horticulturae

MDPI

Article

High Frequency Direct Organogenesis in Five Romanian Tomato (*Lycopersicon esculentum* Mill.) Cultivars

Adela Halmagyi, Ana Coste *, Constantin Deliu and Ioan Băcilă

NIRDBS, Institute of Biological Research Cluj-Napoca, 48 Republicii Street, 400015 Cluj-Napoca, Romania
* Correspondence: ana.coste@icbcluj.ro

Abstract: Tomato (*Lycopersicon esculentum* Mill.) as the most economically important vegetable crop worldwide has been investigated intensively for the development of new and improved varieties. Most of these technologies require efficient protocols for in vitro regeneration and propagation of plant material. In the present study, an efficient and reproducible in vitro regeneration system for five Romanian tomato genotypes (cvs. 'Capriciu', 'Darsirius', 'Kristin', 'Pontica' and 'Siriana') has been established. The tomato genotypes were selected based on their horticultural and economically valuable traits. To study the in vitro morphogenic response, various explants, such as cotyledons, cotyledonary nodes, hypocotyls, leaf explants, internodes, stem nodes and apical buds have been selected. The highest efficiency in terms of direct shoot organogenesis was obtained in cv. 'Capriciu' (98% for apical buds and 94% for stem nodes) on culture media with zeatin and indole-3-butyric acid. One advantage of this regeneration procedure is beside its feasibility in handling, the high percentage of regenerated shoots and their rooting. The present protocol contributes to the existing information regarding the response of tomato cultivars to in vitro culture conditions.

Keywords: genotypes; plant growth regulators; regeneration

Citation: Halmagyi, A.; Coste, A.; Deliu, C.; Băcilă, I. High Frequency Direct Organogenesis in Five Romanian Tomato (*Lycopersicon esculentum* Mill.) Cultivars. *Horticulturae* **2023**, *9*, 411. https://doi.org/10.3390/horticulturae9030411

Academic Editors: Orsolya Borsai, Clapa Doina, Mirela Irina Cordea, Monica Harta, Songling Bai and Zhihui Cheng

Received: 10 February 2023
Revised: 20 March 2023
Accepted: 20 March 2023
Published: 22 March 2023

1. Introduction

Plant tissue cultures are used for various purposes among them clonal multiplication, conservation, international germplasm exchange or to create improved commercial cultivars [1]. Tissue culture technology is an important requisite in breeding programs for development and selection of new cultivars with improved horticultural traits. In recent decades, genetically uniform varieties have replaced cultivars and landraces in traditional agro-ecosystems well adapted to local conditions, therefore it is important to preserve these cultivars which may be further used for selection of quality features [2].

Tomato (*Lycopersicon esculentum* Mill.), an important horticultural crop cultivated all over the world is known as a major source of essential nutrients [3]. It is one of the most extensively studied species not only for their importance as crop species but also as model system for molecular, physiological [4], genetic integrity [5,6], Raman spectroscopy [7], cryopreservation [6], ultrastructural studies [8], genetic transformation [9], and genome editing [10]. In the last decade the interest in tomato research has significantly increased especially due to its anti-cancer and anti-oxidative properties [11]. It is known that the improvement of various traits by conventional breeding requires long-lasting activity, while in vitro techniques can be a potential solution to assist breeding by manipulating desired traits.

In vitro tomato cultures have been successfully used in some biotechnological applications [12–14], for breeding purposes by somatic embryos [15] and to obtain virus-free high-value commercial cultivars [16]. In vitro regeneration of tomato shoots was induced by direct and indirect organogenesis [17–19] or through somatic embryogenesis [20]. There are many factors that affect the in vitro regeneration capacity of tomato plants, of which the most important are: the genotype, explant type, culture media composition, concentration

of plant growth regulators, intensity and quality of light, photoperiod and temperature [14]. Considering the diversity of parameters, optimization of tissue culture system key factors is essential to achieve high efficiency and reproducibility of a certain approach.

Thus, the aim of this research was to establish an efficient direct organogenesis protocol for five Romanian tomato genotypes starting from seeds.

2. Materials and Methods

2.1. Plant Material

Five tomato (*Lycopersicon esculentum* Mill., cvs. 'Capriciu', 'Darsirius', 'Kristin', 'Pontica' and 'Siriana') genotypes (Figure 1a–e) created and approved in Romania have been selected to study the morphogenic response to in vitro cultures of various explant types based on their horticultural and economically valuable traits:

- cv. 'Capriciu'—created at S.C.D.L. Buzău (Romania) and approved in 2007. Is drought tolerant and has good storage resistance. Has indetermined growth is suitable for fresh consumption or trade.
- cv. 'Darsirius'—created at S.C.D.L. Buzău and approved in 2009. Has reduced number of seeds and is resistant to specific tomato diseases and nematodes. Has determined growth, intended for processing.
- cv. 'Kristin'—created at S.C.D.L. Buzău and approved in 2006. Is resistant to transport and storage, has a determined growth and is suited for processing.
- cv. 'Pontica'—created at I.C.D.L.F. Vidra (Romania) approved in 1988 and re-homologated in 2009. Has reduced number of seeds and is considered highly productive. Has a determined growth and is suitable for processing.
- cv. 'Siriana' (F1 hybrid)—created at S.C.D.L. Buzău and approved in 2006. Is a hybrid with high adaptability to environmental conditions and resistant to transport and storage. Has indetermined growth and is suitable for fresh consumption.

The seeds have been provided by the Research and Development Institute for Vegetable and Flower Growing Vidra, Romania.

Figure 1. Tomato genotypes used for initiation of in vitro cultures starting from seeds. (**a**) cv. 'Capriciu'; (**b**) cv. 'Darsirius'; (**c**); cv. 'Kristin'; (**d**) cv. 'Pontica'; (**e**) cv. 'Siriana'. Size bars = 1.0 cm.

2.2. Seed Germination—Establishment of In Vitro Cultures

Seed health status was assessed using a stereomicroscope in order to remove empty, small or damaged seeds. To test the germination capacity, two years old mature seeds were germinated under ex vitro conditions. Seeds were first washed under tap water and transferred to Petri dishes (25 seeds/dish with 10 cm diameter) on moistened filter paper. The seeds in covered Petri dishes were randomly stored in darkness for 5 days (at 24 ± 1 °C) and then transferred (dish lid was removed) under light conditions (16 h light/8 h dark photoperiod and 36 µmol m^{-2} s^{-1} photosynthetic active radiation) at 24 ± 1 °C (Figure 2a).

For the in vitro germination tests, seed surface disinfection was carried out as follows: washing under tap water (1 h), dipping in 75% commercial Clorox (active chlorine content 5%) solution for 15 min and thorough rinsing with sterile distilled water. The seeds were then transferred for germination in 300 mL Erlenmeyer flasks (10 seeds/flask) on half-strength culture medium (1/2 MS) [21] supplemented with 20 g L^{-1} sucrose and solidified

with 7.6 g L^{-1} agar (Figure 2b). The medium pH was adjusted to 5.7 before autoclaving (20 min at 121 °C). The in vitro germination conditions were similar to those described for ex vitro germination.

Figure 2. Tomato seed germination. (**a**) ex vitro seed germination (cv. 'Kristin'); (**b**) in vitro seed germination (cv. 'Kristin'). Size bars = 1.0 cm.

2.3. Morphogenic Response of Various Explants

Based on preliminary experiments related to the efficiency of various plant growth regulators on shoot regeneration from various explants, a MS medium supplemented with 1.5 mg L^{-1} zeatin (Z) and 0.2 mg L^{-1} indole-3-butyric acid (IBA), 20 g L^{-1} sucrose and 7.6 g L^{-1} agar (pH as mentioned above) was selected for further studies (unpublished data).

To identify the highest in vitro regeneration frequency the following explants have been studied: (a) cotyledons, (b) cotyledonary nodes, (c) hypocotyls, (d) leaf explants, (e) internodes, (f) stem nodes and (g) apical buds. The explants were excised on 25th day from seedlings resulted from in vitro germinated seeds. The length of cotyledonary nodes, hypocotyls, internodes, stem nodes and apical buds was approximately 1–1.5 cm, while cotyledons and leaf explants (both excised from the central part) had approximately 1 cm^2 (Figure 3). Leaf explants and cotyledons were placed with the abaxial side towards the medium surface, hypocotyls, internodes were placed parallel to the medium surface, while cotyledonary nodes, stem nodes and apical buds were placed vertically into the solid culture medium.

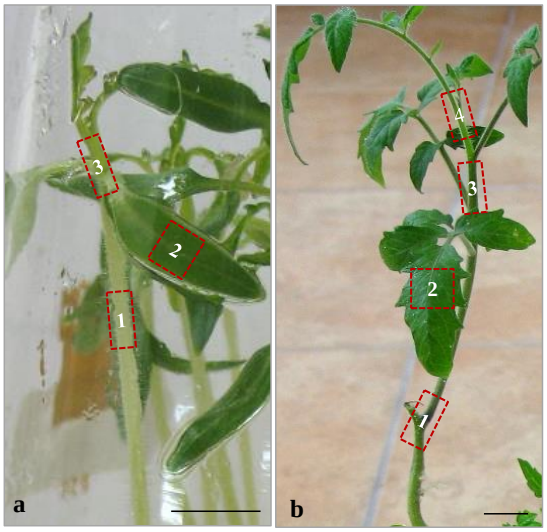

Figure 3. Explants used for in vitro regeneration experiments. (**a**) 1—hypocotyl; 2—cotyledon; 3—cotyledonary node; (**b**) 1—stem node; 2—leaf explant; 3—internode; 4—apical bud. Bars 1 cm.

2.4. Direct Shoot Organogenesis from Stem Nodes and Apical Buds

Due to the promising results obtained in shoot regeneration from stem nodes and apical buds, the effects of various cytokinins on these explants were further studied. For this purpose, seedlings (resulted from in vitro germinated seeds) were micropropagated (two successive subcultures each at 30 days) to serve as source of explants. Stem nodes and apical buds (both approximately 1–1.5 cm in length) were transferred to 100 mL Erlenmeyer flasks on MS culture medium supplemented with the following cytokinins: 1.5 mg L^{-1} thidiazuron (TDZ) (V1), 1.5 mg L^{-1} Z (V2), 1.5 mg L^{-1} kinetin (K) (V3) or 1.5 mg L^{-1} N6-benzyladenine (BA) (V4), each in combination with 0.2 mg L^{-1} IBA, 20 g L^{-1} sucrose and 7.6 g L^{-1} agar at pH 5.7. MS culture medium without PGRs was used as control (V0). For induction of shoots the samples were transferred and maintained in light and temperature conditions as mentioned for seed germination, throughout the experiments.

2.5. Assessment of Germination and Regeneration

The following characteristics have been assessed:

(a) ex vitro and in vitro germination percentage and the mean germination time;

The total germination percentage (TGP) was calculated using the following equation:

$$TGP\ (\%) = G/n \times 100$$

where G = the number of germinated seeds by the end of the experiment; n = the total number of tested seeds.

Mean germination time (MGT) was calculated using the equation:

$$MGT = \sum (n \times d)/N$$

where n = number of seeds germinated every five days for a period up to 25 days; d = number of days from the beginning of the experiment; N = total number of seeds germinated at the end of the experiment [22].

The germination experiments followed a completely randomized design, with five replications of 10 seeds each (50 seeds/cultivar). Germination was considered complete once the protruded radicle reached 1 cm in length [23].

(b) in vitro regeneration rates of various explants;
(c) in vitro shoot regeneration percentages of stem nodes and apical buds on culture media with various cytokinins;
(d) height of shoots (resulted from stem nodes and apical buds) and length of primary roots. The measurements were made using a ruler.
(e) explants (%) forming callus. Visual observations on callus morphology, color, texture were recorded and the callus diameter was measured.

Parameters (b–e) were assessed on the 45th day of culture. For each explant type and culture medium variant, 5 jars (3 explants/jar, 15 explants in total) were used in each of the three replicates per treatment. The experiments were repeated twice. The results were expressed as mean values ± standard deviation (SD).

2.6. Statistical Analyses

Data expressed as percentages were arcsin transformed prior to statistical analysis. Data were subjected to one-way analysis of variance (ANOVA) using SPSS (v.11.5 for Windows). Duncan multiple-range test was used to compare differences between means.

The Student *t*-test was applied to evaluate significant differences between ex vitro and in vitro germination of seeds, and differences in the MGT between the two procedures within a cultivar. Differences were considered statistically significant at $p \leq 0.05$. In addition to the statistical analyses the Pearson's correlation coefficient was determined between the height of shoots and the length of roots for each cultivar on the four culture medium variants using the Excel spreadsheet software (v16.0 Microsoft).

3. Results

3.1. Seed Germination—Establishment of In Vitro Cultures

Although, the TGP among cultivars varied, there were no significant differences between ex vitro and in vitro germination procedures within a cultivar (Table 1). Similarly, no significant differences were recorded in the MGT within the same cultivar between germination conditions tested (Table 1).

Table 1. Ex vitro and in vitro germination of tomato seeds, 25 days after transfer to germination substrate.

	Germination	TGP (% ± SD) *	MGT (Days)
'Capriciu'	ex vitro	91.0 ± 3.5	9.6
	in vitro	87.0 ± 1.7	11.5
'Darsirius'	ex vitro	68.0 ± 4.8	13.1
	in vitro	71.6 ± 3.5	15.4
'Kristin'	ex vitro	77.3 ± 5.5	15.3
	in vitro	78.6 ± 2.1	15.6
'Pontica'	ex vitro	91.3 ± 2.1	12.3
	in vitro	89.0 ± 2.7	14.8
'Siriana'	ex vitro	75.0 ± 3.9	17.9
	in vitro	72.6 ± 3.8	15.7

* Data represent mean values (±SD); TGP—Total germination percentage; MGT—Mean germination time; No significant differences were obtained between treatments within a cultivar (Student *t*-test, $p \leq 0.05$).

3.2. Morphogenic Response of Various Explant Types

All genotypes showed indirect shoot regeneration from hypocotyls, internodes and leaf explants. Hypocotyls and internodes displayed swollen edges with yellowish and respectively green callus formed after approximately 10–15 days (Figure 4a). Leaf explants started to form shoots after 25 days in culture (Figure 4b). In cotyledonary nodes (Figure 4c), stem nodes (Figure 4d–g), and apical buds (Figure 4h) direct shoot regeneration was recorded whereas callus was induced at the base of explants.

Figure 4. Morphogenic response of various explant types after 45 days in culture. (**a**) internodes (i) and hypocotyl (hy) (cv. 'Kristin'); (**b**) leaf explant (cv. 'Kristin'); (**c**) cotyledonary nodes (cv. 'Kristin'); (**d**) stem nodes (cv. 'Kristin'); (**e**–**g**) stem nodes (cvs. 'Capriciu', 'Darsirius' and 'Pontica'); (**h**) apical buds (cv. 'Siriana'). Explants were grown on MS with 1.5 mg L^{-1} Z + 0.2 mg L^{-1} IBA. Size bars = 1.0 cm.

After 45 days in culture the various explants showed significant differences in regeneration percentages within a cultivar (Table 2). Thus, apical buds displayed significantly

higher regeneration percentages in all cultivars compared to most of the explants tested. The cultivar with the highest regeneration rates was cv. 'Kristin' (90% apical buds and 84% stem nodes). The lowest shoot formation rates in all cultivars were obtained for cotyledons (between 8% in cv. 'Siriana' and 11% in cv. 'Capriciu') (Table 2).

Table 2. In vitro shoot regeneration of various explants after 45 days in culture.

Cultivar	Cotyledons	Cotyledonary Nodes	Hypocotyls	Leaf Explants	Internodes	Stem Nodes	Apical Buds
'Capriciu'	11.6 ± 1.6 ^{e,*}	19.6 ± 2.6 ^{d}	15.3 ± 3.5 ^{de}	36.6 ± 3.0 ^{c}	21.3 ± 2.1 ^{d}	73.0 ± 3.5 ^{b}	84.3 ± 3.1 ^{a}
'Darsirius'	9.00 ± 1.6 ^{e}	10.3 ± 1.6 ^{e}	11.6 ± 2.3 ^{e}	34.0 ± 3.0 ^{c}	20.3 ± 2.3 ^{d}	68.3 ± 3.6 ^{b}	78.3 ± 1.9 ^{a}
'Kristin'	10.3 ± 1.7 ^{c}	14.3 ± 1.4 ^{c}	16.3 ± 2.1 ^{c}	25.6 ± 3.1 ^{b}	10.6 ± 1.9 ^{c}	84.6 ± 4.1 ^{a}	90.0 ± 2.1 ^{a}
'Pontica'	9.30 ± 1.6 ^{e}	15.0 ± 1.8 ^{cde}	10.6 ± 2.2 ^{de}	21.3 ± 3.1 ^{c}	16.6 ± 1.7 ^{cd}	70.0 ± 3.8 ^{b}	82.3 ± 3.1 ^{a}
'Siriana'	8.30 ± 2.0 ^{d}	14.6 ± 1.9 ^{d}	11.0 ± 2.8 ^{d}	22.3 ± 2.9 ^{c}	8.00 ± 2.2 ^{d}	67.3 ± 4.8 ^{b}	77.3 ± 2.3 ^{a}

* Data represent mean values (% ± SD). Values followed by the same letter, within a row, are not significantly different ($p \leq 0.05$). Explants were grown on MS medium with 1.5 mg L^{-1} Z + 0.2 mg L^{-1} IBA.

3.3. Direct Shoot Organogenesis from Stem Nodes and Apical Buds

Due to the convincing positive results obtained with stem nodes and apical buds in terms of shoot regeneration, these two explant types were further used to study the effects of various cytokinins on direct shoot organogenesis. A comparison of stem nodes and apical buds grown on various culture media variants showed significant differences regarding the effects of cytokinins on the two explant types (Tables 3 and 4). For instance, in all cultivars the cytokinin that lead to the highest frequency of regenerating stem nodes and apical buds was Z, although, the differences were not always significantly different (Tables 3 and 4), (Figure 5a–j). The height of shoots and length of primary roots varied significantly according to the cytokinin used. The Pearson's correlation coefficients between height of shoot and length of roots are shown in Table 5. In stem nodes, the height of shoots had positive correlation with the length of roots in cv. 'Darsirius' (0.84), and a negative correlation in cv. 'Pontica' (−0.25). In apical buds the correlation was strong positive cv. 'Capriciu' (0.96), and cv. 'Kristin' (0.98) (Table 5). Rooting was observed in all genotypes (for both explant types) even on medium without PGRs (V0) (Figure 5a,b). In both explants a high number of lateral branches was observed in all cultivars; these roots could not be counted and their length could not be measured (Figure 5a–j). The roots were formed regardless of genotype and hormonal treatment always from the explants and not from the callus developed at explant base (Figure 6a,b,h,i). Some cultivars showed adventitious root formation in particular on stems developed from apical buds explants (Figure 6a,g,i).

A common issue observed in both explant types was spontaneous callus formation at the base of the explants (Figure 6a–j). Stem nodes showed a higher percentage of callus formation compared to apical buds (Table 6). The percentage of apical buds forming callus ranged between 9% on medium with K (V3) (cv. 'Pontica') and 35% on medium with BA (V4) (cv. 'Kristin') (Table 6). For stem nodes the highest percentage of callus induction was 40% on V4 (cv. 'Kristin'). The nature and size of callus varied widely according to cultivar and medium composition (Table 6).

Table 3. Direct shoot organogenesis from stem nodes on culture media with different cytokinins.

Cultivar	Medium	Explants Regenerating Shoots (% ± SD) *	Height of Shoots (cm ± SD)	Length of Primary Roots (cm ± SD)
'Capriciu'	V0	65.5 ± 1.4 ^{b}	8.7 ± 0.5 ^{a}	6.1 ± 0.6 ^{b}
	V1	78.8 ± 2.3 ^{b}	7.8 ± 0.7 ^{b}	6.4 ± 0.6 ^{b}
	V2	94.4 ± 1.3 ^{a}	6.2 ± 0.7 ^{c}	3.0 ± 0.7 ^{c}
	V3	73.3 ± 1.6 ^{b}	4.0 ± 0.4 ^{d}	6.2 ± 0.7 ^{b}
	V4	77.7 ± 1.7 ^{b}	7.5 ± 0.5 ^{b}	10.8 ± 0.9 ^{a}
'Darsirius'	V0	63.3 ± 1.4 ^{c}	5.7 ± 0.5 ^{b}	3.8 ± 0.5 ^{d}
	V1	71.1 ± 1.4 ^{bc}	9.3 ± 0.6 ^{a}	15.6 ± 0.7 ^{a}
	V2	82.2 ± 1.1 ^{a}	5.2 ± 0.5 ^{bc}	3.5 ± 0.4 ^{d}
	V3	72.2 ± 0.8 ^{abc}	9.1 ± 0.5 ^{a}	9.8 ± 0.5 ^{b}
	V4	76.7 ± 1.5 ^{ab}	4.5 ± 0.6 ^{c}	5.7 ± 0.8 ^{c}
'Kristin'	V0	52.2 ± 1.0 ^{c}	8.4 ± 0.7 ^{a}	6.6 ± 0.8 ^{a}
	V1	65.6 ± 1.5 ^{b}	4.8 ± 0.5 ^{c}	3.7 ± 0.3 ^{c}
	V2	84.4 ± 1.3 ^{a}	2.7 ± 0.5 ^{e}	3.0 ± 0.3 ^{d}
	V3	68.9 ± 1.8 ^{b}	5.6 ± 1.0 ^{b}	4.4 ± 0.4 ^{b}
	V4	70.0 ± 1.6 ^{b}	3.7 ± 0.2 ^{d}	2.8 ± 0.5 ^{d}
'Pontica'	V0	58.9 ± 0.8 ^{b}	4.7 ± 0.6 ^{c}	2.7 ± 0.8 ^{d}
	V1	81.1 ± 1.5 ^{a}	2.5 ± 0.5 ^{e}	18.2 ± 1.5 ^{a}
	V2	88.9 ± 2.1 ^{a}	3.8 ± 0.4 ^{d}	7.2 ± 0.5 ^{c}
	V3	62.2 ± 1.3 ^{b}	5.9 ± 0.6 ^{b}	11.4 ± 0.6 ^{b}
	V4	80.0 ± 1.6 ^{a}	8.5 ± 0.9 ^{a}	10.3 ± 0.9 ^{b}
'Siriana'	V0	56.7 ± 1.0 ^{b}	9.5 ± 0.6 ^{b}	5.5 ± 0.4 ^{a}
	V1	82.2 ± 1.6 ^{a}	6.5 ± 0.3 ^{c}	6.0 ± 0.4 ^{a}
	V2	84.4 ± 2.2 ^{a}	3.5 ± 0.6 ^{e}	2.4 ± 0.9 ^{b}
	V3	73.3 ± 1.5 ^{a}	12.5 ± 0.6 ^{a}	5.4 ± 0.7 ^{a}
	V4	74.4 ± 1.8 ^{a}	4.8 ± 0.6 ^{d}	6.1 ± 0.7 ^{a}

* Data represent mean values (±SD). Values followed by the same letter, within the same row and studied aspect, were not significantly different ($p \leq 0.05$).

Table 4. Direct shoot organogenesis from apical buds on culture media with different cytokinins.

Cultivar	Medium	Explants Regenerating Shoots (% ± SD) *	Height of Shoots (cm ± SD)	Length of Primary Roots (cm ± SD)
'Capriciu'	V0	68.8 ± 1.8 ^{b}	12.0 ± 0.4 ^{a}	7.2 ± 0.5 ^{a}
	V1	80.0 ± 1.4 ^{b}	2.6 ± 0.3 ^{bc}	5.8 ± 0.4 ^{b}
	V2	97.7 ± 0.5 ^{a}	2.4 ± 1.7 ^{bc}	5.1 ± 1.0 ^{b}
	V3	81.1 ± 0.7 ^{b}	1.5 ± 1.3 ^{c}	1.1 ± 0.9 ^{d}
	V4	80.0 ± 2.0 ^{b}	3.0 ± 0.4 ^{b}	3.2 ± 0.6 ^{c}
'Darsirius'	V0	64.4 ± 1.6 ^{c}	10.0 ± 1.3 ^{b}	6.6 ± 0.9 ^{b}
	V1	76.7 ± 1.0 ^{abc}	4.4 ± 1.2 ^{c}	5.7 ± 2.5 ^{b}
	V2	88.9 ± 2.1 ^{a}	15.7 ± 2.1 ^{a}	4.9 ± 1.2 ^{b}
	V3	74.4 ± 1.2 ^{bc}	8.8 ± 2.4 ^{b}	14.8 ± 4.1 ^{a}
	V4	78.8 ± 2.1 ^{ab}	3.9 ± 1.0 ^{c}	7.6 ± 0.9 ^{b}
'Kristin'	V0	61.1 ± 1.3 ^{c}	10.4 ± 0.9 ^{a}	6.2 ± 0.7 ^{a}
	V1	71.1 ± 1.2 ^{bc}	3.2 ± 1.0 ^{b}	2.3 ± 0.9 ^{b}
	V2	90.0 ± 1.4 ^{a}	3.1 ± 0.6 ^{bc}	2.6 ± 0.7 ^{b}
	V3	76.7 ± 1.4 ^{b}	11.1 ± 1.4 ^{a}	6.2 ± 1.5 ^{a}
	V4	75.5 ± 1.2 ^{b}	2.0 ± 0.4 ^{c}	1.9 ± 0.8 ^{b}
'Pontica'	V0	68.9 ± 2.1 ^{b}	6.3 ± 0.5 ^{a}	6.5 ± 1.2 ^{c}
	V1	84.4 ± 0.8 ^{a}	6.9 ± 0.9 ^{a}	9.5 ± 1.3 ^{a}
	V2	91.1 ± 1.2 ^{a}	5.0 ± 1.4 ^{b}	1.8 ± 0.6 ^{d}
	V3	68.9 ± 1.6 ^{b}	6.7 ± 0.6 ^{a}	7.8 ± 1.1 ^{b}
	V4	81.1 ± 1.7 ^{ab}	6.0 ± 0.9 ^{ab}	2.1 ± 0.7 ^{d}
'Siriana'	V0	62.2 ± 1.2 ^{b}	6.2 ± 0.8 ^{a}	3.9 ± 0.5 ^{b}
	V1	85.6 ± 2.3 ^{a}	4.0 ± 0.7 ^{bc}	1.1 ± 0.6 ^{c}
	V2	87.8 ± 1.7 ^{a}	4.7 ± 1.2 ^{b}	3.4 ± 0.5 ^{b}
	V3	76.7 ± 1.6 ^{ab}	3.2 ± 0.7 ^{cd}	4.9 ± 0.6 ^{a}
	V4	78.8 ± 2.2 ^{a}	2.8 ± 0.8 ^{d}	5.5 ± 0.7 ^{a}

* Data represent mean values (±SD). Values followed by the same letter, within the same row and studied aspect, were not significantly different ($p \leq 0.05$).

Figure 5. Shoot regeneration and rooting of stem nodes and apical buds on culture media with various cytokinins. (**a**) cv. 'Pontica' apex (V0); (**b**) cv. 'Pontica' nodes (V0); (**c**) cv. 'Capriciu' apex (V1); (**d**) cv. 'Capriciu' nodes (V1); (**e**) cv. 'Siriana' apex (V2); (**f**) cv. 'Siriana' nodes (V2); (**g**) cv. 'Kristin' apex (V3); (**h**) cv. 'Kristin' nodes (V3); (**i**) cv. 'Darsirius' apex (V4); (**j**) cv. 'Darsirius' nodes (V4). Arrows represent adventitious roots. Size bars = 1 cm.

Table 5. Correlation coefficients on shoots height and roots length of analyzed tomato genotypes.

Cultivar	Explants	Pearson's Coefficient
'Capriciu'	stem nodes	0.25
	apical buds	0.96
'Darsirius'	stem nodes	0.84
	apical buds	−0.55
'Kristin'	stem nodes	0.78
	apical buds	0.98
'Pontica'	stem nodes	−0.25
	apical buds	0.87
'Siriana'	stem nodes	0.50
	apical buds	−0.12

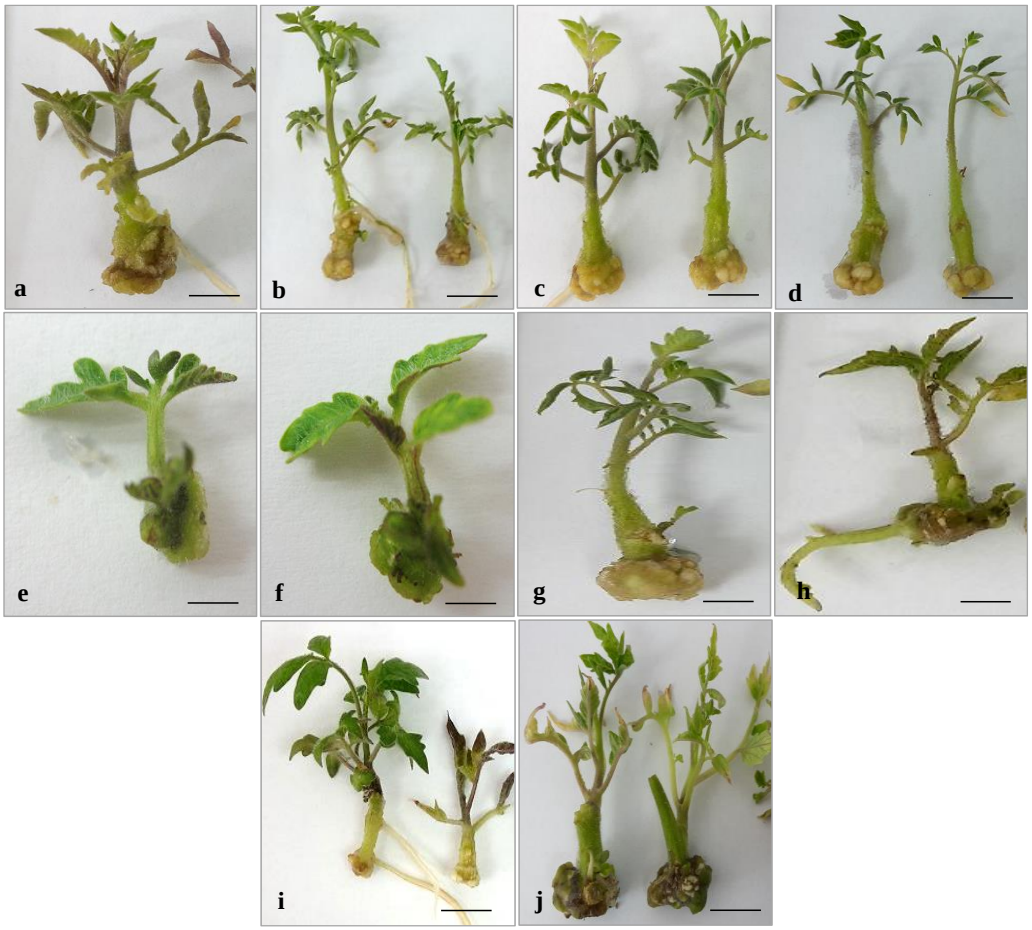

Figure 6. Callus development on culture media with different cytokinins. (**a**) cv. 'Capriciu' apical bud (V1); (**b**) cv. 'Capriciu' stem node (V1); (**c**) cv. 'Darsirius' apical bud (V2); (**d**) cv. 'Darsirius' stem node (V2); (**e**) cv. 'Kristin' apical bud (V4); (**f**) cv. 'Kristin' stem node (V4); (**g**) cv. 'Pontica' apical bud (V4); (**h**) cv. 'Pontica' stem node (V4); (**i**) cv. 'Siriana' apical bud (V3); (**j**) cv. 'Siriana' stem node (V3). Bars 1 cm.

A common issue observed in both explant types (on media with cytokinins) was spontaneous callus formation at the explant base (Figure 6a–j). No callus development was observed in none of the tested explant types on culture medium without PGRs (V0). Various cytokinins lead to significant differences regarding the percentages of explants forming callus (Table 6). In stem nodes of cv. 'Kristin' 39% of explants showed callus formation on medium with BA (V4), whereas in apical buds the percentage of explants forming callus ranged between 9% on medium with K (V3) (cv. 'Pontica') and 34% on medium with BA (V4) (cv. 'Kristin') (Table 6). The nature and size of callus varied widely according to cultivar and medium composition (Table 6). Especially on medium with TDZ (V1), calluses of cv. 'Capriciu' were nodular, extremely compact, almost lignified with a yellow-green colour (Figure 6a,b). Callus with the same appearance but less compact was present in cv. 'Siriana' on culture medium with K (V3) (Figure 6i,j). Cultivars 'Kristin' and 'Pontica' displayed friable callus with a dark-brown colour (Figure 6e–h).

Table 6. Callus formation from stem nodes and apical buds grown on culture media with different cytokinins.

Cultivar	Medium	Stem Nodes		Apical Buds	
		Callus Formation (% ± SD) *	Callus Diameter (cm ± SD)	Callus Formation (% ± SD) *	Callus Diameter (cm ± SD)
'Capriciu'	V0	0 ^{e}		0 ^{e}	
	V1	14.0 ± 2.2 ^{d}	<0.5	10.4 ± 2.10 ^{d}	<0.5
	V2	38.8 ± 2.6 ^{a}	0.5–1.0	25.4 ± 4.6 ^{b}	0.5–1.0
	V3	25.8 ± 4.9 ^{b}	0.5–1.0	21.0 ± 2.9 ^{bc}	0.5–1.0
	V4	25.6 ± 3.6 ^{b}	>1.0	19.6 ± 5.1 ^{c}	>1.0
'Darsirius'	V0	0 ^{f}		0^{f}	
	V1	22.6 ± 3.2 ^{bc}	<0.5	13.2 ± 3.3 ^{e}	<0.5
	V2	18.6 ± 4.0 ^{cd}	0.5–1.0	12.6 ± 3.0 ^{e}	0.5–1.0
	V3	18.4 ± 4.3 ^{cd}	<0.5	15.2 ± 3.3 ^{de}	<0.5
	V4	26.0 ± 3.7 ^{a}	0.5–1.0	18.6 ± 2.7 ^{cd}	0.5–1.0
'Kristin'	V0	0 ^{f}		0^{f}	
	V1	20.4 ± 3.3 ^{d}	<0.5	17.6 ± 4.3 ^{de}	<0.5
	V2	21.4 ± 3.9 ^{d}	0.5–1.0	15.6 ± 3.8 ^{e}	<0.5
	V3	31.4 ± 3.8 ^{c}	<0.5	20.6 ± 3.2 ^{d}	0.5–1.0
	V4	39.6 ± 3.4 ^{a}	>1.0	34.8 ± 4.4 ^{ab}	>1.0
'Pontica'	V0	0 ^{e}		0 ^{e}	
	V1	30.4 ± 4.3 ^{a}	<0.5	25.8 ± 3.5 ^{ab}	<0.5
	V2	30.0 ± 3.1 ^{a}	0.5–1.0	22.0 ± 4.1 ^{b}	0.5–1.0
	V3	22.0 ± 4.7 ^{b}	>1.0	9.80 ± 1.9 ^{d}	0.5–1.0
	V4	20.6 ± 5.1 ^{b}	>1.0	14.4 ± 2.1 ^{c}	>1.0
'Siriana'	V0	0 ^{f}		0 ^{f}	
	V1	17.6 ± 4.7 ^{d}	<0.5	12.8 ± 3.2 ^{e}	<0.5
	V2	29.6 ± 2.3 ^{b}	>1.0	24.6 ± 4.2 ^{c}	0.5–1.0
	V3	35.2 ± 3.5 ^{a}	0.5–1.0	24.4 ± 3.4 ^{c}	0.5–1.0
	V4	22.4 ± 3.8 ^{c}	>1.0	16.0 ± 2.1 ^{de}	>1.0

* Data represent mean values (±SD). Values followed by the same letter, within the same row were not significantly different ($p \leq 0.05$) (comparison was performed between stem nodes and apical buds within the same cultivar).

4. Discussion

Considering the economic value of the targeted Romanian cultivars that had not been previously studied, the development of an efficient regeneration protocol is essential. Therefore, in the present study, the morphogenic response (shoot, root, and callus formation) of various explants (cotyledons, cotyledonary nodes, hypocotyls, leaf explants, internodes, stem nodes and apical buds) to in vitro culture conditions was investigated. Based on the obtained results, subsequently a direct shoot organogenesis protocol from stem nodes and apical buds was established after testing the explants reaction on culture media with various cytokinins. Development of effective in vitro micropropagation protocols to obtain high-quality tomato plants could significantly reduce the market value of seedlings, which became expensive, especially for the valuable cultivars. Such plant biotechnology tools opened great opportunities for genetic engineering of tomatoes [24]. Plant regeneration from cultured tissues is genetically controlled and factors such as the age and physiological condition influences the response of explants to in vitro culture conditions [25]. It was shown that high germination rates are important to obtain homogenous sets of shoots used for the induction of tissue cultures [26]. There were no significant differences in TGP and MGT between ex vitro and in vitro germination conditions within the same cultivar. However, overall high germination percentages were registered, varying between 68–91% (cvs. 'Darsirius' and 'Capriciu') for ex vitro conditions and 71–89% (cv. 'Darsirius' and cv. 'Pontica' for in vitro germination tests. The shortest MGT was obtained in cv. 'Capriciu' for both ex vitro (9 days) and in vitro (11 days) germination procedures. It was

reported that the MGT in tomato under ex vitro conditions was 20% lower when zinc oxide nanoparticles with a certain size and concentration were used [27] or when priming treatments were applied [28].

There are a broad range of studies showing that in vitro tomato regeneration was dependent on the genotype, type of explants, age, type and concentration of plant growth regulators, and growth conditions [29–31]. For example, high proliferation efficiency was reported for hypocotyls [4,32,33], nodal explants [34], cotyledonary leaves and nodes [35,36], and petiole explants [37]. Our results confirmed that among the explant types studied, apical buds and stem nodes displayed the highest percentage of response in all cultivars. On the other hand, we obtained lower regeneration percentages, for hypocotyls (between 10% in cv. 'Pontica' and 16% cv. in 'Kristin'), internodes (between 8% cv. 'Siriana' and 21% cv. 'Capriciu'), and cotyledonary nodes (up to 19% cv. 'Capriciu'), results that confirm that the explant type influences the in vitro regeneration efficiency. A successful plant regeneration system has been reported from different tomato explant types on medium supplemented with TDZ and auxin [38]. In contrast, the medium fortified with TDZ did not improve regeneration in various explants in tomato, while Z showed the best results concerning multiple shoot induction [39].

In our study, we obtained the highest efficiency in terms of direct shoot organogenesis from apical buds (98% cv. 'Capriciu') on culture media with Z and IBA. Some authors reported 80% direct shoot regeneration from tomato shoot tips grown on MS medium with Z and IAA [40]. Other studies showed high shoot regeneration of apical buds on media supplemented with K and BA [41]. It was underlined that TDZ inhibits root formation in in vitro grown tomato [36]. In the studied cultivars rooting occurred for stem nodes and apical buds on media containing TDZ and IBA. Some authors reported high number of roots per shoot on culture media without PGRs [42]. Similarly, we obtained rooting in all genotypes (for both explant types) even on medium without PGRs. Some cultivars showed adventitious root formation mainly on stems developed from apical buds. It was shown that IBA, the most common exogenously applied plant growth regulator, has a greater ability to promote adventitious root formation than IAA [43].

Indirect organogenesis via callus in tomato was reported [30]. Callus induction for various purposes from different explants, such as internodes [44], cotyledon explants [45], and hypocotyls [46] was studied. In the present study, callus formation at the explants base was a spontaneous effect of the in vitro culture (on media with a cytokinin and IBA), which did not influence direct shoot organogenesis. It was shown that callus formed at the base of nodal explants did not suppress regeneration of shoots and had no further consequences on growth of tomato plants [17]. Nevertheless, direct organogenesis from explants is the best option for multiplication as it leads to the generation of true-to-type plants [47]. To preserve all the traits of the cultivars genetic uniformity is of paramount importance especially for micropropagation. However, further studies are necessary to assess if morphological changes that could arise following ex vitro acclimatization of seedlings till ripening.

5. Conclusions

The present study describes a direct organogenesis protocol from stem nodes and apical buds in five Romanian tomato cultivars. It can be concluded that the response of different tomato genotypes and explants on various culture media was different in terms of organogenesis. Optimal direct shoot bud induction was obtained in cv. 'Capriciu' using a solid MS medium supplemented with 1.5 mg L^{-1} Z and 0.2 mg L^{-1} IBA for both apical buds and stem nodes. Establishment of highly efficient regeneration protocol for these commercially valuable cultivars is definitely justified. Based on the promising results in all mentioned parameters we anticipate that these five Romanian tomato genotypes will represent new resources for improved breeding varieties and approaches and research towards preserving local cultivars.

Author Contributions: Conceptualization: A.H., A.C., C.D. and I.B.; Methodology: A.C., A.H. and C.D.; Validation: A.H. and A.C.; Formal analysis: A.C., A.H. and I.B.; Investigation: A.H., A.C., C.D. and I.B.; Data curation: A.H. and A.C.; Original draft preparation: A.H. and A.C.; Writing—review and editing: A.H., A.C., C.D. and I.B. Funding acquisition: A.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by partial funding from the Ministry of Research, Innovation and Digitization through the Core Project BIORESGREEN, subproject BioClimpact no. 7/30.12.2022, code 23020401 and Projects for Excellence Financing in RDI, project 2PFE/2021.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to Gabriela Șovărel (Research and Development Institute for Vegetable and Flower Growing Vidra, Romania) for providing the tomato seeds.

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

References

1. Larkin, P. Somaclonal variation: Origin and causes. In *Encyclopedia of Plant and Crop Science*; Goodman, R.M., Ed.; Marcel Dekker: New York, NY, USA, 2004; pp. 1158–1161.
2. Rao, K.V.; Kiranmayee, K.; Pavan, U.; Sree, T.J.; Rao, A.V.; Sadanandam, A. Induction of multiple shoots from leaf segments, in vitro-flowering and fruiting of a dwarf tomato (*Lycopersicon esculentum*). *J. Plant Physiol.* **2005**, *162*, 959–962. [CrossRef]
3. Story, E.N.; Kopec, R.E.; Schwartz, S.J.; Harris, G.K. An update on the health effects of tomato lycopene. *Ann. Rev. Food Sci. Technol.* **2010**, *1*, 189–210. [CrossRef] [PubMed]
4. Gubiš, J.; Lajchová, Z.; Faragó, J.; Jureková, Z. Effect of growth regulators on shoot induction and plant regeneration in tomato (*Lycopersicon esculentum* Mill.). *Biologia* **2004**, *59*, 405–408.
5. Coste, A.; Suteu, D.; Bacila, I.; Deliu, C.; Valimareanu, S.; Halmagyi, A. Genetic stability monitoring in micropropagated tomato cultivars. *Olten. Stud. Comun. Stiintele Nat.* **2014**, *30*, 38–43.
6. Coste, A.; Şuteu, D.; Băcilă, I.; Deliu, C.; Vălimăreanu, S.; Halmagyi, A. Genetic integrity assessment of cryopreserved tomato (*Lycopersicon esculentum* Mill.) genotypes. *Turk. J. Biol.* **2015**, *39*, 638–648. [CrossRef]
7. Muntean, C.M.; Leopold, N.; Tripon, C.; Coste, A.; Halmagyi, A. Surface-enhanced raman spectroscopy of genomic DNA from in vitro grown tomato (*Lycopersicon esculentum* Mill.) cultivars before and after plant cryopreservation. *Spectrochim. Acta Part A Mol. Biomol. Spect.* **2015**, *144*, 107–114. [CrossRef] [PubMed]
8. Halmagyi, A.; Coste, A.; Tripon, S.; Craciun, C. Low temperature induced alterations in tomato (*Lycopersicon esculentum* Mill.) shoot apex cells. *Sci. Hortic.* **2017**, *222*, 22–31. [CrossRef]
9. Di Matteo, A.; Rigano, M.M.; Sacco, A.; Frusciante, L.; Barone, A. Genetic transformation in tomato: Novel tools to improve fruit quality and pharmaceutical production. In *Genetic Transformation*; Alvarez, M., Ed.; InTech Europe: London, UK, 2011; pp. 55–80.
10. Xia, X.; Cheng, X.; Li, R.; Yao, J.; Li, Z.; Cheng, Y. Advances in application of genome editing in tomato and recent development of genome editing technology. *Theor. Appl. Gen.* **2021**, *134*, 2727–2747. [CrossRef]
11. Ali, M.Y.; Sina, A.A.I.; Khandker, S.S.; Neesa, L.; Tanvir, E.M.; Kabir, A.; Khalil, M.I.; Gan, S.H. Nutritional composition and bioactive compounds in tomatoes and their impact on human health and disease: A review. *Foods* **2021**, *10*, 45. [CrossRef]
12. Raza, M.A.; Nawaz, A.; Ali, M.; Zaynab, M.; Muntha, S.T.; Zaidi, S.H.R.; Khan, A.R.; Zheng, X.L. In-vitro regeneration and development for the conservation and propagation of tomato plant (*Solanum lycopersicum*) and currant tomato (*S. pimpinellifolium*) from two different explants. *Appl. Ecol. Environ. Res.* **2020**, *18*, 879–888. [CrossRef]
13. Magdoleen, G.; Osman, M.G.; Mutasim, M.; Khalafalla, M.M. Promotion of in vitro shoot formation from shoot tip of tomato (*Lycopersicon esculentum* Mill. cv. Omdurman) by ethylene inhibitors. *Int. J. Curr. Res.* **2010**, *4*, 82–86.
14. Bhatia, P.; Ashwath, N.; Senaratna, T.; Midmore, D. Tissue culture studies of tomato (*Lycopersicon esculentum*). *Plant Cell Tissue Organ Cult.* **2004**, *78*, 1–21. [CrossRef]
15. Saeed, W.; Naseem, S.; Gohar, D.; Ali, Z. Efficient and reproducible somatic embryogenesis and micropropagation in tomato via novel structures—Rhizoid tubers. *PLoS ONE* **2019**, *14*, e0215929. [CrossRef] [PubMed]
16. 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]
17. Rashid, R.; Bal, S.S. Effect of hormones on direct shoot regeneration in hypocotyl explants of tomato. *Not. Sci. Biol.* **2010**, *2*, 70–73. [CrossRef]
18. Mohamed, A.N.; Ismail, M.R.; Rahman, M.H. In vitro response from cotyledon and hypocotyls explants in tomato by inducing 6-benzylaminopurine. *Afr. J. Biotech.* **2010**, *9*, 4802–4807.

19. Chaudhary, Z.; Afroz, A.; Rashid, H. Effect of variety and plant growth regulators on callus proliferation and regeneration response of three tomato cultivars (*Lycopersicon esculentum*). *Pak. J. Bot.* **2007**, *39*, 857–869.
20. Godishala, V.; Mangamoori, L.; Nanna, R. Plant regeneration via somatic embryogenesis in cultivated tomato (*Solanum lycopersicum* L.). *J. Cell Tissue Res.* **2011**, *11*, 2521–2528.
21. Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
22. Ellis, R.H.; Roberts, E.H. The quantification of ageing and survival in orthodox seeds. *Seed Sci. Technol.* **1981**, *9*, 373–409.
23. International Seed Testing Association. *Rules Proposal for the International Rules for Seed Testing*; International Seed Testing Association (ISTA): Zurich, Switzerland, 2016.
24. 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]
25. Delporte, F.; Pretova, A.; Du Jardin, P.; Watillon, B. Morpho-histology and genotype dependence of in vitro morphogenesis in mature embryo cultures of wheat. *Protoplasma* **2014**, *251*, 1455–1470. [CrossRef] [PubMed]
26. Ishag, S.; Osman, M.G.; Khalafalla, M.M. Effects of growth regulators, explant and genotype on shoot regeneration in tomato (*Lycopersicon esculentum* cv. Omdurman). *Int. J. Sustain. Crop Prod.* **2009**, *4*, 7–13.
27. Włodarczyk, K.; Smolińska, B. New method of tomato fertilization with the use of chosen nanoparticles. *Chem. Proc.* **2022**, *10*, 92. [CrossRef]
28. Delian, E.; Lupu, C.; Săvulescu, E. Effect of different priming treatments on seeds germination and early seedlings growth of tomato. *Curr. Trends Nat. Sci.* **2018**, *7*, 38–46.
29. Mamidala, P.; Nanna, R.S. Effect of genotype, explant source and medium on in vitro regeneration of tomato. *Int. J. Gen. Mol. Biol.* **2011**, *3*, 5–50.
30. Gerszberg, A.; Hnatuszko-Konka, K.; Kowalczyk, T.; Kononowicz, A.K. Efficient in vitro callus induction and plant regeneration protocol for different polish tomato cultivars. *Not. Bot. Horti Agrobot.* **2016**, *44*, 452–458. [CrossRef]
31. 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. [CrossRef]
32. Gubiš, J.; Lajchová, Z.; Faragó, J.; Jureková, Z. Effect of genotype and explant type on shoot regeneration in tomato (*Lycopersicon esculentum* Mill.) in vitro. *Czech J. Genet. Plant Breed.* **2003**, *39*, 9–14. [CrossRef]
33. Hanur, V.S.; Krishnareddy, B. In vitro organogenesis in tomato (*Solanum lycopersicum*) using kinetin. *Adv. Plants Agric. Res.* **2016**, *4*, 397–401. [CrossRef]
34. Soressi, G.P.; Cammareri, G.; Picarella, M.E. Improvement of in vitro vegetative propagation technique in tomato (*Solanum lycopersicum*). *Acta Hortic.* **2009**, *812*, 283–288. [CrossRef]
35. Alatar, A.A.; Faisal, M.; Abdel-Salam, E.M.; Canto, T.; Saquib, Q.; Javed, S.B.; El-Sheikh, M.A.; Al-Khedhairy, A.A. Efficient and reproducible in vitro regeneration of *Solanum lycopersicum* and assessment genetic uniformity using flow cytometry and SPAR methods. *Saudi J. Biol. Sci.* **2017**, *24*, 1430–1436. [CrossRef]
36. Ashakiran, K.; Sivankalyani, V.; Jayanthi, M.; Govindasamy, V.; Girija, S. Genotype specific shoots regeneration from different explants of tomato (*Solanum lycopersicum* L.) using TDZ. *Asian J. Plant Sci. Res.* **2011**, *1*, 107–113.
37. Sharma, C.; Srivastava, D.K. In vitro plant regeneration from seedlings-derived explants of tomato (*Lycopersicon esculentum* Mill.). *Int. J. Agric. Environ. Biotechnol.* **2014**, *7*, 421–432. [CrossRef]
38. Vinoth, R.; Kumaravel, S.; Ranganathan, R. Anatomical and physiological adaptation of mangrove wetlands in east coast of Tamil Nadu. *World Sci. News* **2019**, *129*, 161–179.
39. Sandhya, D.; Jogam, P.; Venkatapuram, A.P.; Savitikadi, P.; Peddaboina, V.; Allini, V.R.; Abbagani, S. Highly efficient *Agrobacterium*-mediated transformation and plant regeneration system for genome engineering in tomato. *Saudi J. Biol. Sci.* **2022**, *29*, 103292. [CrossRef]
40. Jabeen, N.; Chaudhry, Z.; Rashid, H.; Mirza, B. Effect of genotype and explant type on in vitro shoot regeneration of tomato (*Lycopersicon esculentum* Mill.). *Pak. J. Bot.* **2005**, *37*, 899–903.
41. Jamous, F.; Abu-Qaoud, H. In vitro regeneration of tomato (*Lycopersicon esculentum* Mill). *Plant Cell Biotech. Mol. Biol.* **2015**, *16*, 181–190.
42. Baye, E.; Matewos, T.; Belew, D. Optimization of in vitro rooting protocol for tomato (*Lycopersicon esculentum* Mill.) varieties. *J. App. Nat. Sci.* **2020**, *12*, 365–371. [CrossRef]
43. De Klerk, G.J.; Van der Krieken, W.; De Jong, J.C. The formation of adventitious roots: New concepts, new possibilities. *In Vitro Cell Dev. Biol. Plant* **1999**, *35*, 189–199. [CrossRef]
44. Karim, M.A.; Kayuml, M.A. In vitro regeneration of tomato plant from leaf and internode segments. *J. Bangladesh Agric. Univ.* **2007**, *5*, 213–216. [CrossRef]
45. Arulananthu, G.; Bhat, S.G.; Ramesh, N. Callus induction and in-vitro regeneration of tomato (*Lycopersicon esculentum* Mill.). *Res. J. Life Sci. Bioinform. Pharm. Chem. Sci.* **2019**, *5*, 491.

46. Manawadu, I.P.; Nilanthi, D.; Senanayake, S.G.J.N. Callus formation and organogenesis of tomato (*Lycopersicon esculentum* Mill variety Thilina). *Trop. Agric. Res. Ext.* **2014**, *17*, 86–94. [CrossRef]
47. Verma, V.; Zinta, G.; Kanwar, K. Optimization of efficient direct organogenesis protocol for *Punica granatum* L. cv. Kandhari Kabuli from mature leaf explants. *In Vitro Cell. Dev. Biol. Plant* **2021**, *57*, 48–59. [CrossRef]