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Salt Stress and Tomato Resilience: From Somatic to Intergenerational Priming Memory

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Abstract: To ameliorate plants' response to environmental stresses, seed priming can be a useful tool; it consists of the pre-exposure of the seeds to mild stress, which improves plant adaptation to future exposure to adverse growth conditions. In our previous studies, seed priming with polyamines (2.5 mM putrescine, 2.5 mM spermine, and 2.5 mM spermidine) and salt acclimation have been proven to be an effective treatment in enhancing salt tolerance of tomato cultivars since they induced a better physiological response to salt stressful condition. The persistence of the memory of the first (priming) stress and retrieval of such remembered information upon exposure to later new stress play an important role in the applicability of seed priming in agriculture. Therefore, the aim of this work was the detection of the persistence of a stress memory induced by polyamine priming in tomatoes. Primed and not-primed seeds were stored at +4 °C for 2 years after the original priming treatment; then, germinated seeds were sown in non-saline soil and irrigated with 80 and 160 mM NaCl salt solutions until fruit production. The results confirm the increase in salt tolerance in primed plants compared to not-primed ones, indicating the presence of long-term somatic memory. In comparison with not primed, the primed plants produced better quality fruits, i.e., higher weight, water content, and higher amount of carotenoids, soluble sugars, and phenols. To determine if the memory can be inherited by the offspring, seeds were then collected from primed and not-primed plants (generation G1), and further experiments were undertaken by growing G1 plants under the same irrigation regime as the parental generation. After 45 days of growth, both antioxidants and osmolyte amounts were enhanced, leading to an improvement in the tolerance to saline conditions in the offspring of primed plants and confirming the results already observed in the parental generation. These results demonstrate, for the first time, the presence of both long-term somatic and intergenerational priming memory in tomatoes and may pave the pathway to future agricultural application of seed priming.

Keywords: tomato fruits; salt stress; seed priming; somatic memory; intergenerational priming memory

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1. Introduction

Plants respond to environmental stresses by activating different responses that allow the plants to cope with stressful conditions [1,2]; activation of stress responses involves changes in DNA methylation, acetylation and deacetylation of histone tails, chromatin remodeling, alternative splicing of transcripts, and accumulation of metabolites. Some

changes are retained and may be transferred to the offspring, thus maintaining partial protection against a possible following stress [3]. Heritability of chromatin modifications, through mitosis and meiosis, represents a useful mechanism for the long-term storage of information on environmental events during the life of an individual (somatic memory) and across generations (inter- and transgenerational memory) [4]. The ability to transfer and reactivate defense responses to stressful conditions, such as salinity, is of enormous importance for plant survival when they are re-exposed to the same environmental stressor [1,2], thus providing a better capacity to cope with stress.

Seed priming protocol foresees a pre-sowing seed dipping in a priming agent solution selected by preliminary screening, which allows controlled hydration of seeds to imbibe water, but it does not allow radicle protrusion through the seed coat [3]. Such treatment hastens the germination process and consequently the rate of seedling emergence even under extreme environmental conditions, i.e., drought, high temperature, salinity, etc. Seed priming provides extensive crop benefits, and it is divided into different types depending on the priming agent used [4]. The area of research on seed priming is particularly fascinating because of the possible wide application in the development of environmentally friendly strategies for the improvement of crop yield and plant fitness in stressful conditions [5].

The success of seed priming in enhancing plant stress tolerance is based on the persistence of the stress memory against different adverse conditions (such as salinity or drought), which leads to efficient stress response when primed seedlings are again exposed to a stressor [6]; several molecular mechanisms seem to be involved in the development of stress-priming memory, but the mechanisms underlying this phenomenon in crops are not fully understood due to the involvement of many pathways [7]. So far, it is known that the responses of stress-related genes require the activity of transcription factors and post-transcriptional regulation, but also alternative splicing, RNA silencing [2], DNA methylation, and chromatin modifications have been identified in stress memory [1,8], in particular, plant exposure to saline conditions can modify chromatin at the genomic level and in specific loci [7–9]; the transmission of these modifications through mitosis and growth is considered stable. Thus, these priming-induced changes could survive the physiological turnover and be transmitted to the next generation or maintained by the same individual [6,9].

The existence of significant epigenetic changes in salt stress somatic memory has been demonstrated in *Arabidopsis* and soybean; these variations were detected in the expression of genes involved in acetylation, deacetylation, and methylation of histones [10–12]. Important genes responsible for the induction of somatic memory are the trans-membrane receptor-like kinases (RLKs) that recognize priming agents [13] and the somatic transcriptional memory-associated Repressor of Silencing-1 (AtROS1), whose role is the demethylation of the promoter of genes involved in the biosynthesis of flavonoids [14]. Further evidence suggests that epigenetic alterations are regulated by the activity of myeloblastosis (MYB) family transcription factors and microRNAs [10]. All these data provide the basis for the development of research on long-term somatic memory in plants [1].

Besides the identification of somatic memory, which is maintained through mitosis in the same generation, an inter- and transgenerational memory has been reported [2,11]. The latter is long-term and involves the transfer of memory to following generations, called offspring or generations 1, 2, 3 (G1, G2, G3), etc. (Figure 1) [2]. The effects of inter- and transgenerational priming memory on crops are still poorly understood, with the few available data coming from studies on *Arabidopsis*, common bean, rapeseed, wheat, and rice [2,15–17].

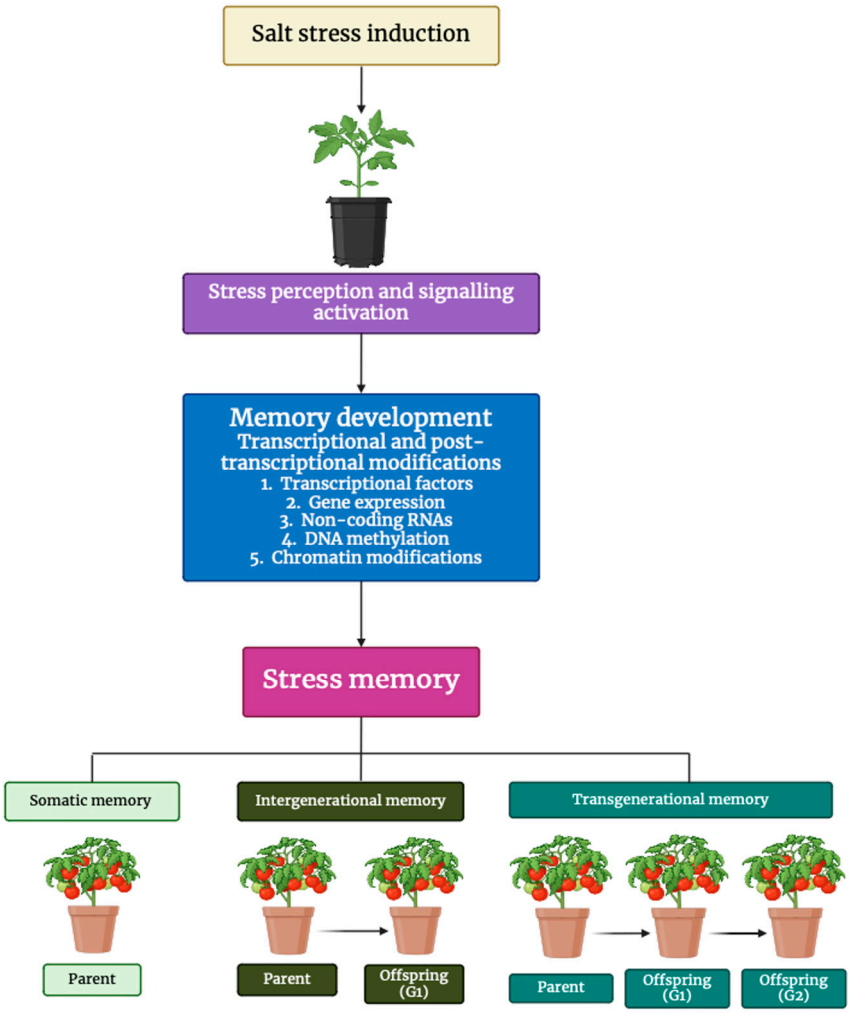

Figure 1. Schematic representation of stress memory development in plant. Created in Biorender.com.

As reported by Suter and colleagues [17], salt stress exposure over multiple generations can lead to phenotypic changes in subsequent generations of *A. thaliana*; however, these effects would be determined by both the plant genotype and the number of generations exposed to the stress. Nevertheless, the results of this study support the hypothesis that epigenetic modifications caused by exposure to stress over multiple generations can induce transgenerational phenotypic alterations [17].

The understanding of stress memory persistence and its regulation in response to stress can be a breakthrough with an enormous potential for agriculture, crop improvement, and environmental sustainability. In our previous studies, seed priming with polyamines (putrescine, spermine, and spermidine) and salt acclimation has been proven to be an efficient treatment in tomato cultivars, sensitive to salt [18]. Due to the importance of this crop for human diet and health and its elevated sensitivity to salt, study of the persistence of stress memory over time and its possible transfer to the next generation would represent one further step to foreseeing the performance of this crop in the field and in the preservation of the seeds. Therefore, the aim of this study was to determine the persistence of priming memory in tomatoes at somatic and intergenerational levels.

2. Materials and Methods

The reagents were analytical grade or equivalent and purchased from Merck (Burlington, MA, USA). In each set of experiments, all working solutions were prepared immediately before the use of stock reagents.

2.1. Persistence of Priming Treatment During the Storage and Seed Germination

Due to the economic importance of tomatoes, a greenhouse trial was performed using a salt-sensitive cherry tomato that is very common and marketed in Italy. Seeds of *Solanum lycopersicum* L., cv. Principe Borghese, were bought from Blumen Group S.p.A, Piacenza, Italy. The original seed priming treatment was performed, as reported by Borromeo et al. [18]. Briefly, tomato seeds were primed in 15 mL of the following solutions: 2.5 mM putrescine (PUT), 2.5 mM spermine (SPM), and 2.5 mM spermidine (SPD) for 24 h at room temperature (RT); then, the seeds were dried for 48 h at RT. To verify the maintenance of priming memory over time, primed and not-primed tomato seeds were stored in the dark at +4 °C for up to 2 years. After this storage period, the viability of the seeds was evaluated, and the germination rates (%) were recorded after 7 days of incubation in the dark at RT [19,20].

2.2. Growth Conditions, Soil, and Fruits Analyses of Primed and Not-Primed Plants

Two years after priming, germinated seeds, either primed or not primed, were sown in non-saline soil (COMPO SANA® COMPACT, Münster, Germany). Soil characteristics were as follows: pH 6.5; dry bulk density 150 kg/m^3; electrical conductivity 0.50 dS/m; porosity 90% *v*/*v*. Soil components were as follows: neutral sphagnum peat, perlite (<5%), and composted green soil improver [21].

The greenhouse experiment, carried out at the University of Rome Tor Vergata, lasted 6 months (from 15 January 2024 to 2 July 2024). All seedlings were grown for 14 days before beginning salt stress; then, the plants were divided into 4 experimental sets: (1) not-primed plants irrigated with tap water, (2) not-primed plants irrigated with saline solutions, (3) primed plants irrigated with tap water and (4) primed plants irrigated with saline solutions. Salt concentrations of the solutions were selected based on our previous data [18].

The irrigation regime was modified according to the growth phase of the plants: (1) during the first 2 weeks of growth, all plants (primed and not primed) were watered with 80 mL of tap water every 72 h, (2) from the 3rd to 8th week of growth, the plants were watered with 80 mL of tap water or salt solution every 72 h, (3) from the 9th week of growth until the end of experiment, plants were watered with 50 mL of tap water or salt solution every 24 h. The electrical conductivity (EC) values of the irrigation water were, respectively, as follows: 0.6 dS/m (0 mM NaCl, non-saline), 8.8 dS/m (80 mM NaCl, saline), and 16.2 dS/m (160 mM NaCl, high saline). Light intensity and temperature were measured daily using a multi-parameter sensor (FlowerCare-HHCCJCY01HHCC-HHCC Plant Technology Co., Ltd., Stuttgart, Germany) (Figures 2 and 3). To verify the increase in soil salinity, at the end of the experiment, the EC of the soil was evaluated [20] using an EC meter (HANNA Instrument 98312 DiST®5 and DiST®6, Padova, Italy).

Observations were performed after 45 days (before anthesis) and then after 60, 90, and 120 days of growth. Growth was evaluated by considering the following parameters: shoot length and flower development. In addition, fruit production and ripening were determined according to the following: (1) fruit developmental stage; (2) days to ripening, evaluated considering the pigmentation of the epicarp (from yellow to completely red) and the softness of the fruit; and (3) harvest of ripe fruit.

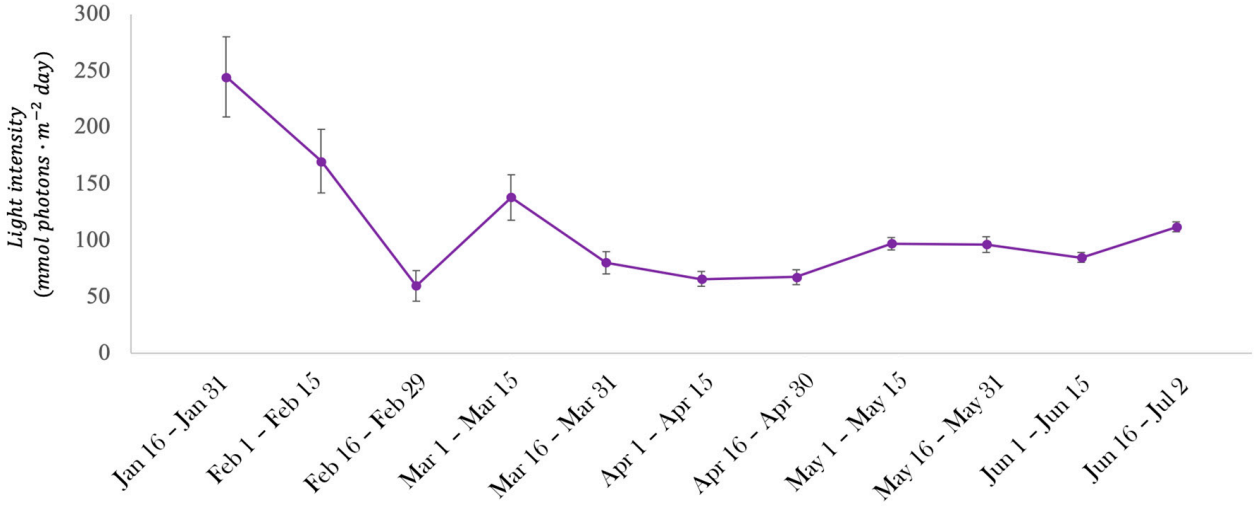

Figure 2. Trend of light intensity, expressed as mmol photons m^{-2} day, during the experimental period. Data are expressed as mean ± SE. The average values correspond to a time of 15 days ± 1 day.

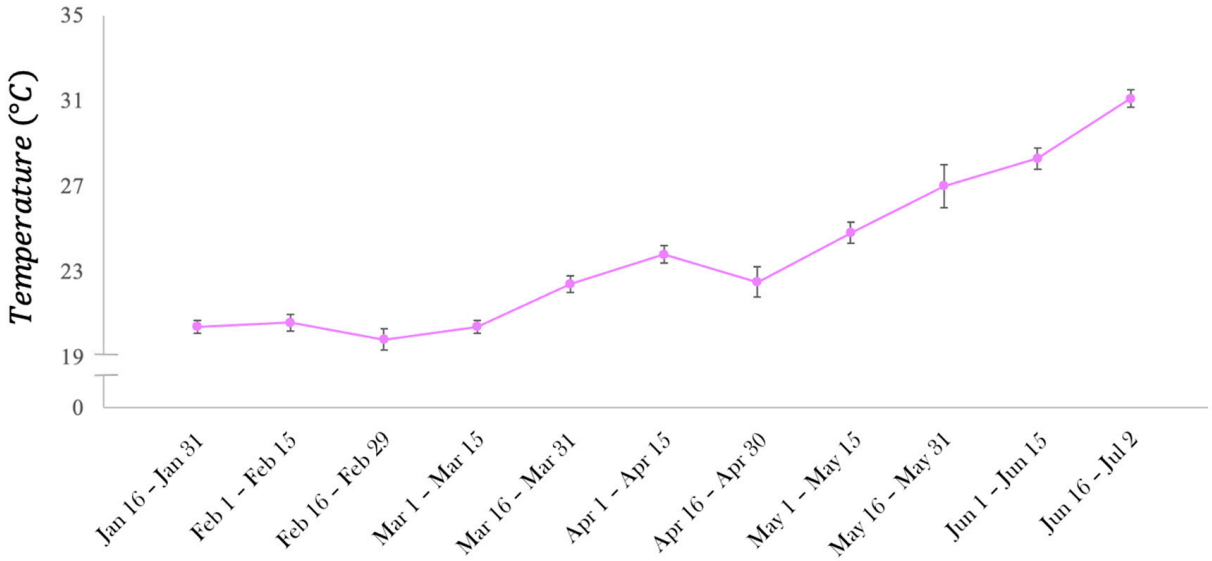

Figure 3. Trend of temperature, expressed as °C, during the experiment. Data are expressed as mean ± SE. The average values correspond to a time of 15 days ± 1 day.

Fruit evaluation was based on the weight and area of the fruit, number of seeds per fruit, and water content. This latter was determined according to the method reported by Santangeli et al. [22]. Each fruit was considered an ellipse, and the area was calculated using the following formula:

$$Area\ of\ ellipse = \pi \cdot a \cdot b$$

where a = length of semi-major axis and b = length of semi-minor axis.

These parameters were estimated in both primed and not-primed plants (controls, CTRL), either or not exposed to saltwater. The fruits were harvested, weighted (fresh and dry weights), and sampled for biochemical tests. To avoid changes in the nutritional and organoleptic properties of the fruits, biochemical tests were performed just after the sampling.

2.3. Intergenerational Priming Memory

After harvesting, the seeds were removed from the locular gel and washed for one minute under tap water. They were then placed in glass tubes, previously washed with 1% sodium hypochlorite, and filled with 15 mL of double-distilled water. The seeds were kept under these conditions until the complete detachment of the locular gel residues (approximately after 5–7 days), then transferred onto a filter paper and left to dry for 24 h at RT and stored in new sterilized glass tubes at RT. Germinated seeds, obtained from parental fruits, were sown in non-saline soil, and a new experiment was carried out to determine the transfer of priming memory to the next generation. The new plants, called generation 1 (G1), were grown and irrigated as described in Table 1 for 45 days. At the end of the growth period, the morphology and biomass of G1 plants were determined; then, these plants were collected and stored at −20 °C until further tests.

Table 1. Germination rates (%) of tomato seeds, 2 years after the original seed priming treatment. Data are expressed as mean ± SE (n = 3). Mean values in the column marked by different letters are significantly different within the same group ($p < 0.05$; ANOVA and Tukey–Kramer test). CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

Priming Solution	Germination (%)
CTRL	25 ± 4 [a]
2.5 mM PUT	95 ± 3 [b]
2.5 mM SPM	100 [b]
2.5 mM SPD	100 [b]

2.4. Analyses of Plants and Fruits

For the biochemical analyses, plant samples (shoots) were frozen (1 g of frozen material), while the fruits were harvested and sampled (0.2 g of fresh material) and quickly analyzed. Carotenoids, phenolic compounds, and antioxidant activity (AA) analyses were performed on samples homogenized in 5 mL of 95% ethanol; soluble sugars were detected in samples suspended in 1.5 mL of 1% phosphate-buffered saline (PBS). Extracts of fruits and plants were incubated, centrifuged, and stored until analyses.

2.5. Biochemical Determinations on Fruits and Plants

The analysis of carotenoids of tomato fruits was carried out according to Borromeo et al. [18] with a small modification, i.e., 0.5 mL of extract were diluted in 0.5 mL of 95% ethanol, while chlorophylls of G1 plants were quantified using 0.1 mL of supernatant diluted in 0.9 mL of 95% ethanol. Carotenoid and chlorophyll concentrations were determined according to Lichtenthaler (1987) [23] and expressed as μg g f.w.$^{-1}$.

The total phenolic content of fruits and plants was quantified according to Santangeli et al. [22]. Samples absorbances were measured at 724 nm with a spectrophotometer (VARIAN Cary 50 Bio, Santa Clara, CA, USA); the concentration of phenols was evaluated using a calibration curve made with a known concentration of chlorogenic acid (10 μg mL^{-1}, 40 μg mL^{-1} and 50 μg mL^{-1}) ($y = 0.0038x + 0.0025$; $R^2 = 0.9952$). The data were expressed as μg chlorogenic acid equivalent g f.w.$^{-1}$.

Flavonoids of fruits and plants were quantified according to the method described by Chang et al. [24]. The absorbance was measured at 415 nm with a spectrophotometer (VARIAN Cary 50 Bio, Santa Clara, CA, USA) and calculated using a calibration curve of quercetin as standard (10 μg mL^{-1}, 20 μg mL^{-1}, 40 μg mL^{-1} and 80 μg mL^{-1}) ($y = 0.0013x - 0.0007$; $R^2 = 0.9989$). Flavonoids were expressed as μg of quercetin equivalent mg f.w.$^{-1}$.

The quantification of soluble sugars of fruits was performed using the anthrone protocol, reported by Chun and Yin [25]. Samples absorbances were measured at 625 nm with a spectrophotometer (VARIAN Cary 50 Bio, Santa Clara, CA, USA). The concentration of sugars was calculated according to a calibration curve of glucose, carried out with solutions of 20 mg L^{-1}, 40 mg L^{-1}, 60 mg L^{-1}, 80 mg L^{-1}, and 100 mg L^{-1} ($y = 0.008x + 0.0068$; $R^2 = 0.9992$). The data were expressed as mg glucose equivalent g $f.w.^{-1}$.

The AA of tomato fruits and plants was tested by 2,2-Diphenyl-1-Picryl-Hydrazyl-Hydrate (DPPH) assay [19], using a fresh solution of 0.5 mM of DPPH and samples at different concentrations (40 mg mL^{-1} for tomato fruits, 40–200 mg mL^{-1} for G1 plants). Samples absorbances were recorded at 517 nm using a spectrophotometer (VARIAN Cary 50 Bio, Santa Clara, CA, USA). The AA was determined based on the formula reported by Garcia et al. [26]. AA was expressed as % (fruit samples) or as IC_{50} value (mg mL^{-1} for G1 plant samples).

The quantification of proline was carried out according to the method by Santangeli et al. [22]. Proline concentration was detected at 520 nm with a spectrophotometer (VARIAN Cary 50 Bio, Santa Clara, CA, USA) and calculated using a calibration curve made with standard solutions of L-Proline (5 µg mL^{-1}, 10 µg mL^{-1}, 15 µg mL^{-1}, and 20 µg mL^{-1} ($y = 0.0378x - 0.0063$; $R^2 = 0.9971$)). Data were expressed as µg proline g $f.w.^{-1}$.

2.6. Statistical Analysis

Data are reported as mean ± standard error (SE). The graphs were made with GraphPad Prism 10.2.2. One-way analysis of variance (ANOVA) was performed with Past 4.15. The Tukey–Kramer method was applied to determine the difference of significance among groups. All analyses were significant at $p < 0.05$. Mean values in the column marked by different letters are significantly different within the same group ($p < 0.05$; ANOVA and Tukey–Kramer test). When comparing primed groups to non-primed ones, the significance was *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

3. Results

3.1. Persistence of Somatic Memory During Seed Germination and Plant Growth

Primed and not-primed seeds were preserved for 2 years as described above. Then, seed germinability was tested by detecting the germination rates (%); the latter showed significant differences between primed and not-primed seeds (Table 1). Plant irrigation with salt water led to a progressive decrease in plant development after 45 days of growth (Figure 4a–c). Significant differences were observed between primed and not-primed plants irrigated with 160 mM NaCl (Figure 4c). The best growth rate was observed in SPD-primed plants (Figure 4c).

After 2 months of growth, the vegetative phase ended and the anthesis started (Figure S1a). In not-primed plants, irrigation with 80 mM NaCl led to early fruit production and ripening (Figure S1b,c). In primed plants, anthesis was observed already at 2 months of growth, and, similarly to CTRLs, salt exposure improved the rate of fruit production (Figure S1a–c). Most significant results were obtained in SPD-primed plants: i.e., after 4 months of salt irrigation, fruits were fully ripe (2 weeks earlier than CTRLs) (Figure S1c).

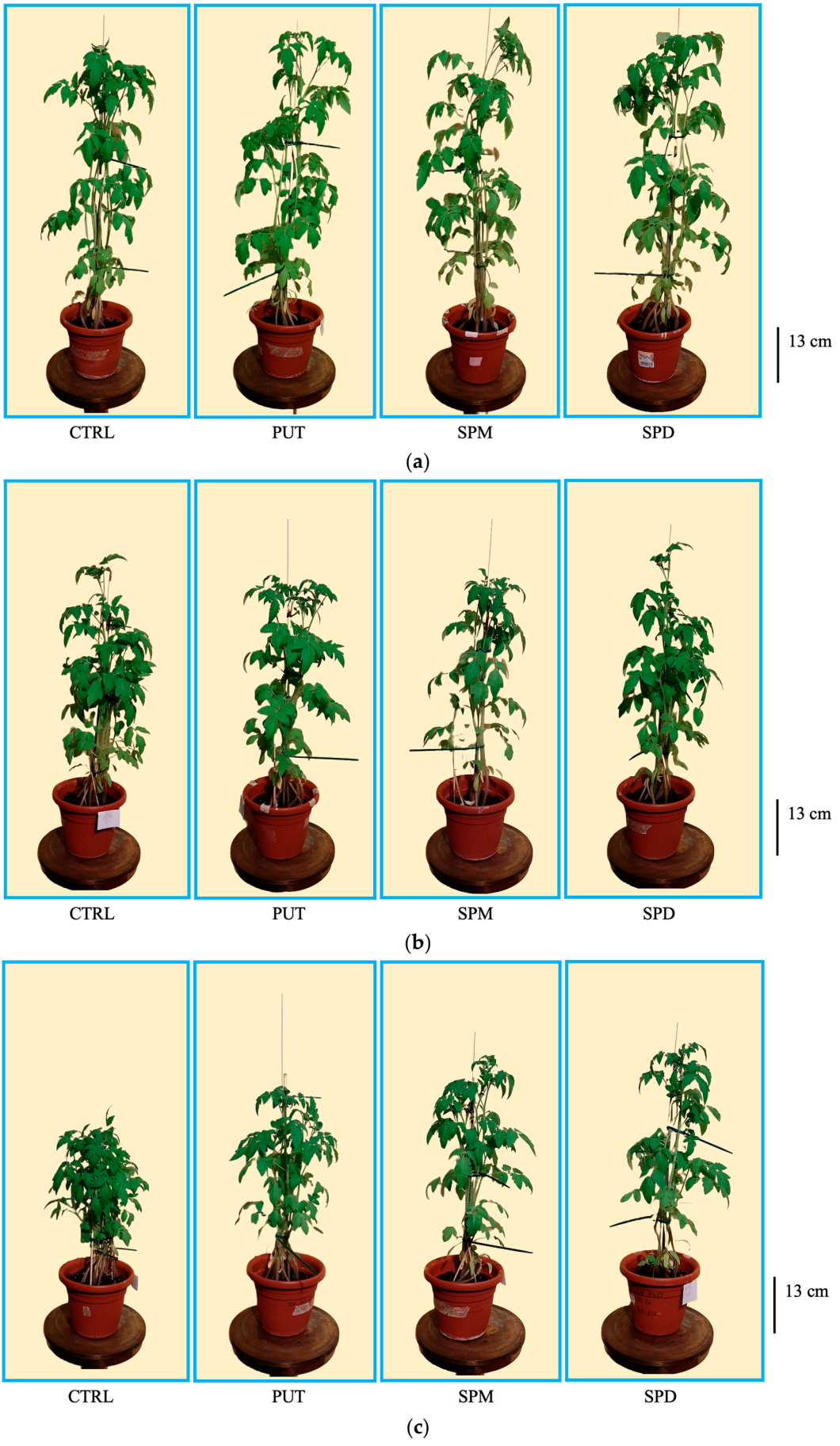

Figure 4. Tomato plants after 45 days of growth, irrigated with non-saline water (**a**), 80 mM NaCl (**b**), and 160 mM NaCl (**c**). CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

3.2. Fruit Ripening and Quality

Saline irrigation accelerated fruit production and maturation in both CTRLs and primed plants (Tables 2 and 3). Polyamines (PAs) priming, in particular SPD, led to an earlier fruit ripening in plants irrigated with 160 mM NaCl (the first red fruit was observed 17 days earlier than the corresponding CTRL). In general, all primed plants irrigated with 160 mM NaCl showed faster fruit ripening in comparison to CTRL, grown under the same conditions (Figure S1c) (Table 3).

Table 2. Fruit production in primed and not-primed plants. All data are expressed as days post-sowing (DPS). CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

NaCl (mM)	Priming Solution	First Yellow Tomato (DPS)	First Red Tomato (DPS)	End of Harvest (DPS)
0	CTRL	139	147	169
80	CTRL	113	119	169
160	CTRL	127	131	167
0	PUT	154	157	169
80	PUT	114	120	146
160	PUT	113	119	161
0	SPM	142	148	166
80	SPM	122	125	169
160	SPM	115	120	169
0	SPD	139	152	167
80	SPD	108	114	162
160	SPD	107	114	165

Table 3. Analysis of morpho-physiological parameters of tomato fruits at the end of harvest. Data are expressed as the mean ± SE; for the analysis of the EC, the number of replicates is $n = 3$. Mean values in the column marked by different letters are significantly different within the same group ($p < 0.05$; ANOVA and Tukey–Kramer test). Significant differences to CTRL are reported as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

NaCl (mM)	Priming Solution	EC of Soil (dS/m)	Fruit Weight (g)	Fruit Area (cm^2)	Water Content (%)	n. Seeds Per Fruit	Days for Ripening
0	CTRL	0.41 ± 0.03^{a}	13.3 ± 0.9^{a}	6.1 ± 0.7^{a}	68.0 ± 6.8^{a}	61 ± 2^{a}	7 ± 2^{a}
80	CTRL	0.93 ± 0.12^{b}	3.8 ± 0.4^{b}	2.8 ± 0.3^{b}	19.6 ± 3.7^{b}	17 ± 3^{b}	6 ± 1^{a}
160	CTRL	2.51 ± 0.10^{c}	1.7 ± 0.2^{c}	1.6 ± 0.2^{c}	8.3 ± 2.0^{c}	5 ± 2^{c}	12 ± 1^{b}
0	PUT	0.39 ± 0.04^{a}	13.7 ± 1.5^{a}	6.8 ± 0.7^{a}	67.3 ± 3.7^{a}	55 ± 3^{a}	10 ± 3^{a}
80	PUT	0.97 ± 0.02^{b}	4.1 ± 0.4^{b}	2.8 ± 0.2^{b}	23.8 ± 2.3^{b}	10 ± 2^{b}	$4 \pm 0.3^{b*}$
160	PUT	$1.33 \pm 0.04^{c***}$	2.6 ± 0.4^{c}	$2.4 \pm 0.2^{b*}$	13.1 ± 2.6^{c}	11 ± 2^{b}	$8 \pm 1^{a***}$
0	SPM	0.33 ± 0.04^{a}	13.4 ± 3.1^{a}	6.8 ± 1.2^{a}	62.6 ± 7.3^{a}	48 ± 2^{a}	8 ± 1^{a}
80	SPM	1.15 ± 0.20^{b}	$5.3 \pm 0.2^{b**}$	4.0 ± 0.5^{b}	$33.4 \pm 3.6^{b*}$	$27 \pm 3^{b*}$	7 ± 1^{a}
160	SPM	$2.15 \pm 0.06^{c*}$	$3.7 \pm 0.1^{c***}$	$3.2 \pm 0.4^{b*}$	$19.6 \pm 2.9^{c*}$	$20 \pm 3^{b*}$	$8 \pm 0.4^{a*}$
0	SPD	0.37 ± 0.03^{a}	15.5 ± 2.2^{a}	7.5 ± 0.3^{a}	57.2 ± 3.1^{a}	51 ± 7^{a}	8 ± 1^{a}
80	SPD	1.05 ± 0.01^{b}	$5.6 \pm 0.4^{b**}$	3.8 ± 0.5^{b}	$34.7 \pm 5.7^{b*}$	$37 \pm 3^{b***}$	6 ± 1^{a}
160	SPD	2.33 ± 0.11^{c}	$4.2 \pm 0.1^{c***}$	$3.6 \pm 0.3^{b***}$	$21.2 \pm 3.7^{b*}$	$26 \pm 3^{c***}$	$8 \pm 0.3^{a**}$

Both salt irrigation and priming caused alterations in fruit pigmentation (Figure 5), which was very pronounced in CTRLs irrigated with 160 mM NaCl. The increase in salt in the soil caused a decrease in weight, size, water content, and number of seeds in CTRL fruits (Table 3); on the contrary, fruits of primed and stressed plants showed an improvement in

all morphological parameters, especially in fruits produced by SPM- and SPD-primed and stressed plants (Figure 5 and Table 3).

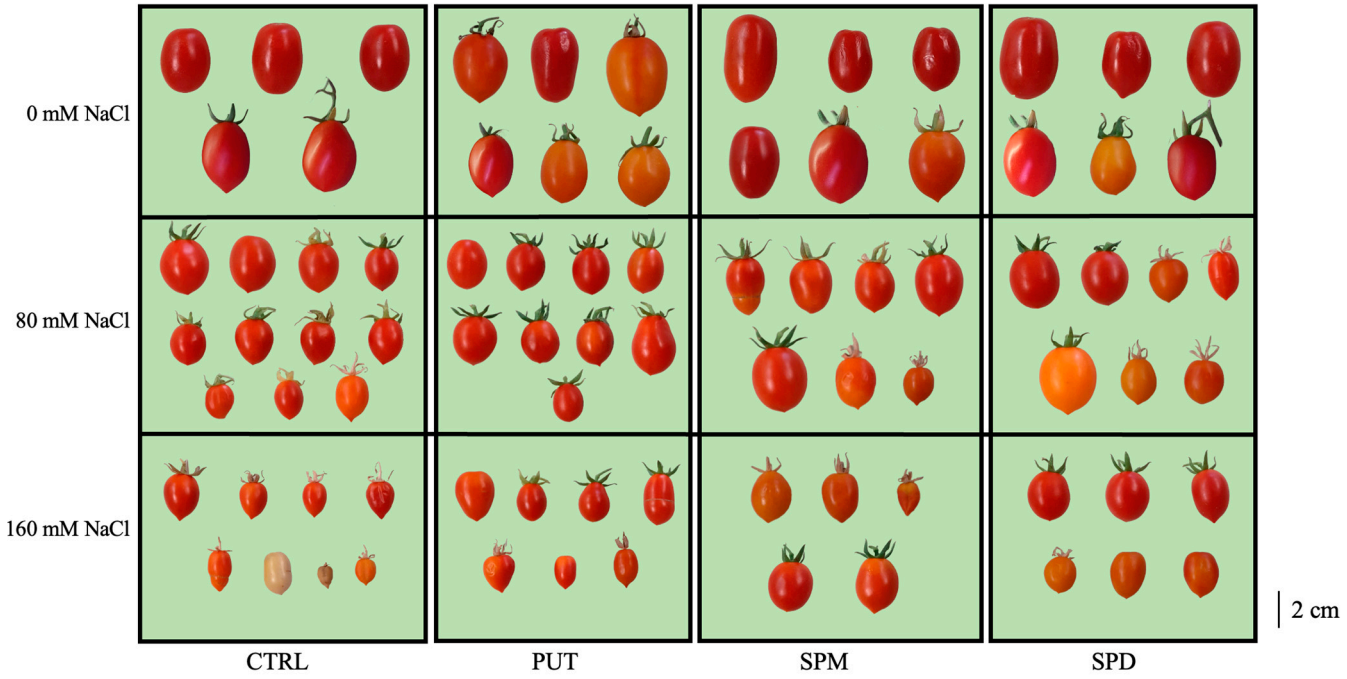

Figure 5. Yield of tomatoes obtained from primed and not-primed plants at the end of the experiment. All tomatoes were used for subsequent morphological and biochemical analyses. CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

Saline irrigation, particularly at 160 mM NaCl, significantly reduced the amount of carotenoids in CTRLs. This trend was not observed in fruits produced by primed plants (Figure 6), where a marked increase in carotenoid content was observed (Figure 6). Moreover, salinity enhanced sugar content in all tomatoes (primed and not primed). In particular, fruits obtained from primed plants exhibited significantly higher sugar concentrations than CTRLs, either under saline or non-saline irrigation (Figure 7). SPM was found to be the PA that most improved sugar concentration in response to salt (+56.3%) (Figure 7).

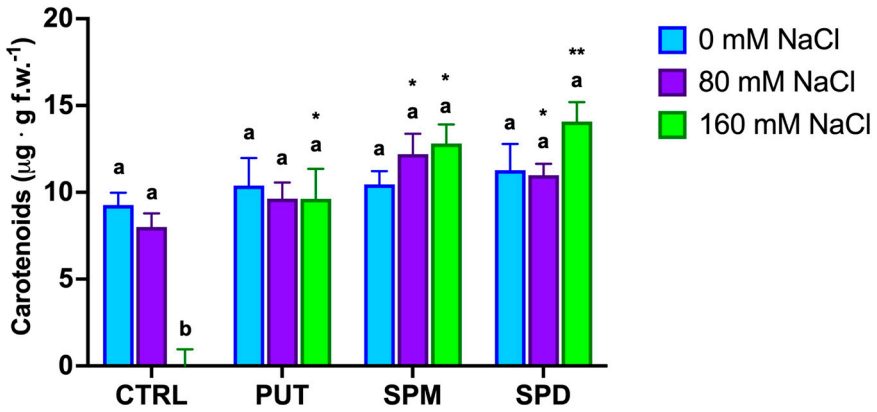

Figure 6. Amounts of carotenoids in tomato fruits. Data are expressed as mean ± SE. Mean values in the column marked by different letters are significantly different within the same group ($p < 0.05$; ANOVA and Tukey–Kramer test). Significant differences to CTRL are reported as * $p < 0.05$; ** $p < 0.01$. CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

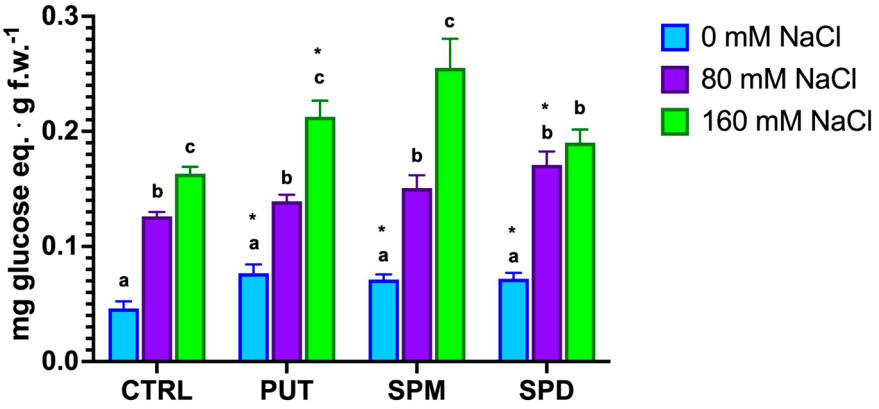

Figure 7. Soluble sugar content in tomato fruits. Data are expressed as the mean ± SE. Mean values in the column marked with different letters are significantly different within the same group ($p < 0.05$; ANOVA and Tukey–Kramer test). Significant differences to CTRL are reported as * $p < 0.05$. CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

The AA of fruit extracts was evaluated by DPPH assay and quantification of antioxidant secondary metabolites (phenolic compounds). Salinity decreased AA only in CTRLs (Table 4). In fruits from primed plants, complex results were observed: all PAs increased AA (+79.8%, +87.4%, and +76.5% for PUT, SPM, and SPD, respectively) in response to salt, but only PUT enhanced the content of both phenols and flavonoids (Table 4).

Table 4. Antioxidant activity, phenol, and flavonoid content of tomato fruits. Data are expressed as the mean ± SE. Mean values in the column marked with different letters are significantly different within the same group ($p < 0.05$; ANOVA and Tukey–Kramer test). Significant differences to CTRL are reported as * $p < 0.05$; ** $p < 0.01$. CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

NaCl (mM)	Priming Solution	Antioxidant Activity (%)	Phenols (µg Chlorogenic Acid Eq. g f.w.$^{-1}$)	Flavonoids (µg Quercetin Eq. mg f.w.$^{-1}$)
0	CTRL	21.2 ± 2.4 ^{a}	747.0 ± 22.6 ^{a}	0.68 ± 0.08 ^{a}
80	CTRL	19.9 ± 0.9 ^{a}	945.9 ± 48.9 ^{b}	0.63 ± 0.05 ^{a}
160	CTRL	11.9 ± 1.2 ^{b}	929.5 ± 66.3 ^{ab}	0.83 ± 0.11 ^{a}
0	PUT	14.7 ± 1.8 ^{a}	546.8 ± 67.7 ^{a*}	0.44 ± 0.04 ^{a}
80	PUT	18.6 ± 0.6 ^{ab}	909.8 ± 53.0 ^{b}	1.08 ± 0.08 ^{b**}
160	PUT	21.4 ± 1.3 ^{b*}	1281.7 ± 86.1 ^{c**}	0.97 ± 0.10 ^{b}
0	SPM	21.5 ± 2.1 ^{a}	704.7 ± 43.0 ^{a}	0.92 ± 0.06 ^{a}
80	SPM	21.3 ± 1.9 ^{a}	777.9 ± 77.1 ^{a}	0.56 ± 0.08 ^{b}
160	SPM	22.3 ± 2.5 ^{a**}	806.6 ± 71.5 ^{a}	0.73 ± 0.07 ^{ab}
0	SPD	15.9 ± 2.4 ^{a}	718.8 ± 42.6 ^{a}	0.79 ± 0.06 ^{a}
80	SPD	16.9 ± 1.1 ^{a}	925.3 ± 42.0 ^{b}	0.91 ± 0.09 ^{a}
160	SPD	21.0 ± 1.8 ^{a*}	1253.9 ± 51.6 ^{c*}	0.41 ± 0.03 ^{b*}

3.3. Intergenerational Priming Memory During Germination and Growth

The germination rate of G1 seeds seems to confirm the positive effects of priming through the generation (Figure S2 and Table 5); in particular, seeds of fruits produced by primed plants irrigated with 160 mM NaCl showed a significant increase in germination rate compared to CTRL (+34.5%, +63.8%, and +58.6%, respectively, with PUT, SPM, and SPD).

Table 5. Germination rate of seeds obtained from fruits produced by primed and not-primed tomato plants subjected to saline and non-saline irrigation regime. Data are expressed as the mean ± SE (n = 4). Mean values in the column marked with different letters are significantly different within the same group ($p < 0.05$; ANOVA and Tukey–Kramer test). Significant differences to CTRL are reported as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

NaCl (mM)	Priming Solution	Germination (%)
0	CTRL	84 ± 3 ^{a}
80	CTRL	98 ± 2 ^{a}
160	CTRL	58 ± 3 ^{b}
0	PUT	92 ± 3 ^{a}
80	PUT	98 ± 2 ^{a}
160	PUT	78 ± 5 ^{a*}
0	SPM	79 ± 3 ^{a}
80	SPM	94 ± 5 ^{a}
160	SPM	95 ± 3 ^{a***}
0	SPD	89 ± 2 ^{a}
80	SPD	77 ± 5 ^{a**}
160	SPD	92 ± 4 ^{a***}

During the vegetative growth, the persistence of priming memory was assessed by observing morphological parameters and detecting the biomass after 4 weeks of saline and non-saline irrigation. The offspring of primed plants showed a higher tolerance to salt (in particular, at 160 mM NaCl) than the offspring of not-primed ones (Figure S3a–c and Table 6).

Table 6. Morphological parameters and biomass of G1 plants after 45 days of growth. Data are expressed as the mean ± SE (n = 18). Mean values in the column marked with different letters are significantly different within the same group ($p < 0.05$; ANOVA and Tukey–Kramer test). Significant differences to CTRL are reported as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

NaCl (mM)	Priming Solution	n. Leaves	Shoot Length (cm)	Root Length (cm)	Plant Biomass (g)
0	CTRL	16 ± 1.0 ^{a}	21 ± 0.8 ^{a}	4 ± 0.6 ^{a}	0.76 ± 0.10 ^{a}
80	CTRL	12 ± 1.2 ^{a}	13 ± 1.1 ^{b}	4 ± 0.4 ^{a}	0.45 ± 0.07 ^{ab}
160	CTRL	6 ± 1.0 ^{b}	6 ± 1.0 ^{c}	2 ± 0.5 ^{b}	0.17 ± 0.04 ^{b}
0	PUT	13 ± 0.9 ^{a}	19 ± 0.8 ^{a}	3 ± 0.2 ^{a}	0.64 ± 0.07 ^{a}
80	PUT	15 ± 1.2 ^{a}	16 ± 1.1 ^{ab}	4 ± 0.5 ^{a}	0.56 ± 0.08 ^{a}
160	PUT	13 ± 1.3 ^{a***}	13 ± 1.0 ^{b***}	4 ± 0.5 ^{a}	0.43 ± 0.05 ^{a**}
0	SPM	16 ± 1.2 ^{a}	22 ± 1.4 ^{a}	3 ± 0.2 ^{a}	0.62 ± 0.10 ^{a}
80	SPM	14 ± 1.3 ^{a}	16 ± 0.7 ^{b}	4 ± 0.5 ^{a}	0.56 ± 0.08 ^{a}
160	SPM	13 ± 1.1 ^{a**}	14 ± 0.7 ^{b***}	4 ± 0.4 ^{a}	0.53 ± 0.07 ^{a***}
0	SPD	17 ± 1.2 ^{a}	22 ± 1.1 ^{a}	3 ± 0.2 ^{a}	0.72 ± 0.08 ^{a}
80	SPD	16 ± 1.0 ^{a}	18 ± 0.5 ^{b*}	5 ± 0.5 ^{b}	0.61 ± 0.08 ^{a}
160	SPD	10 ± 0.8 ^{b**}	12 ± 0.6 ^{c***}	4 ± 0.6 ^{ab}	0.34 ± 0.03 ^{b**}

At the end of growth, Chl a, b, and the total chlorophyll content were quantified in the offspring. The highest salinity of irrigation solution decreased the chlorophyll content of CTRL plants, while an opposite trend was observed in the offspring of PUT- and SPM-primed plants (Table 7). All the offspring of primed plants exhibited a significant increase in

total chlorophyll content with respect to CTRL when irrigated with 160 mM NaCl (+175%, +170%, and +89% with PUT, SPM, and SPD, respectively) (Table 7).

Table 7. Chlorophyll concentration of G1 plants after 45 days of growth. Data are expressed as the mean ± SE (n = 3). Mean values in the column marked with different letters are significantly different within the same group ($p < 0.05$; ANOVA and Tukey–Kramer test). Significant differences to CTRL are reported as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

NaCl (mM)	Priming Solution	Chl a (μg g f.w.$^{-1}$)	Chl b (μg g f.w.$^{-1}$)	Total Chl (μg g f.w.$^{-1}$)
0	CTRL	108.6 ± 5.2 ^{a}	42.6 ± 5.5 ^{a}	151.3 ± 9.2 ^{a}
80	CTRL	94.4 ± 2.3 ^{a}	38.9 ± 1.8 ^{a}	133.3 ± 1.5 ^{a}
160	CTRL	41.7 ± 2.5 ^{b}	22.6 ± 6.1 ^{a}	64.3 ± 7.5 ^{b}
0	PUT	79.8 ± 1.5 ^{a***}	33.5 ± 3.1 ^{a}	113.3 ± 1.8 ^{a*}
80	PUT	112.2 ± 3.8 ^{b*}	54.0 ± 3.2 ^{ab}	166.2 ± 5.8 ^{b**}
160	PUT	119.9 ± 5.7 ^{b***}	56.9 ± 1.7 ^{b***}	176.7 ± 4.2 ^{b***}
0	SPM	94.1 ± 2.4 ^{a}	43.0 ± 4.1 ^{a}	137.1 ± 4.4 ^{a}
80	SPM	92.2 ± 3.1 ^{a}	45.2 ± 5.0 ^{a}	137.4 ± 6.8 ^{a}
160	SPM	120.3 ± 5.2 ^{b***}	53.4 ± 2.6 ^{a**}	173.7 ± 6.1 ^{b***}
0	SPD	111.1 ± 1.6 ^{a}	51.2 ± 7.1 ^{a}	162.2 ± 8.7 ^{a}
80	SPD	90.4 ± 4.0 ^{b}	47.5 ± 2.2 ^{a}	137.9 ± 6.0 ^{ab}
160	SPD	80.7 ± 5.0 ^{b***}	40.8 ± 4.8 ^{a}	121.4 ± 9.6 ^{b***}

3.4. Antioxidant Activity and Osmolyte Metabolism of G1 Plants

The analysis of the half maximal inhibitory concentration (IC_{50}) value revealed that the offspring of PUT- and SPM-primed plants showed the highest increases of AA (Figure 8a–d) when irrigated with elevated salinity solution (Figure 8c,d). These data were confirmed by the analysis of phenolic compounds: i.e., the most significant results were found in the offspring of PUT- and SPM-primed plants (Table 8) irrigated with 160 mM NaCl.

Table 8. Phenols and flavonoids of G1 plants after 45 days of growth. Data are expressed as the mean ± SE (n = 3). Mean values in the column marked with different letters are significantly different within the same group ($p < 0.05$; ANOVA and Tukey–Kramer test). Significant differences to CTRL are reported as * $p < 0.05$; *** $p < 0.001$. CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

NaCl (mM)	Priming Solution	Phenols (μg Chlorogenic Acid Eq. g f.w.$^{-1}$)	Flavonoids (μg Quercetin Eq. mg f.w.$^{-1}$)
0	CTRL	1203.7 ± 43.2 ^{a}	1.30 ± 0.02 ^{a}
80	CTRL	1403.7 ± 54.3 ^{a}	1.29 ± 0.04 ^{a}
160	CTRL	908.0 ± 73.6 ^{b}	0.72 ± 0.05 ^{b}
0	PUT	1027.6 ± 58.1 ^{a}	1.08 ± 0.05 ^{a*}
80	PUT	1314.1 ± 85.7 ^{ab}	1.39 ± 0.02 ^{b}
160	PUT	1465.9 ± 56.8 ^{b***}	1.50 ± 0.04 ^{b***}
0	SPM	1011.7 ± 46.9 ^{a}	1.17 ± 0.03 ^{a}
80	SPM	903.0 ± 41.1 ^{a***}	1.22 ± 0.05 ^{a}
160	SPM	1457.2 ± 30.0 ^{b***}	1.57 ± 0.04 ^{b***}
0	SPD	1023.9 ± 61.3 ^{ab}	1.31 ± 0.02 ^{a}
80	SPD	1257.0 ± 62.9 ^{a}	1.21 ± 0.06 ^{ab}
160	SPD	821.9 ± 38.0 ^{b}	1.08 ± 0.02 ^{b***}

Both the offspring of primed and not-primed plants showed an enhancement of proline synthesis with the increasing salt stress (Figure 9). In the offspring of PUT- and SPM-primed plants, a significant improvement in osmolyte concentration (+30.2% and +24.1% with PUT and SPM, respectively) was recorded in samples irrigated with 160 mM NaCl (Figure 9).

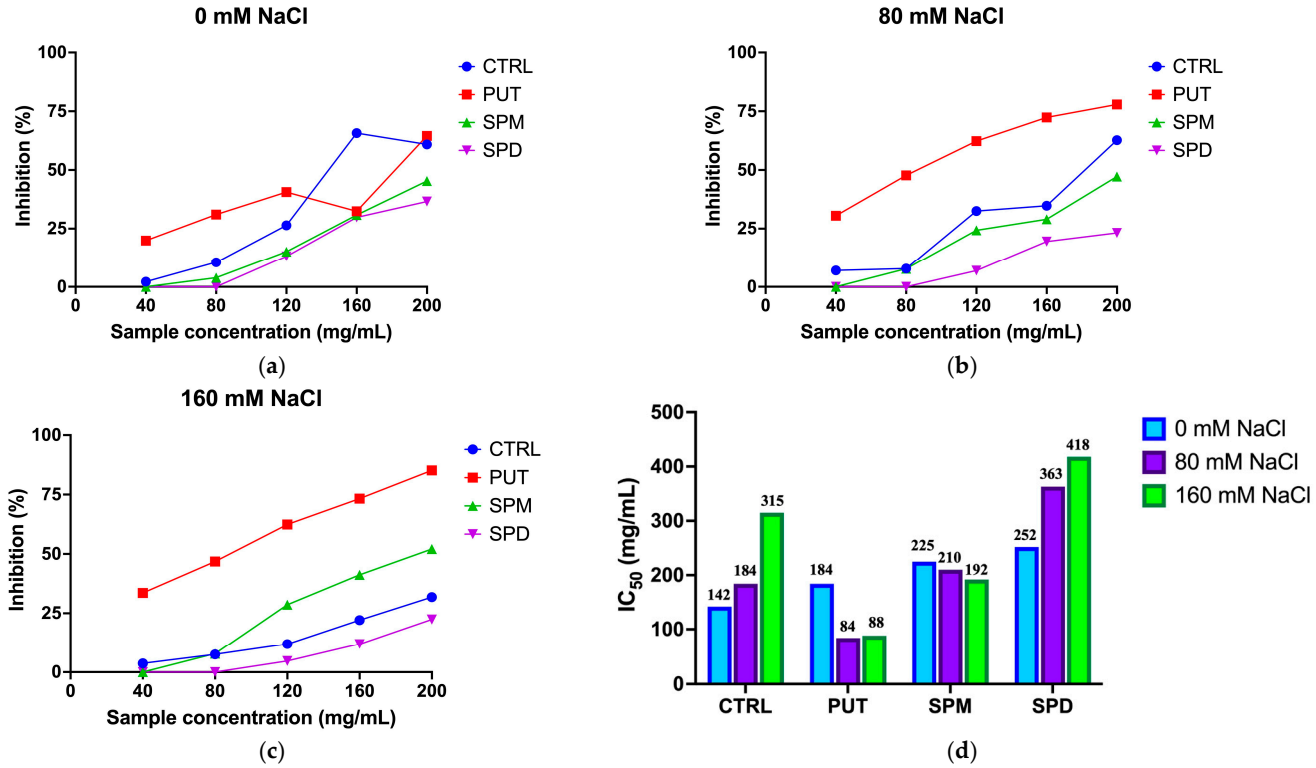

Figure 8. DPPH free radical inhibiting activity (%) at various concentrations of leaf extract (**a**–**c**) and half maximal inhibitory concentration (IC_{50}) values of G1 plants after 45 days of growth (**d**). CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

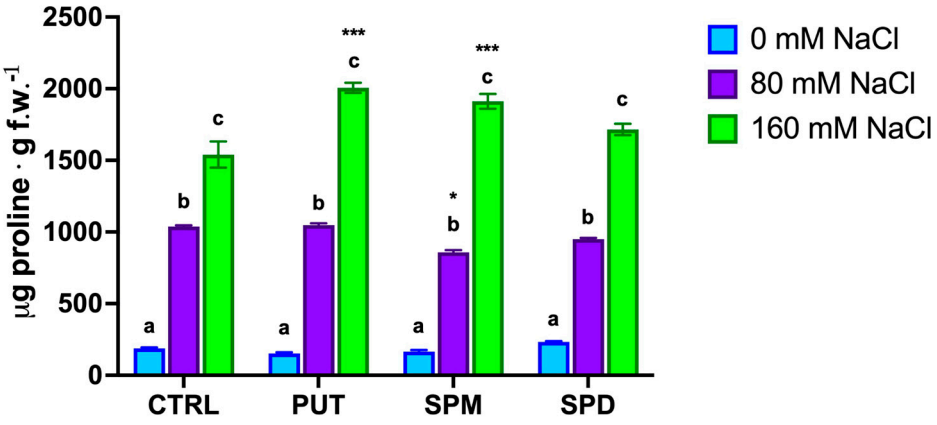

Figure 9. Proline concentration of G1 plants after 45 days of growth. Data are expressed as the mean ± SE (n = 3). Mean values in the column marked with different letters are significantly different within the same group ($p < 0.05$; ANOVA and Tukey–Kramer test). Significant differences to CTRL are reported as * $p < 0.05$; *** $p < 0.001$. CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

4. Discussion

Plants can develop stress memory as a survival mechanism; however, the genetic basis of this process has been mostly studied in the model plant *Arabidopsis* [2,9,14]. The phenomenon of stress memory can enhance plant adaptation to future stress events, and the development of this memory, by altering metabolic pathways, may cause transient or non-transient epigenetic changes, which can be transferred to following generations [1,2,27].

4.1. Evidence of Long-Term Somatic Memory

According to Lagiotis et al. [2], plants possess somatic, inter- and transgenerational memory. There are many literature reports concerning stress memory of priming [11,12], but there are no works on the persistence and transfer of priming memory in tomato plants.

To evaluate the long-term persistence of priming memory, PA-treated tomato seeds stored for 2 years (from 2022 to 2024) were germinated and exposed to stress. Our data show a significant difference in primed seed compared to the control; the germination rate was higher than 50% with respect to the controls; therefore, these data provide further evidence supporting the persistence of priming memory in tomatoes after a long time of storage, as reported by Racette et al. [28] in other species, and represent a valuable step toward future application.

Based on these promising results, we decided to sow the germinated seeds and observe the life cycle of the plants, exposed once more to saline conditions. Former primed plants were able to tolerate saline irrigation better than CTRLs, supporting the theory of long-term somatic memory, hypothesized by Lagiotis et al. [2]. These results are the first report on the long persistence of priming treatment in tomatoes, i.e., over a 2-year storage period, differently from the reports on other species [28,29].

The long-lasting effect of priming was even more evident in the reproductive stage of the plants, where a positive correlation between priming and fruit ripening time, number of seeds/fruit, and quality were recorded. According to Pascale et al. [30] and Maggio et al. [31], the irrigation of tomato plants, not primed, with saline water (EC_{wi} between 3.5 dS/m and 6.0 dS/m) increased the concentration of carotenoids, vitamin C and reducing sugars in tomato fruits. Our data confirm the influence of salinity on the earlier anthesis and fruit production [31], nevertheless, the quality of fruit produced by not-primed plants was negatively affected, differently from the data by Maggio et al. [31], in fact, pigment alteration of epicarp was observed, and physiological changes were also noted, such as a decrease in the weight and water content of the fruit.

All our data support the priming memory theory since the fruits of primed plants (even though small variations were observed according to the PA used as the priming agent) had a higher concentration of carotenoids and sugars compared to CTRL fruits, where a decrease in carotenoids was detected, resulting in the alterations of the epicarp coloration, reported above. So far, there are no literature reports on the antioxidant activity of fruits produced by plants developed from primed seed stored for a long period; an increase in total antioxidant activity was detected in fruits of primed plants compared to CTRL fruits, as already observed in our previous study [19]. Higher antioxidant power was related to an enhancement of phenolic compounds only after PUT and SPD priming. Further studies are in progress.

4.2. Evidence of Intergenerational Priming Memory

Interesting results were observed during germination and vegetative growth of G1. Seeds extracted from tomatoes, produced by primed plants, irrigated with 160 mM NaCl, showed higher germination rate (i.e., 77.6%, 94.8%, and 91.7% with PUT, SPM, and SPD, respectively) than seeds from fruits produced by not-primed plants (which germination

rate was 57.7%). These results suggest that low concentrations of PAs, used as seed priming agents, presented a hormetic effect on the viability and germination rate of G1 seeds; as reported by Jalal et al. [32], hormesis contributes to the adaptation and protection of plants from stress, such as salinity, and improves crop productivity by enhancing plants tolerance responses under stressful conditions. Hormesis is, therefore, an advantage that allows crops to survive and maintain their biomass and yield production.

Due to the paucity of data on the inter- and transgenerational memory of horticultural crops [33], this work can be considered a pioneer in this research field of intergenerational priming memory, revealing part of the unexplored aspects of such memory in this horticultural crop.

Further evidence supporting the hypothesis of intergenerational priming memory was found by analyzing the morphology and biomass of tomato plants after 45 days of growth in saline conditions: by comparing the growth of the offspring of primed plants with the offspring of not-primed ones, irrigated with 160 mM NaCl, better performance was observed in the primed, i.e., higher tolerance to saline conditions, increased production of new leaves, shoot length, and biomass compared to the not-primed plants. These data confirm the importance of hormopriming with PAs [34] and provide further support for the existence and transfer of priming memory from the parental plant to the offspring.

Furthermore, G1 behavior toward salinity was similar to that of primed parental generation, e.g., increased growth compared to their controls and higher chlorophyll and proline concentration, particularly in the offspring of PUT- and SPM-primed plants; this similarity between parental generation and offspring significantly highlighted the transfer of priming memory to G1.

Similarly, antioxidant activity and phenolic compounds of G1 were higher with respect to the CTRL, providing further support for the intergenerational priming memory thesis. A decrease in antioxidant activity and phenolic compounds was observed in the offspring of not-primed plants irrigated with saline solutions, confirming previous data.

The different PA agents lead to different levels of tolerance; the progeny of PUT- and SPM-primed plants seem to possess a higher stress memory, showing better salt tolerance, vice versa, the offspring of SPD-primed plants appear to behave differently with respect to the other primed plants since these plants showed lower tolerance, especially at the highest salinity level. These data suggest the importance of screening to detect the best priming agent, not only in the first phases of stress exposure but also in the following phases, in order to validate the persistence of somatic and intergenerational priming.

All the results open new perspectives concerning the use of seed priming as a technique to counteract salt stress.

5. Conclusions

Climate change is significantly affecting agriculture. To compensate for the lack of fresh water, farmers irrigate crops with poor quality or saline water, causing damage to depleted soil and plants. Some techniques, i.e., acclimation and seed priming, can increase salt tolerance. In the present work, significant results were obtained concerning somatic and intergenerational stress memory in tomatoes. Regarding somatic memory, high germination rates of primed seeds were maintained after 2 years of seed storage. We may say that seed priming can be a useful tool to increase not only vegetative growth but also fruit production by improving their organoleptic and nutritional properties, making them richer in nutrients than the not-primed counterparts. The behavior of the G1-primed plants was also even better than the controls when exposed to salt. The progeny of PUT- and SPM-primed plants seem to possess a higher stress memory with respect to SPD-primed, thus showing a better tolerance to salinity. Although further studies on the persistence

and transfer of stress memory will be needed, this work opens future perspectives for the application of priming treatment in seed preservation.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae11030236/s1, Figure S1. Development of tomato plants after 2 months (a), 3 months (b), and 4 months (c) of saline and non-saline irrigation. The pictures refer to anthesis and fruit ripening stages. CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine. Figure S2. Germinating seeds from fruits produced by primed and not-primed plants (stressed and not stressed) after 7 days of incubation in the dark at RT. CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine. Figure S3. Plants from G1, irrigated for 4 weeks, with 0 mM NaCl (a), 80 mM NaCl (b), and 160 mM NaCl (c). CTRL = control; PUT = putrescine; SPM = spermine; SPD = spermidine.

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