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Effect of Vegetative Propagation Materials on Globe Artichoke Production in Semi-Arid Developing Countries: Agronomic, Marketable and Qualitative Traits

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Abstract: In Tunisia, globe artichoke is mainly propagated by underground dormant axillary buds (ovoli), which are removed from the field in August during the quiescence period. The high cost of in vitro-plants and the absence of specialized nurseries were among the reasons for the rise of heterogeneity and spread of diseases. The aim was to help farmers to improve artichoke yield and quality by ameliorating their vegetative propagation technique with low cost methods. Three plant cuttings management methods were tested: summer ovoli (T0); spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation (T1); and offshoots nursery's cuttings not forced (T2). The cuttings management can affect both yield and qualitative traits of artichoke. T1 nursery plants produced the heaviest primary heads, 7% and 23% higher than T2 and T0, respectively. T1 plants exhibited the highest yield during the harvest season, with +17.7% and +12.2% compared to T0 and T2, respectively. T0 and T1 showed the highest total antioxidant capacity and inulin content; the propagation method also affected the short-chain sugars ratio. T1 is a viable and sustainable alternative to the traditional one that does not heavily impact on growing costs and improves yield and quality of artichoke.

Keywords: *Cynara scolymus*; cuttings; yield; antioxidant; phenolic acids; fructans; sugars

1. Introduction

Globe artichoke (*Cinara cardunculus* L. subsp. *scolymus* (L.) Hegi) is one of the most important cultivated species in the Mediterranean Basin and is continuing to be planted and adapted in other parts of the world due to its health benefits [1,2]. In Tunisia, the cultivation of globe artichoke is mainly concentrated in the low Madjerda valley. In 2016, the total area involved with this crop reached 3850 hectares, and the total production was approximately 19,000 tons [3]. Around 75% of this area is represented by the cultivar "Violet d'Hyères" which is the most appreciated purple variety in Tunisia for its fast commercialization in local and international markets. In the last five years, Tunisian globe artichoke exportations have increased in a remarkable way, and reached 1222 tons in 2014 [4]. Due to the large quantity of propagation material present in a plant, globe artichokes are generally propagated vegetatively by offshoots, stumps, or dried shoots harvested from commercial fields at the end of the growing cycle [5]. In recent years, the propagation of artichokes has undergone considerable evolution

to avoid soil borne diseases following the development of micro-propagation systems [6,7], grafting techniques [8–10], and by using hybrid seeds [11]. All of these agronomic innovations are commonly applied in developed countries, where there are nursery techniques and technological equipment. In other contexts, such as Tunisia, vegetative propagation is the most widely used technique that can easily be adopted by producers.

For Tunisian farmers, earliness is one of the most important factors for the production, and it is directly linked to the export period which extends from November to January. The absence of specialized nurseries for artichoke plantlet production, especially for the local varieties, has lead to the growers producing their cuttings by themselves, in inappropriate conditions. As a consequence of this uncontrolled practice, the national average yield of globe artichoke has never exceeded 7 t ha^{-1} during the last years [3]. Yet, many researchers in some countries, such as Italy [5,12,13], Turkey [14,15], and the US [16], conducted several experiments to improve the vegetative propagation materials and production techniques of globe artichoke; however, they did not refer to the available vegetative material of the farmers, but they focused their attention on the use of micro-propagated plants. Then, they started to promote the nurseries' establishment, and to schedule the cuttings' propagation. This strategy seems to be quite difficult and inadequate in the case of Tunisia, for several reasons. The first one is linked to the absence of nurseries for the in vitro-plant production. The second reason is related to the harvest time, that starts at the end of February or in March. As a result, Tunisian farmers are not able to export their production and this affects negatively their incomes. In addition to that, head production of micro-propagated artichokes could be lower than the ordinary plants and instable from one season to another [17,18].

In the last three decades, artichoke breeding programs have produced some hybrids, which started to be considered in the main artichoke producing areas as an alternative to the traditional ones [19]. Yet, hybrids are free from the main endemic diseases like viruses and fungi, and showed good yields [20–22], but they start to produce heads later if compared to the standard cultivars [23] and they are still not widely commercialized. Indeed, the cost of hybrid seeds is often very high and not all farmers can deal with it.

Considering the qualitative aspects of artichoke, there are many sources which clearly describe the noticeable health properties and quality of this vegetable [1,24,25]. Until now, the main research lines evaluating the effects of agricultural practices on artichoke quality, are concerned with seed propagation [21,26,27], stress effects [28], different fertilization techniques [29,30], mycorrhiza use [31], the application of bio-regulators [32], and the use of sustainable farming systems [33]. Regarding the management of vegetative propagation material and artichoke quality, there is little information.

Earliness, high yield, and good quality of the production are the main criteria targeted by the farmers. These objectives could be reached just by improving the quality of cuttings by upgrading the rooting of offshoots in the nursery [12], scaling the production of early transplants [34], or conditioning the transplants to mitigate heat, drought stress, and biotic stresses [15].

The main objective of this work is to improve the quality of artichoke cuttings on the base of the simple tools that Tunisian farmers can afford. In this trial, plants derived from nursery's cuttings were evaluated for their agronomic, marketable, and qualitative traits, compared to the traditional plants originated from field's ovoli (underground dormant axillary buds).

2. Results

2.1. Vegetative Growth and Yield Parameters

Following the plants' vegetative growth 70 to 190 days after planting, nursery transplants of 163 days (T2) showed the lowest elongation rhythm (Figure 1) but the highest extension width compared to the plants deriving from the two types of ovoli: the traditional, and the nursery's ones (Figure 2). The pre-germination of ovoli was efficient for T0 and T1 after a rest period for the activation of the root system and lateral buds, obtaining a good start for vegetative growth.

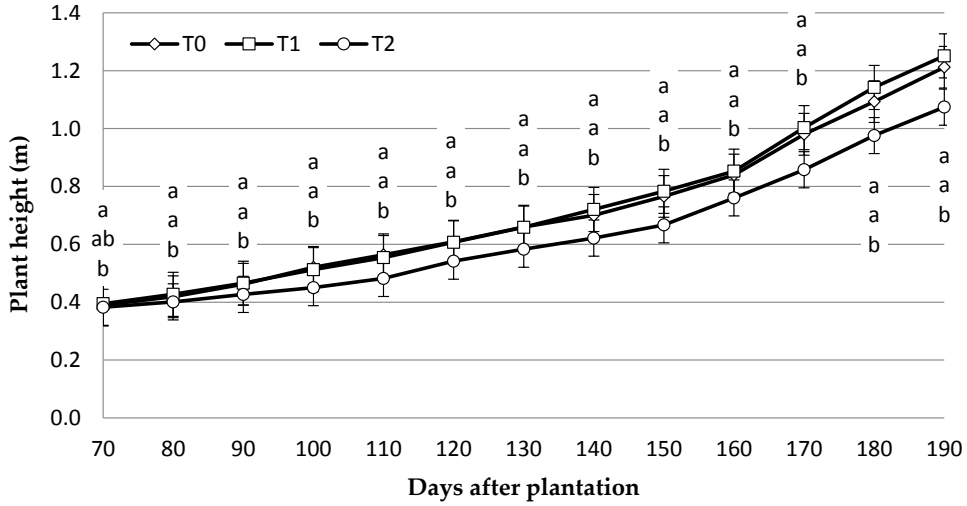

Figure 1. Plant height evolution according to the cuttings' type used for the artichoke vegetative propagation. Within the same period, data were analyzed by ANOVA, and values with no letter in common are significantly different at $p < 0.05$ according to Tukey HSD Test. Standard errors are reported. T0: summer ovoli; T1: spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation; T2: spring offshoots nursery's cuttings not forced.

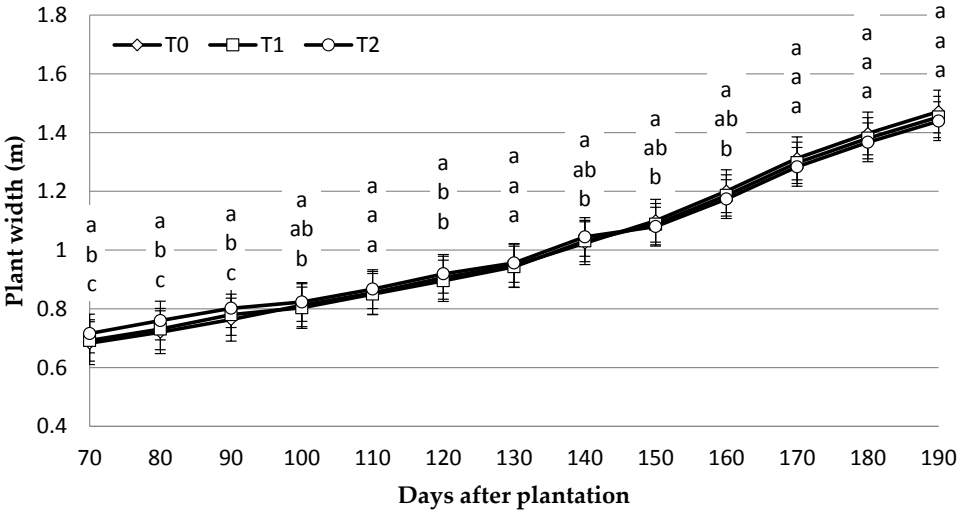

Figure 2. Plant width evolution according to the cuttings type used for the artichoke vegetative propagation. Within the same period, data were analyzed by ANOVA and values with no letter in common are significantly different at $p < 0.05$ according to Tukey HSD Test. Standard errors are reported. T0: summer ovoli; T1: spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation; T2: spring offshoots nursery's cuttings not forced.

The monitoring of offshoot formation revealed significant differences between the treatments. At the end of January (150 days after plantation), the emission of new offshoots started. T1 and T0 were fairly close, and showed the highest number of offshoots with an average of almost 5 shoots at the end of the harvesting season (Figure 3). T2 displayed the lower offshoots production, 14.7% less than T1.

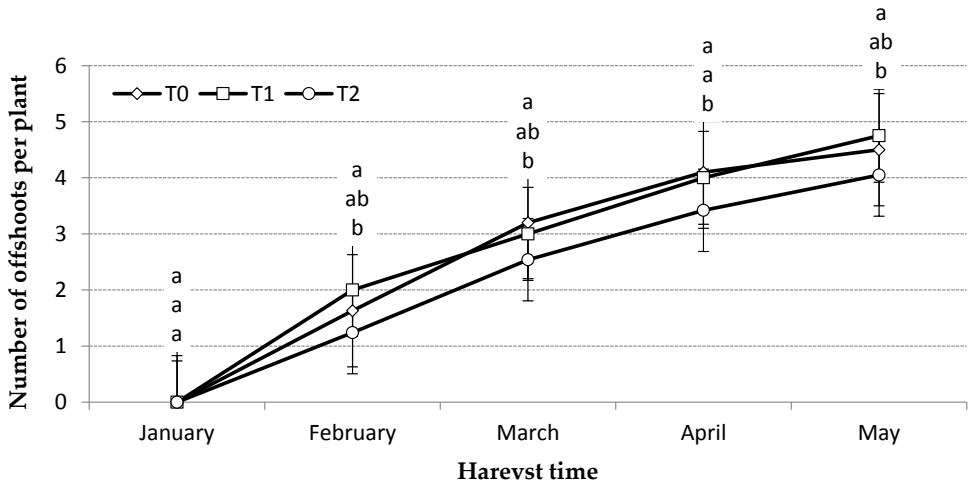

Figure 3. Number of offshoots formed according to the cuttings type used for the artichoke vegetative propagation. Within the same period, data were analyzed by ANOVA, and values with no letter in common are significantly different at $p < 0.05$, according to Tukey HSD Test. Standard errors are reported. T0: summer ovoli; T1: spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation; T2: spring offshoots nursery's cuttings not forced.

First heads were harvested on 14 January 2016, and their height, diameter, and fresh weight were measured (Table 1). T1 nursery plants produced the heaviest primary heads with a mean weight of 269 g, 7% and 23% higher than T2 and T0, respectively. In terms of height and diameter, primary heads produced by plants deriving from nursery's ovoli and traditional field's ovoli were similar. The flower initiation of T1 plants begun earlier than the other plants' treatments, and this result was proved by the estimation of the percentage of early yield, so the primary heads were advantageous to grow and to gain more weight before the first time of harvest.

Table 1. Characterization of primary harvested heads according to the cutting type used for the artichoke vegetative propagation.

Treatment	Height (mm)	Diameter (mm)	Weight (g)
T0	95.3 ± 1.5 a	92.5 ± 0.6 ab	208 ± 8 c
T1	96.9 ± 1.9 a	94.8 ± 0.7 a	269 ± 11 a
T2	89.9 ± 2.2 b	89.2 ± 0.4 b	249 ± 6 b

Within each parameter, data were analyzed by ANOVA and different letters indicate significant differences according to the Tukey's HSD test at $p < 0.05$. Standard errors are reported. T0: summer ovoli; T1: spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation; T2: spring offshoots nursery's cuttings not forced.

The yield of the local cultivar "Violet d'Hyères" was evaluated progressively through the measurement of heads weight per plant in each harvest for all treatments. As a consequence, cumulative yield per hectare (Figure 4) was monthly estimated along the growing cycle, from 14 January to 15 May 2016. T1 plants, deriving from nursery's ovoli, exhibited the highest head production during the harvest season, with a final cumulative yield of 29.21 t ha^{-1} against 24.03 t ha^{-1} (−17.7%) and 25.65 t ha^{-1} (−12.2%), respectively, for T0 and T2.

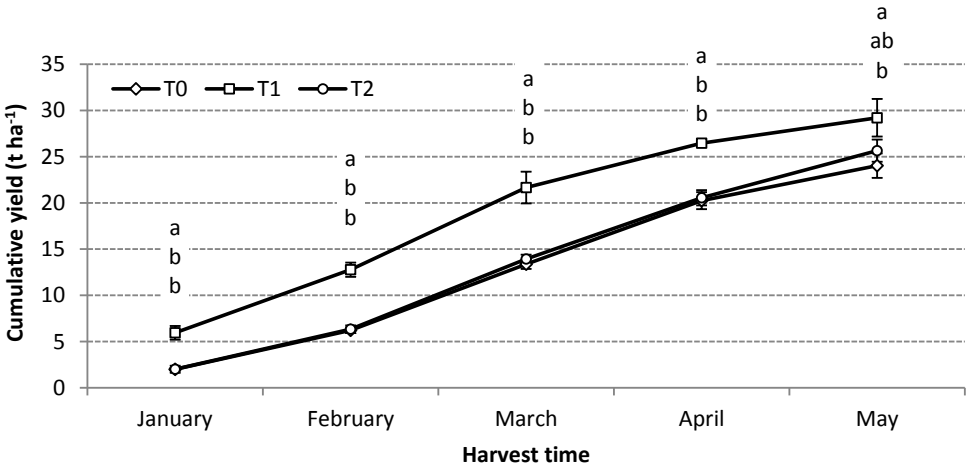

Figure 4. Cumulative yield evolution according to the cuttings type used for the artichoke vegetative propagation. Within the same period, data were analyzed by ANOVA and values with no letter in common are significantly different at $p < 0.05$ according to Tukey's HSD Test. Standard errors are reported. T0: summer ovoli; T1: spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation; T2: spring offshoots nursery's cuttings not forced.

The precocity assessment (Table 2) through the three periods: early (before 15 January), mid-early (between 16 January and 15 March), and late (after 15 March) revealed that the relative yield rate obtained before mid-January was relatively low for all treatments, and not higher than 5.2% of total yield in the best case (T1).

Table 2. Yield distribution (t ha^{-1}) and percentage per treatment according to the cutting type used for the artichoke vegetative propagation.

Treatment / Harvest Period	T0		T1		T2	
	t ha^{-1}	%	t ha^{-1}	%	t ha^{-1}	%
Early yield (<Jan 2015	0.60 ± 0.15 b	2.5	1.52 ± 0.11 a	5.2	1.00 ± 0.12 c	3.9
Mid-Early yield (Jan–Mar 2015)	11.63 ± 0.23 b	48.4	13.55 ± 0.29 a	46.4	12.12 ± 0.31 c	47.3
Late yield (>Mar 2015)	11.80 ± 0.19 b	49.1	14.14 ± 0.17 a	48.4	12.53 ± 0.16 c	48.8
Total yield	24.03± 0.15 b	100	29.21± 0.15 a	100	25.65± 0.15 c	100

Within the same period, data were analyzed by ANOVA and values with no letter in common are significantly different at $p < 0.05$ Tukey's HSD Test. Standard errors are reported. T0: summer ovoli; T1: spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation; T2: spring offshoots nursery's cuttings not forced.

The mid-early yield recorded between 16 January and 15 March was 46% higher than total yield for all treatments, with the highest head production in T1 treatment (13.55 t ha^{-1} against 12.12 t ha^{-1} and 11.63 t ha^{-1}, respectively, for T2 and T0). The same tendency was observed in the late yield, where T1 has the highest production in terms of tons per hectare, but not the biggest percentage of the total yield compared to the relative portion of T0 yield in the same period that reached 49.1%. As consequence of these different rates, total yields cumulated at the end of harvest season were always significantly higher for plants deriving from nursery's ovoli.

The calibration of the harvested heads (Table 3) was done on the base of precocity scale (early, mid-early, and late). In the three periods, the harvested heads of T1 treatment were the heaviest ones compared to T0 and T2, and they ranged from 304 g in the early harvest to 260 g in the late one. In addition, the highest mean head weight was recorded in the earliest harvest period before mid-January, independently from the treatments.

Table 3. Mean weight (g) and mean diameter (mm) of heads per treatment according to the cutting type used for the artichoke vegetative propagation.

Harvest Period \ Treatment	T0	T1	T2
Mean head weight (g)			
Early yield (<Jan 2015)	304 ± 5 b	322 ± 2 a	287± 6 c
Mid-Early yield (Jan–Mar 2015)	299 ± 2 b	314 ± 4 a	285 ± 4 c
Late yield (>Mar 2015)	260 ± 3 b	277 ± 5 a	247 ± 3 c
Mean head diameter (mm)			
Early yield (<Jan 2015)	99 ± 3 ab	109 ± 2 a	89 ± 1 b
Mid-Early yield (Jan–Mar 2015)	85± 2 ab	92 ± 3 a	73 ± 1 b
Late yield (>Mar 2015)	69 ± 1 ab	74 ± 2 a	62 ± 2 b

Within the same period, data were analyzed by ANOVA, and values with no letter in common are significantly different at $p < 0.05$, according to Tukey's HSD Test. Standard errors are reported. T0: summer ovoli; T1: spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation; T2: spring offshoots nursery's cuttings not forced.

The biggest head size was obtained in the early harvest season, with a maximum of 109 mm of diameter in the treatment T1, and the smaller one in the late harvest, with an average of 62 mm found in the treatment T2. According to Table 4, more than 65% of harvested heads were classified between the two sizes 75 mm and 110 mm of diameter in all treatments. T2 was the most advantageous, with a rate of 71%, followed by T0 (69%) and T1 (68%) of heads. For the smallest size, T2 presented about 4.10 t ha^{-1} with the highest rate of the production (16%). According to UNECE standard classification of commercial quality artichokes (Table 5), more than 50% of the production was classified as an extra quality only for plants deriving from nursery and traditional ovoli. T1 was the most advantageous treatment, with more than 18 t ha^{-1} for the class Extra, followed by T0 with 12.25 t ha^{-1}, and so far, T2 with 3.09 t ha^{-1}. For the class I, 34% of the T2 total production was the highest rate compared to T0 and T1. Concerning the lowest quality class, T1 expressed the lowest total yield with 4.38 t ha^{-1} conversely to T2, that showed the highest portion of the total yield with an average of 13.84 t ha^{-1}.

Table 4. Yield distribution (t ha^{-1}) and its percentage classified by diameter according to the cutting type used for the artichoke vegetative propagation.

Head Diameter \ Treatment	T0		T1		T2	
	t ha^{-1}	%	t ha^{-1}	%	t ha^{-1}	%
D ≥ 130 m	1.44 ± 0.01 a	6	0.51± 0.05 c	2	0.77 ± 0.01 b	3
110 m ≤ D < 130 mm	3.84 ± 0.07 ab	16	4.62 ± 0.30 a	18	2.57 ± 0.31 b	10
90 mm ≤ D < 110 mm	8.65 ± 0.18 c	36	11.39 ± 0.23 a	39	9.49 ± 0.07 b	37
75 mm ≤ D < 90 mm	7.93 ± 0.28 b	33	8.47 ± 0.23 c	29	8.72 ± 0.15 a	34
6 mm ≤ D < 75 mm	2.16 ± 0.08 b	9	3.51 ± 0.05 b	12	4.10 ± 0.06 a	16

Within the same diameter, data were analyzed by ANOVA and values with no letter in common are significantly different at $p < 0.05$ according to Tukey's HSD Test. Standard errors are reported. T0: summer ovoli; T1: spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation; T2: spring offshoots nursery's cuttings not forced.

Table 5. Total yield distribution (t ha^{-1}) and its percentage according to the cutting type used for the artichoke vegetative propagation in relation to UNECE classification.

Marketable Classes \ Treatment	T0		T1		T2	
	t ha^{-1}	%	t ha^{-1}	%	t ha^{-1}	%
Class Extra	12.25 ± 0.06 b	51	18.11 ± 0.24 a	62	3.09 ± 0.17 c	12
Class I	7.68 ± 0.03 b	32	6.72 ± 0.14 c	23	8.72 ± 0.17 a	34
Class II	4.09 ± 0.02 b	17	4.38 ± 0.04 c	15	13.84 ± 0.17 a	54

Within the same marketable class, data were analyzed by ANOVA and values with no letter in common are significantly different at $p < 0.05$ according to Tukey's HSD Test. Standard errors are reported. T0: summer ovoli; T1: spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation; T2: spring offshoots nursery's cuttings not forced.

2.2. Qualitative Traits

During the intermediate period (mid-early) of head harvest, the qualitative traits of marketable product and leaves were evaluated for each treatment, in order to highlight any changes in the nutritional profile of the plant. The data obtained demonstrate that the plant propagation method can significantly affect qualitative aspects.

Regarding pigment content (Table 6), T1 and T2 had significantly higher values than the control treatment. In particular, the content of chlorophyll *a*, *b* and xanthophyll + carotenoids in T1 was higher of 24.5%, 24.7%, and 18.0%, respectively, if compared to T0. T0 and T1 showed the highest total antioxidant capacity values in contrast to T2 that also presented the lowest levels of total phenols. Regarding the phenolic acid content, the chemical analyses identified the presence of chlorogenic acid and caffeic acid. For the first one, the highest values were recorded in T0 and T1, respectively 29.6% and 30.2% greater than T2. No significant differences were found with caffeic acid. The qualitative parameters so far reported were also assessed in the plant leaves (data not reported), however, no significant differences were observed, with the exception of the chlorogenic acid content. The latter was higher in T0 (91.2 mg kg^{-1} dw) and T2 (89.0 mg kg^{-1} dw), whereas T1 presented the lower values (53.6 mg kg^{-1} dw). The chlorogenic acid content in the leaves was much lower than that of the head (−97.7% on average).

Table 6. Effect of cutting type used for the artichoke vegetative propagation on pigments and antioxidant compounds.

	Chlorophyll *a*	Chlorophyll *b*	Xanthofills + Carotenoids	TP	TAC	Chlorogenic Acid	Caffeic Acid
	μg g^{-1} dw	μg g^{-1} dw	μg g^{-1} dw	(mg GAE kg^{-1} dw)	(mg Fe^{2+} kg^{-1} dw)	(mg kg^{-1} dw)	(mg kg^{-1} dw)
				artichoke head			
T0	221± 3 b	92.5 ± 2 b	29.5 ± 1.5 b	8361 ± 56 a	7636 ± 43 a	3873 ± 25 a	79.4
T1	293 ± 4 a	122 ± 5 a	37.4 ± 2.4 a	9068 ± 78 a	6827 ± 72 ab	3905 ± 48 a	69.2
T2	293 ± 6 a	124 ± 7 a	35.4 ± 2.1 a	6569 ± 29 b	6033 ± 37 b	2724 ± 32 b	54.3
	**	*	*	***	*	***	n.s.

Within the same trait, data were analyzed by ANOVA and values with no letter in common are significantly different at $p < 0.05$ according to Tukey's HSD Test. Standard errors are reported. T0: summer ovoli; T1: spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation; T2: spring offshoots nursery's cuttings not forced; TP: total phenols; TAC: total antioxidant activity. n.s.: not significant; *: significant at $p < 0.05$; **: significant at $p < 0.01$; ***: significant at $p < 0.001$.

Also, sugar content was affected by the plant's propagation mode. In general, inulin concentration was significantly higher than the other sugars considered (Figure 5), and was found only in the marketable product.

Concerning the marketable heads (Figure 5A), the sucrose and inulin contents were conditioned by the treatments evaluated in this experiment. T0 and T1 showed inulin values higher than 180 g kg^{-1} dw, whereas T2 significantly differed from T0, presenting 12.8% less. Always in the marketable head, even the sucrose content was affected by propagation method, and T1 and T2 showed the highest concentrations; no significant differences were observed in glucose and fructose load. Interestingly, the propagation mode also affected the short-chain sugars ratio. The sucrose/glucose ratio ranged from 0.35 to 0.44, respectively, for T0 and T1, whereas fructose/glucose ratio (0.7) was rather constant. The leaf biomass (Figure 5B) presented significantly higher concentrations of glucose than fructose and sucrose; moreover, treatments significantly influenced the glucose content. T2 supplied the higher responses, with 19.7% and 25.0% more than T0 and T1, respectively.

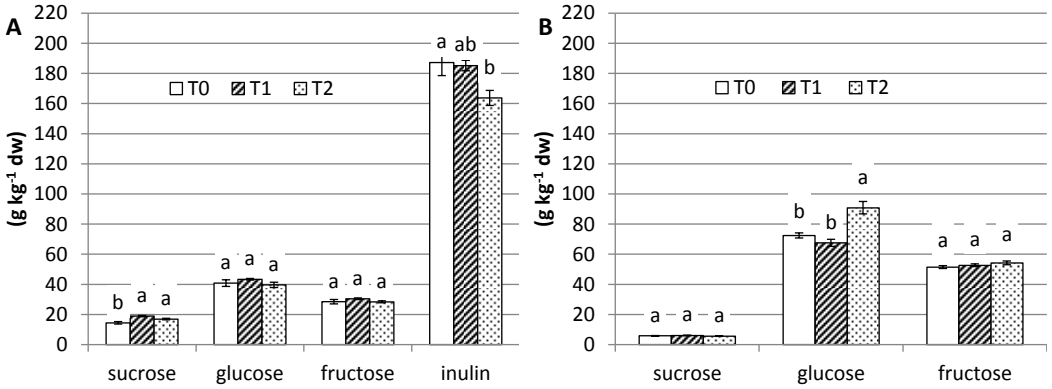

Figure 5. Effect of cutting type used for the vegetative propagation on sugar content in artichoke head (**A**) and leaves (**B**). Within the same trait, data were analyzed by ANOVA, and values with no letter in common are significantly different at $p < 0.05$, according to Tukey's HSD Test. Standard errors are reported. T0: summer ovoli; T1: spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation; T2: spring offshoots nursery's cuttings not forced.

The mineral profile of the plant is another parameter that was affected by the propagation mode of artichoke. Regarding marketable head (Table 7), chlorides, phosphates, sulfates, sodium, potassium, and calcium were significantly different. T0 expressed the higher results for all the components listed above, T1 was not different from T0 except for chlorides and sodium content, whereas T2 and T0 showed the same content of phosphates. As part of the leaves, the content of sodium, potassium, and magnesium nitrate was significantly lower in T2. Regarding nitrates, T0 presented the highest concentration.

Table 7. Effect of cuttings type used for the vegetative propagation on mineral content in artichoke head and leaves.

	Cl^-	PO_4^{3-}	SO_4^{2-}	Na^{2+}	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	NO_3^-
	(g kg^{-1} dw)								
Artichoke head									
T0	12.4 a	6.87 a	2.69 a	2.58 a	0.331	30.2 a	1.81	3.42 a	nd
T1	12.3 a	5.41 b	2.16 b	2.67 a	0.347	28.3 b	1.75	2.78 b	nd
T2	10.6 b	6.69 a	2.28 b	2.03 b	0.385	29.2 ab	1.85	2.39 b	nd
	***	***	***	***	n.s.	*	n.s.	***	
Artichoke leaf									
T0	61.9	3.32	4.30	19.0 a	1.30	42.1 a	2.98 a	8.52	7.34 a
T1	57.1	3.20	5.00	17.8 a	1.29	41.0 ab	2.97 ab	9.61	5.72 b
T2	49.2	3.45	4.30	15.7 b	1.23	39.1 b	2.53 b	8.22	5.22 b
	n.s.	n.s.	n.s.	***	n.s.	*	*	n.s.	***

Within the same trait, data were analyzed by ANOVA, and values with no letter in common are significantly different at $p < 0.05$, according to Tukey's HSD Test. T0: summer ovoli; T1: spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation; T2: spring offshoots nursery's cuttings not forced. nd: not detected; n.s.: not significant; *: significant at $p < 0.05$; **: significant at $p < 0.01$; ***: significant at $p < 0.001$.

3. Discussion

Vegetative propagation methods on globe artichoke significantly affected the yield, and the marketable and qualitative characteristics of the product. Under the production point of view, the lowest growth rhythm, registered in T2, could be due to the transplant age [27]. This can influence the vegetative development of artichoke plants, modifying the development stage of the auxiliary buds that were activated during the nursery phase and the aerial part of T2 cuttings grew up laterally using

the accumulated reserves. The height development of T2 plants was affected by the slow emission of new adventitious roots, and the damage of the existed roots during the nursery phase, after the removal of plants from the nursery to the field. In fact, tissue age can affect the ability of cuttings to form new adventitious roots, and it is also linked to ontogenetic phases; these mark the differentiation of the tissue from meristem that is getting physiologically older and more mature [35]. The slow growth rate of T2 plant also affected the reproductive phase of the crop, and this result is confirmed by T1, which produced the earliest and the heaviest primary heads compared to other treatments. These results prove that ovoli typology can influence the number of early harvested heads and head weight, as reported in literature [36]. Overall, T1 provided the highest yield results, with more than 29 t ha^{-1}, which are in line with those obtained from other experiments. In 2013, an experimental essay was conducted in Kaâlat El Andalous in Ariana governate in the north of Tunisia (37° N, 10° E, altitude 238 m). As suggested in other studies [37], the biggest head size was obtained in the early harvest season. In addition, the harvest period significantly influenced the physical quality of heads. The lowest mean weight and mean diameter of heads were obtained in the late harvesting period. Similar results were presented by another experiment [38], where the highest head weight (253 g) and head diameter (74 mm) were obtained on 15 January, whereas the lowest values (179 g for head weight and 67 mm for head diameter) were registered on 15 March.

As reported for agronomic and marketable parameters, the qualitative ones were influenced by the vegetative propagation methods. Taking into consideration head color characteristics, different pigment contents were observed. As known, pigment content depends on the photosynthetic capacity of leaves and leaf area [39]. This information can be linked to our experiment, where leaves of T1 and T2 plants were wider than T0, in fact, significant differences were observed in plant width during the vegetative phase. The values measured for antioxidant capacity were generally lower than those observed for other artichoke varieties, like "Violetto di Chioggia" and "Violetto di S. Erasmo" [2]; if we consider "Violetto di Toscana", instead "Violet d'Hyères", the total antioxidant capacity (TAC) values are on average 50% higher. This variability in the concentration of antioxidant compounds is mainly due to genetic aspects, agronomic practices, and environmental conditions [1,40]. Furthermore, the high content of antioxidants and total phenols in T0 and T1 may also be connected to more stressful conditions that the plant cuttings have experienced before transplanting in the experimental field. The drought stress, that was induced for 60 days, at least for T0 and T1 plants to obtain cuttings with quiescent buds, has influenced the quality of produced heads during the growing cycle. As previously reported [36], ovoli management has an important impact on the head production. As known, a stress experienced by the plant during the growing season can significantly influence the antioxidant amount. In fact, plant stress tolerance may be improved by the enhancement of in vivo levels of antioxidant [41,42]. The lower or slower root system development reduced, for some periods, the nutrient and water availability to the plant, by inducing such responses. The low antioxidant content in T2 is probably due to the lower stress condition that the plant has suffered during the crop cycle. As a result, the concentration of chlorogenic acid (caffeoylquinic acid) was lower in T2 by 29.6% and 30.2% compared to T0 and T1. Based on total caffeoylquinic acid contents, caffeoylquinic acid is the most abundant single component (39%), followed by 1,5-*O*-dicaffeoylquinic acid (21%), and 3,4-O-dicaffeoylquinic acid (11%). Cynarin (1,3-*O*-dicaffeoylquinic acid) content is usually very low (about 1.5%) in methanolic extracts of artichoke [1]. The chlorogenic acid content in the leaves was much lower than that of the head. This result is similar to what reported for the "Violetto di Toscana" artichoke variety (−82.9%) [43]. Moreover, it should be remembered that the caffeoylquinic derivatives content in artichoke tissues highly depends on the physiological stages of the plant. The total caffeoylquinic acid content can range from about 8% in young tissues to less than 1% in senescent tissues on dry matter basis [44].

As known, the artichoke is a species characterized by a high amount of long and short chain of sugars. In addition to simple sugars, the fructan group is of growing interest as a functional food ingredient, because of their potential benefits for human health [45–47]. As human enzymes cannot

digest fructans, they reach the colon and serve as a substrate for enterobacterial growth [1]. Fructan containing diets selectively stimulate bifidobacteria and make them the predominant species [48]. Consequently, an increased fecal content of short-chain fatty acids and a decreased concentration of tumor-promoting substances, such as ammonia, were observed [49]. The inulin values measured for "Violet d'Hyères" artichoke, commonly grown in Tunisia, are in line with those obtained for other varieties of artichoke (Le Castel and Green Globe) [47]. The sugar ratio is also greatly influenced by the varietal characteristics. The sucrose/glucose ratio ranges from 0.46 to 2.29 for the varieties Poivrade and Buette, respectively [47]. Other authors, instead, found lower sucrose/glucose ratios (0.07–0.1), respectively, for "Violetto di S. Erasmo" and "Violetto di Chioggia" [2]. The high concentration of glucose, compared to fructose and sucrose in leaf biomass, were significant. Treatments remarkably influenced the glucose content, and T2 supplied the highest outcomes. This result is mainly linked to the bigger size of the leaves in T2 in the first part of the crop cycle, thus promoting the photosynthetic activity. This confirms that the high artichoke leaf area positively influenced the photosynthesis activity, as already reported [50].

The mineral profile was also affected by the plant propagation mode. In general, most of results are attributable to the different ability of offshoots and ovoli to form new roots, and to their ability to explore the soil. Some significant changes have been recorded for potassium and sodium. In fact, these minerals are the main indicators for plant tolerance to mitigate drought stress [51,52]. During the nursery phase, the cuttings of T2 were continuously irrigated until their transplantation in the experimental field, whereas cuttings of T1 and T0 were forced to enter in a vegetative rest period by causing drought stress through stopping of irrigation for more than 2 months. After 80 days from field transplantation, T2 plants emitted new secondary roots, mainly in the 0.25 m of the topsoil, whereas T0 and T1 plants developed deeper secondary roots. This could explain why the absorption of mineral nutrients was lower in T2 than the other treatments. In general, the mineral content was significantly higher in the leaves compared to the marketable yield. Such a response is in agreement with other findings [43]. In the head, the most abundant element was potassium, with values close to 30 g kg^{-1} dw and to 40 g kg^{-1} dw in the leaves. On the other hand, the content of chlorides is particularly high, probably due to their persistence in the soil, because of the use of potassium chloride as a fertilizer for previous cultures. Compared to the mineral composition of other vegetables reported in the literature, the globe artichoke represents a good source of K [53]. In addition, the Na/K ratio of globe artichoke is one of the lowest ratios, following potato. This parameter is very important, since a high level is involved in increasing blood pressure and cardiovascular diseases [54]. Therefore, the consumption of globe artichoke might also be suggested to overcome these diseases. Moreover, the increased mineral content in the leaves can be explained by the capacity of globe artichoke to compartmentalize excess ions in the vegetative, rather than the reproductive, parts of the plant [55].

4. Materials and Methods

4.1. Experimental Site

The experiment was conducted in the Support Station of Manouba (SAM), which is under the supervision of the Inter-professional Group of Vegetables (GIL). It is located in Manouba region, situated in the north of Tunisia (36°48′ N; 10°03′ E, altitude 469 m). The local climate of this area is characterized by an upper semi-arid stage, with an average daily temperature ranging from 9 °C as minimum in January, to 25 °C as maximum in August. The rainfall is irregular, with an average of 450 mm per year. Meteorological data recorded during the trial period are reported in Figure 6.

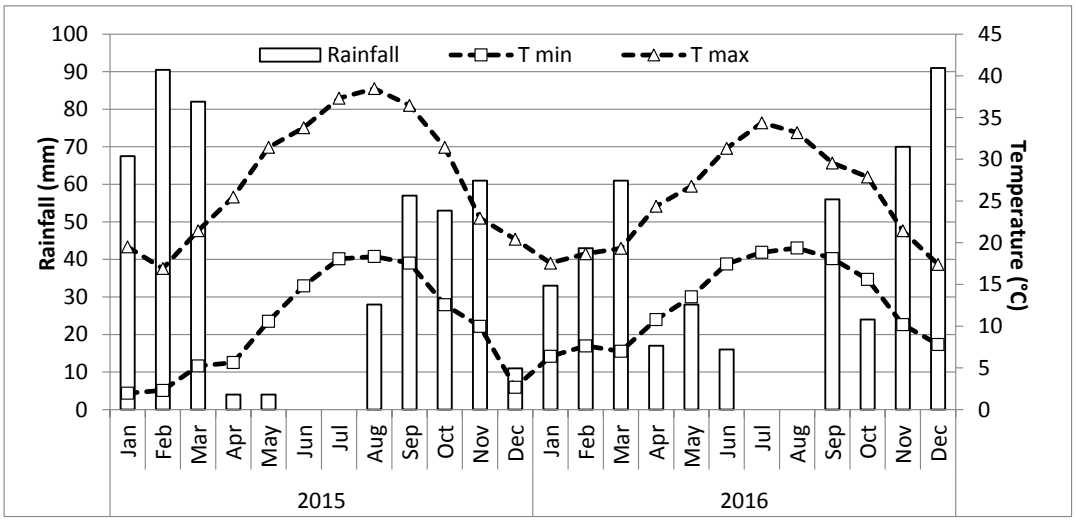

Figure 6. Monthly averages of maximum and minimum air temperatures and cumulative rainfall registered during artichoke growing cycle.

4.2. Plant Material and Offshoots Nursery Phase

All the artichoke (cv. Violet d'Hyères) cuttings were selected and collected from the artichoke field of the agricultural development company "ESAADA" situated in Manouba governorate (36°50′ N; 9°51′ E). Three cutting management method were considered (Figure 7): summer field's ovoli (T0), spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation (T1), and offshoots nursery's cuttings not forced (T2).

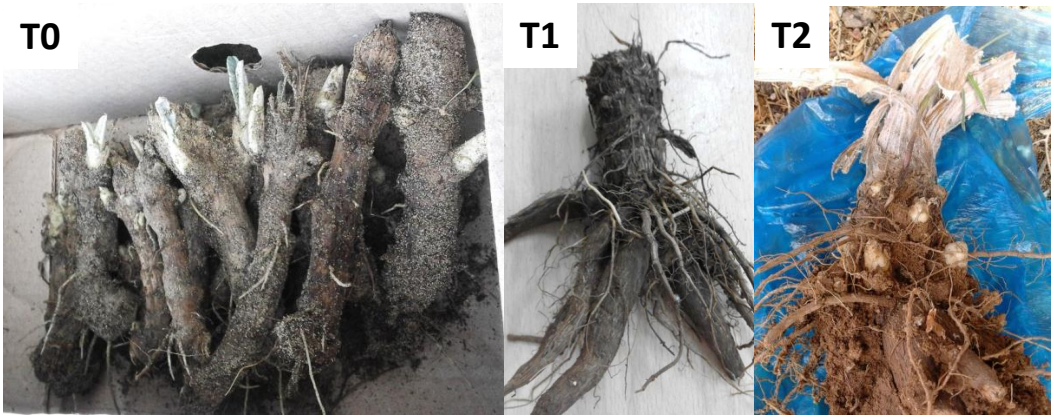

Figure 7. Cutting management considered in the experiment: summer field's ovoli (T0), spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation (T1), and offshoots nursery's cuttings not forced (T2).

All the plant material used in this trial was obtained from 200 mother plants vegetatively propagated in ESSAADA field. For the T0 treatment, ovoli were directly removed from the field of ESSAADA on 5 September. Then, they were selected according to their visual phytosanitary aspect and their rhizome diameter (15 mm to 20 mm). Successively, they were pre-germinated 10 days in watered sand before the plantation in the experimental field. Concerning offshoots, characterized by 5 to 7 leaves, they were removed from mother plants that were controlled since November 2014 for their morpho-phytosanitary aspects and homogeneity. After removal, they were trimmed 0.15 m from the base, eliminating the apical part of leaves to reduce transpiration, then dipped in a copper

hydroxide solution (50%) for 2 min to disinfect them. An artichoke nursery was established on 4 April 2015 in SAM. Before that, the soil (clay 28%; silt 48%; sand 24%) was plowed by offset, then prepared by rotative herse on 20 March. After that, ridges were made respecting a spacement of 0.6 m among the rows, and a drip irrigation system was installed. In the nursery, the planting was carried out on four twinned lines 22 m long, by placing the offshoots in a staggered pattern on the two blanks of the ridge, and respecting gaps of 0.15 m between the plants. The plant density in the nursery was about 22 plants m^{-2}. The T1 nursery offshoots were differently treated compared to those of treatment T2. T1 cuttings were grown from 1 April to 15 June 2015 (75 days), then forced to pass a vegetative rest period (83 days) by stopping irrigation. Then, they were removed from the soil in 5 September and pre-germinated for 10 days before field transplantation (15 September). T2 cuttings were grown for 163 days (1 April to 10 September) without vegetative rest, removed from the soil in 15 September, the aerial part cut-down and washed, then the root part was dipped in a copper hydroxide solution (50%) for 2 min and planted in the experimental field. For the fertilization, 47 kg of N ha^{-1}, 25 kg of P_2O_5 ha^{-1} and 42.5 kg K_2O ha^{-1} were fractionated and distributed during the nursery phase.

4.3. Field Establishment

The cuttings coming from the different treatments were set up in the field in 15 September 2015, following a randomized block design with three replications for each treatment. All the treatments and other experimental traits are reported in Table 8 for an easier overview. Each plot (36 m^2) inside the randomized block was made by 30 plants (90 plants in total for each treatment) spaced 0.8 m apart in the row, and 1.5 m between the plantation lines. The crop requirements in terms of water and mineral nutrient needs were achieved following the programs of irrigation and fertilization elaborated by SAM and CTPTA. The irrigation was performed by drip irrigation system using drilling water characterized by 1.4 g L^{-1} of dry residue. The total consumption of water during all the crop cycles was about 730 mm. For the fertilization, 320 kg ha^{-1} of N, 160 kg ha^{-1} P_2O_5, and 210 kg ha^{-1} K_2O were fractionated, depending on the requirements of the crop in each phase.

Table 8. Treatment overview and plot characteristics in the experiment.

Treatments	Cutting Type	Nursery Phase	Forcing Period	Plot Area	Plants/Plot
		(Days)	(Days)	(m^2)	(n°)
T0	summer field's ovoli (underground dormant axillary buds)	-	-	36	30
T1	spring offshoots nursery's cuttings forced to pass a vegetative rest period by stopping irrigation	75	83		
T2	spring offshoots nursery's cuttings not forced	163	-		

4.4. Crop Measured Parameters

Ten plants from each plot were considered for measurements, and samplings from 70 to 190 days after planting. The measured parameters for vegetative growth were plant height (m), width (m), and number of shoots per plant. Total yield was recorded since the beginning of the harvest until the end of the production season, considering the number of harvested heads per plant and the yield of each harvest. Starting from the first harvest, primary heads were characterized by their height (mm), diameter (mm), and weight (g). Then, the cumulative yield per hectare was recorded monthly until the last heads were produced. Early yield was calculated taking into account the first harvest until the one before the mid of January. While, the mid-early yield was considered during the period from 16 January until the 15 March; the rest of harvests were treated as late yield. In the meantime, precocity was evaluated for each treatment. The calibration of harvested heads was based on the diameter measurements. The commercial quality of globe artichoke was estimated according to

UNECE standard. For quality traits, a representative sample for each plot was harvested and stored at −80 °C for further freeze drying.

4.5. Pigment Extraction

Chlorophyll *a*, *b* and xanthophylls + carotenoids content was determined on 0.2 g of freeze-dried artichoke sample, and extracted in ethanol for 48 h in the dark at +4 °C [56]. The following formulas were used for pigments' quantification (1–3):

$$\text{Chl } a = 13.95 \times \text{A665} - 6.88 \times \text{A649} \quad (1)$$

$$\text{Chl } b = 24.96 \times \text{A649} - 7.32 \times \text{A665} \quad (2)$$

$$\text{Xan + Car} = (1000 \times \text{A470} - 2.05 \times \text{Chl } a - 114.8 \times \text{Chl } b)/245 \quad (3)$$

where A is the absorbance obtained at each wavelength (665, 649 and 470 nm).

4.6. Extraction of Phenols for Analysis

Artichoke freeze-dried sample (0.2 g) were homogenized in methanol (20 mL) with an Ultra Turrax T25 until uniform consistency at 13,500 rpm. Samples were filtered (filter paper, 589 Schleicher) and appropriate aliquots of extracts were assayed by Folin–Ciocalteu (FC) assay for total phenol (TP) content and by ferric reducing antioxidant power (FRAP) assay for total antioxidant activity. Concerning HPLC analysis, extracts were further filtered through cellulose acetate syringe filters (0.45 µm). For each sample, triplicate extractions and analyses were carried out.

4.7. Determination of Total Phenols by the Folin–Ciocalteu Assay

The content of TP was determined using the FC assay with gallic acid as calibration standard, by a Shimadzu UV-1800 spectrophotometer (Shimadzu Scientific Instruments Inc, Columbia, MD, USA). The FC assay was carried out by pipetting 200 µL of extract into a 10 mL polypropylene tube. This was followed by addition of 1 mL of FC reagent. The mixture was vortexed for 30 s, and 800 µL of filtered 20% sodium carbonate solution was added after 1 min, and before 8 min from addition of the FC reagent. This was recorded as time zero; the mixture was then vortexed for 30 s after addition of sodium carbonate. After 2 h at room temperature, the absorbance of the colored reaction product was measured at 765 nm. The content of TP in the extracts was calculated from a standard calibration curve, built with different concentrations of gallic acid, ranging from 0 to 600 µg mL^{-1} (correlation coefficient: R^2: 0.9982). Results were expressed on the basis of mg of gallic acid equivalent per kg (mg GAE kg^{-1}) of dry matter [57].

4.8. Determination of Total Antioxidant Activity by Ferric Reducing Antioxidant Power

The Ferric Reducing Antioxidant Power (FRAP) reagent was prepared fresh, so that it contained 1 mM 2,4,6-tripyridyl-2-triazine and 2 mM ferric chloride in 0.25 M sodium acetate at pH 3.6 [58]. A 100 µL aliquot of the methanol extract, prepared as above, was added to 1900 µL of FRAP reagent and throughly mixed. After leaving the mixture at 20 °C for 4 min, the absorbance at 593 nm was determined. Calibration was against a standard curve (0–1200 µg mL^{-1} ferrous ion) produced by the addition of freshly prepared ammonium ferrous sulfate. FRAP values were calculated as mg mL^{-1} ferrous ion (ferric reducing power) from three determinations, and are presented as mg of $Fe^{2+}E$ (ferrous ion equivalent) kg^{-1} dw.

4.9. Separation and Analysis of Free Phenolic Acids by HPLC

The phenolic acids were separated and quantified using a HPLC-DAD constituted of a Jasco X-LC system (Jasco Co., Tokyo, Japan), consisting of a model PU-2080 pump, a multiwavelength detector (model. MD-2015, Jasco X.LC system, Jasco Co., Tokyo, Japan), an autosampler (mod. AS-2055, Jasco

X.LC system, Jasco Co., Tokyo, Japan), and a column oven (mod. CO-2060, Jasco X.LC system, Jasco Co., Tokyo, Japan). ChromNAV Chromatography Data System software (Jasco Inc., Tokyo, Japan) was used for result analyses. The separation of phenolic acids was achieved on a Tracer Extrasil OSD2 column (5 µm, 250 × 4.6 mm), operating at 35 °C, at a flow rate of 1 mL min^{-1}. The mobile phase consisted of two solvents: 0.1% acid formic (A) and methanol (B). Gradient elution was as follows: 0–100% B over 50 min, and held at 100% B for an additional 10 min to clean up the column. Two wavelengths (310 and 325 nm) were used to detect eluent composition. HPLC analysis at 325 nm was used for quantification of chlorogenic and caffeic acids. Phenolic acids were quantified following a calibration method. Standards ranging from 0.3 to 30 mg L^{-1} were used.

4.10. Quantitative Determination of Sugars by HPLC

The liquid chromatography apparatus utilized in these analyses was a Jasco X.LC system consisting of a model PU-2080 pump, a model RI-2031 refractive index detector, a model AS-2055 autosampler, and a model CO-2060 column. ChromNAV Chromatography Data System was used as software. The separation of sugars was achieved on a Hyper-Rez XP Carbohydrate Pb^{++} analytical column (7.7 mm × 300 mm, Thermo Fisher Scientific, Waltham, MA USA), operating at 80 °C. Isocratic elution was effected using water at a flow rate of 0.6 mL min^{-1}. D-(+)-glucose, D-(−)-fructose and sucrose were quantified following a calibration method. All standards utilized in the experiments were accurately weighed, dissolved in water, and the calibration curves were generated with concentrations ranging from 100 mg L^{-1} to 1000 mg L^{-1} for the standards. The inulin determination was performed according to a reported method [59].

4.11. Quantitative Determination of Anions and Cations by Ion Cromatography (IC)

The IC was performed using an ICS-900 Ion Chromatography system (Dionex Corp., Milan, Italy) equipped with a dual piston pump, a model AS-DV autosampler, an isocratic column at room temperature, a DS5 conductivity detector, and an AMMS 300 suppressor (4 mm) for anions, and CMMS 300 suppressor (4 mm) for cations. Chromeleon 6.5 Chromatography Management Software (Dionex Corp., Milan, Italy) was used for system control and data processing. A Dionex Ion- Pac AS23 (Dionex Corp., Milan, Italy) analytical column (4 mm × 250 mm) and a guard column (4 mm × 50 mm) were used for anion separations, whereas a Dionex IonPac CS12A 23 (Dionex Corp., Milan, Italy) analytical column (4 mm × 250 mm) and a guard column (4 mm × 50 mm) were used for cation separations. The eluent consisted of 4.5 mM sodium carbonate and 0.8 mM sodium bicarbonate at a flow rate of 1 mL min^{-1} for anions and of 20 mM metansolfonic acid for cations at the same flow rate. Anions and cations were quantified following a calibration method. Dionex solutions containing seven anions at different concentrations and five cations were taken as standards, and the calibration curves were generated with concentrations ranging from 0.4 mg L^{-1} to 20 mg L^{-1}, and from 0.5 mg L^{-1} to 50 mg L^{-1} of the standards, respectively.

4.12. Statistical Analysis

Concerning agronomic parameters and marketable traits, statistical analysis was performed according to the randomized block design with three treatments (T0, T1, T2) and three replications. For the three treatments, 10 plants were considered from each plot. About qualitative traits, three representative samples from each treatment (one for each plot) were considered and analyzed in triplicate. Data were analyzed by analysis of variance (ANOVA). In the case of a significant *F*-value, the means were compared with Tukey's Honestly Significant Difference test at the significance level of $p < 0.05$. For all figures and tables, standard errors were reported.

5. Conclusions

The presented results clearly showed that the vegetative propagation methods can affect both yield and qualitative traits of artichoke. Spring offshoots nursery, that were forced to pass a vegetative

rest period by stopping irrigation (T1), was the best method under the yield point of view. T1 produced the biggest heads, the highest cumulative production, and the highest rate of extra product category. Also for quality traits, T1 provided the best outcomes in several aspects, such as the antioxidant contents. It can be affirmed that T1 is a viable and sustainable alternative to traditional agronomic practice usually adopted by farmers that does not heavily affect the costs, and helps in improving the yield and quality aspects of the product.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis Of Variance
CTPTA	Centre Technique de Pomme de Terre et d'Artichaut
FC	Folin–Ciocalteu
FRAP	ferric reducing antioxidant power
GAE	gallic acid equivalent
GIL	Inter-professional Group of Vegetables
SAM	Support Station of Manouba
T0	classic dormant buds that were removed directly from the field in august, pre-germinated for10 days before plantation
T1	nursery's dormant buds obtained from offshoots that were grown for 75 days
T2	nursery plants deriving from offshoots grown for 163 days until the summer plantation
TAC	total antioxidant capacity
TP	total phenols

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