agriculture

MDPI

Article

Development of a Simple and Low-Resource Regeneration System of Two Greek Tomato Varieties

Vaia Styliani Titeli [1], Ioannis Zafeiriou [1], Angeliki Laskaridou [1], Georgios Menexes [2], Panagiotis Madesis [3,4], Evangelia Stavridou [1,4] and Irini Nianiou-Obeidat [1,*]

[1] Laboratory of Genetics and Plant Breeding, School of Agriculture Forestry and Natural Environment, Aristotle University of Thessaloniki, P.O. Box 261, 54124 Thessaloniki, Greece; vgtiteli@gmail.com (V.S.T.); gianniszafeir93@gmail.com (I.Z.); ang.laskaridou@gmail.com (A.L.); estavrid@certh.gr (E.S.)
[2] Laboratory of Agronomy, School of Agriculture Forestry and Natural Environment, Aristotle University of Thessaloniki, P.O. Box 261, 54124 Thessaloniki, Greece; gmenexes@agro.auth.gr
[3] Laboratory of Molecular Biology of Plants, School of Agricultural Sciences, University of Thessaly, 38446 Thessaly, Greece; pmadesis@uth.gr
[4] Centre for Research and Technology Hellas, Institute of Applied Biosciences, 6th km Charilaou-Thermi Rd., Thermi, 57001 Thessaloniki, Greece
* Correspondence: nianiou@agro.auth.gr

Abstract: Native tomato landrace varieties (*Solanum lycopersicum* L.) are sources of high genetic diversity and are adaptable to local environmental conditions. The in vitro propagation system can be used as a tool to produce disease-free, high-quality propagation material and preserve the unique characteristics of commercial Greek tomato varieties, such as the variety Areti and the traditional landrace variety Makedonia, both of exceptional quality and economic importance. Twenty- and 27-day-old cotyledon and leaf explants were cultivated in 4 regeneration media supplemented with 0 or 0.1 mg·L^{-1} indole-3-acetic acid and 0.5 or 1 mg·L^{-1} zeatin. Regeneration efficiency was dependent on variety, explant type and age, and regeneration media. Areti was a more appropriate target genetic material, exhibiting a six-fold greater response to regeneration compared to Makedonia, regardless of media and explant age. The regenerated shoots of both varieties were successfully rooted (60%) and acclimatized (98%). This regeneration protocol would be valuable in the production of propagation material for commercial and conservation practices and in breeding programs for genetic improvement.

Keywords: ex situ propagation; indole-3-acetic acid; traditional tomato varieties; shoot regeneration; zeatin

Citation: Titeli, V.S.; Zafeiriou, I.; Laskaridou, A.; Menexes, G.; Madesis, P.; Stavridou, E.; Nianiou-Obeidat, I. Development of a Simple and Low-Resource Regeneration System of Two Greek Tomato Varieties. *Agriculture* **2021**, *11*, 412. https://doi.org/10.3390/agriculture11050412

Academic Editor: Rodomiro Ortiz

Received: 19 March 2021
Accepted: 29 April 2021
Published: 3 May 2021

1. Introduction

Tomato (*Solanum lycopersicum* L.) is a model system in classical, cellular, and molecular genetics for studying the molecular basis of fruit development and composition [1]. However, tomato faces environmental factors, which are constraints to yield potential [2]. Developing plants with improved genotypes and desirable traits is necessary to achieve higher yields, better fruit quality, and improvement of other morphophysiological traits. Aside from cultivated species *S. lycopersicum* L. and the wild species *S. pimpinellifolium* (L.), there are eight related wild species with large genetic variability [1]. The reference genome sequence of cultivated tomato, along with genomes of wild species *S. pimpinellifolium* (L.) and *S. pennellii* (Correll), are sources of valuable horticultural traits [3–5].

Native varieties can be sources of high genetic diversity and are used to preserve and protect important genetic resources for the development of robust and adaptive plants of high agricultural value. Native tomato and relatives grown in the Mediterranean region have been studied in terms of genetic and phenotypic diversity [3,6–8]. Seven out of 33 native Greek tomato varieties comprise 27 morphotypes [6].

Plant tissue culture has contributed to the advancement of agricultural sciences [9]. In vitro plant tissue culture especially for recalcitrant solanaceous crops, such as tomato, which have variable regeneration efficiency, has been employed for the mass propagation and conservation of tomato in conventional or molecular breeding [10]. The advantages of micropropagation are related to high multiplication capacity for the production of pathogen-free plants and the cloning of elite stock material. Several applications of in vitro methods in tomato have been developed, including virus-free mass propagation [11,12]; genetic transformation [13]; the impact of epigenetics on tissue culture [14] for introducing, through somaclonal variation, variants with desirable traits in breeding programs for crop improvement; and the ex situ conservation of genetic resources in tissue banks using in vitro slow-growth storage [15].

To optimize the micropropagation process, research has been directed to the development of efficient regeneration and acclimatization protocols and automation of the workflow [16]. The development of disease-free, robust, plant propagation material via regeneration and micropropagation is an efficient way to increase production, with reduced cost [17], for genetic transformation and micrografting purposes. Protocols of in vitro regeneration, coupled with micropropagation and grafting methods, have been developed to produce high-quality stress-resistant propagation material via tissue culture [18]. In vitro regeneration is also an essential tool in breeding programs using the gene transfer technology into elite tomato germplasm producing tolerant phenotypes without modifying the genetic background [19].

The concept behind this study was to understand the thus far unknown regeneration ability of two Greek commercial tomato varieties, which exhibit indeterminate, robust growth with exceptional qualitative traits, high yield, and adaptability to local environmental conditions. Areti is a commercial variety derived from individual selection in the F_2 segregating generation and was released in 1988 and is ideal for field and greenhouse cultivation, whereas Makedonia is a traditional variety derived from traditional genetic material and was released in 1985 and is mainly cultivated as a field crop. Both varieties are of high economic importance for the Greek vegetable market and are mainly maintained by the Greek Gene Bank (GGB) at the Institute of Plant Breeding and Genetic Resources (IPB&GR) of the Hellenic Agricultural Organization-Demeter.

Therefore, the aim of this research was to develop an efficient, simple, low-resource-input, regeneration protocol for these two varieties that could be used as a basis for the further mass production of high-quality disease-free propagation material. The development of this type of regeneration system is a prerequisite in breeding programs using genetic transformation protocols and in micrografting for the development of tolerant varieties to stress factors and improved qualitative traits.

2. Materials and Methods

Seeds were supplied by the Hellenic Agricultural Organization, DEMETER, Thessaloniki, Greece. Treatments were applied in vitro under sterile conditions in a laminar flow workstation (Microflow, MDH Limited, Andover, UK). Nutrient regeneration media and tools were sterilized at 1.2 atm and 121 °C for 20 min to avoid potential contamination. After transferring explants to regeneration media, the total duration of the experiment (from regeneration to acclimatization) was 6 weeks (Figure S1).

Seeds were surface sterilized with a mix of 2.5% NaOCl and Triton^{TM} (Merck KGaA, Darmstadt, Germany) for 10 min and placed on Murashige and Skoog (MS) basal medium [20] supplemented with 3% sucrose and 0.6% agar. Seed germination was assessed 12 days after sowing. As explants, sections of cotyledons and leaves, excised from 20- or 27-day-old seedlings, were used for both varieties (Figure 1 and Figure S2). The explants were placed in MS regeneration media (MSR) with the growth regulator combinations: MSR1 (0.1 mg·L^{-1} auxin (indole-3-acetic acid (IAA)) and 0.5 mg·L^{-1} zeatin cytokinin (Z)), MSR2 (0 mg·L^{-1} IAA, 1 mg·L^{-1} Z), MSR3 (0.1 mg·L^{-1} IAA, 1 mg·L^{-1} Z), and MSR4 (0 mg·L^{-1} IAA, 0.5 mg·L^{-1} Z).

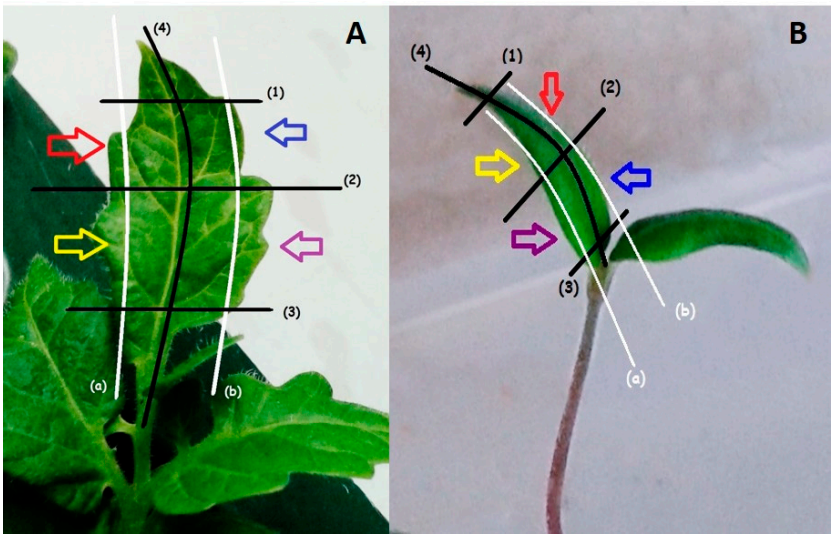

Figure 1. Schematic presentation of explants cuttings used in the regeneration experiment: (**A**) leaf and (**B**) cotyledon. Horizontal lines 1–3 represent incisions on leaves and cotyledons, while Line 4 was made across the central neuron of each explant type. Vertical Line 4 represents the vertical incision across the central nerve of the leaf and Lines A and B the vertical incisions that ultimately produced the four excised cuttings (indicated with arrows).

Regeneration procedures took place in a growth chamber with constant temperature at 23 ± 0.5 °C and a 16 h (h) light/8 h dark photoperiod under cool white fluorescent tubes (60 μmol·m^{-2}·s^{-1}), for 45 days. For each experimental module, 11 explants were used in each of the 3 replicates per treatment. After 30 days, the numbers of regenerated shoots per explant were determined.

Newly formed shoots were transferred into MS nutrient medium supplemented with 1 mg·L^{-1} IAA for further development and rooting. Rooted plantlets of about 6 cm in length were transplanted into pots containing 2:1 peat/perlite. Acclimatization was under greenhouse conditions in 2 phases: initially, during the first week, rooted plants were exposed to 90% relative humidity (RH) for reduced transpiration, and later, during the second week, plantlets were introduced progressively into controlled growing conditions, with approximately 23 ± 0.5 °C and 60–70% RH for hardening (Figure S1).

The effectiveness of regeneration media, age, and explant type was studied based on the frequency of shoot formation. The regeneration index was defined as the number of regenerated shoots of each explant per treatment [21]. The mean number and percent of rooted and acclimatized plants were determined.

Data relative to the number of regenerated shoots and number of rooted and acclimatized plantlets were analyzed with ANOVA using the general linear model [22] with the effect of variety (Areti and Makedonia), explant type (cotyledons and leaves), regeneration media (MSR1–4), and explant age (20 and 27 days). The 2 × 2 × 4 × 2 factorial experiment was arranged in a completely randomized design. For each treatment combination, there were 3 replications with 11 explants each. Prior to the ANOVA, mean comparison data were $\log_{10}(X + 1)$ transformed to achieve homoscedasticity and normality of model residuals. Mean values of treatment combinations (main effects and/or interaction effects), where appropriate, were compared with the least significant difference (LSD) [23]. All statistical analyses were performed with SPSS (version 23, IBM, New York, NY, USA).

3. Results

According to the ANOVA (Table 1), shoot regeneration was affected significantly by the variety (V) × regeneration media (M) × age of explant (A) interaction ($p = 0.018$) and the main effect of the explant type (E). All other significant responses are controlled by the main effects L, E, and A ($p < 0.001$). The ANOVA for rooting and acclimatization (Table 1)

showed that only the main effects of variety (V), type of explant (E), and age of explant (A) were significant in all hypotheses tested ($p < 0.05$). Thus, the mean values for rooting and acclimatization were compared only for the main effects.

Table 1. ANOVA results regarding sources of variation and their interactions for shoot regeneration, rooting, and acclimatization.

Source of Variation	Significance		
	Shoot Regeneration	Rooting	Acclimatization
Variety (V)	*	*	*
Type of Explant (E)	*	*	*
Age of explant (A)	*	*	*
Regeneration medium (M)	ns	ns	ns
(V) × (E)	ns	ns	ns
(V) × (A)	ns	ns	ns
(E) × (A)	ns	ns	ns
(A) × (M)	ns	ns	ns
(V) × (M)	ns	ns	ns
(E) × (M)	ns	ns	ns
(V) × (E) × (M)	ns	ns	ns
(V) × (E) × (A)	ns	ns	ns
(V) × (A) × (M)	*	ns	ns
(E) × (A) × (M)	ns	ns	ns
(V) × (E) × (A) × (M)	ns	ns	ns

ns, * nonsignificant or significant at $p < 0.05$, ANOVA.

3.1. In Vitro Regeneration

Direct organogenesis from intact cotyledons and leaves of 20- and 27-day-old explants was assessed in four regeneration media, supplemented with different concentrations in auxin and zeatin, to evaluate the regeneration efficiency. The new shoots were formed after 14 days (Figure 2A,D). The two varieties showed statistically significant differences regarding the mean number of regenerated shoots among the different explants (Tables 2 and 3). Statistically significant differences were also observed between (i) the two different types of explants with the leaves showing the highest regeneration capacity and (ii) the age of explants with the best being the 20-day-old explants (Table 3). Areti exhibited greater regeneration capacity in both explant types and age groups in all the regeneration media when compared to Makedonia (Tables 2 and 3).

Table 2. Mean value (x) of shoot regeneration in Areti and Makedonia per explant and treatment. The regenerated shoots were derived from cotyledon and leaf explants (20 and 27 days old) in 4 media (MSR1, MSR2, MSR3, and MSR4). Numerical values outside the parentheses are the mean values of raw data, and values in the parentheses are the mean values * of $\log_{10}$-transformed data. Mean values were compared with the least significant difference (LSD).

Variety	Type of Explant	Age of Explants	Regeneration Media			
			MSR1	MSR2	MSR3	MSR4
Areti	Cotyledon	20 days	4.7 (0.72)	4.0 (0.46)	11.7 (1.10)	1.3 (0.23)
		27 days	0.7 (0.16)	1.3 (0.23)	1.3 (0.30)	1.0 (0.20)
	Leaf	20 days	5.3 (0.76)	4.0 (0.69)	28.7 (1.44)	8.3 (0.92)
		27 days	6.7 (0.85)	4.7 (0.54)	4.3 (0.57)	9.3 (1.00)
Makedonia	Cotyledon	20 days	3.7 (0.62)	0.0 (0.00)	0.3 (0.10)	0.0 (0.00)
		27 days	0.0 (0.00)	0.7 (0.16)	0.3 (0.10)	0.0 (0.00)
	Leaf	20 days	4.7 (0.68)	2.3 (0.42)	3.0 (0.46)	5.0 (0.74)
		27 days	1.3 (0.30)	1.0 (0.39)	1.7 (0.36)	4.0 (0.46)

LSD 0.05 = 0.50 (for log transformed data). The mean values represent cumulative mean values of the 3 replicates.

Table 3. Mean value (x) of the regeneration, rooting of regenerated shoots, acclimatization of rooted shoots affected by the main factor variety, and the type and age of explants. Mean values followed by different letters are statistically different, at significance level a = 0.05, according to the results of the ANOVA. Numerical values outside the parentheses are the mean values of raw data and values in the parentheses indicate the mean values of log_{10}-transformed data.

Factors		Regeneration	Rooting	Acclimatization
Variety	Areti	6.1 (0.63a)	4.6 (0.58a)	4.0 (0.53a)
	Makedonia	1.8 (0.30b)	1.7 (0.29b)	1.4 (0.27b)
Type of explant	Leaf	5.9 (0.66a)	4.7 (0.61a)	3.9 (0.55a)
	Cotyledon	1.9 (0.27b)	1.6 (0.26b)	1.5 (0.25a)
Age of explants	20 days	5.4 (0.58a)	4.3 (0.54a)	3.0 (0.51a)
	27 days	2.4(0.35b)	2.0 (0.33b)	1.6 (0.29b)

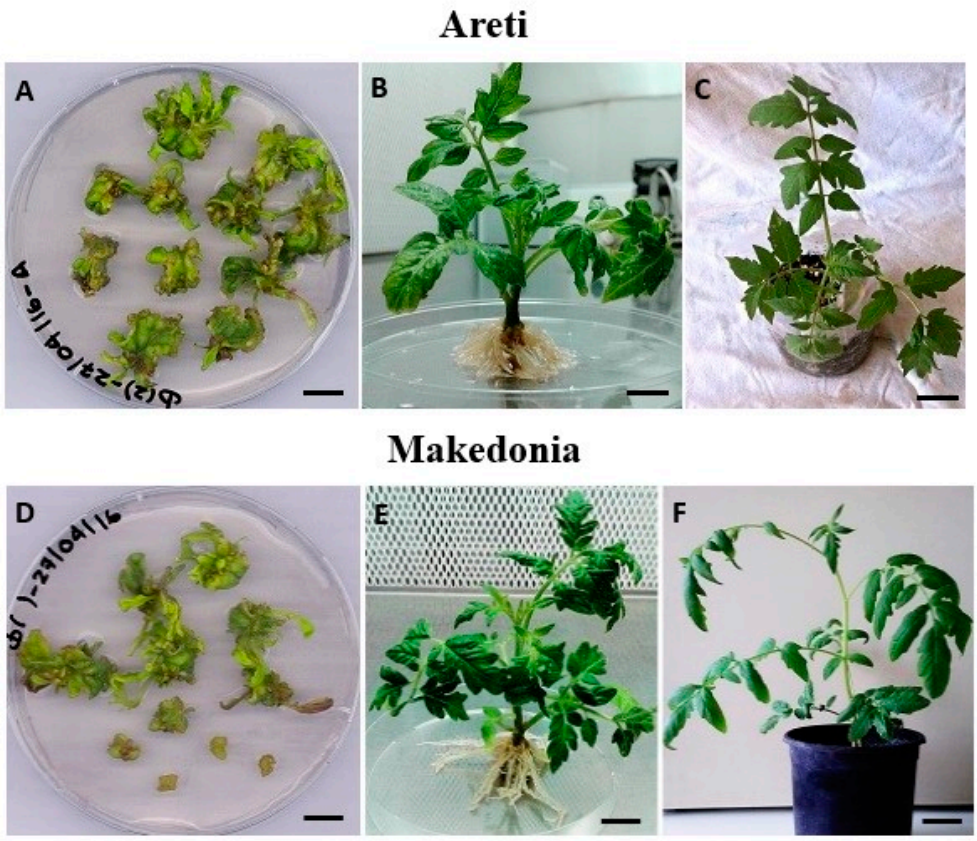

Figure 2. Regeneration, rooting, and acclimatization of var. Areti and Makedonia: (**A**,**D**) shoot regeneration of leaf explants on MSR3 and MSR1 media, respectively, bar = 2 mm; (**B**,**E**) rooting in MS supplemented with 1 mg·L^{-1} IAA, bar = 11 mm; (**C**,**F**) successfully acclimatized plants, bar = 126 mm.

More specifically, 20-day-old cotyledon explants of Areti in MSR3 were significantly different from MSR2 (mean number: 4.0) and MSR4 (mean number: 1.3) but not from MSR1 (mean number: 4.7) (Table 2). It is worth mentioning that older cotyledons (27 days old) had the lowest regeneration without any statistically significant differences among the regeneration media (Table 2). In contrast, for Makedonia, only the 20-day-old cotyledons in MSR1 induced shoot regeneration, with a mean number of 3.7 (Table 2), which was statistically different from all the other media in both the 20- and 27-day-old explants.

The best regeneration efficiency of leaf explants in Areti (20-day-old leaves in MSR3) was approximately six-fold greater compared to that of Makedonia (20-day-old leaves in MSR4) (Table 2). The highest number of regenerated shoots in Areti was observed in 20-day-old leaf explants in MSR3 (Figure 2A), with a mean number of 28.7 (Table 2), showing

statistically significant differences among the different combinations of media and age groups, except for the 27-day-old leaf explants in MSR4 (mean number: 9.3) (Table 2). Leaf explants of Makedonia of both explant ages exhibited low regeneration capacity without any significant differences in all media, although the 20-day-old leaf explants in MSR4 would be preferable for this particular landrace variety (mean and total number: 5 and 15, respectively) (Table 2).

3.2. In Vitro Rooting and Acclimatization

The majority of the regenerated shoots were successfully rooted (Figure 2B,E) and acclimatized for both varieties in the selected regeneration medium (Figure 2C,F). Comparisons between the two varieties showed that Areti outperformed Makedonia with a mean number of rooting of 4.6 against 1.7 and a mean number of acclimatization of 4.0 against 1.4 (Table 3), respectively. Between the types of explants, the highest mean value of rooting (4.7) and acclimatization (3.9) was observed in regenerated shoots derived from leaf explants with significant differences against cotyledons (mean number of rooted shoots: 1.6; mean number of acclimatized plants: 1.5) (Table 3). The analysis of the age groups showed that 20-day-old explants responded better to rooting and acclimatization with mean numbers of 4.3 and 3.0, compared to 27-day-old explants with 2.0 and 1.6, respectively (Table 3).

Overall, the best result in Areti was observed in 20-day-old leaf explants in MSR3, with 28.7 regenerated shoots (Table 2), of which approximately 60% had developed roots (Figure 2A,B). Additionally, 20-day-old cotyledons regenerated 11.7 shoots in MSR3 (Table 2), of which 66% were rooted. Regarding Makedonia, the regenerated shoots from 20-day-old leaves in MSR4 (mean number: 5.0; Table 2) developed roots in 93.3% (Figure 2D,E). Additionally, all the rooted plantlets from both varieties were successfully acclimatized despite the success in the regeneration and rooting processes (Figure 2C,F).

4. Discussion

More than 90% of Greek landraces are either extinct or threatened with extinction. The protection of agricultural biodiversity and ecosystems, along with the genetic diversity between species and within species, is important for the sustainable management of plant genetic resources. A tissue culture system was employed to promote and, in parallel, preserve the Greek tomato traditional variety Makedonia that shows good adaptability to local environmental conditions with high economic value for the local producers, and the commercial variety Areti, to assist in the production of disease-free material. A prerequisite for the preservation and mass production of traditional tomato landraces is to maintain their unique characteristics, which is possible via in vitro direct regeneration.

It is well stated that a successful in vitro regeneration system for tomato varies with the nutrient media, a combination and concentration of growth regulators and nutrients, and genotype and explant type [18,24]. In the present study, Areti was a more appropriate genetic target material by responding more effectively in the regeneration media compared to Makedonia. The best regeneration ability in Areti was observed in 20-day-old cotyledon (11.7) and leaf explants (28.7) in MSR3 (Table 2). Relatively, in Makedonia, the best results were obtained in 20-day-old cotyledon explants in MSR1 (3.7) and leaf explants in MSR1 (4.7) and MSR4 (5) (Table 2).

The regeneration ability was observed to be substantially dependent not only on the landraces but also on the different types of explants [25]. Herein, the leaf explants of both varieties and age groups performed better when compared to the cotyledons. The regeneration process depends also on the age of explants, with the young explants having a better morphogenic response compared to the older ones [26]. In our study, the highest regeneration capacity overall was indeed observed in the younger explants (20 days old) regardless of the media, explant type, and variety. Similar results were observed by Shah et al. (2015) [27], showing that the in vitro shoot regeneration frequency was significantly higher when leaf parts were used as explants.

In relation to the growth regulators, the regeneration ability is enhanced in the presence of both auxin and cytokinin [28]. High ratios of cytokinin/auxin stimulate shoot regeneration, while high ratios of auxin/cytokinin promote root regeneration [29,30]. Herein, the highest regeneration ability (~3 shoots per explant) was observed in 20-day-old leaf explants in Areti in MSR3 supplemented with 0.1 mg L^{-1} IAA and 1 mg L^{-1} Z. A similar effect was observed on cv. Micro Tom using trans-zeatin (TZ) (1 mg L^{-1}) combined with IAA (0.1 mg L^{-1}) [31] and on three Nigerian tomato landraces with 64–97% shoot regeneration from cotyledon explants in MS supplemented with 0.1 mg L^{-1} IAA and 1 mg/L Z [32]. Shoot regeneration from 7–10-day-old cotyledon explants was stimulated in MS supplemented with an even lower IAA concentration (0.05 mg L^{-1}) and 1 mg L^{-1} Z [33]. The media supplemented with a higher concentration of zeatin (MSR3) were the most suitable in both explant types in Areti. Pawar et al. (2012) [34] also observed that in MS medium supplemented with a high concentration of Z (2.0 mg L^{-1}) and 0.2 mg L^{-1} IAA, the regeneration efficiency, days to shoot initiation, and number of shoots per explant were enhanced. This is probably due to the high concentration of zeatin, which supports shoot regeneration in in vitro tomato cultures [35]. The genotype plays a determinative role in relation to the concentration of the growth regulators in the media, leading to the genotype regeneration potential, as it is observed in this study, between Areti and Makedonia.

Several factors affect the rooting process in tissue culture, such as the physiological status of plantlets, medium composition, and growth regulators. As reviewed in Gerszberg et al. (2015) [25], root formation in most cases is achieved with auxins alone with concentrations ranging from 0.1 to 1 mg L^{-1}. Herein, the rooting in MS supplemented with 1 mg L^{-1} IAA was successful for further development of the newly formed shoots of all regenerated plants from both varieties. Similar results were observed by Gupta and Van Eck (2016) [36], indicating that the addition of 1 mg L^{-1} IAA to the root induction medium resulted in earlier root development. Effective rooting was also observed on MS medium supplemented with auxins, which supports the promotive effect of auxins on root initials when compared with culturing on an auxin-free medium [10].

The development of an in vitro regeneration protocol, especially for the recalcitrant species *S. lycopersicum* L., which has variable regeneration efficiency, may offer new prospects for the production of high-quality reproductive material and contribute to further the improvement of important commercial tomato varieties [37,38]. The molecular mechanisms involved in the process of regeneration induction are indistinct and still being studied [39]. Research is lately enhanced with the acknowledgment of basic genes that take part in tissue regeneration and are involved in hormonal biosynthesis, transport, signaling, and other hormone interactions [40]. Based on the above, the in vitro system developed herein could be used as a baseline to carry further research on molecular breeding of these tomato varieties and simultaneously enable the conservation and protection of the gene pool found in the local genetic material.

Tomato is a functional food crop of high nutritional value, and thus, it is crucial to food security and quality, as well as the nutraceutical and pharmaceutical industry [41]. Conservation of agrobiodiversity and sustainable development are two interrelated disciplines focusing on environmental protection and ecosystem conservation towards social progress and economic development [42]. Tomato landraces are less sensitive to environmental factors and are an important asset of local agriculture due to quality issues and the special demands of consumers [43]. Landraces can contain valuable alleles [44] and therefore it is essential to collect, preserve, evaluate and exchange the valuable sources of genetic traits that can be implemented into breeding programs for the improvement of tomato crop [15].

5. Conclusions

Our study confirms that the genotype and the type and the age of explants significantly affect the regenerative capacity of these two varieties. Overall, Areti was a more responsive genetic material, exhibiting high regeneration capacity compared to Makedonia. Additionally, the rooting medium used in this study was effective for all regenerated

plants of both varieties. However, due to the higher regenerative capacity of the Areti, the number of rooted plants that were also acclimatized was greater when compared to that of Makedonia. This research might be useful in the genetic transformation and/or grafting for improved breeding approaches of tomato varieties. In the frame of promoting sustainable agriculture to the local agricultural communities, the results of this study are considered as an initiative towards preserving traditional varieties and, thus, the biodiversity of the local agroecosystems.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11050412/s1, Figure S1: Experimental pipeline of in vitro tissue culture (regeneration and rooting) and acclimatization of Areti and Makedonia tomato varieties. MS, IAA, and Z indicate Murashige and Skoog basal medium, indole-3-acetic acid, and zeatin, respectively. The letters d, h, and RH indicate days, hours, and relative humidity, respectively. Figure S2: In vitro seed culture and seedling development of the traditional variety Makedonia. (**A**) Ungerminated seeds placed on MS supplemented with 3% sucrose and 0.6% agar. (**B**) Emergence of cotyledons on MS approximately 10 days after sowing. (**C**) Seedling growth 20 days after sowing with both cotyledons and leaves present.

Author Contributions: Conceptualization, P.M., E.S. and I.N.-O.; data curation, V.S.T., I.Z., A.L. and E.S.; formal analysis, V.S.T. and G.M.; funding acquisition, P.M. and I.N.-O.; Investigation, V.S.T., I.Z. and A.L.; methodology, I.N.-O.; project administration, P.M. and I.N.-O.; resources, P.M. and I.N.-O.; software, V.S.T., G.M. and E.S.; supervision, P.M. and I.N.-O.; validation, E.S. and I.N.-O.; visualization, V.S.T. and E.S.; writing—original draft preparation, V.S.T. and E.S.; writing—review and editing, G.M., E.S. and I.N.-O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by private company MISSIRIAN SA, Conventional and molecular techniques in tobacco breeding (PAR. 044555).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article.

Acknowledgments: The authors would like to acknowledge the A.U.Th. Research Committee (Special Account for Research Funds) Aristotle University of Thessaloniki, Greece, for the financial support and the Hellenic Agricultural Organization—Demeter, Thessaloniki, Greece, for providing the Greek tomato variety seed.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Gebhardt, C. The historical role of species from the Solanaceae plant family in genetic research. *Theor. Appl. Genet.* **2016**, *129*, 2281–2294. [CrossRef]
2. Gerszberg, A.; Hnatuszko-Konka, K. Tomato tolerance to abiotic stress: A review of most often engineered target sequences. *Plant. Growth Regul.* **2017**, *83*, 175–198. [CrossRef]
3. Terzopoulos, P.J.; Bebeli, P.J. Phenotypic diversity in Greek tomato (*Solanum lycopersicum* L.) landraces. *Sci. Hortic.* **2010**, *126*, 138–144. [CrossRef]
4. Sato, S.; Tabata, S.; Hirakawa, H.; Asamizu, E.; Shirasawa, K.; Isobe, S.; Kaneko, T.; Nakamura, Y.; Shibata, D.; Aoki, K.; et al. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* **2012**, *485*, 635–641. [CrossRef]
5. Bolger, A.; Scossa, F.; Bolger, M.E.; Lanz, C.; Maumus, F.; Tohge, T.; Quesneville, H.; Alseekh, S.; Sørensen, I.; Lichtenstein, G.; et al. The genome of the stress-tolerant wild tomato species Solanum pennellii. *Nat. Genet.* **2014**, *46*, 1034–1038. [CrossRef]
6. Terzopoulos, P.J.; Bebeli, P.J. DNA and morphological diversity of selected Greek tomato (*Solanum lycopersicum* L.) landraces. *Sci. Hortic.* **2008**, *116*, 354–361. [CrossRef]
7. Cebolla-Cornejo, J.; Roselló, S.; Nuez, F. Phenotypic and genetic diversity of Spanish tomato landraces. *Sci. Hortic.* **2013**, *162*, 150–164. [CrossRef]
8. Corrado, G.; Caramante, M.; Piffanelli, P.; Rao, R. Genetic diversity in Italian tomato landraces: Implications for the development of a core collection. *Sci. Hortic.* **2014**, *168*, 138–144. [CrossRef]

9. García-Gonzáles, R.; Quiroz, K.; Carrasco, B.; Caligari, P. Plant tissue culture: Current status, opportunities and challenges. *Cienc. Investig. Agrar.* **2010**, *37*, 5–30. [CrossRef]
10. Vikram, G.; Madhusudhan, K.; Srikanth, K.; Laxminarasu, M.; Swamy, N.R. Zeatin induced direct multiple shoots development and plant regeneration from cotyledon explants of cultivated tomato (*Solanum lycopersicum* L.). *Aust. J. Crop. Sci.* **2012**, *6*, 31–35.
11. Koeda, S.; Takisawa, R.; Nabeshima, T.; Tanaka, Y.; Kitajima, A. Production of Tomato yellow leaf curl virus-free parthenocarpic tomato plants by leaf primordia-free shoot apical meristem culture combined with In vitro grafting. *Hortic. J.* **2015**, *84*, 327–333. [CrossRef]
12. Koeda, S.; Matsumoto, S.; Matsumoto, Y.; Takisawa, R.; Nishikawa, K.; Kataoka, K. Medium-term in vitro conservation of virus-free parthenocarpic tomato plants. *Vitr. Cell. Dev. Biol. Plant.* **2018**, *54*, 392–398. [CrossRef]
13. Fan, P.; Miller, A.M.; Schilmiller, A.L.; Liu, X.; Ofner, I.; Jones, A.D.; Zamir, D.; Last, R.L. In vitro reconstruction and analysis of evolutionary variation of the tomato acylsucrose metabolic network. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E239–E248. [CrossRef] [PubMed]
14. Us-Camas, R.; Rivera-Solís, G.; Duarte-Aké, F.; De-la-Peña, C. In vitro culture: An epigenetic challenge for plants. *Plant Cell. Tissue Organ Cult.* **2014**, *118*, 187–201. [CrossRef]
15. Kulus, D. Genetic resources and selected conservation methods of tomato. *J. Appl. Bot. Food Qual.* **2018**, *91*, 135–144. [CrossRef]
16. Loyola-Vargas, V.M.; Ochoa-Alejo, N. An introduction to plant cell culture: The future ahead. *Methods Mol. Biol.* **2012**, *877*, 1–8. [CrossRef] [PubMed]
17. Dong, O.X.; Ronald, P.C. Genetic engineering for disease resistance in plants: Recent progress and future perspectives. *Plant. Physiol.* **2019**, *180*, 26–38. [CrossRef]
18. Stavridou, E.; Tzioutziou, N.A.; Madesis, P.; Labrou, N.E.; Nianiou-Obeidat, I. Effect of different factors on regeneration and transformation efficiency of tomato (*Lycopersicum esculentum*) hybrids. *Czech. J. Genet. Plant. Breed.* **2019**, *55*, 120–127. [CrossRef]
19. Senapati, S.K. A review on research progress on in vitro regeneration and transformation of tomato. *Annu. Res. Rev. Biol.* **2016**, *9*, 1–9. [CrossRef]
20. Murashige, T.; Skoog, F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
21. Grigoriadis, I.; Nianiou-Obeidat, I.; Tsaftaris, A.S. Shoot regeneration and micrografting of micropropagated hybrid tomatoes. *J. Hortic. Sci. Biotechnol.* **2005**, *80*, 183–186. [CrossRef]
22. Calinski, T.; Steel, R.G.D.; Torrie, J.H. Principles and Procedures of Statistics: A Biometrical Approach. *Biometrics* **1981**, *37*, 859. [CrossRef]
23. Toothaker, L. Multiple Comparison Procedures. In *Paper Series on Quantitative Applications in the Social Sciences, Series No. 07-089*; Sage University: Newbury Park, CA, USA, 1993.
24. Liza, L.N.; Ashrafuzzaman, M. *In Vitro* Growth Media Effect for Regeneration of Tomato (*Lycopersicon esculentum*) and Evaluation of the Salt Tolerance Activity of Callus. *J. Agric. Sustain.* **2013**, *3*, 132–143.
25. Gerszberg, A.; Hnatuszko-Konka, K.; Kowalczyk, T.; Kononowicz, A.K. Tomato (*Solanum lycopersicum* L.) in the service of biotechnology. *Plant. Cell. Tissue Organ. Cult.* **2015**, *120*, 881–902. [CrossRef]
26. Harish, M.C.; Rajeevkumar, S.; Sathishkumar, R. Efficient in vitro callus induction and regeneration of different Tomato landraces of India. *Asian J. Biotechnol.* **2010**, *2*, 178–184. [CrossRef]
27. Shah, S.H.; Ali, S.; Jan, S.A.; Din, J.; Ali, G.M. Callus induction, in vitro shoot regeneration and hairy root formation by the assessment of various plant growth regulators in tomato (*Solanum lycopersicum* Mill.). *J. Anim. Plant. Sci.* **2015**, *25*, 528–538.
28. Schaller, G.E.; Bishopp, A.; Kieber, J.J. The yin-yang of hormones: Cytokinin and auxin interactions in plant development. *Plant. Cell* **2015**, *27*, 44–63. [CrossRef]
29. Skoog, F.; Miller, C. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symp. Soc. Exp. Biol.* **1957**, *54*, 118–130.
30. Ikeuchi, M.; Ogawa, Y.; Iwase, A.; Sugimoto, K. Plant regeneration: Cellular origins and molecular mechanisms. *Development* **2016**, *143*, 1442–1451. [CrossRef] [PubMed]
31. Khuong, T.T.H.; Crété, P.; Robaglia, C.; Caffarri, S. Optimisation of tomato Micro-tom regeneration and selection on glufosinate/Basta and dependency of gene silencing on transgene copy number. *Plant Cell Rep.* **2013**, *32*, 1441–1454. [CrossRef] [PubMed]
32. Ajenifujah-Solebo, S.; Isu, N.; Olorode, O.; Ingelbrecht, I.; Abiade, O. Tissue culture regeneration of three Nigerian cultivars of tomatoes. *Afr. J. Plant. Sci.* **2012**, *6*, 370–375. [CrossRef]
33. Kantor, M.; Sestras, R.; Chowdhury, K. Identification of the most organogenic-responsive variety of tomato using the variety × medium interaction. *Rom. Biotechnol. Lett.* **2010**, *15*, 5640–5645.
34. Pawar, B.; Jadhav, A.; Kale, A.; Chimote, V.; Pawar, S. Zeatin induced direct in vitro shoot regeneration in tomato (*Solanum lycopersicum* L.). *Bioscan* **2012**, *7*, 247–250.
35. Pino, L.E.; Lombardi-Crestana, S.; Azevedo, M.S.; Scotton, D.C.; Borgo, L.; Quecini, V.; Figueira, A.; Peres, L.E.P. The Rg1 allele as a valuable tool for genetic transformation of the tomato 'Micro-Tom' model system. *Plant Methods* **2010**, *6*, 1–11. [CrossRef]
36. Gupta, S.; Van Eck, J. Modification of plant regeneration medium decreases the time for recovery of *Solanum lycopersicum* cultivar M82 stable transgenic lines. *Plant Cell. Tissue Organ. Cult.* **2016**, *127*, 417–423. [CrossRef]

37. Tadeu De Faria, R.; Destro, D.; Filho, J.C.B.; Illg, R.D. Introgression of in vitro regeneration capability of *Lycopersicon pimpinellifolium* Mill. into recalcitrant tomato cultivars. *Euphytica* **2002**, *124*, 59–63. [CrossRef]
38. Prihatna, C.; Chen, R.; Barbetti, M.J.; Barker, S.J. Optimisation of regeneration parameters improves transformation efficiency of recalcitrant tomato. *Plant Cell. Tissue Organ. Cult.* **2019**, *137*, 473–483. [CrossRef]
39. Motte, H.; Vereecke, D.; Geelen, D.; Werbrouck, S. The molecular path to *in vitro* shoot regeneration. *Biotechnol. Adv.* **2014**, *32*, 107–121. [CrossRef]
40. Su, Y.H.; Zhang, X.S. *The Hormonal Control of Regeneration in Plants*, 1st ed.; Elsevier Inc.: Amsterdam, The Netherlands, 2014; Volume 108, ISBN 9780123914989.
41. Edesi, J.; Maria, A.; Hely, P. Modified light spectral conditions prior to cryopreservation alter growth characteristics and cryopreservation success of potato (*Solanum tuberosum* L.) shoot tips in vitro. *Plant Cell Tissue Organ. Cult.* **2017**, *128*, 409–421. [CrossRef]
42. Bianchi, F.J.J.A.; Mikos, V.; Brussaard, L.; Delbaere, B.; Pulleman, M.M. Opportunities and limitations for functional agrobiodiversity in the European context. *Environ. Sci. Policy* **2013**, *27*, 223–231. [CrossRef]
43. Al Shaye, N.; Migdadi, H.; Charbaji, A.; Alsayegh, S.; Daoud, S.; AL-Anazi, W.; Alghamdi, S. Genetic variation among Saudi tomato (*Solanum lycopersicum* L.) landraces studied using SDS-PAGE and SRAP markers. *Saudi J. Biol. Sci.* **2018**, *25*, 1007–1015. [CrossRef] [PubMed]
44. Sardaro, M.L.S.; Marmiroli, M.; Maestri, E.; Marmiroli, N. Genetic characterization of Italian tomato varieties and their traceability in tomato food products. *Food Sci. Nutr.* **2013**, *1*, 54–62. [CrossRef] [PubMed]