

# An Innovative Vegetative Propagation System for Large-scale Production of Globe Artichoke Transplants.

## Part I. Propagation System Setup

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**ADDITIONAL INDEX WORDS.** *Cynara cardunculus* var. *scolymus*, offshoot, soilless, benzylaminopurine, cold storage, rooting

**SUMMARY.** A research project was conducted at the University of Tuscia, Viterbo (central Italy), to set up a vegetative propagation system for producing disease-free artichoke transplants (*Cynara cardunculus* var. *scolymus*) of the Romanesco type (cultivar C3). The system included the following steps: 1) micropropagated plantlets were grown in a soilless culture year-round in greenhouse conditions, starting at the end of August; 2) stock plants were periodically treated with a chemical growth regulator [6-benzylamino purine (BA)] and then cut back at the collar level to promote offshoot production; 3) offshoots were periodically harvested and cold stored; and 4) cuttings were rooted at the end of spring under conditions of high humidity in multi-pack trays so as to be ready for summer transplanting. Results showed that the foliar application of BA to the stock plants increased the offshoot number quadratically to 200 mg·L<sup>-1</sup>. The rooting percentages of cuttings and root growth were enhanced by raising the cutting weight class (30–45 g) and by the application of naphthaleneacetic acid (NAA) to the cutting root zone at a rate of 2000 mg·L<sup>-1</sup>. The percent rotten cuttings increased as the 2 °C cold-storage time increased from 30 to 150 days. Similarly, the percentage of rooting and root growth decreased approximately from 60 to 150 days.

Globe artichoke is a field-grown vegetable crop widely used in the Mediterranean region, but it is also gaining popularity in other parts of the world such as California, Argentina, and New Zealand. Italy is the world leader in globe artichoke production, with about 50,000 ha produced annually (Istituto Nazionale di Statistica, 2004). The major production areas for artichokes are located in southern Italy (Puglia, Sicilia, Sardegna) and in central Italy (Lazio, Campania, Toscana). Globe artichokes are generally planted from July through September and grown year-round as perennials (4 to 5 years). In southern Italy, the globe artichoke is grown for autumn, winter, and early spring harvest (e.g., ‘Violetto di Sicilia’, ‘Spinoso Sardo’, ‘Brindisino’), while in central Italy globe artichokes are cultivated for

early spring harvest (e.g., ‘Campagnano’, ‘Castellamare’, ‘C3’, ‘Terom’, ‘Violetto di Toscana’). Due to the lack of suitable seed-planted cultivars and the large quantity of propagation material present in a plant (Snyder, 1981), globe artichokes are generally propagated vegetatively by offshoots, stumps, or dried shoots harvested from commercial fields at the end of the production cycle. Although this vegetative propagation seems to be economically profitable, the potential for the spread of disease is very high. Many pests [e.g., nematodes (*Meloidogyne* spp.)] and pathogens, like fungi (e.g., *Verticillium dahliae*) and viruses, frequently have been observed in globe

artichoke fields (Cirulli et al., 2000; Greco et al., 2000; Martinelli et al., 1981; Pasquini and Barba, 2000). Field surveys in artichoke fields in central Italy (Pasquini et al., 2004) have reported a high incidence of viral infections in late artichoke cultivars (up to 100%), especially artichoke latent virus (ArLV) (83%), artichoke mottled crinkle virus (AMCV) (18.2%), tomato spotted wilt virus (TSWV) (6.5%) and Italian artichoke latent virus (IALV) (3.5%). These pathogens can be spread by vegetative propagation material, hence leading to plant damage (e.g., chlorosis and necrosis of leaves, stunting, wilting, and collapsing of plants) and resulting in significant economic losses. In vitro propagation of the globe artichoke has solved many of the disease problems related to traditional vegetative propagation (Harbaoui, 1982; Morzadec and Hourmant, 1997). However, in vitro propagation is expensive, and the resulting plants are often not identical to the parent (untrue-to-type), particularly in early cultivars (Pécaut and Martin, 1993).

In 2000, research was started at the Department of Plant Production of the University of Tuscia (Italy) to develop a vegetative propagation system that preserves the in vitro propagation advantages (disease-free plants, plant uniformity) and reduces the plant costs for growers. The proposed system involved the following steps: 1) pathogen-free micropropagated plantlets were transplanted in a soilless culture at the end of the summer and grown year-round under greenhouse conditions; 2) stock plants were periodically treated with a chemical growth regulator (BA) and then cut back at the collar level to promote offshoot production; 3) offshoots were periodically harvested and cold stored; and 4) cuttings were rooted at the end of the spring under high humidity conditions in multi-pack trays so as to be ready for summer transplanting.

The purpose of these steps was

### Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
3.7854	gal	L	0.2642
2.5400	inch(es)	cm	0.3937
28.3495	oz	g	0.0353
28,350	oz	mg	3.5274 × 10 <sup>-5</sup>
7.4892	oz/gal	g·L <sup>-1</sup>	0.1335
1	ppm	mg·L <sup>-1</sup>	1
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

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to set up a propagation system for the successful production of artichoke transplants. Four experiments were conducted: 1) to evaluate the effects on offshoot production from the application of foliar BA to stock plants; 2) to determine the effects of offshoot size on rooting; 3) to evaluate the influence of the application of NAA on rooting; and 4) to study the influence of the length of cold storage of cuttings on survival and rooting.

## Materials and methods

**EXPT. 1.** Pathogen-free micropropagated plantlets of the 'C3' artichoke were obtained from a commercial source (Vitroplant, Cesena, Italy) in 8-cm plastic containers at the four-leaf stage (Romanesco type) and transplanted into 20-cm plastic pots filled with pumice (with a particle diameter ranging from 1 to 3 mm) on 25 Aug. 2000. Stock plants were placed at a plant density of 5.1 plants/m<sup>2</sup> in a polyethylene-covered greenhouse equipped with insect-proof nets on the vent openings to prevent plant virus vectors [e.g., aphids (*Brachycaudus cardui*, *Myzus persicae*, *Protrama radicum*)] from entering the greenhouse. The greenhouse was located at the Tuscia University experimental farm in central Italy (lat. 42°25'N, long. 12°08'E). Inside the greenhouse, the air temperature was maintained between 10 and 25 °C by using artificial heating and ventilation. Relative humidity (RH) ranged from 45% to 85%. Light was provided by natural solar radiation and the midday photosynthetically active light intensity in the greenhouse was between 350 and 1000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Nutrients were applied through fertigation at the following concentrations: 210 mg·L<sup>-1</sup> of N (15 mM), 31 mg·L<sup>-1</sup> of P (1 mM), 234 mg·L<sup>-1</sup> of K (6 mM), 200 mg·L<sup>-1</sup> of Ca (5 mM), 48 mg·L<sup>-1</sup> of Mg (2 mM), 5 mg·L<sup>-1</sup> of Fe (89  $\mu\text{M}$ ), 0.5 mg·L<sup>-1</sup> of B (45  $\mu\text{M}$ ), 0.5 mg·L<sup>-1</sup> of Mn (9  $\mu\text{M}$ ), 0.05 mg·L<sup>-1</sup> of Zn (0.8  $\mu\text{M}$ ), 0.02 mg·L<sup>-1</sup> of Cu (0.3  $\mu\text{M}$ ), 0.01 mg·L<sup>-1</sup> of Mo (0.1  $\mu\text{M}$ ). Plants were drip-irrigated two to seven times per day, as needed to avoid moisture stress. Mites (*Tetranychus urticae*) and powdery mildew (*Leveillula taurica* f.sp. *cynarae*) were controlled with fenson (Acarol; Isagro, Milano, Italy) at 0.3 g·L<sup>-1</sup> and sulfur (Zolfo Bagnabile; Bayer CropScience, Milano, Italy) at 1.6 g·L<sup>-1</sup>, respectively. Stock plants were grown until the end of Dec.

2000. Five BA concentrations (0, 50, 100, 150, 200 mg·L<sup>-1</sup>) were tested in a randomized complete-block design with four replicates. The experimental unit was a group of 90 stock plants, leading to a total of 1800 stock plants (90 stock plants  $\times$  four replicates  $\times$  five BA concentrations). BA solutions were prepared by dissolving BA crystals in 20 mL of 1 N hydrochloric acid plus 100 mL tap water with constant stirring. The volume of the solution was increased to 1 L and two drops of Tween 20 were added as a wetting agent. On 20 Oct. 2000, BA treatments were applied to runoff in the form of foliar sprays, about 50 mL of BA was required per plant. Control plants were sprayed with a solution without BA. One week after the treatment, the plants were cut back to remove the dominant shoot and to promote axillary and rhizomic bud swelling. The aboveground biomass of the stock plants was weighed and oven-dried at 80 °C for 6 d to quantify the dry matter. The offshoots of each plant were counted on 11 Nov. and 26 Nov. 2000.

**EXPT. 2.** The offshoots derived from the 200 BA treatment from Expt. 1 were harvested, weighed, and divided into four cutting weight classes: 0–15 g (fewer than two leaves); 15–30 g (about three to four leaves); 30–45 g (about four to five leaves); 45–60 g (more than five leaves). Cuttings were trimmed (removing the upper half of the leaves), and immersed for 3 min in a watery solution containing 1 g·L<sup>-1</sup> carbendazim (Bavistin; Basf Agro, Milano, Italy). A randomized complete-block design with three replicates was used to evaluate the effect of the four cutting weight classes on rooting. Each experimental unit consisted of 40 offshoots leading to a total of 480 offshoots (40 offshoots  $\times$  three replicates  $\times$  four cutting weight classes). Cuttings were rooted in commercial potting soil (Metro-mix 360; W.R. Grace and Co., Cambridge, Mass.) in high humidity tents and in light and temperature conditions similar to those found in a propagation greenhouse (irradiance level 120–250  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; mean air temperature of 20 °C). After 30 d, the following data were recorded for each cutting: number of rooted cuttings, total number of primary roots, length of longest root, and total dry root weight.

**EXPT. 3.** Offshoots derived from the 200 BA treatment from Expt. 1

were harvested when they had four to six leaves. Cuttings were weighed, trimmed (removing the upper half of the leaves), and immersed for 3 min in a watery solution containing 1 g·L<sup>-1</sup> carbendazim (Bavistin). A randomized complete-block design with three replicates was used to evaluate the effect of four NAA concentrations (0, 1000, 2000, 3000 mg·L<sup>-1</sup>) on rooting. Each experimental unit consisted of 40 offshoots leading to a total of 480 offshoots (40 offshoots  $\times$  three replicates  $\times$  four NAA concentrations). NAA was dissolved in a solution containing 50% ethanol and 50% water. The basal end of the cuttings was dipped in a NAA solution for 10 to 15 s. Control cuttings were treated with a solution without NAA. Cuttings were rooted in commercial potting soil (Metro-mix 360) under climatic conditions similar to Expt. 2. After 30 d, the following data were recorded for each cutting: number of rooted cuttings; total number of primary roots, length of longest root, and total dry root weight.

**EXPT. 4.** Offshoots derived from the 200 BA treatment from Expt. 1 were harvested when they had four to six leaves. Cuttings were used to evaluate the effects of the length of cold-storage on rooting. Cuttings were weighed, trimmed (removing the upper half of the leaves), immersed for 3 min in a watery solution containing 1 g·L<sup>-1</sup> carbendazim (Bavistin), and cold stored in plastic bags at 2  $\pm$  0.5 °C, and 90  $\pm$  5% RH. At 0, 30, 60, 90, 120, and 150 d of cold storage, three bags were removed from the cold store and the number of completely rotten cuttings were counted. Each experimental unit consisted of one plastic bag containing 30 cuttings leading to a total of 540 cuttings (30 cuttings  $\times$  three replicates  $\times$  six cold storage periods). The cuttings that were not rotten were rooted in high humidity tents in a growth chamber (12-h photoperiod, with corresponding 22 °C light/18 °C night, with an irradiance level of 250  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) to avoid the influence of climatic variation resulting from the different planting times. After 30 d, the following data were recorded for each cutting: number of rooted cuttings, total number of primary roots, length of longest root, and total dry root weight.

**STATISTICAL ANALYSIS.** The data from Expt. 2 and Expt. 3 were subjected to analysis of variance and Duncan's

multiple range test to determine the statistical significance of differences between treatments. Regression analysis was performed on the data from Expt. 1 and Expt. 4 and Schwarz's Bayesian model-selection criterion (SBC) was used to select the most appropriate model (i.e., linear or quadratic) from the data (Schwarz, 1978). All statistical analyses were performed using SPSS (version 10 for Windows; SPSS, Chicago).

### Results and discussion

In Expt. 1, 7 d after the BA applications, the stock plant aboveground dry weight and leaf number were not significantly affected by the BA treatment. The average stock plant dry weight and leaf number were 26 g/plant and 16.2 no./plant, respectively. On both sampling dates, the number of offshoots increased by increasing the BA concentration, indicating that BA concentration has a beneficial effect on the number of offshoots (Fig. 1). During the first sampling (15 d after cutting back the stock plant), the number of offshoots from stock plants receiving BA concentrations of 50, 100, 150, and 200 mg·L<sup>-1</sup> was 140%, 188%, 244%, and 224%, respectively, higher than that of control plants with an optimal foliar BA concentration around 165 mg·L<sup>-1</sup>. A similar trend in offshoot number per stock plant was also observed on the second sampling date (30 d), with the highest values observed at 200 mg·L<sup>-1</sup> of BA. Moreover, in all BA treatments, the number of offshoots counted on both sampling dates was similar, demonstrating that BA application has an early effect on offshoot emission. Despite the preliminary results (Temperini et al., 2000a, 2000b) that showed the ineffectiveness of lower rates of foliar BA applications (5, 10, and 20 mg·L<sup>-1</sup>) in increasing the total number of globe artichoke offshoots, we observed that BA had a beneficial effect on promoting axillary and rhizomic bud swelling. BA is considered to be a potent growth regulator with cytokinin activity and has been successfully used as a foliar spray to stimulate axillary shoot development in ornamental crops, improve the branching of potted plants, and increase stock plants' cutting production (Browne et al., 2001; Henny, 1986; Wang, 1990).

In Expt. 2, rooting was strongly affected by cutting weight (Table 1).

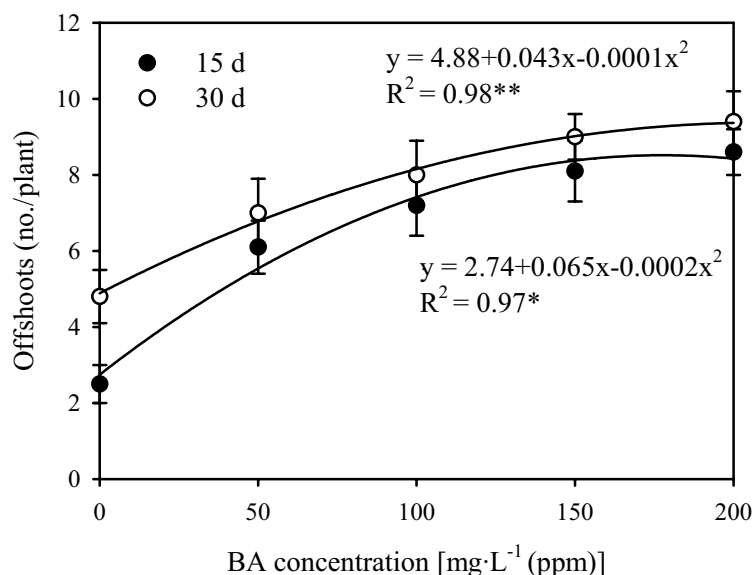


Fig. 1. Effect of 6-benzylamino purine (BA) concentration on offshoot number of globe artichoke at 15 and 30 d after cut back of stock plants (Expt. 1). Bars indicate standard errors. \*, \*\* Significant at  $P \leq 0.05$  or  $0.01$ , respectively.

Table 1. Effect of cutting weight class on rooting frequency, number of roots per cutting, longest root length and on root dry weight of globe artichoke (Expt. 2). Values are the mean of three replicate samples.

Cutting wt class (g) <sup>z</sup>	Rooting (%)	Roots		
		No. (no./plantlet)	Length (cm) <sup>y</sup>	Dry wt (mg/plantlet) <sup>x</sup>
0–15	26.1 c <sup>w</sup>	2.2 c	6.0 b	71 c
15–30	50.2 b	3.5 b	7.1 ab	158 b
30–45	68.9 a	4.7 a	8.7 a	230 a
45–60	70.1 a	5.1 a	9.2 a	271 a

<sup>z</sup>1 g = 0.0353 oz.

<sup>y</sup>1 cm = 0.3937 inch.

<sup>x</sup>1 mg = 3.5274 × 10<sup>-5</sup> oz.

<sup>w</sup>Significant difference by Duncan's multiple range test ( $P = 0.05$ ) for the values within a column.

Table 2. Effect of naphthaleneacetic acid (NAA) application on rooting frequency, number of roots per cutting, longest root length and on root dry weight of globe artichoke (Expt. 3). Values are the mean of three replicate samples.

NAA concn (mg·L <sup>-1</sup> ) <sup>z</sup>	Rooting (%)	Roots		
		No. (no./plantlet)	Length (cm) <sup>y</sup>	Dry wt (mg/plantlet) <sup>x</sup>
0	65.1 b <sup>w</sup>	4.5 b	8.5 b	232 b
1000	67.0 b	4.6 b	8.2 b	224 b
2000	89.0 a	5.6 a	9.3 a	299 a
3000	72.1 b	4.9 b	7.8 b	209 b

<sup>z</sup>1 mg·L<sup>-1</sup> = 1 ppm.

<sup>y</sup>1 cm = 0.3937 inch.

<sup>x</sup>1 mg = 3.5274 × 10<sup>-5</sup> oz.

<sup>w</sup>Significant difference by Duncan's multiple range test ( $P = 0.05$ ) for the values within a column.

The highest rooting percentage was recorded on cuttings heavier than 30 g (average 70%) followed by cuttings of 15–30 g, while the lowest percentage of rooting was observed on cuttings weighing less than 15 g (Table

1). Plantlets obtained from cuttings heavier than 30 g exhibited the highest root number, root length, and root dry weight (Table 1). The presence of a small rhizome was more evident on cuttings heavier than 30 g (about



four to five leaves). Heavier cuttings may have had more rhizome adventitious root primordia and carbohydrate storage, which led to an improvement in their rooting capability. Our results confirmed that an improvement can be achieved in the efficiency of rooting by increasing the size of cuttings (Rosati et al., 2000; Tesi et al., 2003).

In Expt. 3, the application of 2000 mg·L<sup>-1</sup> of NAA to globe artichoke cuttings enhanced both the rooting percentages and root development (Table 2). This had important implications for subsequent performances of the cuttings. It is well established that exogenous auxin promotes root formation in many plants (Bredmose et al., 2004; Browne et al., 2000; Rosier et al., 2004). In vitro experiments on artichoke have also proved that NAA is an effective auxin for promoting rhizogenesis (Brutti et al., 2000).

In Expt. 4, rotten cuttings increased as the cold storage period was increased. For example, as much as 13% of the cuttings were rotten after 150 d of cold storage (Fig. 2). The percentage of rooting decreased quadratically by increasing the cold-storage period of cuttings (Fig. 3). After 150 d of cold storage, the percentage of rooted cuttings was reduced by 35% as compared to the control treatment (Fig. 3). A similar trend was observed for root growth and development. However, root dry weight increased by 24% after 30 d of cold storage as compared to the control treatment, suggesting that a short period of cold storage (30 d) can increase root growth. Cold storage of unrooted and rooted cuttings of some crops [e.g., carnation (*Dianthus caryophyllus*), chrysanthemum (*Dendranthema grandiflorum*), strawberry (*Fragaria ananassa*)] has been reported (Dole and Wilkins, 1999; Rajapakse et al., 1996) as an important tool for crop propagation scheduling or production. The success of the storage of cuttings depends on cutting factors (genotype, carbohydrate status, nitrogen concentration) and storage conditions, including temperature, relative humidity, and atmospheric composition (Druege et al., 2000). A short cold storage period had a positive effect on carnation rooting due to a temporary accumulation of endogenous auxin in the rooting zone of the cuttings, while the opposite trend was observed when a long storage period was imposed (Garrido

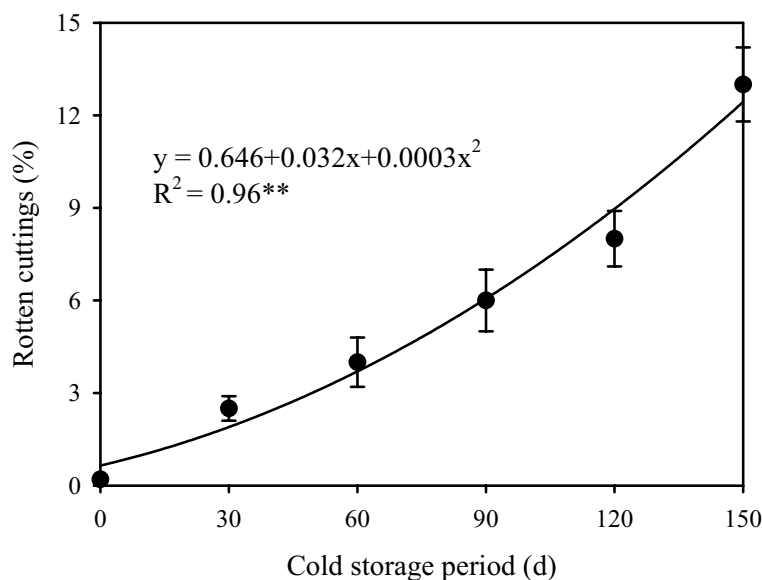


Fig. 2. Effect of the cold storage period on the percentage of rotten cuttings of globe artichoke (Expt. 4). Bars indicate standard errors. \*\*Significant at  $P \leq 0.01$ .

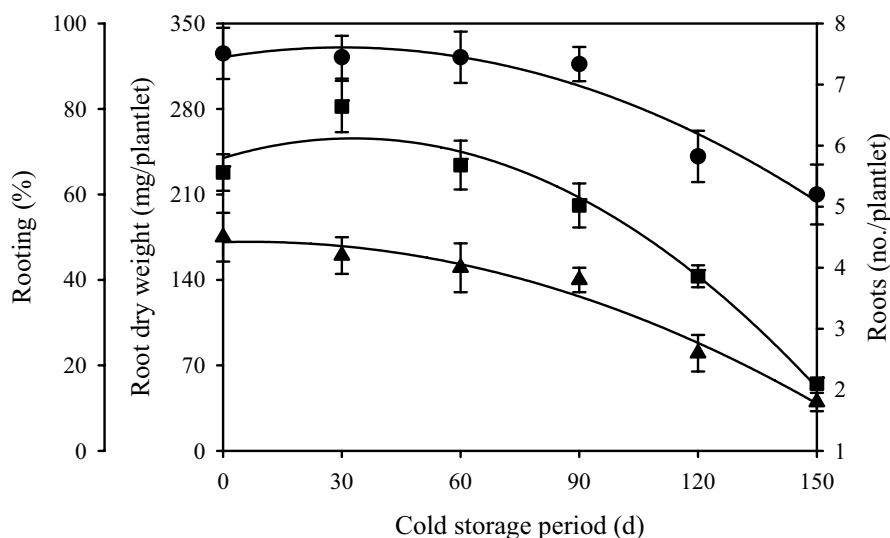


Fig. 3. Effect of the cold storage period on the number of roots ( $\blacktriangle$ ), the root dry weight ( $\blacksquare$ ) and the rooting frequency ( $\bullet$ ). Fitted curves are (—):  $y = 92.11 + 0.151x - 0.002x^2$  ( $R^2 = 0.94^*$ ) for rooting frequency;  $y = 239.8 + 0.979x - 0.015x^2$  ( $R^2 = 0.97^{**}$ ) for root dry weight;  $y = 4.42 + 0.002x - 0.0001x^2$  ( $R^2 = 0.97^{**}$ ) for root number (Expt. 4). Bars indicate standard errors. 1 mg = 3.5274  $\times 10^{-5}$  oz. \*, \*\*Significant at  $P \leq 0.05$ , or 0.01, respectively.

et al., 1998). Moreover, Rajapakse et al. (1996) observed the depletion of soluble sugars and a fructan-containing substance during the low-temperature storage of chrysanthemum cuttings.

To summarize, we can conclude that the efficiency of the production of artichoke transplants using our propagation system can be improved in the following ways: 1) by foliar application of BA (200 mg·L<sup>-1</sup>) to stock plants; 2)

by using cuttings with weight heavier than 30 g and by the application of NAA to the basal part of the cutting at a rate of 2000 mg·L<sup>-1</sup>; and 3) by cold storing the cuttings for less than 3 months (optimal length of cold storage = 30 d), until the field planting period, accepting the fact that percent rotten cuttings will increase and percent rooting will decrease somewhat for those cuttings stored longest.

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