Production of Micropropagated Melon Plantlets Adapted to Saline Environment

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Abstract

An experiment was carried out to evaluate the behaviour on salt stress induced by sodium chloride of two melon clones obtained from micropropagation. Since arbuscular mycorrhizal (AM) fungi seem to increase salt tolerance in some crops, at acclimatisation melon plantlets were mycorrhizal with an AM fungus. The root systems of both mycorrhizal and non mycorrhizal plants were exposed to increasing salt concentrations and then placed in distilled water to study the wilting response and the recovery of plants. Observations were made on several morphological parameters, in particular to verify the modification of root system morphology caused by AM fungus, as demonstrated already by several studies. The analysis of infected roots was carried out following Phillips and Hayman's protocol (1970). Afterwards, the specimen was read using an optical microscope.

INTRODUCTION

Under natural conditions the root system of numerous plants is in arbuscular mycorrhizal (AM) symbiosis with positive influence on mineral nutrition, water uptake, hormone production and resistance to root disease.

Several studies have demonstrated the effectiveness of mycorrhizal colonisation on plants under different stress situations (Sylvia et al. 1992, Augè 2001, Entry et al., 2002). In some crops such as onion and bell pepper arbuscolar mycorrhizal fungi seems to increase salt tolerance (Hirrel et al, 1980), while in tomato the use of mycorrhizae to overcome the salt stress is still controversial (Prud et al., 1984, Copeman et al., 1996).

Melon has been described by various authors as a moderately tolerant crop to salinity, but it has been shown that the effects of salinity are more harmful during the first stages of growth relative to further stages of plant growth and development (Nukaya et al. 1985, Franco et al. 1993).

For the micropropagated plantlets, moreover, the transfer to ex vitro conditions is one of the most critical steps of the micropropagation process. The limitations in resisting the trasplant stress include a poorly developed cuticle, non-functional stomata, a heterotrophic habit, and a weak root system.

The aim of this study was to verify the possible different behaviour of plantlets obtained from micropropagation of two melon clones on salt stress induced by sodium chloride and if the inoculation of AM fungi early on in acclimatisation increases salt tolerance.

MATERIALS AND METHODS

The experiment was carried out on two melon clones that we obtained through micropropagation of the cultivar "Pynionet" from Valencia and a selected Fusarium oxysporum-resistant accession from the Apulia population supplied from Dipartimento di Biologia e Patologia Vegetale of Bari University. Microplants with three nodes were transplanted into pots with a substratum composed of sterile soil-agriperlite mixed (2:1 v/v) and crude AM inoculum (5 g/pot) and compared with untreated plants. Crude AM inoculum consisted of soil which contained spores, external mycelium and infected root fragments obtained from alfalfa pot-cultures inoculated with *Glomus viscosum* strain A6. Plants were acclimatised in greenhouse conditions under mist in 90% U.R and 20-25 °C T for two weeks and then watered by hand regularly and maintained under natural light

supplemented with fluorescent lamps (800 w m^{-2})(16 h/day) for four weeks.

The root systems of two melon clones, either inoculated or not, were carefully washed to remove the substratum. Four replicate plants for each treatment were used. Each plantlet was placed in a box containing 200 ml of sodium chloride solution (NaCl 0 mM (test), 30 mM, 50 mM or 100 mM). When the wilting had stabilized, the plant roots were washed in distilled water for a few seconds and then transferred to boxes with distilled water until the recovery of the plantlets. To study the wilting trend, wilting indexes were defined based on the following characteristics: 0, no wilting; 1 leaves soft; 2, leaves and stem soft; 3, stem belt; 4, the whole plant is soft and hangs (Rosendahl et al, 1991). The rate of increase in the wilting index and in recovery for each treatment was calculated as the regression coefficient between the square root of time and wilting index.

The morphological parameters measured to quantify the growth of the VA and control plants were the number of leaves, the leaf area index, fresh and dry weight of shoots and fresh weight of roots. To characterize root system morphology root length and total density were evaluated using the modified line intersect method (Tennant, 1975). Root tips were examined by light microscopy.

The presence of a mycorrhizal infection was determined by clearing the roots in 10% KOH and staining them with trypan blue in lactic acid (Phillips and Hayman, 1970). Afterwards, the specimen was read using the optical microscope

The statistical analysis of data was based on the analysis of variance. Comparisons among the treatments were made by Student Newman Keuls test.

RESULTS AND DISCUSSION

The results of salt stress induced by sodium chloride on two melon clones either inoculated or not, are shown in table 1. To simplify matters, the clone obtained from the cultivar is referred to as 2 and the selected accession as 64.

From an examination of data relative to clone 2, it appears evident that the rate of increase in wilting index rises in line with the concentration of the sodium chloride solution. Thus, the higher the saline concentration, the faster the rate of increase in wilting index. In the mycorrhizal plantlets, these values are lower compared to the control as can be seen by the higher tolerance level. It is also possible in the mycorrhizal plantlets to note a significant increase in the time necessary to reach wilting in all the concentrations used. It is of particular interest to note that the time necessary for maximum wilting in mycorrhizal plantlets in a solution of 50mM of NaCl is the same as that of the non-mycorrhizal plantlets in 30mM of NaCl. The same can be seen in mycorrhizal plantlets in a concentration of 100mM and non-mycorrhizal plantlets in 50mM of solution; the relative rate of increase in wilting index is identical. Thus mycorrhizal symbiosis would appear to be particularly effective because it allows to use irrigation water with a higher saline concentration.

The comparison between the two clones showed clone 64 had a higher tolerance to saline conditions, a characteristic that can be based on the low rate of increase in wilting index and the correspondingly longer wilting time necessary verified in non-mycorrhizal plantlets. This characteristic determines a low reactivity to the treatment with mycorrhiza. When compared to the mycorrhizal plantlets, in fact, no significative differences can be seen except for a tendency for the tolerance to increase.

As regards the recovery rate (table 2), the lowest values correspond with the lowest time of recovery. From an analysis of recovery rates it is not possible to make a correlation between data and treatments. However, it should be noted that in clone 2, the mycorrhizal plants, with the exception of the experiment using 50mM of NaCl, recovered faster than the non-mycorrhizal plants.

A study of morphological parameters, made 45 days after the inoculation, showed the positive effect of mycorrhizal inoculation on the epigeal and ipogeal growth of the plantlets. (table 3). In the clones 2 the values relative to the leaf area and fresh and dry epigeal weight (83 cm^2, 2.1 g, 0.14 g) were doubled with respect to plantlets that were not treated (41.6, 1.1, 0.07 respectively) while the root characteristics – fresh weight, total

length and density (fig.1-2)- were more or less triplicated : 0.68, 133.8 e 0.67 compared with 0.16, 37.9, 0.19. Less notable were the differences in the clones 64, where however a significant increase could be seen in the number of leaves, the fresh epigeal weight and the total length of the root.

A study of the apexes under the microscope did not show any morphological or histological changes.

An image analysis of the clone 2 indicated the presence of the characteristic vesicles and longitudinally running internal hyphae on the roots of inoculated plantlets with AM fungus (fig.3) and their total absence in the non-inoculated test plantlets (fig.4). However, only a slight infection was found in the clone 64 where a delay in the onset of plant-fungus symbiosis was evident.

CONCLUSIONS

The two clones that were considered have different characteristics; indeed, clone 64 shows a greater development of the epigeal and hypogeal parts and showed more tolerance to saline conditions.

In accordance with what has been indicated in other researches (Chavez et al 1990, Gianninazzi et al.,1990, Gribaudo et al.1996) micropropagated plants responded positively to mycorrhizal inoculation. The values recorded for various morphological parameters showed a faster growth rate in mycorrhizal plants, which determines a higher tolerance to salt stress. The more evident development of root systems, and therefore of the absorbent surfaces, is of particular note, and could be correlate to a higher tolerance to salt stress.

The two clones displayed a different reaction to mycorrhizal colonisation. 45 days after inoculation clone 2 was full symbiosis between plant and fungus, while clone 64 displayed only slight signs of infection. It seems plausible to conclude that the differing behaviour of the two clones with respect to salt stress during the experiment derives from this. Data taken 45 days after inoculation shows that the mycorrhizal plantlets of clone 64 differed very little from the non-mycorrhizal plantlets of the same clone. There was no significant difference in their response to salt stress. The mycorrhizal plantlets of clone 2, however, were already completely infected, their growth rate was greater than the non-mycorrhizal plantlets and they proved more resistant to salt stress.

In conclusion, the results confirm the importance of mycorrhizal inoculation to obtain plantlets that are more efficient for nutrient acquisition and better equipped to cope with the stress situations that are not only typical of micropropagation but also of environmental stress such as salt. This cooperation between two biotechnological approaches, those of micropropagation and mycorrhizal inoculation, is an important tool in more sustainable horticultural production.

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Tables

Table 1. Rates of wilting increase and time necessary for maximum wilting from salt stress caused by NaCl (mM) on melon.

Clones	Treatment	Rate of increase in wilting index (wilting/min$^{-0.5}$)				Maximum of wilting (min)			
		30 mM	50 mM	100 mM	mean	30 mM	50 mM	100 mM	mean
2	control	0,278 a	0,344 a	0,637 a	0,419 a	330 b	255	105 b	230 b
	+ VA	0,118 b	0,268 b	0,344 b	0,243 b	1050 a	330	255 a	545 a
Mean		0,198	0,306	0,491	0,331	690	293	180	388
64	control	0,101	0,131	0,333	0,189	1050	1020	230	767
	+ VA	0,098	0,116	0,266	0,160	1080	1080	270	810
Mean		0,099	0,123	0,300	0,174	1065	1050	250	788

Rates of increase were calculated as the regression coefficient between the square root of time and the wilting index
Values followed by the same letter are not statistically different at 0.05 level (Test SNK)

Table 2. Rates of recovery from salt stress caused by NaCl (mM) on melon.

Clones	Treatment	Rate of recovery in distillate water (wilting/min$^{-0.5}$)			
		30 mM	50 mM	100 mM	mean
2	control	-0.162 a	-1.297 b	-0,387 a	-0,615
	+ AM fungus	-0.796 b	-0.477 a	-1.102 b	-0.637
Mean		-0.479	-0.887	-0.745	-0.626
64	control	-0.796	-0.706	-0.936	-0.813
	+ AM fungus	-0.900	-0.900	-1.313	-1.038
Mean		-0.848	-0.803	-1.125	-0.926

Rates of recovery were calculated as the regression coefficient between the square root of time and the wilting index

Values followed by the same letter are not statistically different at 0.01 level (Test SNK)

Table 3. Effect of mycorrhization on melon plants growth.

Clones	Treatments	Leaves (n.)	Leaf area (cm^{-2})	Shoot (f.w.) (g)	Shoot (d.w.) (g)	Root weight (g)	Root length (cm)	Root density (cm cm^{-3})
2	control	5.3 b	41.6 b	1,1 b	0.07 b	0.16 b	37.9 b	0.19 b
	+ AM fungus	7.1 a	83.0 a	2,1 a	0.14 a	0.68 a	133.8 a	0.67 a
mean		6.2	62.3	1,6	0.1	0.42	85.9	0.43
64	control	5.8 b	56.7 b	1.8	0.12	0.65	51.7 b	0.26
	+ AM fungus	7.2 a	76.4 a	1.9	0.13	0.79	74.3 a	0.37
mean		6.5	66.5	1.8	0.12	0.72	63.0	0.31

Values followed by the same letter are not statistically different at 0.01 level (Test SNK)

Figures

Fig. 1. Clones 2: Root system of mycorrhizal (left) and non-mycorrhizal (right) plantlet.

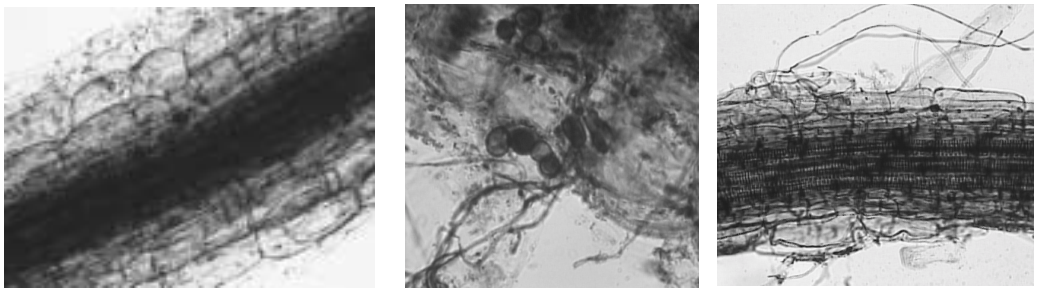

Fig. 2. Inoculated roots of melon clones 2: vesicles and hyphae (left), longitudinally running hyphae in root (center) and roots of non-mycorrhizal melon (right).