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Micropropagation Supports Reintroduction of an Apulian Artichoke Landrace in Sustainable Cropping Systems

Simona Ancona [1], Giuseppe De Mastro [1], Maria M. Jenderek [2] and Claudia Ruta [1,*]

[1] Department of Agricultural and Environmental Science, Università degli Studi di Bari Aldo Moro, via Amendola 165/a, 70125 Bari, Italy; ancona.simona@gmail.com (S.A.); giuseppe.demastro@uniba.it (G.D.M.)
[2] National Laboratory for Genetic Resources Preservation, USDA, ARS, Fort Collins, CO 80521, USA; maria.jenderek@usda.gov
* Correspondence: claudia.ruta@uniba.it

Abstract: Artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori) is a perennial plant of the Asteraceae's family native to the Mediterranean basin. Italy has rich artichoke biodiversity, but many landraces are subjected to genetic erosion caused by increasing use of commercial varieties that are more homogenous in production. An Apulian landrace 'Troianella' was established in vitro to valorize and provide high-quality material for propagation in nurseries and, subsequently, for cultivation in production fields. The shoot proliferation was tested on four different growth media, adding cytokinin (-6-benzylamminopurine (BAP-0.05 mg L^{-1}). Among these, the best results were achieved on MS519-A and BM media in which MS macronutrients were supplemented with additional doses of $CaCl_2$ and $MgSO_4$ (plus 120 mg L^{-1} and 190 mg L^{-1}, respectively). In vitro root induction was obtained with 10 mg L^{-1} of Indole-3-acetic acid (IAA) and 30 g L^{-1} of sucrose. Plants derived from tissue culture were acclimatized in greenhouse using mycorrhizal symbiosis to increase survival during the acclimatization phase and to improve their performance after transplanting in field. Three arbuscular mycorrhizal (AM) fungi (*Septoglomus viscosum*, *Funelliformis mosseae*, and Symbivit, a commercial mix) were added to a sterile substrate and compared to a sterile control without any AM fungal inocula. After 3 months, the best growth and plant appearance were on substrates with *S. viscosum* fungus or the commercial mycorrhizal fungi mix. The results supported a development of an efficient micropropagation protocol and a production of high quality plant material for sustainable farming of the endangered 'Troianella' landrace.

Keywords: shoot proliferation; rooting; ex vitro establishing; mycorrhiza

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1. Introduction

Artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori) is a perennial plant of the Asteraceae's family, mainly used for consumption of its edible immature flower heads. Of interest are also the leaves, stems, and roots for possible uses in dietary and pharmaceutical products due to their bioactive compounds, such as inulin from the roots, oil for biodiesel production from the seeds, as well as energy from plant biomass [1]. It is native to the Mediterranean basin, where Italy and Spain are the world's leading producers, followed by Egypt [2]. In Italy, its production is important for the economy and social development, especially in the South districts and the Puglia region, traditionally rich in artichoke biodiversity. During recent years, the use of few commercial varieties, more productive and homogenous, increased genetic erosion of the landraces and globe artichoke was included in the regional list as one of the vegetable species recognized at risk of genetic depletion [3]. The key role of agrobiodiversity to support modern agriculture is universally recognized [4]. In this context, the exploitation of artichoke biodiversity can be fundamental for maintaining and increasing food security [5] and improving the varietal range enhancing of the local germplasm. The reintroduction of the landraces in the modern

cultivation should be considered under different points of view aiming improvement of the cropping system sustainability under low inputs [6]. Indeed, the starting point is a production of high quality propagative plant material to be transferred in the field.

Typically, the artichoke landraces are propagated vegetatively by offshoots (basal shoots), underground dormant axillary buds (commonly called "ovoli") or by a division of mature plants. The effectiveness of these propagation techniques can be very limited if not performed in nurseries producing artichoke plantlets [7].

To increase the number of produced plants maintaining a sanitary status, the nursery sector depends more and more on a tissue culture propagation system. In vitro cloning of artichoke tissue culture is the key to a rapid, large, and homogenous propagation of material on a mass scale, in limited space, producing healthy and genetically uniform plants [8,9]. Once the material is micropropagated to the desired level and established ex vitro, the nursery might produce plants fast and in a sustainable way using mycorrhizal infection of the roots. The mycorrhiza (from Greek μύκης mýkēs, "fungus", and ῥίζα rhiza, "root") is a symbiotic association between a fungus and the roots of a vascular host plant. The fungus colonizes the plant's root system either intracellularly as in arbuscular mycorrhizal (AM) fungi, or extracellularly in ectomycorrhizal fungi [10]. The former is mainly used in nurseries, providing a prompt mutualistic association that is maintained by the plants even after transplantation in the field [11]. The fungus has constant and direct access to carbohydrates, such as glucose and sucrose, from the plant, while the plants benefit from the mycelium's higher absorptive capacity for water and mineral nutrients. This happens partly due to the large surface area of fungal hyphae, which are much longer and finer than plant root hairs, and partly as such fungi can mobilize minerals unavailable to the plant's roots from the soil [11,12]. Thus, the effect improves the plant mineral absorption capabilities, so mycorrhizas play essential roles in soil biology and soil chemistry [13]. Another role of the mycorrhiza could be a competition with fungal diseases due to the fungi mutualistic association; in artichoke, e.g., with *Verticilium dahalie*, [14]. The advantages of the symbiosis are also in improving the productivity and quality of field crops [15], that is particularly visible in artichoke [9,16].

The objective of the study was to establish an efficient propagation protocol using in vitro culture and arbuscular mycorrhizal symbiosis for 'Troianella' landrace that is a typical spring artichoke from the countryside of Troia (Foggia province) located in the North of the Puglia region in Italy. This local variety is endangered; today only a few plants are maintained in old family farms for its organoleptic characteristics. The technology may enhance the autochthonous genetic resource of the 'Troianella' landrace and supply high quality plants to nurseries, making the artichoke production chain modern and sustainable.

2. Materials and Methods

2.1. Introduction of Explants in In Vitro Culture

During the spring, selected offshoots of the 'Troianella' landrace (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori) were taken from a field in the Troia countryside (41°21′51; 15°19′2 E) (Puglia Region, Italy), where the landrace was maintained in situ, to initiate in vitro tissue culture.

The offshoots were cleaned from residual soil, reduced to 7 mm, and surface sterilized (Figure 1). Shoot tips were washed under running water for a few minutes, sterilized under laminar flow hood in 70% (*v*/*v*) ethyl alcohol for 30 seconds, and then rinsed with sterile distilled water. Sterilization continued with 0.05% (*w*/*v*) mercuric chloride ($HgCl_2$) solution for 15 min followed by four rinses in sterile distilled water. The cultures were initiated on a basal medium (BM: Murashige and Skoog (MS) [17] macro nutrients modified, Nitsch and Nitsch (NN) [18] micro nutrients, FeNaEDTA (40 mg L^{-1}), thiamine HCl (0.4 mg L^{-1}) (Table 1)) enriched with sucrose (20 g L^{-1}), agar (7 g L^{-1}), N6-(2-Isopentenyl) adenine (2iP 1 mg L^{-1}), Indole-3-acetic acid (IAA 1 mg L^{-1}) and Gibberellic acid (GA_3 0.025 mg L^{-1}) [19].

(**a**) (**b**) (**c**)

Figure 1. (**a**) Offshoot from the field cleaned from residual soil; (**b**) reduction of the size of the offshoot; (**c**) shoot tips ready for surface sterilization.

Table 1. Composition of growth media tested for shoot multiplication of 'Troianella' landrace artichoke.

	Growth Media (mg L^{-1})			
Compounds	**MS519**	**MS519-A**	**LS452**	**BM**
Ammonium nitrate	1650	1650	1650	1650
Calcium Chloride, Anhydrous	332.2	450	332.2	450
Cobalt Chloride × $6H_2O$	0.025	0.025	0.025	
Cupric Sulfate × $5H_2O$	0.025	0.025	0.025	0.025
FeNaEDTA				40.0
Na_2EDTA × $2H_2O$	37.26	37.26	37.26	
Ferrous Sulfate × $7H_2O$	27.8	27.8	27.8	
Magnesium Sulfate, Anhydrous	180.7	370	180.7	370
Manganese Sulfate × H_2O	16.9	16.9	16.9	25
Molybdic Acid (Sodium Salt) × $2H_2O$	0.25	0.25	0.25	0. 25
Potassium Iodide	0.83	0.83	0.83	
Potassium Nitrate	1900	1900	1900	1900
Potassium Phosphate, Monobasic	170	170	170	170
Potassium Hydroxide			100	
Zinc Sulfate × $7H_2O$	8.6	8.6	8.6	10
Boric Acid	6.2	6.2	6.2	10
Glycine (Free Base)	2.0	2.0		
Nicotinic Acid (Free Acid)	0.5	0.5		
Pyridoxine × HCl	0.5	0.5		
Thiamine × HCl	0.1	0.1	0.4	0.4
Myo-Inositol	100	100	100	100
MES (Free Acid)			1000	
Agar	7000	7000	7000	7000
sucrose	20,000	20,000	30,000	20,000
BAP	0.05	0.05	0.05	0.05

After 3 weeks, plantlets were ready for induction to proliferation (Figure 2).

(**a**) (**b**) (**c**)

Figure 2. 'Troianella' landrace artichoke: (**a**) Plantlet on culture initiation medium after 3 weeks; (**b**) plantlet after 3 weeks on proliferation medium; (**c**) five (5) new plantlets ready for the second subculture.

Four growth media were tested (Table 1):

1. BM, regularly used at the Laboratory of Micropropagation and Microscopy-Department of Agricultural and Environmental Science-University of Bari Aldo Moro (Puglia, Italy), for routine culture produced by stocks [19];
2. Linsmaier & Skoog Modified Basal Medium (commercial product LS452-PhytoTech Labs, Inc. 14610 W. 106th St, Lenexa KS 66215) [20];
3. Murashige and Skoog basal medium (commercial product MS519-PhytoTech Labs, Inc. 14610 W. 106th St, Lenexa KS 66215) [17];
4. MS519 modified by the authors increasing calcium chloride and magnesium sulphate content (plus 120 mg L^{-1} and 190 mg L^{-1} respectively) and named by the authors MS519-A (A as artichoke).

These three last media were chosen based on some similarities to the BM medium. All the media were enriched with 6-benzylamminopurine (BAP 0.05 mg L^{-1}), and agar (7 g L^{-1} (Agar No. 1) Oxoid Agar Bacteriological). Sucrose (20 g L^{-1}) was added to BM, MS519 and MS519-A while LS452 already included sucrose (30 g L^{-1}).

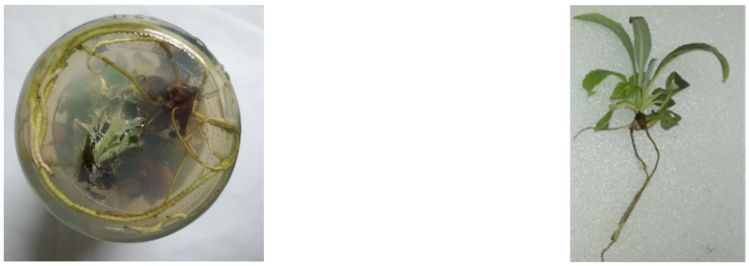
The multiplication was implemented with three subcultures each lasting 3 weeks. At the end of each subculture, the length of the shoots, including the leaves, and the number of newly formed axillary shoots was observed on each growth media.

The pH of the media was adjusted to 5.6–5.8 before 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. Rooting and Acclimatization with Mycorrhizal Fungi Inoculation

After three micropropagation cycles, 1000 shoots were transferred to solidified BM rooting induction medium with a higher sucrose concentration (30 g L^{-1}) and supplemented with IAA 10 mg L^{-1} [19].

During the root induction, artichoke shoots were maintained in a growth chamber with similar parameters used for shoot proliferation (22 ± 1 °C with a photoperiod of 16 h light under a light intensity of 50 µE s^{-1} m^{-2}). After 5 weeks, a sample of 200 rooted microplants (Figure 3) was transplanted in a climatic greenhouse at the Department of Agricultural and Environmental Science-University of Bari Aldo Moro, Puglia, Italy (41°7′31″ N, 16°52′0″ E).



(**a**) (**b**)

Figure 3. (**a**) In vitro roots of artichoke after 5 weeks; (**b**) plant ready for acclimatization in a greenhouse.

Acclimatization conditions in the greenhouse were 18–25 °C and a reduced humidity from 85–90% to 50–60% for over 20 days. At the time of transplanting to the greenhouse, roots were inoculated with different arbuscular mycorrhizal (AM) fungus species: *Septoglomus viscosum* (syn. *Glomus viscosum*) (H.T. Nicolson) C. Walker, D. Redecker, Stiller and A. Schüßler, *Funelliformis mosseae* (syn. *Glomus mosseae*) (T.H. Nicolson and Gerd.) C. Walker and A. Schüßler, and a commercial mix of mycorrhizas (Symbivit purchased from MYBATEC srl-Novara-Italy) consisting of six species of *Glomus* fungi (*G. etunicatum*, *G. microaggregatum*, *G. intraradices*, *G. claroideum*, *G. mosseae*, and *G. geosporum*). The plants were then transplanted in organic Jiffypots® square planting pots (8 cm^2) filled with a substrate composed of sterile peat (46% organic carbon, 1–2% organic nitrogen, and 80% organic matter; pH 6.5) and perlite (2:1, *v*/*v* ratio). For each pot, about 300 spores of

S. viscosum (30 g), *F. mosseae* (30 g), or Symbivit (3 g) were distributed immediately below the roots [21] (Figure 4). For this experiment, 50 plants for each mycorrhizal treatment were used, while 50 plants not inoculated were used as control.

(**a**) (**b**) (**c**) (**d**)

Figure 4. Four organic planting pots with progressive phase of transplants of the mycorrhizal treatment: (**a**) mycorrhizal inoculum; (**b**) root system in direct contact with the mycorrhizal inoculum; (**c**) root system covered with peat; and (**d**) plant from the in vitro culture covered with a plastic cup to keep high relative humidity.

Before transplanting the rooted plantlets, pure inocula of *S. viscosum* and *F. mosseae* were multiplied on onion (*Allium cepa* L.) plants selected as a host crop due to their high mycotrophy, according to Dalpè and Monreal [22]. These inocula contained sand, soil, spores, external mycelium, and infected root fragments.

To evaluate the mycorrhizal effect, the substrate was sterilized before transplanting, and only tap water was used to keep the substrate moist.

At the time of planting in a greenhouse, and after 3 months from this time, the length and the number of the leaves and the root length were measured. At this time, survival (percent) of plants, ratio between root and leaf total length (calculated as sum of the length of the leaves) and mycorrhizal colonization were also evaluated.

2.3. Evaluation of the Mycorrhizal Infection

After 1 month of acclimatization in the greenhouse, mycorrhizal plants, originating from the in vitro culture and inoculated with three AM fungi, were progressively transferred outside. Plants from the peat pots were transplanted to bigger pots (28 cm × 28 cm) (Figure 5). The pots were filled with new peat and inert soil to reach the pot volume. Outside, the plants were evaluated for biometrical parameters at 1 and 3 months.

Figure 5. From the left to the right, a control plant and plants with root system inoculated with spores of *F. mosseae*, *S. viscosum*, and a commercial mycorrhiza mix after 1 month of outside cultivation.

After 3 months, plants were transplanted from the pots to the field. At the transplanting time, samples of roots from control and for each mycorrhizal treatment were evaluated

for a colonization index. Roots from each treatment were cleaned from residual soil under running water, treated with a base (KOH 10%) in warm water for 10 min, and then exposed to an acid (HCl 3%) for 5 min. At this point roots were stained with Trypan Blue 0.05% in lactic acid. The staining with Trypan Blue was performed in warm water for 2 min, then roots were washed with clean lactic acid and stored at 4 °C in the dark [23] (Figure 6a). The following day, in order to evaluate the colonization rate (expressed in percentage), roots from each mycorrhizal treatment were cut into 100 samples, 10 per slide, each 1 cm long (Figure 6b), and checked under a microscope (Leica DMLB100, Milan, Italy) equipped with an imaging system program (X-PRO Analysis Image X-Pro analytical software (Alexasoft, Florence, Italy) designed to optimize image capture with Nikon DXM 1200 Digital Camera (Tokyo, Japan).

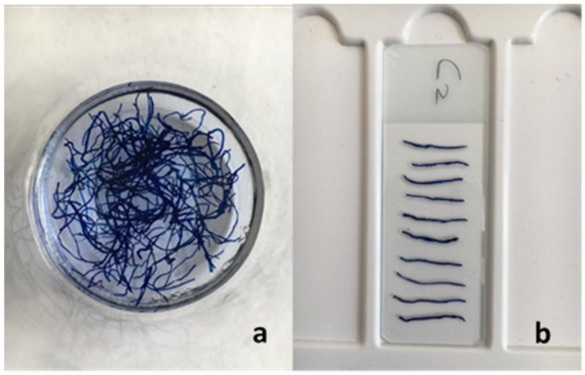


Figure 6. Roots staining for microscopic analysis: (**a**) roots stained with Trypan Blue stored at 4 °C in dark; (**b**) 10 samples, 1 cm length on slide ready to be scanned under microscope.

2.4. Statistical Analysis

Data were subjected to analysis of variance (ANOVA), using the CoStat software. The Student Newman Keuls (SNK) test ($p \leq 0.001$) was used to compare the means of the different treatments. Before ANOVA analysis, percentage data were subjected to the angular transformation.

3. Results

3.1. Introduction of Explants in In Vitro Culture

The percentage of surviving shoot tips after the sterilization and establishment in the in vitro condition during 3 weeks was 70%.

Shoot proliferation on MS519, MS519-A, and LS452, in comparison to the BM medium used as a control, was performed in 3 week cycles, and three subcultures were considered for each medium. No significant differences were observed in the length of shoots between the control (BM medium) and the other three proliferation media. The higher shoot number was observed on the MS519-A medium; however, the value was not significantly different from the control (BM). The recorded number of shoots grown on the two other media (MS519 and LS452) was significantly lower than in the MS519-A and the control media (Figure 7).

3.2. Rooting

Rooting of shoots on BM medium with IAA 10 mg L^{-1} and sucrose 30 g L^{-1} was linear over time. At 4 weeks, 61.5% of cultures were rooted (Figure 8).

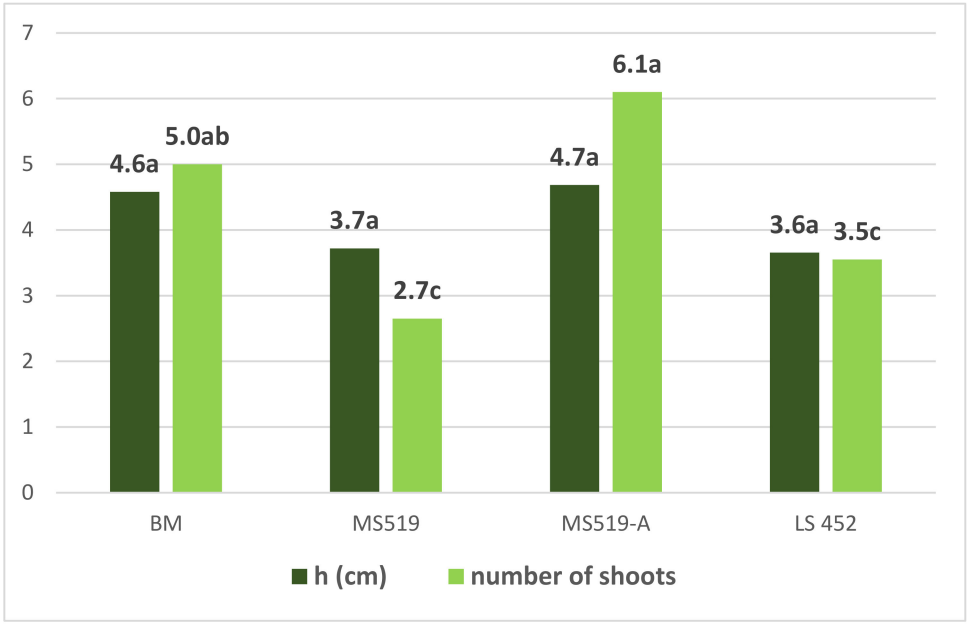


Figure 7. Effects of different media on shoots proliferation and elongation of 'Troianella' landrace. Data were recorded 3 weeks after each subculture on multiplication media and were a mean of three subcultures. Different letters for each column indicate significant differences between the different media tested (SNK test at $p \leq 0.001$). No significant differences were observed in the shoot length.

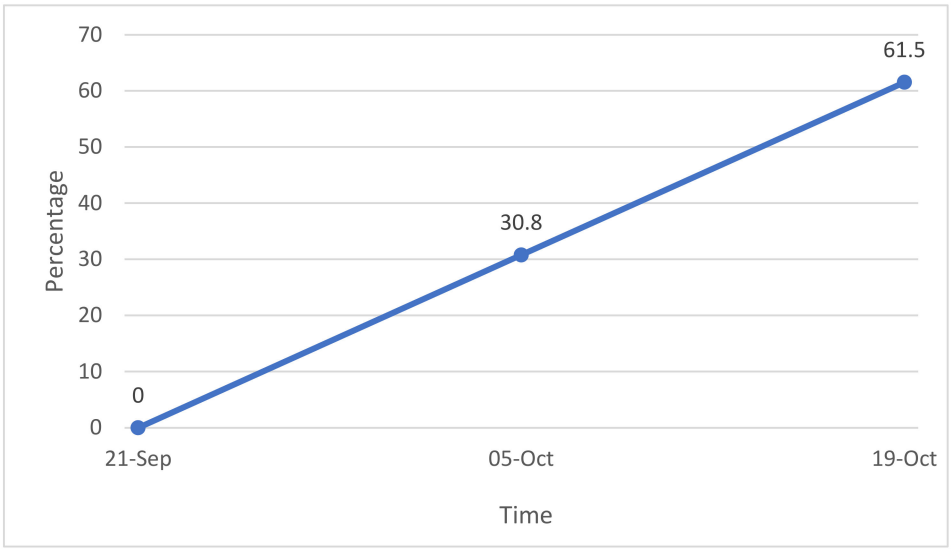


Figure 8. Linear growth of root system after 2 and 4 weeks on culture medium (BM) with IAA 10 mg L^{-1} and sucrose 30 g L^{-1}.

Observations on the rooting induction showed that 31% of the plantlets already had roots after 2 weeks (Figure 8). The number of the rooted plants doubled after the next 2 weeks (61.5%) (Figure 8). Plants were cultivated additional 15 days for root elongation.

At the time of planting in a greenhouse, the average length of transplants was approximately 8 cm, with 8.5 leaves, and a total root length of approximately 4.5 cm.

Data for the percentage of survival and the biometric parameters (length of leaves, number of leaves, length of roots, ratio between root and leaf length, and mycorrhizal colonization rate) were taken at 3 months (Table 2).

Significant differences between the mycorrhizal treatments in terms of number of leaves were observed, while a significant positive effect of mycorrhiza on the plant growth

was visible in the length of the leaves. The best results were observed with *S. viscosum* and the commercial mix (more than 15.00 cm longer than control) followed by *F. mosseae*.

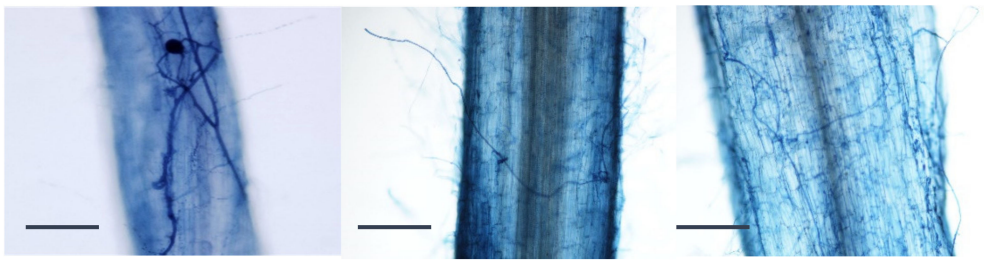
Table 2. Biometric parameters of 'Troianella' landrace plantlets inoculated with different AM fungi (*S. viscosum*, *F. mosseae*, and commercial mix) or not inoculated (Control) at transplanting (T) and after 3 months (3M) of inoculation.

Treatment	Ex Vitro Survival		Leaves						Roots					Root/Leaf *
			Number (n)				Length (cm)			Length (cm)			Colonization (%)	
	%		T		3M		T		3M	T		3M	3M	3M
Control	79	c	8.08	a	10.75	a	8.0	a	17.90	c	4.60	a	35.73	c	0b	0.19
S. viscosum	95	a	8.05	a	13.90	a	8.3	a	33.40	a	4.50	a	104.25	a	90a	0.22
F. mosseae	85	b	8.09	a	11.87	a	7.6	a	26.50	b	4.50	a	61.86	bc	80a	0.20
Comm. mix	90	ab	8.08	a	13.00	a	7.7	a	33.10	a	4.60	a	89.53	ab	90a	0.21

* ratio between root and leaf length. Different letters for each row indicate significant differences between different AM fungi tested (SNK test at $p \leq 0.001$).

After 3 months of inoculation *S. viscosum* seemed to induce also the highest length of the roots, reaching about 104.00 cm, but not significantly different from the root length inoculated with the commercial mix (89.53 cm), while lower values were observed for *F. mosseae* and the control (61.86 and 35.73 cm, respectively). It is interesting to underline that the symbiosis between 'Troianella' roots and *S. viscosum* resulted in a length three times longer than in the control. The ratio root/leaf length confirmed the increase in the roots due to the *S. viscosum* infection. On the other hand, even with differences in absolute values, the ratio root/leaf length remained almost constant in all the treatments.

The highest mycorrhiza colonization rate in root samples was observed for *S. viscosum* and the commercial mix (90% of roots with hyphae colonization) (Figure 9; Table 2); however, the rate was not statistically different between the three inoculates.



(**a**) (**b**) (**c**)

Figure 9. Detail 'Troianella' landrace root colonized by AM fungi after 4 weeks from inoculation: (**a**) *S. viscosum*; (**b**) *F. mosseae*; (**c**) Commercial mix (Light microscopy images; Bar (a) = 0.1 mm; Bars (b) and (c) = 1.0 mm).

The positive role of mycorrhizal symbiosis on the growth of 'Troianella' artichoke plantlets was validated by the high percentage of colonization evaluated by the root analyses (Table 2).

4. Discussion

The disadvantages of conventional propagation methods have highly constrained the development of effective procedures for producing artichoke plantlets in nurseries. The application of the micropropagation technique for its large-scale propagation has been extensively utilized from the beginning of 1980 [24,25], being the most promising way to overcome these limits.

In recent years, there have been several varieties of globe artichoke multiplied by micropropagation from commercial laboratories; nevertheless, there are still many studies regarding the different steps of artichoke micropropagation.

Based on the previous experiences raised on the in vitro culture of autumn artichoke varieties [19], in this study a protocol in sustaining the micropropagation of the 'Troianella' spring landrace was tested.

As suggested by Harbaoui and Debergh [26], adding a mix of growth regulators (IAA, 2ip, and GA_3) to the nutrient medium in the first phase, to establish the in vitro conditions, allowed us to obtain elongated explants very suitable and very reactive to the in vitro multiplication. A very low concentration of cytokinin (BAP 0.05 mg L^{-1}) was sufficient to raise a mean of 5–6 shoots for each subculture, avoiding the formation of adventitious buds that could be expression of somaclonal variation. This becomes highly significant when considering the reintroduction of endangered plants.

During the multiplication cycles, corresponding to the most extended phase of in vitro culture, three different growth media (MS519, MS519-A, and LS452) were tested as an alternative to the BM, already successfully used on several autumn Apulian landraces [9,19,27]. Among them, the most effective for the shoot proliferation index was MS519-A, despite the fact that the results obtained with BM were not statistically different. With the increase in the amount of calcium chloride and magnesium sulfate, MS519-A has the same concentration of the macroelements of BM, which in turn is different from the two commercial growth media tested (MS519 and LS452).

However, the most difficulties underlined by researchers for applying micropropagation to the artichoke arise during the root induction, strictly depending on the different genotypes, where the rooting percentage may range from 1 to 92% [28–30].

In order to improve in vitro artichoke rooting, various investigations were done on the use of different types and concentrations of growth regulators [26,29–36], the addition of supplements such as activated charcoal [36–38], cyclodextrin [39], or pretreatments (darkness, two-phase) [30] have been carried out. In the present research, such as already tested in several different artichoke genotypes [9,19,40,41], the addition of IAA at high concentration (10 mg L^{-1}), in combination with the increase in sucrose concentration from 20 to 30 g L^{-1} allowed us to achieve a satisfactory percentage of rooting and a root system characterized by a strong root length of 4.5 cm at the moment of plantlet transplanting into greenhouse. The same concentration of IAA was also applied to induce rooting by Ozsan and Onus [38,42] on open-pollinated cultivars and F1 hybrids and Tavazza et al. [43] on cv. Spinoso Sardo.

Moreover, through mycorrhizal inoculation with *S. viscosum* and the commercial mix, the percentage of survival in a greenhouse was very high. The acclimatization phase of micropropagated artichoke plantlets is particularly stressful [39,44]. The success of this phase strictly depends on the quality of the material coming from the in vitro culture in terms of well-developed plants (3–4 cm in height) than with a good root system. In this research, the well-developed and rooted plantlets were also supported by AM fungi inocula. Concerning ex vitro survival, all the mycorrhizal treatments showed higher ex vitro survival than the control plantlets, which is in line with earlier findings [45–47]. Adding AM fungi during the acclimatization stage of micropropagation enhances the growth and development of micropropagated plantlets [48,49]. The AM fungi help the host plants to overcome transplanting stress, ensuring greater survival, better development of the roots, and a more rapid growth, due to the improvement of the nutrient uptake, the water conducting capacity, and photosynthetic rates, as well as protecting the plant from root diseases [50]. In this study, among the different treatments, *S. viscosum* and the commercial mix were found to sustain ex vitro establishment more than *F. mosseae* (Table 2). The positive effect of the pure mycorrhizal inoculum *S. viscosum* on the growth of the leaves and the roots of 'Troianella' landrace, measured after 3 months, was in agreement with previous research on 'Catanese' landrace [19,27]. At the same time, the good results obtained using commercial arbuscular mycorrhizal inoculants in promoting mycorrhizal colonization and in increasing plant growth during the phase of acclimatization in nursery conditions confirmed the effectiveness of these products readily available on the market, as reported from previous studies on other cultivars [51,52]. This aspect is particularly interesting considering that, although AM fungi do not have a strict host specificity, fungus and plant can show a functional specificity that influences the symbiotic interaction [53]. Therefore, to find the affinity between commercial arbuscular mycorrhizal inoculants and

this species may be a great opportunity to raise higher productivity from the reintroduction of artichoke landraces under a sustainable cropping system.

5. Conclusions

The present study was aimed to evaluate the possibility of reintroducing the endangered Apulian artichoke 'Troianella' in a sustainable cropping system. For this purpose, it was very important to both increase the number of high-quality plantlets through micropropagation technique, and to evaluate the effectiveness of AM fungi in ensuring a higher survival, better development of the roots, a rapid growth during the acclimatization stage, and supporting the subsequent sustainable cultivation in the field.

The results obtained showed a good response of 'Troianella' landrace to the in vitro culture. The comparison among different growth media tested revealed that, using the same cytokinin in a very low concentration (BAP 0.05 mg L^{-1}), they could influence the shoot multiplication rate. In this study, the most effective for the shoot proliferation index was MS519-A, despite the fact that the results obtained with BM were not statistically different. Mycorrhizal inoculation of the rooted plantlets at the time of transplanting in the greenhouse confirmed its effectiveness for obtaining high-quality nursery plant materials in comparison with the non-inoculated plants. Among the three inoculants tested, the most effective ones were the pure inoculum *S. viscosum* and the commercial mix Symbivit.

In conclusion, the micropropagation technique, combined with mycorrhizal symbiosis, can support the reintroduction of the 'Troianella' artichoke under a sustainable cropping system. Further research will focus on the evaluation of the agronomic behavior of the multiplied clones and developing a possible selection program to increase the productivity of this landrace.

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