported root densities ranging between 0.1 and 1.0 cm cm^{-3}. These values are lower than those provided for herbaceous crops and some deciduous orchards, although olive root systems can be extensive and deep.

It appears from this short review that fundamental research on this subject is of prime interest: when and where the roots grow is crucial to understanding the functioning of the root system and its relationship with the above-ground organs. In fact, without precise information on root distribution, we cannot expect to efficiently manage the irrigation of the orchard. For these purposes, we have carried out the present study in order to have a comprehensive view of the root system behavior of young olive trees cultivated under field conditions.

In this work, we examine how roots explore the soil volume during the first years after plantation. The relationship between root and canopy development was also investigated. The last section of this paper proposes a methodological approach to determine irrigation requirements of young olive trees and considers how water supply could be linked to the development of canopy and root system during the first years of cultivation when ground cover and the root system are not yet completely developed.

2. Materials and Methods

Olive orchard

The study was carried out during the period 1998-2003 at the experimental farm of the Institut National Agronomique de Tunisie, located 15 km south of the capital Tunis (36.5ºN, 10.2ºE), northern Tunisia. In this region, climate is Mediterranean with yearly averages of 450 mm rainfall and 1200 mm reference evapotranspiration. It is dry and hot from May to September. The orchard, of 1.6 ha, was planted in 1998 at 6x6 m^2 spacing on a textural clay soil (29%C, 49%L, 23%S) of about 2 m depth. The volumetric soil water content was measured in the laboratory at field capacity (50%) and at the wilting point (26%). Crop management practices carried out in the orchard, i.e. pruning, fertilizer (Masmoudi-Charfi and Ben Mechlia, 2009) and pest management practices, were similar to those applied in intensive orchards (Masmoudi-Charfi, 2006; Masmoudi-Charfi *et al*., 2006). The trial concerned trees of cultivar Chétoui, which is the main oil variety of northern Tunisia.

Climatic data and irrigation management

Daily crop evapotranspiration (ET_c) was determined according to Allen *et al*. (1998) for the non-standard conditions such as: $ET_c = ET_o \times K_c \times K_r$, K_c ranging between 0.3 and 0.5 according to age, while K_r values were determined experimentally and varied between 0.69 and 0.75. For this purpose, a large white grilled (10 cm/10 cm) sheet was used. It was placed below the tree and the shade squares were counted and compared to the total number of squares (those lighted by sun and those shaded by leaves). This percentage represents the K_r value. Daily reference evapotranspiration (ET_o) was computed according to the Penman-Monteith equation, with maximum and minimum yearly values of 1320 mm (1999) and 1212 mm (2003), respectively. Data relative to rainfall, ET_o and temperature are reported in Table 1. All climatic data were recorded continuously with an automatic weather station located about 150 m from the young olive orchard.

During the six years of the study, rainfall amounts varied from 327 mm (2001) to 790 mm (2003), while effective rainfall amounts ranged between 226 mm and 546 mm. These values were determined according to the USDA-SCS method (FAO, 1976).

Accounting for these conditions, olive trees were irrigated every year during the spring-summer season. Water flows were programmed four times per season regardless of the critical stages and water availability. Irrigation was supplied by furrows (basin and drain) during the four first years and then by a drip system (2002 and 2003). Two parallel drip lines were fixed on the soil surface at about 0.5 m from trunks. There were four emitters per tree, two at each side of the tree trunk, separated 1 m from each other; each having a 4-L h^{-1} flow rate. The area wetted by irrigation application varied between 1 m^2 (1st year) and 6 m^2 (6th year). Watering conditions for the whole period are given in Table 2.

Water requirements were covered at levels varying between 0.3 ET_c and 1.1 ET_c according to year and water availability.

Measurements

Soil water content. The volumetric water content of the soil was measured with a neutron probe (SOLO 25) which was previously calibrated for the soil in question (Masmoudi-Charfi, 2008). Twenty-eight access tubes, 1.5 m long, were placed at the corners of a square of 2 m^2 below the canopy but also within the tree line and between tree lines. The soil moisture in each of these tubes was recorded frequently during the irrigation period every 0.3 m to 1.2 m depth, and the mean calculated separately for each position: below the canopy, far from the emitters, along and between the lines of tree (unpublished data). For the top 0.2 m soil layer, soil water content was determined by gravimetry. More details are given in Masmoudi-Charfi (2008).

Table 1 - Climatic data recorded during experimentation (1998-2003)

	1998	1999	2000	2001	2002	2003
Annual rainfall (mm)	376	440	410	327	345	790
Effective annual rainfall (mm)	260	304	283	226	238	546
Absolute T_{max} (°C)	47.0	41.0	44.0	42.0	43.0	46.0
Absolute T_{min} (°C)	3.0	1.0	4.0	3.0	3.0	3.0
Average T_{max} (°C)	25.0	23.7	25.2	25.8	25.6	24.9
Average T_{min} (°C)	13.3	15.0	14.8	15.8	15.5	14.9
Annual ET_o (mm)	1313	1320	1293	1282	1231	1212

Table 2 - Water requirement and irrigation application for young olive trees of cultivar Chétoui during the experimental period

	1998	1999	2000	2001	2002	2003
Irrigation system	Basin	Basin	Drain	Drain	Drip	Drip
First irrigation	March	May	April	April	March	May
Last irrigation	August	September	September	September	August	September
Dose (m^3/tree)	0.12	0.18	0.22	0.44	0.7-1.7	0.3-1.0
Irrigation amount (m^3/tree/year)	0.84	0.72	0.88	1.76	4.98	5.41
$I + P_e$ (mm)*	140	61	180	141	248	389
ET_c (mm)*	243	241	291	287	273	368
$I+P_e / ET_c$	0.6	0.3	0.6	0.5	0.9	1.1

(*) indicates that values are determined for the irrigation period.

P_e is the effective rainfall determined according to the USDA-SCS method (FAO, 1976) and I is the irrigation amount.

The ratio $I+P_e / ET_c$ was calculated for the irrigation period.

The tree downward projection canopy flat area varied between 2% (first year) and 33% (sixth year).

Root distribution. Distribution of the root system was studied during the rest period (November-December) on the same Chétoui olive trees by extensive observations of their root system. The trench method was used as described by Fernandez *et al*. (1991). For this purpose, a large pit was opened at 0.4 m from the trunks and roots were counted on the internal trench wall, which was divided into five layers of 0.2 m width each and down to 1.0 - 1.2 m depth. Root diameter was measured by means of a caliper 1/100. Maximum distance of roots from trunk was determined at each soil layer in order to estimate lateral root extension. Total volume of soil and the area explored by the root system were determined assuming central symmetry to the trunk.

Root density. Root densities were determined on the same Chétoui olive trees by using the cylinder method as described by Fernandez *et al*. (1991). Soil samples were taken during the rest period by a conventional auger at 0.4 m, 0.8 m and 1.2 m from trunks in order to quantitatively assess the importance of the root system through an estimation of root densities as described by Tennant (1975). Samples were taken within layers of 0.2 m width, down to 1.0 - 1.2 m depth, following east and south directions, along the line of drippers (south) as well as perpendicular to this. They were then washed out abundantly and sieved through a 0.5 mm screen. Extracted roots were counted by adopting a reference scale (Tennant, 1975). Root length was then derived from the average root density value for each of the six trees. Figure 1 presents details on both protocols. With this scheme, it was possible to obtain information on root distribution in the zones affected and not affected by irrigation.

Canopy measurements. Canopy diameter measurements were monitored at the same time as the study of the root system and on the same experimented trees. The maximum projected canopy area (S_c) was determined for each of the six trees assuming a circular shape. These measurements were used to set a typical model of growth and to examine the relationship between root and canopy development.

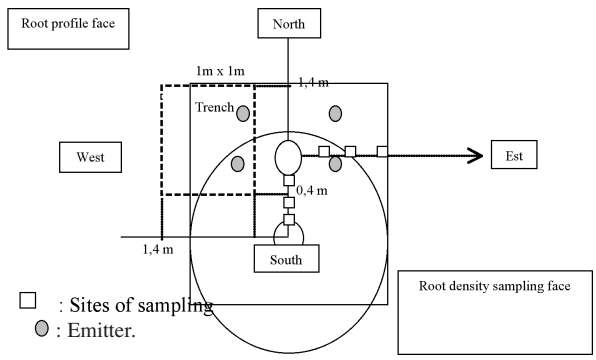

Fig. 1 - Scheme of sampling to determine root distribution and root densities for young olive trees aged one to six years. Root profiles were mode following to NW direction while samples of root density determination were taken at 0.4 m, 0.8 m and 1.2 m from trunks to 1.2 depth following to SE direction.

Canopy leaf area was determined for the six-year-old olive tree by computing the number of leaves on representative shoots and estimating its specific leaf area. It reached 14 m^2 on May 2003. This value was adopted to calculate the root length/leaf canopy ratio.

Methodological approach to determine irrigation requirements of young olive trees. This section proposes a methodological approach to determine irrigation requirements of young olive trees and how the water supply can be linked to the development of the canopy and root system during the first years of cultivation when ground cover and root system are incompletely developed. Determination of water requirements according to the FAO method (Allen *et al*., 1998) is adequate for standard conditions, i.e. when soil coverage reaches 60% or more. However, when the coverage area is less, a reductive coefficient K_r is introduced (COI, 1997; Allen *et al*., 1998). In some cases, particularly for young and new orchards (low tree canopy cover), this coefficient may not be precise enough to allow good estimation of water needs. In addition to problems estimating K_r values, the K_c is strongly affected by conditions that influence evaporation from the soil surface (Orgaz *et al*., 2006). Recently, Testi *et al*. (2004) proposed a simple linear relationship between the olive ground cover (and Leaf Area Index) and the average K_c of the summer months, valid for ground cover fractions up to 0.25, along with its variation when wet surface soil spots are present. These authors indicate that this relationship does not apply outside a rainless summer, and the contribution to soil evaporation from the drip system depends on the surface area and location of the wet spots and is not scalable.

Thus, we developed the following approach which is designed to determine the consumptive use of olive trees in relation to their canopy growth and root development during the first six years after planting.

Before full development of the root system, only a fraction of rainfall water is accessible to trees. Thus, the water balance equation should consider the area concerned by tree transpiration *i.e*. where roots are active (S_r); S_r is assumed to be circular and to increase following a logistic-shaped curve.

Root extension, as well as canopy increase, seems to coincide with a logistic growth curve as given by the following equation:

$$L(t)=Lo+\frac{Lx-Lo}{1+\exp[\alpha(t-\beta)]}$$

where (t) is the number of years from planting; Lo, Lx dimensions of interest, respectively, at planting and at maximum growth; α, β are adjustment parameters.

In order to link the water supplied to trees to the evaporative demand, a supply ratio (K_{supply}) that takes into account only the tree-related quantities is defined by this equation:

$$K_{supply} = (P^* + I^*) / ET^*$$

Considering that irrigation (I*, m^3) is supplied by a localized system or in small basins around the trunk, only a small surface is wetted and affected by soil evaporation and transpiration. Irrigation water is therefore assumed to be fully accessible to the root system of the tree. On the other hand, effective rainfall for a single tree (P*) is taken as the volume of rainfall water available to the root system which could be approximated by the following equation:

$$P^* (m^3) = P (m) \times S_r (m^2)$$

P is rainfall, considered here as total rainfall.

The evapotranspiration volume of an individual tree (ET*) can be estimated from the root area of the tree as:

$$ET^* (m^3) = K_c \times ET_o (m) \times S_r (m^2).$$

Different water supply ratios are determined as K_c - FAO, I/ET_o, P*+I*/ET*and I*/ET*. The ratio I/ET_o is the irrigation supply, P*+I*/ET* is the volumetric total supply and I*/ET* is the volumetric irrigation supply. These ratios are for the period April-August over the first six years of olive tree cultivation. Values are represented in the same figure to compare results.

3. Results

Soil water status

Simultaneous monitoring of soil moisture carried out during the 2003 campaign at the canopy limit and near the emitters showed that soil water contents vary from 15 to 39% according to depth and distance to trunk (Fig. 2). Low values of soil water content were observed in the upper layers, while minimums were recorded within the superficial strata (0-20 cm) as a result of soil water evaporation and root absorption. This result confirms the concordance between root development and soil water depletion. The results showed large variation between measurements at the limit of the canopy, while low variation of soil moisture was observed near the emitters with values ranging between 32 and 38% according to depth (Fig. 2).

Root system drawings

Root profiles for the tagged trees show two or three types of roots according to age (Fig. 3). During the first years after planting, trees developed fine roots in the upper 0.2 m of the soil layer, which then extended rapidly in lateral and vertical directions with inclinations varying from 30° to 60° depending on their size and position. For older plants, larger roots were observed beyond the first 0.3 m and they developed horizontally with numerous fine roots.

The number and diameter of roots which emerged from the lateral face of the trench are summarized in Table 3.

Results indicate that most roots (70%) are localized in the first 0.6 m of soil. The maximum number is found in the top layers, with diameters ranging between 2 mm (one-year-old tree) and 32 mm (four-year-old tree). Some roots developed in deeper strata, reaching 1.0 m depth. Very few roots were found below this depth even for the oldest tree.

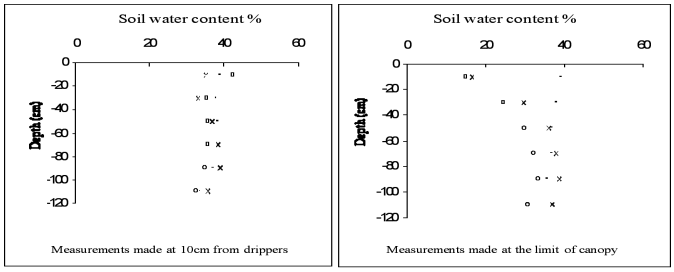

Fig. 2 - Soil water content (%) measured at two sites: on the left at 10 cm from the emitters and on the right at the limit of the canopy during the 2003 campaign.

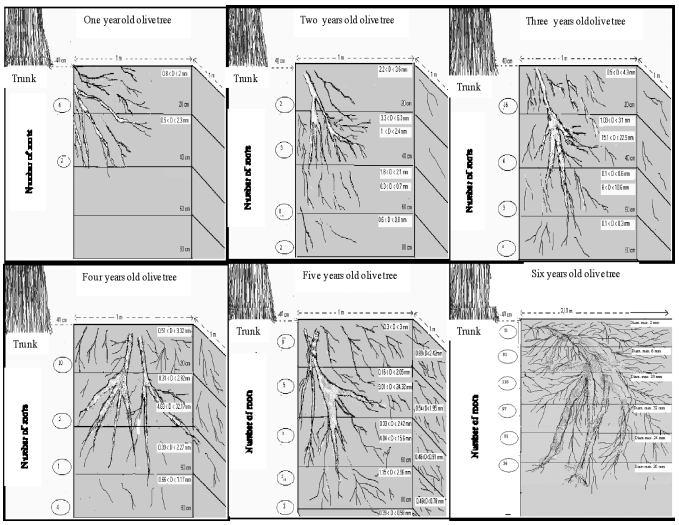

Fig. 3 - Drawings of the root system of young olive trees of cultivar Chétoui aged one to six years. Roots were counted on the internal trench wall, down to 1.0 - 1.2 m depth depending on age.

Table 3 - Maximum number of roots and root diameter emerging from the trench face for each soil layer for olive trees aged one to six years

	Age (year)					
Soil layer (cm)	1	2	3	4	5	6
0-20	6	2	16	10	9	51
20-40	2	3	6	5	5	91
40-60	0	8	3	1	3	116
60-80	0	2	4	4	5	97
80-100	0	0	0	0	3	81
Total number of roots	8	15	29	20	25	472
Maximum root diameter (mm)	2	6	23	32	24	27

Extension of the root system

Results presented in Table 4 show that the main development of the root system occurred during the first two to four years of cultivation, horizontally and within the top layers (0.2-0.3m). During this period, the soil volume explored by roots increased at a regular rate of about 1.0 m^3 yearly. For the three-year-old tree, roots explored a volume of 3.65 m^3. The soil volume explored by the root system of the five-year-old-tree represents 47% of that reached by the older tree (six-year-old tree).

Root density

Results relative to root density estimation are reported in figure 4. A noticeable root concentration is observed for both east and south directions and close to trunk in the top layers around each of the six trees. Average values varied between 0.001 cm cm^{-3} and 0.670 cm cm^{-3} depending on depth, distance to trunk, direction and tree age.

Greater values, by up to 0.5 cm cm^{-3}, were recorded in the first 60 cm and at 0.4 m from trunk. These values decreased significantly as the distance to trunk increased (except some measurements for two- and three-year-old plants). Roots were less frequent at all depths outside the canopy limit and particularly for the deeper layers. At these depths, however, it should be mentioned that root densities rarely exceed 0.4 cm cm^{-3} for both directions, while average values ranged between 0.067 cm cm^{-3} and 0.303 cm cm^{-3} (Table 5).

Root system length

The overall length of the root system varied from 1.0 km to 33.9 km depending on age (Table 6).

A significant increase of the overall length of the root system was observed for the six-year-old tree. It was 4.8 times greater than that recorded the previous year. The lowest value was recorded for the four-year-old tree. There was no apparent cause which could explain this result.

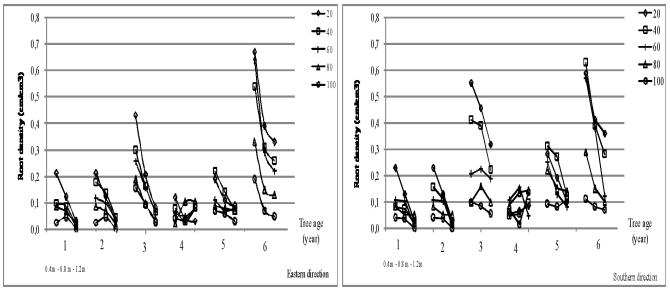

Fig. 4 - Root densities (cm cm^{-3}) recorded for olive trees of cultivar Chétoui aged one to six years based on direction and depth. For each tree, three measurements were carried out for both directions at different distances from trunk; the first observation was made at 0.4 m, the second at 0.8 m and the third at 1.2 m.

Root development and canopy growth

Results presented in figure 5 showed for tree aged one to four years that roots grew at higher rates than

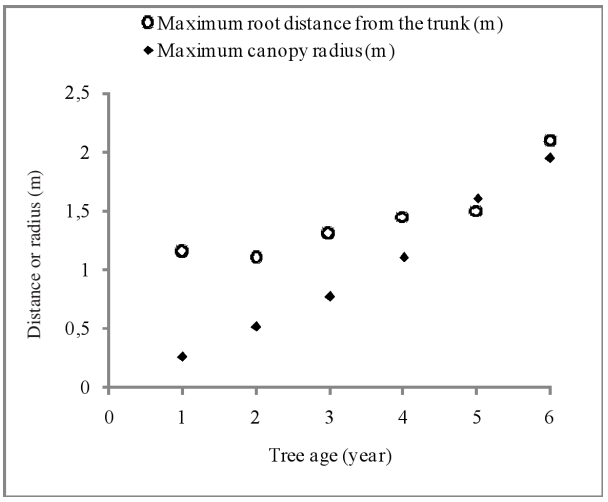

Fig. 5 - Maximum root distance from the trunk (m) and Maximum canopy radius (m) following to age for olive trees Chétoui ages one to six years.

Table 4 - Maximum distance of roots to trunk (m) and volume of soil explored by the root system (m^3) for olive trees aged one to six years

Depth (cm)	Age (year)					
	1	2	3	4	5	6
0-20	1.05	1.05	1.25	1.45	1.50	2.12
20-40	1.15	1.10	1.30	1.45	1.45	1.95
40-60	0	1.00	1.25	1.25	1.25	1.80
60-80	0	0.80	1.00	1.25	1.25	1.65
80-100	0	0	0	0	1.00	1.55
Explored soil volume (m^3)	1.45	2.55	3.65	4.60	5.30	11.2

Table 5 - Average root densities (D_r, cm cm^{-3}) determined for trees aged one to six years

	1	2	3	4	5	6
D_r (cm cm^{-3})	0.067	0.079	0.196	0.075	0.133	0.303

Table 6 - The overall length of root system (L_r, km) for trees aged one to six years

	1	2	3	4	5	6
L_r	1.005	1.975	7.056	3.450	7.049	33.936

canopy radius. Then, differences between the canopy radius and the root-to-trunk-distance decreased. Roots reached for the six-year-old tree a maximum distance to trunk of 2.10 m, while the canopy limit was observed at 1.95 m. The projected canopy area (S_c) increased slowly after planting to reach 0.21 m^2 for the one-year-old tree and 11.94 m^2 for the six-year-old-tree (Table 7), while the root area progressed at a constant rate of 1.2 m^2 per year to reach 13.8 m^2 for the six-year-old-tree.

A significant relationship was found between canopy (S_c, m^2) and root (S_r, m^2) areas, which can be approximated by a linear model with a correlation coefficient r of 0.94, as illustrated by Figure 6, where

$$S_c = 1.183\ S_r - 3.602 \qquad (R^2 = 0.876)$$

The S_r/S_c ratio derived from both canopy and root areas decreased significantly from 20 to 0.9 depending on tree age. For the four-, five- and six-year-old trees, this ratio approximated the unit.

A decrease of the S_r/S_c ratio implies a tendency to equilibrium between the under-ground and above-ground organs beginning from the fourth year after planting, which apparently results from the establishment of competition between shoots, roots and fruits (and explains the decrease of this ratio). In fact, trees began to produce olives within the second year after planting and the first commercial crop arrived in year four (6.5 kg / tree).

Results indicate also that plants seem to be able to adjust their root systems to the larger above-ground development during the winter rest. This feature is well represented by the root length/leaf canopy area ratio. A value of 2.3 km m^{-2} of leaves for the six-year-old tree was found in the present study, a value which is considered optimum for such conditions.

Irrigation supply as a function of canopy and root development

In order to link the water supplied to trees to the evaporative demand, a supply ratio (K_{supply}) that takes into account only the tree-related quantities is defined as developed in section 'Measurements. Methodological approach to determine irrigation requirements of young olive trees'. This ratio could be considered as a crop coefficient for young trees when reference evapotranspiration, rainfall and irrigation amounts are computed according to the previous equations and expressed in m^3/tree. Adoption of such a ratio allows estimation of irrigation requirements for different rainfall and evapotranspiration regimes. The different water supply ratios, K_c - FAO, I/ET_o, P*+I*/ET* and I*/ET*, determined for each of the six olive trees are given in Figure 7 for comparative purposes.

Results show that the ratio of applied irrigation (I, mm) to reference evapotranspiration (ET_o, mm) during the dry season from April to August was very low. It increased from 0.02 to 0.14 when trees grew from one to six years. When using the volume method to calculate the irrigation and precipitation falling on the area covered by roots, K_{supply} comes very close to the K_c-FAO. Estimation of effective precipitation remains however big challenge for using the proposed method.

Table 7 - Canopy and root area estimations (m^2) of olive trees aged one to six years

	1	2	3	4	5	6
Root area (S_r)	4.20	3.80	5.30	6.60	7.10	13.80
Canopy area (S_c)	0.21	0.82	1.86	3.79	8.04	11.94
S_r / S_c	20.00	4.60	2.80	1.70	0.90	1.20

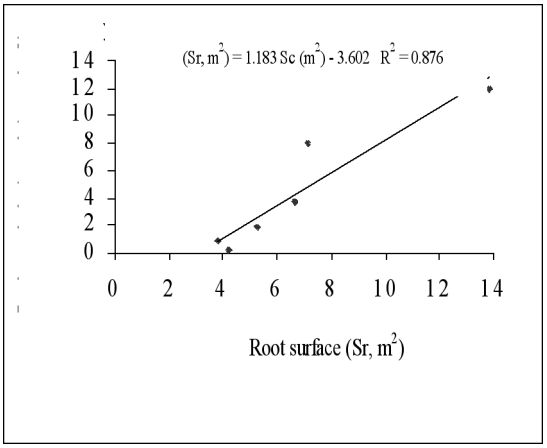

Fig. 6 - Relationship between canopy and root areas for young olive trees aged one to six years.

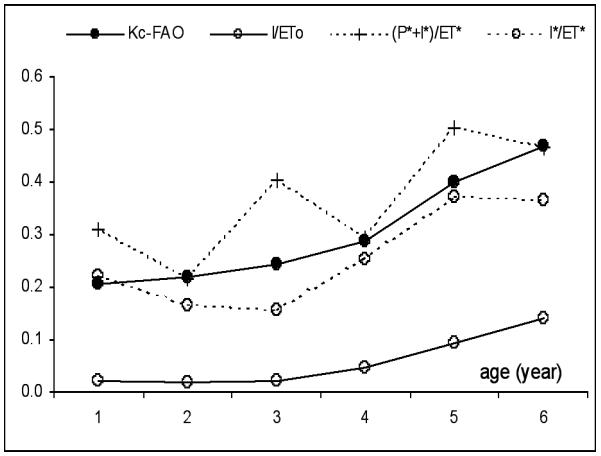

Fig. 7 - Variation of K_c-FAO, irrigation supply (I/ET_o), volumetric total supply (P*+I*/ET*) and volumetric irrigation supply (I*/ET*) ratios calculated for the period April-August over the first six years of olive tree cultivation, 1998-2003, Mornag - Tunisia.

4. Discussion and Conclusions

This study provides preliminary results on root distribution of young olive trees of cultivar Chétoui, which could be exploited to manage young olive orchards efficiently. Root profiles for trees aged one to six years show rapid extension of the root system during the first two to four years of cultivation following to horizontal direction. Most roots (70%) are localized in the first 0.6 m of soil with a maximum number developed in the top layers. Some roots developed in deeper strata, reaching 1.0 m depth, but at this age very few roots were found below this depth. The largest roots were observed beyond the first 0.3 m with maximum diameters between 2 mm and 32 mm according to age. Results indicate also that lateral fine roots are abundant and give rise to a fibrous root system which represents the main absorbing surface as it was reported by Palease *et al*. (2000). These roots originate from the branching of a parent root and constitute their ramifications, generally at right angles, as indicated by Doussan *et al*. (2003), who classified the roots into three main categories according to their ontogenesis: primary, adventitious and lateral. In our case, the primary root constitutes the main root of the cutting. It was not dominated at the outset by a principal axis as it occurs in trees grown from seedlings. Rather, many adventitious roots are produced from the base of the cutting. Similar results were found in the literature for young olive trees, although studies were carried out under different conditions. Abd-El-Rahman *et al*. (1966) reported for young trees, aged seven years and grown under 150 mm of rainfall, that roots are contained in the shallow tillage (0.15-0.30 m) to approximately 0.3 m from trunk. In Sardinia, Pisanu and Corrias (1971) observed a very shallow root system in the roots of excavated trees. Their photographs and drawings clearly illustrate the horizontal development of the roots and the fact that roots of contiguous trees avoid competition by developing outwards from the tree row. In Spain, Nunuez-Aguilar *et al*. (1980) observed for 12-year-old 'Manzanilla' olive trees, that most roots are localized in the outer layers at 0.45 m from the trunk with diameter less than 0.5 mm. Mickelakis and Vougioucalou (1988) observed for five-year-old 'Kalamon' olive trees cultivated in Create, a maximum number of roots at a depth of 0.4 m. Later, Bongi and Palliotti (1994) indicated that the root system of young olive trees is mainly confined to the top meter of soil, growing at depths between 0.15 and 0.40 m at a maximum distance of 0.30-0.40 m from the trunk.

Results relative to soil volume exploration showed a regular increase of about 1.0 m^3 yearly but this rate is apparently lower than that reported in other studies. For three-year-old trees cultivated on loamy soil in southern Italy, Dichio *et al*. (2002) found volumes of about 8.6 m^3 for the irrigated trees and 5.1 m^3 for those cultivated under rain-fed conditions (670 mm/year of rainfall). In our case and for trees of the same age, roots explored a volume of 3.65 m^3 only. This extension represents, according to Fernandez and Moreno (1999), Doussan *et al*. (2003), Fernandez *et al*. (2003) and Connor and Fereres (2005), the plants' evolutionary response to the spatio- temporal variability. It explains, in our case, the lateral spread of roots and the depths they achieve; soil characteristics (clay) and its mechanical resistance may adversely affected root exploration. Increases in soil strength during the summer months, as a consequence of occasional water shortage (interval between irrigations varying between 20 and 50 days), may have reduced the average number of laterals developed on the primary axes. During the following years (fifth and sixth years) the application of drip irrigation led trees to limit their root development, confining most roots to the upper layers with a noticeable root concentration observed for both east and south directions close to the trunk. An increase of root density is however observed with average values varying between 0.001 and 0.670 cm cm^{-3} depending on depth, distance to trunk, direction and tree age. Similar values ranging between 0.1 and 1.0 cm cm^{-3} were reported by Connor and Fereres (2005). Greater values of up to 0.5 cm cm^{-3} were recorded in the first 60 cm and at 0.4 m from the trunk. Values of root density then decreased, significantly as distance to trunk increased (except some measurements for two- and three-year-old trees). Roots were less frequent at all depths outside the canopy limit and particularly for the deeper layers. Nunez-Aguilar *et al*. (1980) observed similar results for 12-year-old 'Manzanilla' olive trees with highest values of about 0.7 cm cm^{-3} at 0.45 m from the trunk. For seven-year-old olive trees growing with only 150 mm mean annual rainfall, Abd-El-Rahman *et al*. (1966) also found maximum root densities in the top layers at 0.15 - 0.30 m and up to 0.3 m from the trunk. These results show good concordance between soil profiles made for the six experimental trees and their root density distribution, having agronomic applications, since they could be used to manage more efficiently irrigation and also fertilization. Water and fertilizer supplies should be given at these distances from trunks for young trees to guarantee their efficacy.

Many factors are cited to explain root density distribution (Fernandez and Moreno, 1999) amongst the cultural practices are reported in most papers. In our case, the six-year-old tree provided the highest values with average density of 0.303 cm cm^{-3}, however the root densities recorded for the three-year-old plant were greater than those observed for the older trees. Genetic factors inherent to the potentialities of that tree may be involved (Michelakis and Vougioucalou, 1988). However, it seems that the most influential factor that affected root density is the heterogeneous distribution of water in the orchard. Results showed spatial variability of soil moisture with lower differences between measurements near the emitters (values ranging between 32

and 38% depending on depth) and larger variation between measurements at the limit of the canopy. This result was unexpected, but it may indicate lower rates of root absorption around the emitter despite the high densities observed at this distance from trunk (0.4 m). For such a situation, Fernandez and Moreno (1999) indicated that sites of maximum root density may coincide with low root activity as a compensation mechanism; thus root activity may be higher in zones of low root density than in zones of high density.

The influence of soil water content on root distribution is reported by Fernandez *et al*. (1991), who observed that adequate watering makes roots continue to grow during the dry season, thus, increasing the period of their activity and preventing their shrinking during this period. Palease *et al*. (2000) and Bongi and Palliotti, (1994) indicated that root extension depends largely on the distributed water amounts and the irrigation frequency. Larger volumes of water would favor the existence of wider wet bulbs and could increase root length density. In opposite, low water availability can slow down root growth because roots are able to sense the soil dryness and order stomata to close; thereby reducing water losses and preventing excessive water stress. Water shortage may also increase mortality of fine roots even in the irrigated orchards; roots developed outside the wetted area during the rainy period may die.

Root distribution and densities are also highly dependent on leaf area and canopy development. In fact, this trial shows that olive tree establishes equilibrium between root and canopy development rapidly, around the fourth year after planting despite the larger extension of roots observed during the first two years in comparison to canopy growth. Such increases in root area could be explained as a need to adequate the root system to a more vigorous canopy development. Inversely, greater leaf area could provide greater total carbohydrates reserve for root activity. This relationship between leaf and root is very important to consider because dry soil conditions determine a cumulative effect over the years which indirectly affected root activity through an integrated chemical and hydraulic signaling mechanism controlling leaf water relationships, as stated by Fernandez and Moreno (1999). It could be represented by the under-/above-ground ratio. Our results show high values of this ratio during the first year after planting, indicating a greater availability of water per unit of leaf area. However, beginning from the second year after planting this ratio decreased rapidly to attain a minimum value of 0.9. Dichio *et al*. (2002) explains that a decrease of root-canopy ratio is a consequence of lack of water during the growing phase, which led plants to several physiological modifications; thus it can be used as an indicator of tree adaptation to water shortage.

Other reasons could be evoked to explain the decrease of this ratio such as the establishment of competition for nutrients between shoots, roots and fruits, which are considered the strongest sinks. The establishment of such competition is important to insure a balanced development of the tree, once it begins to set fruits. During this period of youth and first-fruit-set, the tree re-orientates the mobilization of carbohydrates (Proietti and Tombesi, 1996; Palease *et al*., 2002) and high amounts of assimilates are drain to growing olives against the competing demand of the growing roots and shoots. As a results, the number of roots and their length could be reduced because their growth remain highly dependent of the available assimilates.

Under adequate watering conditions, olive trees seem to be able to adjust their root systems to the larger above-ground development during the winter rest essentially when no (or low) competition with other organs occurs. Such result was reported by Palease *et al*. (2002) and Connor and Fereres (2005) who indicate that this feature is well represented by the root length /leaf canopy area ratio. In our experiment we found a value of 2.3 km m^{-2} of leaves for the six-year-old tree. This ratio is concordant with the optimum values of 2.2 - 2.9 km m^{-2} which were determined for intensive plantations (Connor and Fereres, 2005), and this result is very important for the current work because it indicates that olive trees were adequately irrigated. Such management of water ensured good development of the root system and the canopy despite the difficulties involved in fixing the irrigation amounts for such young trees with regard to the incomplete soil coverage and root development.

For such young orchards, it is known that only a fraction of rainfall water is accessible to trees. Thus, the water balance equation should consider only the area concerned by tree transpiration *i.e*. where roots are active. This area is assumed to be circular and results show that it increases following a logistic-shaped curve (Masmoudi *et al*., 2007). This factor was taken into account to develop a mathematical model which allows estimation of irrigation needs of young trees, based on the study of the root/canopy over a long period of time. In this model a supply ratio was determined as shown previously in order to link the water supplied to the evaporative demand that takes into account only the tree-related quantities. Results show that the ratio of applied irrigation to reference evapotranspiration during the dry season from April to August was very low. It increased from 0.02 to 0.14 when trees grew from one to six years. When using the volume method to calculate the irrigation and precipitation falling on the area covered by roots, the supply ratio comes very close to K_c-FAO. Estimation of effective precipitation remains, however, a big challenge for using the proposed method.

The present study provides preliminary results on root distribution of young olive trees of cultivar Chétoui, which could give insight into the efficient management of intensive orchards. However, these results

should be enhanced by root activity observations. Furthermore, development of a more detailed study on young trees would be useful to get more information on the relationship between root activity and root distribution because sites of heavy root density may present lower root activity, even in young trees. In such study, the root system should be viewed as a population of roots with varying, although coordinated, morphological and physiological properties. Measurements of carbohydrate status at different stages of development at both root and canopy levels would also improve these results and give us more valuable information on the relationship between the rooting system distribution and canopy development, essential for irrigation requirement estimation as they determine evapotranspiration and water available for the root system. More knowledge is needed on root growth in young trees because they are more vulnerable to water shortages. For such trees, and particularly plants obtained from rooted cuttings, water uptake remains highly dependent on the effective areas of transpiration and water absorption. Thus, it is probably more convenient to consider evapotranspiration, rainfall and irrigation in terms of volume of water/tree instead of mm. Our progress in the future will be measured by our capacity to integrate knowledge on water supply, evaporative demand and the soil volume explored by the root system for different locations and planting densities.

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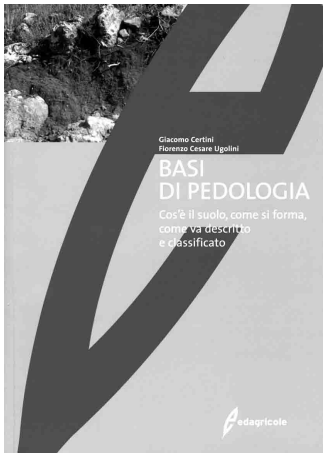
BOOK REVIEWS



BASI DI PEDOLOGIA. Cos'è il suolo, come si forma, come va descritto e classificato. *Certini G.*, and *F.C. Ugolini*. Edagricole, Edizioni Agricole Il Sole 24 Ore, Bologna, 2010. pp. 196. ISBN 978-88-506-5286-0. € 19.00.

At a time when the study of crops is increasingly refers to the soil characteristics in which they grow, the text by Giacomo Certini and Fiorenzo Cesare Ugolini is unanimous and enthusiastically welcomed by the scientific world, and agricultural world in general. Knowing soil texture, its constitution and origin is the first step to set up and rationally manage each crop. Furthermore, in the context of fruit tree species, the optimal balance between vegetation and production depends largely on the relationships between rootstock and soil and, therefore, between plant and peculiar soil characteristics which for many years will host the root system. The work of the aforementioned authors is, therefore, a long-awaited, scientific and technical reference for those who want to deepen their knowledge in the "horizon" of soil.

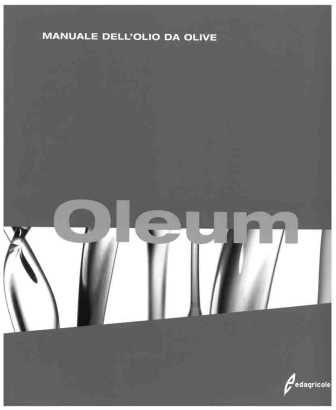
The text is well articulated and the essential themes, accurately and deeply treated, reflect the rigorous expertise of the authors in the field. The volume includes the following eight chapters. 1. *Che cos'è il suolo, a cosa serve e come si forma* (What is soil, what is it for how does it forms); 2. *Le fasi del suolo* (Soil phases); 3. *Il profilo del suolo e la sua descrizione* (The soil profile and its description); 4. *Le forme di humus* (The forms of humus); 5. *I fattori della formazione del suolo* (Factors of soil formation); 6. *I processi pedogenetici* (Pedogenic processes); 7. *La Soil Taxonomy. Gli orizzonti diagnostici e i regimi pedoclimatici* (Soil Taxonomy. Diagnostic horizons and soil and climate regimes); 8. *I dodici ordini della Soil Taxonomy* (The Twelve Orders of Soil Taxonomy).

The volume is completed by an ample and rich list of references and a useful analytic index.

Furthermore, the wide range of tables, figures in black and white and color make it easy to read and support the display of topics. The text is an indispensable tool for students, to whom is addressed, but there is no doubt about the value it can bring to those working in agriculture, either as scholars or direct operators.

Cinzia Silori

OLEUM. Manuale dell'olio da olive. *Ricci A.* (ed.) Edagricole, Edizioni Agricole Il Sole 24 Ore, Bologna, 2011. pp. 320. ISBN 978-88-506-5276-1. € 45.00.



With the release of "Oleum", this interesting and qualified volume adds to those concerning the complex and brood Agro-Industries topic. The text, written in the specific context of the processing of olives into oil, has makes use of the collaboration, synergistic and additive, of the most talented scholars in the field. The result obtained by the editor, Antonio Ricci, is that of a harmonic fusion of scientific knowledge that, right from the first chapter, gives the reader a broad and updated view of Knowledge and acquisitions. Each chapter, cleverly arranged in a crescendo of technical and scientific horizons, deals with the specific issues by providing, in addition to an up-to-date view of the topic, also a large repertoire of bibliographic references. The topics are organized as follows. 1. *La chimica dell'olio da oliv*e (Chemistry of olive oil) (G. Lercker); 2. *Normativa sui requisiti chimici, fisici e organolettici degli oli di oliva* (Regulatory requirements on chemical, physical and organoleptic characteristics of olive oil) (L. Conte); 3. *I contaminanti e la filiera produttiva degli oli di oliva* (Contaminants and the olive oil production process) (L. Conte, S. Moret, G. Purcaro, T. Populin, A. Ermacora, and M. Marega); 4. *Caratteristiche chimico-fisiche dell'oliva e dell'olio con riferimenti alle cultivar più diffuse* (Physical and chemical characteristics of olive and oil with reference to the most cultivated cultivars) (G. Panelli); 5. *L'olivo e l'olio nell'emisfero sud* (Olive and oil in the southern hemisphere) (G. Panelli); 6. *Caratterizzazione molecolare delle varietà di olive e degli oli di oliva* (Molecular characterization of olive varieties and of olive oils (L. Baldoni, R. Mariotti, and N.G.M. Cultrera); 7. *Tecnologie di estrazione, conservazione, packaging e loro riflessi sulla qualità dell'olio vergine di oliva* (Extraction, storage and packaging technologies and their impact on virgin olive oil quality) (M. Servili, S. Esposto, A. Taticchi and R. Sacchi); 8. *Utilizzazione dei reflui oleari* (Use of oil wastewater production) (M. Servili, S. Esposto, S. Urbani, A. Taticchi, and M. Petruccioli); 9. *Caratteristiche meccaniche e funzionali degli impianti di estrazione dell'olio di oliva* (Mechanical and functional characteristics of olive oil extraction machinery and wastewater mills) (P. Amirante); 10. *Modelli strutturali di frantoi e collocazione logistica degli impianti* (Structural models of mills and logistic location of facilities) (P. Amirante); 11. *Oli aromatizzati* (Aromatic oils) (L. Cerretani); 12. *Utilizzo dell'olio nella preparazione dei prodotti agroalimentari* (Using oil in the preparation of foodstuffs) (L. Cerretani); 13. *Valutazione sensoriale degli oli vergini di oliva e loro classificazione* (Sensory evaluation of virgin olive oils and their classification) (A. Giomo); 14. *La valutazione sensoriale degli oli vergini di oliva* (Sensory evaluation of virgin olive oils) (B. Alfei); 15. *Normativa relativa alla commercializzazione dell'olio di oliva* (Legislation on olive oil marketing) (R. Filo della Torre).

The elegant typographical presentation and the impressive range of tables and images in black and white and color make the work not only attractive but also make reading it a pleasent and fuild experience. For its peculiar characteristics, the text addresses a broad spectrum of readers: from student to scientists of the subject, from the technical crusher to the manufacturer of machinery for olive oil extraction, and from traders to the legislators.

Enrico Rinaldelli