Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Scientia Horticulturae 106 (2005) 472–483
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

The effect of mycorrhizal symbiosis on the development of micropropagated artichokes

Irene Morone Fortunato [a,*], Claudia Ruta [a], Annamaria Castrignanò [b], Francesco Saccardo [c]

[a] *Dipartimento di Scienze delle Produzioni Vegetali, Università via Amendola 165A, I-70125 Bari, Italy*
[b] *CRA-Istituto Sperimentale Agronomico, via Celso Ulpiani 5, I-70125 Bari, Italy*
[c] *Dipartimento di Produzione Vegetale, Università, via San Camillo de Lellis, I-01100 Viterbo, Italy*

Received 8 November 2004; received in revised form 22 March 2005; accepted 4 May 2005

Abstract

In this work, microrosettes of *Cynara cardunculus* L. var. *scolymus* Fiori of the ''catanese'' type were subcultured in a medium supplemented with 6-benzylaminopurine (BAP) (0.05 mg l^{-1}). For root induction, indoleacetic acid (IAA), α-naphthalene acetic acid (NAA) and indole-3-butyrric acid (IBA) were used at three concentrations: 2, 5 and 10 mg l^{-1}. The highest percentage of rooted shoots was aided by the presence of 10 mg l^{-1} IAA.

Once transplanted in pots, the plantlets were inoculated with 10 g *Glomus viscosum* strain A6 (AM fungus). Acclimatisation was clearly facilitated by the addition of the AM fungus. Indeed, the mycorrhizal plantlets registered a survival of between 90 and 95% for the rooting shoots and 60% for the non-rooting shoots.

The botanical characterization of the material produced was carried out in field and was based on several morphological and productive parameters. Data collected confirm the characteristics of the original cultivar, the efficiency of the in vitro propagation material and the possibility of using this technique in early types of artichoke.

Keywords: *Cynara cardunculus* L. var. *scolymus* Fiori; In vitro rooting; Acclimatisation; Botanical characterisation; Fungus AM *Glomus viscosum*

* Corresponding author. Present address: Dipartimento di Scienze delle Produzioni Vegetali, Facoltà di Agraria, Via Amendola 165/a, Bari, Italy. Tel.: +39 080 5442975; fax: +39 080 5442976.
E-mail address: irene.morone@agr.uniba.it (I.M. Fortunato).

0304-4238/$ – see front matter
doi:10.1016/j.scienta.2005.05.006

1. Introduction

Studies on mycorrhizal symbiosis have made it possible to acquire a deeper understanding of the relationship between fungus and host plant. It has been established that mycorrhizal plants are aided by symbiosis through greater and faster root structure development as well as through numerous mechanisms such as greater water absorption and a higher resistance to both parasite attacks on the root structure and different types of stress (Azcòn-Aguilar and Barea, 1997; Gianinazzi et al., 1990; Giovannetti, 1990; Gribaudo et al., 1996; Sylvia and William, 1992). Moreover, the earlier the plant is inoculated, the more evident the effects will be (Fortuna et al., 1992).

The mycorrhizal plant has a greater growth capacity and is able to overcome adverse environmental conditions more easily, and thus, produce larger quantities and overcome stress. The acclimatisation phase of micropropagated plantlets is one of the most delicate phases of micropropagation and can create high levels of stress due to the unique characteristics of in vitro plantlets. Micropropagated artichokes are particularly sensitive to acclimatisation (Brutti et al., 2000; Ordas et al., 1990).

After improving in vitro rooting protocol, the principal objective was to study the effects of mycorrhiza on the growth trend of micropropagated early artichokes of the ''catanese'' type during acclimatisation as well as their in-field botanical characterization.

2. Materials and methods

Four *Cynara cardunculus* L. var. *scolymus* Fiori of the ''catanese'' type mother plants from a 2-year-old crop were used to establish micropropagation.

2.1. In vitro

Excised artichoke shoot tip explants, 5–6 mm in length, were transferred into a 70 ml glass tube (one explant per tube) containing 20 ml of basal medium (BM: macronutrients Murashige and Skoog (1962), micronutrients of Nitsch and Nitsch (1969), FeEDTA (25 mg l^{-1}), thiamine HCl (0.4 mg l^{-1}), myoinositol (100 mg l^{-1}) and agar (7 g l^{-1})) containing sucrose (20 g l^{-1}) and supplemented with γ·γ dimethylallilminopurine (2ip) (1 mg l^{-1}), indoleacetic acid (1 mg l^{-1}), gibberellic acid (GA_3) (0.025 mg l^{-1}) to establish the in vitro conditions (Harbaoui and Debergh, 1980).

After 3 weeks, the shoots were transferred into 500 ml jars containing 100 ml of proliferation medium and subcultured three times for proliferation. The proliferation medium was composed of BM, sucrose (20 g l^{-1}), and 0.05 mg l^{-1} 6-benzylaminopurine (BAP) (Morone Fortunato and Ruta, 2003). Each jar contained four shoots.

Each regenerated shoot was rooted in a 175 ml glass vessel containing 40 ml of BM added with a higher sucrose concentration (30 g l^{-1}) and supplemented with different auxins, indoleacetic acid, or α-naphthalene acetic acid (NAA) or indole-3-butirric acid (IBA) of differing concentrations (2–5–10 mg l^{-1}). For each treatment, 40 shoots were used.

The pH of the medium was adjusted to 5.6–5.8 prior to autoclaving. During the trial, artichoke explants were maintained in a growth chamber at 22 ± 1 °C with a photoperiod of 16 h light under a light intensity of 50 μE s^{-1} m^{-2}.

2.2. In nursery

The rooted and unrooted microplants obtained were transferred to a greenhouse. Acclimatisation took place in greenhouse conditions at 15–18 °C with mist and a humidity level reduced from 85–90% to 55–60% over 20–25 days. In order to aid acclimatisation, once transferred to commercial peat mixture soil the plantlets were inoculated with mycorrhiza fungus AM *Glomus viscosum* strain A6.

The peat mixture was enriched with nutrients (organic carbon 46%, organic nitrogen 1–2%, organic matter 80%) and mixed with perlite at a 2:1 (v/v) ratio. This was then sterilized and used to fill 10 cm diameter pots. Ten grams of Crude AM inoculum was then added to each pot at the base of both rooted and unrooted microplants. Crude AM inoculum consisted of infested soil which contained spores, external mycelium and infected root fragments obtained from strawberry pot-cultures inoculated with *G. viscosum* A6.

At the time of transplant and after 30 and 60 days the following parameters were studied: plant height (cm), fresh and dry leaf weight (g), leaf area (cm^2), number of roots, length of roots (cm), fresh and dry root weight (g). The leaf area measures were made using Licor LAI Area Meter 3100.

The analysis of infected roots was carried out following the protocol of Phillips and Hayman (1970) and the samples were then observed using an optical microscope.

2.3. In field

The botanical characterization of the material produced was carried out in field. The experiment took place at *La Pietra* farm in the coutryside of Monopoli, Bari, South Italy. The soil is characterised by a red colour and is of a medium depth with good structure and fertility. After 60 days of acclimatisation, 100 micropropagated and inoculated plantlets of *C. cardunculus* L. var. *scolymus* (L.) Fiori, of the ''catanese'' type were used for the trial. The distance between the plantlets was 120 cm between the rows and 100 cm within the rows.

The characteristics taken into consideration and descriptions of the variety were based on the morphological and productive parameters of Dellacecca et al. (1976) and were taken from 100 plants.

2.3.1. Morphological parameters

2.3.1.1. Vegetative phase. Plant: size reached by the plant in full yield, growth habit determined by the different angle of the leaves on the stem.

Leaf: colour, presence of thorns, dimensions with reference to the observations carried out on the fifth and seventh leaves, heterophylly, lamina: entire margin, lobed or pinnatisected.

2.3.2. Reproductive phase

Head: shape, compactness, dimensions (height and diameter) (cm), colour of the external bracts, presence of thorns on the bracts, shape of the bracts, apex of the bracts, superior margin of the bracts, dimensions of the bracts (cm).

2.3.3. Productive parameters

Number of heads per plant, fresh and dry weight (g) of heads, thickness receptacle (cm), fresh and dry weight edible (g).

3. Statistical analysis

3.1. In vitro

The experiment has two factors: the type of auxins used and their concentration and comprises a conventional 3×3 factorial arrangement. With three-fold replication this gave 27 experimental units, which were completely randomized. Each replication was a culture medium tube formed by the number of rooting shoots compared to the total number of tested shoots (40) for every treatment, and was expressed as a percentage. Since the data were expressed as percentages of rooting shoots, to overcome the difficulties of irregularities, we transformed the measured values according to the angular transformation. ANOVA analysis and multiple comparisons of means, using the Student–Newman–Keuls (SNK) method (Miller, 1981), were performed on the transformed data and the results were finally transformed to the original scale at the end.

3.2. In nursery

The variables were: plant height, fresh and dry leaf weight, leaf area, number of roots, length of roots and fresh and dry root weight. These were observed in a sample of 18 plants, split into 9 mycorrhizal and 9 non-mycorrhizal inoculated plantlets, at three successive times over unequal intervals, to see how the differences between treatments changed over time during the acclimatization period.

In experiments with repeated measurements the time factor cannot be randomised, moreover there is likely to be a greater correlation between observations that are made on successive occasions than between those separated by longer times.

Therefore, the conventional statistical analysis of variance could not be used directly with the repeated observations and we performed a multivariate approach of analysis of variance (Cole and Grizzle, 1966) to provide significance tests for the treatment effects. To perform the multivariate analysis, it was necessary to convert the univariate form of repeated measures data for each variable to multivariate form, where the measurements taken at time 1, 2 and 3 were treated as independent variables.

In the repeated measures analysis of variance, the three effects of interest were:

‘‘between-subject’’ effect (mycorrhiza);

''within-subject'' effect (time);
interaction between the two types of effects (mycorrhiza × time).

For the tests that involved only between-subjects effect, the analysis of variance was performed on the sum of the dependent variables divided by the square root of their number, which gives a one-way analysis of variance. On the contrary, for within-subjects effect and for within-subject-by-between-subject interaction effects multivariate tests were performed, which were Wilks' Lambda, Pillai's Trace, Hotelling-Lawely trace and Roy's maximum root (Morrison, 1976).

4. Results

4.1. In vitro

The results of analysis of variance on the transformed data (Table 1) show the high statistical significance of the auxin effect and its interaction with the concentration, whereas in Tables 2 and 3, the results of multiple tests for the only significant effects are reported.

4.2. In nursery

During acclimatisation, the mycorrhizal plantlets registered a survival of between 90 and 95% for the rooting shoots and 60% for the non-rooting shoots. The percentages of

Table 1
Analysis of variance of auxin and concentration effects and their interaction on the angular transformed values of shoot rooting

Source	DF	Rooted shoots (%)		Probability
		Mean square	*F*	
Growth regulators (gr)	2	0.042	58.63	<0.0001
Concentration (*c*)	2	0.0001	0.20	0.8202
gr × *c*	4	0.288	401.25	<0.0001
Error	18	0.0007		
Total	26			

DF: degrees of freedom; growth regulators: IAA, NAA, IBA; concentration 2, 5, 10 mg l^{-1}.

Table 2
Means of shoot rooting relative to the different types of auxins

Growth regulators (gr)	Rooted shoots (%)
IAA	49a
NAA	44b
IBA	36c

Values with different letters are signicantly different according to SNK' test (probability level < 0.05). Error mean square = 0.000718. Critical differences as a function of the distance between the means: 0.0265, 0.0322.

Table 3
Means of shoot rooting relative to the interaction between different auxin and concentration

Growth regulators (gr)	Concentration (mg l^{-1})		
	2	5	10
IAA	23d	37c	86a
NAA	53b	52b	26d
IBA	52b	40c	17e

Values with different letters are significantly different according to SNK' test (probability level < 0.05). Error mean square = 0.000718. Critical differences as a function of the distance between the means: 0.0460, 0.0559, 0.0618, 0.0662, 0.0695, 0.0723, 0.0767.

survival for non-mycorrhizal plantlets were much lower; 30–35% for in vitro rooting shoots and 0% for non-rooting shoot.

Table 4 gives the probability levels of multivariate test for between-subject (between plantlet) effect and for within subject (time) effect and its related interaction. As regards the multivariate tests, only the results of Wilks' test are reported, as the other tests produced similar results. The table shows that the between-subject effect was not significant only for the leaf number and the root length, whereas the within-effect was always highly significant. The interaction was not significant for the leaf number, the leaf height and the root length.

In Table 5, the means and standard deviations of the two treatments of the between-subject effect are reported, from which it can be seen that, for all variables for which the effect was statistically significant, mycorrhiza produced a notably positive effect.

The effect of the interaction mycorrhiza × time is displayed in Figs. 1 and 2 which show how the growth rates of the inoculated plantlets were notably faster than those of the control and that the former had a more robust appearance with greater root development. Of particular interest in the micropropagated plantlets was the fact that 60 days after the transplant, the leaf area had doubled (Fig. 1); in addition, there was an increase in both fresh and dry weight measurements.

The histological analysis on 60-day-old infected roots shows the presence of the characteristic vesicles and longitudinally running internal hyphae on roots of the plantlets inoculated with AM fungus and their total absence in those not inoculated (Fig. 3).

Table 4
Probability levels for testing "between-subject" effect (mycorrhiza), "within-subject" effect (time) and their interaction

Source	Variables								
	Leaves					Roots			
	n	*h* (cm)	fw (g)	dw (g)	Area (cm^2)	*n*	*l* (cm)	fw (g)	dw (g)
Mycorrhiza	0.2051	0.0001	0.0035	0.0004	0.0001	0.0022	0.3930	0.0005	0.0002
Time	0.0442	0.0006	0.0004	0.0001	0.0002	0.0015	0.0075	0.0041	0.0022
Time × mycorrhiza	0.0894	0.098	0.0136	0.0037	0.0020	0.0030	0.4873	0.0077	0.0048

Leaves: *n* = mean number of leaves per plant; *h* = mean height of leaves per plant; fw = mean fresh weight of leaves per plant; dw = mean dry weight of leaves per plant; area = mean leaf area per plant. Roots: *n* = mean number of roots per plant; *l* = mean length of roots per plant; fw = mean fresh weight of roots per plant; dw = mean dry weight of roots per plant.

Table 5
Mean and standard deviations of epigean and hypogean parameters of mycorrhizal plantlets at 60 days from transplant

	Leaves								Roots					
	h (cm)		fw (g)		dw (g)		Area (cm^2)		*n*		fw (g)		dw (g)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Myc+	33.82	1.04	20.38	1.05	1.61	0.07	390.49	17.80	11.67	1.53	3.05	0.51	0.25	0.030
Myc−	17	3.00	12.87	2.50	0.92	0.08	177.4	24.67	3	1.00	0.62	0.09	0.05	0.002

Leaves: *h* = height of leaves per plant; fw = fresh weight of leaves per plant; dw = dry weight of leaves per plant; area = leaf area per plant. Roots: *n* = number of roots per plant; fw = fresh weight of roots per plant; dw = dry weight of roots per plant. Myc^+ = mycorrhizal plantlets; Myc^- = non-mycorrhizal plantlets.

4.3. In field

4.3.1. Morphological characteristics

The plant was small with a maximum height of around 70–100 cm and head growth at 35–40 cm. The plant growth habitat was intermediate (Table 6). The leaf had a green-grey colour and was inermous with medium dimensions and high heterophylly due to the presence of entire lamina leaves in the initial growth stages of the plant with lobed and pinnatisected leaves in subsequent stages. The head was elliptical and compact and had small or medium dimensions. The external bracts were oval, of medium dimensions and had a green/violet colour. The apices were rounded, entire or with a slight incision and had

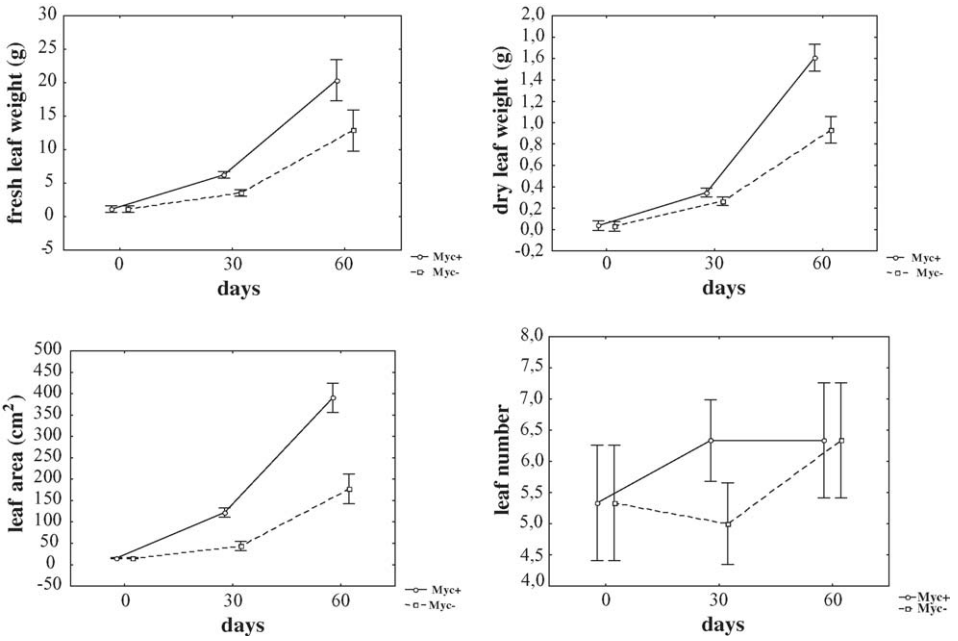

Fig. 1. Acclimatisation: growth epigean system at 0, 30 and 60 days after transplant in nursery.

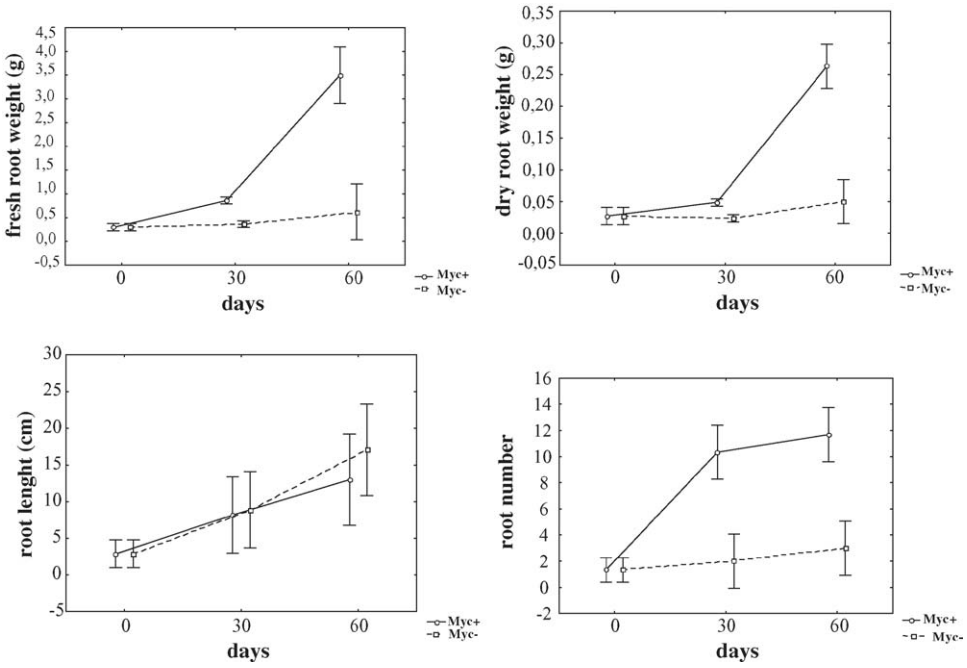

Fig. 2. Acclimatisation: growth hypogean system at 0, 30 and 60 days after transplant in nursery.

a small violet thorn. The internal bracts were of a white/green colour with light violet shading.

4.3.2. Productive characteristics

The number of heads per plant was between 5 and 11 (Table 7). The weight of the heads was on average the same for both principal and axillary heads and was between 100 and 200 g (small or medium size).

5. Discussion

The problems related to the in vitro rooting of the artichoke have been explained in various investigations on the use of only one auxin (Harbaoui and Debergh, 1980; Draoui et al., 1993), mixtures of two auxins (Marras et al., 1985), the addition of activated charcoal (Bigot and Foury, 1984) or the use of cyclodextrin (Brutti et al., 2000) or only giberellic acid (Morzadec and Hourmant, 1997).

In the present study, IAA is the auxin that causes the highest percentage of rooted shoots (49%) (Table 2). Moreover the increase in the concentration of IAA determined an increase in the percentage of rooting shoots, while the other two auxins tested, IBA and NAA, had the opposite effect (Table 3). The reason for this behaviour is that whereas an excess of NAA or IBA inhibits rooting, IAA loses part of its efficiency during in vitro culture because

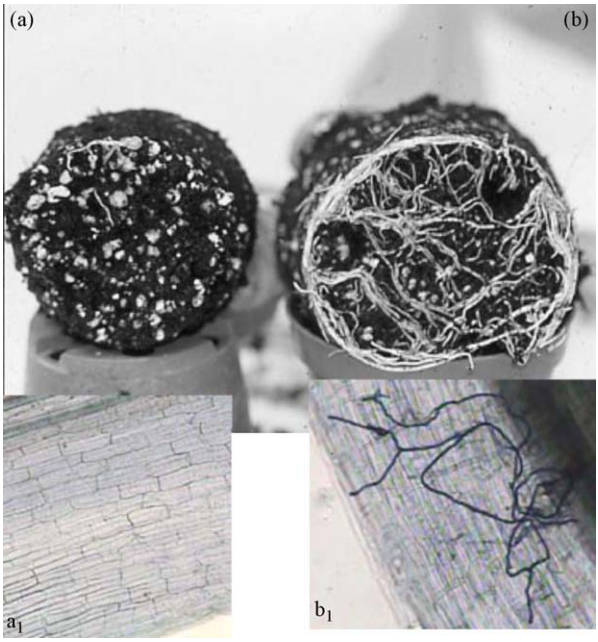

Fig. 3. Differences between not-inoculated (a) and inoculated (b) roots of micropropagated artichoke at 60 days after transplant in nursery. (a_1) Optical microscope particular of longitudinally, section of (a), (b_1) optical microscope particular of longitudinally, section of (b); mycorrhizal structures in root.

Table 6
Morphological characteristics of micropropagated plants inoculated at acclimatation with *Glomus viscosum* after 6 months of planting in the field

Morphological characteristics of heads (cm)	Mean	Minimum	Maximum	S.D.
Height	12.87	10.50	15.50	1.42
Diameter	6.56	5.30	7.80	0.68
External bract height	6.128	5.62	6.56	0.35
External bract length	3.80	2.84	4.56	0.46

Table 7
Productive characteristics of micropropagated plants inoculated at acclimatation with *Glomus viscosum* after 6 months of planting in the field

Productive characteristics of heads	Mean	Minimum	Maximum	S.D.
Heads/plant (*n*)	7.13	5.00	11.00	1.62
Fresh weight (g)	145.17	92.68	215.59	35.26
Dry weight (g)	24.30	13.12	41.02	6.72
Thickness receptacle (cm)	1.130	0.80	2.00	0.18
Fresh weight edible (g)	62.22	38.44	96.53	13.50
Dry weight edible (g)	10.38	5.69	18.73	3.02

it is sensitive to light and is readily inactivated (Hartmann and Kester, 2002); as a result, the concentration active in the medium is lower.

Therefore, the best percentage of rooting is obtained by the presence of 10 mg l^{-1} concentrations of IAA (86% rooted shoots).

The results of our research indicate how mycorrhizal inoculation can induce positive changes in micropropagated artichoke plantlets during the phase of acclimatisation in nursery conditions, confirming the behaviour of other species, for example, the strawberry (Morandi et al., 1979), grapevine and oil palm (Ravolanirina et al., 1989), Prunus cerasifera Ehrh clone MrS 2/5 (Fortuna et al., 1992), the grapevine, the kiwi fruit and the apple tree (Gribaudo et al., 1996). The non-mycorrhizal plantlets show a trend to reduction in leaf numbers, even if the effect is not statistically significant, confirming the stress that follows their transplant from vitro to nursery, whereas the mycorrhizal plantlets show no signs of such stress and after 30 days of acclimatisation had already reached their maximum number of leaves (Fig. 1). The positive influence of the mycorrhiza was even more evident on the hypogean system; Fig. 2 shows the increase of fresh and dry weights; there was a significant rise in the number of roots, however, resulting in a greater number of hair zones which causes an increase in the absorbent surfaces and makes the system more efficient.

The results in field show the behaviour of mycorrhizal micropropagated plants which is evident in a high degree of uniformity which reflects the morphological and productive characteristics of the mother plant.

6. Conclusions

In conclusion, the methodology described in the present study is highly suitable for the micropropagation of *C. Cardunculus* L. var. *scolymus* (L.) Fiori, of the ‘‘catanese’’ type. The existing protocols for this species were not carried out on this ‘‘catanese’’ type of artichoke (Ancora, 1986; Harbaoui and Debergh, 1980; Moncousin, 1981; Ordas et al., 1990; Morzadec and Hourmant, 1997; Brutti et al., 2000).

The results obtained show that an effective mycorrhizal symbiosis between AM fungi and micropropagated plantlets of artichokes is possible. This confirms the importance of mycorrhizal inoculation to obtain plantlets that are more efficient for nutrient acquisition and better equipped to cope with the stress situations typical of micropropagation (Fortuna et al., 1992; Gianinazzi et al., 1990; Gribaudo et al., 1996; Morandi et al., 1979; Ravolanirina et al., 1989).

The artichoke plants in field show uniformity both phenotypically and in their behaviour. Moreover, the morphological and productive parameters pointed out form part of the interval variation reported by Dellacecca et al. (1976). Data collected confirm the characteristics of the original cultivar, the efficiency of the in vitro propagation material and the possibility of using this technique in early types of artichoke.

The application of mycorrhiza on micropropagated plantlets in order to produce the ‘‘catanese’’ type artichoke plants for transplant is highly effective. This cooperation between two biotechnological approaches, those of micropropagation and mycorrhizal inoculation, is an important tool in more sustainable horticultural production.

Acknowledgements

Thanks are due to Miss Anna Tagarelli for her precious technical support in carrying out the experiment.

The authors wish to thank Prof. Manuela Giovannetti of Dipartimento di Chimica e Biotecnologie agrarie, University of Pisa (Italy) for her precious advice and for providing arbuscular-mycorrhizal inoculum.

References

Ancora, G., 1986. Globe artichoke (*Cynara scolymus* L.). In: Bajai, Y.P.S. (Ed.), Biotechnology in Agriculture and Forestry, Crops, vol. 2 Crops I. Springer-Verlag, Berlin, Heideberg, pp. 471–484.

Azcòn-Aguilar, C., Barea, J.M., 1997. Applying mycorrhiza biotechnology to horticulture: significance and potentials. Sci. Hortic. 68, 1–24.

Bigot, C., Foury, C., 1984. Multiplication in vitro d'artichaut (*Cynara scolymus* L.) à partir de semences: comparaison au champ de quelques clones à la lignée dont ils sont issus. Agronomie 4 (8), 699–710.

Brutti, C., Apostolo, N.M., Ferrarotti, S.A., Llorente, B.E., Krymkiewicz, 2000. Micropropagation of *Cynara scolymus* L. employing cyclodextrins to promote rhizogenesis. Sci. Hortic. 83, 1–10.

Cole, J.W.L., Grizzle, J.E., 1966. Applications of multivariate analysis of variance to repeated measures experiments. Biometrics 22, 810–828.

Dellacecca, V., Magnifico, V., Marzi, V., Porceddu, E., Scarascia Mugnozza, G.T., 1976. Contributo alla conoscenza delle varietà di carciofo coltivate nel mondo. Atti II Congresso Internazionale di Studi sul Carciofo. Edizione Minerva Medica Torino, pp. 199–316.

Draoui, N., Ghorbel, A., Kchouk, M.E., 1993. In vitro culture of Globe Artichoke (*Cynara scolymus* L.) in Tunisia: utilization of vitromethods in artichoke inprovement. Agricoltura Mediterranea 123, 139–145.

Fortuna, P., Citernesi, S., Morini, S., Giovanetti, M., Loreti, F., 1992. Infectivity and effectiveness of different species of arbuscular mycorrhizal fungi in micropropagated plants of Mr S2/5 plum rootstock. Agronomie 12, 825–829.

Harbaoui, Y., Debergh, P., 1980. Multiplication in vitro de clones selectionnès l'artichaut (*Cynara scolymus* L.). In: Application de la colture in vitro a l'àmelioration des plantes potageres, Reun. Eucarpia, Versailles, pp. 1–7.

Hartmann, H.T., Kester, D.E., 2002. Plant Propagation Principles and Practices. Prentice Hall (Chapter 9).

Gianinazzi, S., Trouvelot, A., Gianinazzi-Pearson, V., 1990. Role and use of mycorrhizas in horticultural crop production. Adv. Hort. Sci. 4, 25–30.

Giovannetti, M., 1990. Le micorrize come agenti di lotta biologica contro i patogeni delle piante agrarie. Informatore Fitopatologico 10, 17–20.

Gribaudo, R., Zanetti, R., Morte, E., Previati, A., Schubert, A., 1996. Development of mycorrhizal infection in in vitro and in vivo-formed roots of woody fruit plant. Agronomie 16, 621–624.

Marras, F., Foddai, A., Fiori, M., 1985. La selezione sanitaria del carciofo "Spinoso sardo". Informatore fitopatologico, XXXV, 47–50.

Miller Jr., L.R.G., 1981. Simultaneous Statistical Inference. Springer-Verlag, New York.

Moncousin, C., 1981. Multiplication végétative accélérée. Rev. Horticole Suisse 54, 105–116.

Morandi, D., Gianinazzi, S., Gianinazzi, Pearson, V., 1979. Interet de l'endomycorrhization dans la reprise et la croissance des framboisiers issus de multiplication vegetative in vitro. Ann. Amelior Plant 29, 23–30.

Morone Fortunato, I., Ruta, C., 2003. Piantine micropropagate di carciofo precoce con inoculo di funghi micorrizici arbuscolari. Italus Hortus 213–217.

Morrison, D.F., 1976. Multivariate Statistical Methods, second ed. McGraw-Hill Book Co., New York.

Morzadec, J.M., Hourmant, A., 1997. In vitro rooting improvement of Globe artichoke (cv. Camius de Bretagne) by GA_3. Sci. Hortic. 70, 59–62.

Murashige, T., Skoog, R., 1962. A revised medium from rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15, 473–497.

Nitsch, J.P., Nitsch, C., 1969. Haploid plants for pollen grains. Science 63, 85–87.

Ordas, R.J., Tavazza, R., Ancora, G., 1990. In vitro morphogenesis in the globe artichoke (*Cynara scolymus* L.). Plant Sci. 71 (2), 233–237.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–161.

Ravolanirina, F., Blal, B., Gianinazzi, S., Gianinazzi-Pearson, V., 1989. Mise au point d'une méthode rapide d'endomycorrhization de vitroplant. Fruit 44, 165–170.

Sylvia, D.M., William, S.E., 1992. Vesicular–arbuscular mycorrhizae and environmental stress. In: Bethlenfalvay, G.J., Linderman, R.G. (Eds.), Mycorrhiza in Sustainable Agriculture. American Society of Agronomy Special Publication no. 54, Madison, WI, USA, pp. 101–124.