

# *In Vitro* Germination and Early Vegetative Growth of Five Tomato (*Solanum lycopersicum* L.) Varieties under Salt Stress Conditions

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## Abstract

In Senegal, tomato (*Solanum lycopersicum* L.) cultivation is affected by salinity in many agro-ecological zones. The selection of salt tolerant varieties would be an alternative solution to enhance the production. Thus, germination and growth are studied under axenic conditions for five varieties of tomato subjected to increasing concentrations of NaCl [0, 35, 70 and 105 mM], and supplemented in an MS/2 medium for 30 days. The results reveal that salt negatively affects the evaluated parameters. The *Rodeo* and *Lady Nema* varieties have the lowest final germination rates (50%) unlike the *Mongal* variety (55%). These last two varieties have a decrease of 71.78% and 81.28% in the height of the stem, respectively, in the presence of NaCl at [105 mM] while that of the *Rodeo* variety is 70%. The *Xewel* variety has the greatest average number of leaves in the presence of [NaCl 35 mM] (4.95 leaves) and [NaCl 70 mM] (4.77 leaves). The *Lady Nema* variety records the longest taproot length (2.99 cm) unlike the *Rodeo* variety (2.25 cm) at [NaCl 105 mM]. The *Ganila* variety reveals the highest number of secondary roots at the concentrations of [0, 35 and 105 mM] of NaCl with, respectively, 44.12, 29.25, and 4.25 roots. The *Lady Nema* variety records the highest fresh weight of aerial (0.055 g) and root parts (0.014 g) and the lowest root dry weights (0.0023 g). These results allow to conclude that the *Lady Nema* and *Mongal* varieties seem more tolerant, *Ganila* and *Xewel* are moderately tolerant and *Rodeo* is more sensitive to the presence of salt.

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## Keywords

*Solanum lycopersicum*, NaCl, Germination, Growth, Tolerance, In Vitro Conditions

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## 1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops in the world particularly in Senegal. Globally, production and cultivated areas are constantly increasing [1], i.e. a respective increase of 2.55% and 5.08% from 1960 to the present is mentioned [2]. Despite the possibility of cultivating this species on a large scale, tomato yields in Senegal (20.05 t/ha) do not yet reach the values recorded in other countries such as the United States (96.8 t/ha), South Africa (75.5 t/ha) and China (59.4 t/ha) [2]. These insufficient yields are linked to the salinization of agricultural lands, which is one of the major constraints considerably limiting production in the world and in the Sahel in particular [3]. According to [4], 800 Mha of arable lands worldwide is affected by salinity. Indeed, among environmental stresses, salt stress is one of the main abiotic factors leading to an increase in the loss of arable lands and a drastic reduction in agricultural productivity [5]. It is, therefore, an important obstacle to food security and a major cause of land degradation, particularly, in arid and semi-arid regions [6] [7]. Added to this, the consequences of climate change are becoming more and more restrictive for the growth and development of plants, especially in semi-arid and arid areas [8]. Under these conditions of abiotic stress, the physiology of plants is disrupted [9] [10], including in tomatoes [11].

The expression of plants in response to salinity results in both water stress due to the osmotic effects induced by salt, and chemical stress, mainly due to the toxic effects of sodium. Thus, salt stress exerts at the same time osmotic, ionic and nutritional imbalances in plant species, including tomato [3]. According to [12], salinity hinders plant growth in several ways: 1) it increases the osmotic potential of the soil, making it difficult for the plant root system to access water, which provokes osmotic stress [13] [14]; 2) salinity can be associated with high concentrations of ions inside cells, which causes an inhibitory effect on plant metabolism qualified as ionic stress [15] [16]. The altered status of water leads to the reduction of initial growth and limitation of plant productivity. In most plants, growth decreases with increasing salt concentrations in the medium [17], but in others, growth is stimulated by moderate salt concentrations [18]. Salinity is an abiotic constraint that negatively influences almost all stages of the life cycle of tomato plant, leading to a reduction in its yield [19] [20]. This constraint affects the morphological parameters of the tomato as well as the relative water content of the leaves, the photosynthetic pigments, the leaves gas exchange parameters, the fluorescence of chlorophyll and the absorption of essential macronutrients [21]. According to [4], to cope with salinity, the plant implements two

types of responses corresponding to the two types of stress generated by saline stress, *i.e.* osmotic stress and ionic stress: a rapid response resulting from an increase in external osmotic pressure [21] [22] and a slow response due to exclusion of Na<sup>+</sup> ions from the cell [23] or their compartmentalization in cells of certain organs [15] respectively.

In Senegal, land salinization particularly affects coastal areas [24] and lowlands [25]. Consequently, of the 3,800,000 ha of cultivable lands, approximately 1,700,000 ha are affected by salinization [26]. According to [27], one third of irrigated lands are affected by salinization. The salinization of senegalese lands results mainly from the different climatic phases which followed one another in the Quaternary and which provoked the invasion of the continent by marine waters [28]. In addition, there is the effect of climate changes, in particular the rainfall deficit of the 1970s and the rise in sea level (marine intrusion, floods, *etc.*), which have contributed to accentuating the effects of salinity downstream of the main watersheds of rivers or inlets of Senegal rivers, *i.e.* Senegal, Sine, Saloum and Casamance [28] [29].

Under the pressure of strong demographic growth, Senegal must imperatively find ways to increase agricultural yields in order to achieve food self-sufficiency. Hence, it would be strategic to be able to use varieties that are more tolerant to salinity. In this context, we undertook this work, which consists of studying the *in vitro* behavior of five tomato varieties subjected to increasing levels of salinity (NaCl), in order to assess their behavior or even sensitivity to this abiotic stress during the germination process and the early steps of the vegetative growth.

## 2. Material and methods

### 2.1. Plant Material

The plant material consists of seeds of five F1 hybrid tomato varieties (*Solanum lycopersicum* L.) supplied by the company Tropica Sem-Senegal (Technisem Novalliance Group): *Ganila*, *Lady Nema*, *Mongal*, *Rodeo* and *Xewel*. Their characteristics are summarized in **Table 1**. They were harvested and bagged in 2019,

**Table 1.** Characteristics specific to each FI hybrid variety of tomato (*Solanum lycopersicum* L.).

VARIETIES	ORIGIN	CHARACTERISTICS
<i>Ganila</i>	Tropica Sem	Adapted to crops in rainy season determined growth, very productive, fairly good vigor, good fruit set, early (60 to 65 days), tolerant to TYLCV (Tomato Yellow Leaf Curl Virus) and <i>Fusarium</i> , Resistance to TMV (Tobacco Mosaic Virus)
<i>Lady Nema</i>	Tropica Sem	Adapted to crops in rainy and hot season, determined growth, good leaf cover, good yield, earliness of 75 - 80 days, good tolerance to nematodes, CMV (Cucumber Mosaic Virus), TYLCV (Tomato Yellow Leaf Curl Virus), resistant to TMV (Tobacco Mosaic Virus) and <i>Fusarium</i>
<i>Mongal</i>	Tropica Sem	Adapted to the hot season and to wintering, determined growth, very good vigor, excellent fruit set, early (65 days), resistant to: TMV (Tobacco Mosaic Virus), <i>Fusarium</i> , <i>Stemphylium spp</i> , <i>Ralsonia solacerum</i> , <i>Meloidogyne spp</i> .
<i>Rodeo</i>	Tropica Sem	Adapted to the cool dry season and hot dry season, determined growth, very good vigor, good fruit set, excellent productivity, medium early (70 to 80 days), very good tolerance TYLCV (Tomato Yellow Leaf Curled Virus)
<i>Xewel</i>	Tropica Sem	Adapted to crops in rainy season, determined growth, very good productivity, early (60 to 65 days), tolerant to TYLCV (Tomato Yellow Leaf Curl Virus) and <i>Fusarium</i> , Resistance to TMV (Tobacco Mosaic Virus)

with an 85% minimum germination rate and a varietal purity of 99%. They were stored at an average temperature of  $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .

## 2.2. Culture Conditions

The basic culture medium used was that of [30], the macro-elements of which were diluted by half (MS/2). To establish the salt stress, sodium chloride (NaCl) was incorporated into the culture media at final concentrations of 0, 35, 70 and 105 mM for each treatment. The pH of the culture media was adjusted to 5.7 before solidification with agar at  $9\text{ g}\cdot\text{L}^{-1}$ . The culture media corresponding to the treatments described above were distributed in culture tubes ( $25 \times 150\text{ mm}$ ) which were filled up with 20 mL per tube before being sterilized by autoclaving at  $110^{\circ}\text{C}$  for 20 minutes.

## 2.3. Seed Disinfection and Germination Screening

The seeds of each hybrid variety were surface-disinfected with 70% alcohol, followed by soaking in a stirred batch of bleach (NaOCl) at  $8^{\circ}$  chlorometric sodium hypochlorite for 10 minutes. The sodium hypochlorite solution was diluted with sterile distilled water (2v.v). They were then rinsed thoroughly with a 3-time sterile distilled water. Each test tube was aseptically sown with two disinfected tomato seeds. However, after germination, only one seed was maintained in each tube. The experiments were incubated at a temperature of  $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$  in a culture chamber provided with neon incident light. The light intensity corresponded to a synthetically active radiation equivalent to  $75\text{ }\mu\text{E}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ , with a 16 h light/8 h night photoperiod. Thus, germination treatments consisted of 5 tomato varieties subjected to 4 different concentrations of salinity. For each saline treatment, three replicates of 24 test tubes each were used per variety, for a total of 12 replicates per variety.

For each variety and each saline treatment, a daily count of germinated seeds was performed and translated into cumulative germination percentage. The effect of NaCl was studied by calculating the final cumulative rate of germination (%) after 07 days of culture. The breakthrough of the radicle from the seed coats was used as the criterion for germination [31] [32] [33].

## 2.4. Agro-Morphological Parameters of Growth and Biomass Determination

The trials for the *in vitro* growth of tomato seedlings were conducted for 30 days. After 1 month of culture, the seedlings were removed from each tube, thoroughly washed in a sterile deionized water, to remove the agarified medium from the root systems, surface-wiped with blotting paper. The following agro-morphological parameters were determined: the height of the stem, the number of leaves, the length of the taproot, the number of secondary roots and the fresh weight of the aerial and root parts. They were evaluated using a ruler for the measurements and a Sartorius precision scale (accuracy: 0.0001) for fresh

and dry biomass determination. Following these measurements, the parts were separated from each other and dried in an oven (Binder brand) for 72 h at a temperature of  $70^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  before weighing the dry biomass of the aerial and root parts, respectively.

## 2.5. Statistical Analysis

The experiment was set up as a standard randomized design, with salt concentration chosen as a main factor variable and tomato variety as the subfactor variable. The collected data were subjected to a multiple comparison of means and to a variance analysis with two factors (Variety  $\times$  [NaCl]) by the test of Student-Newman-Keuls (SNK). Analyzes were carried out according to a general linear model by the R – 4.0.3 Software using the package “*agricoleae*”. For *in vitro* growth of seedlings under salt stress, differences between means were compared using the Student-Newman and Keuls test, and significance was determined at 95% confidence limits *i.e.* the significantly different means were discriminated by the SNK test at the p value of 5%.

## 3. Results

### 3.1. Effects of NaCl on *in Vitro* Germination of Five Tomato Seed Varieties

The different concentrations of NaCl (**Table 2**) used had a significant effect on the seed germination rate of the different varieties ( $P < 2 \times 10^{-16}$  and  $F = 68.543$ ). In addition, the results of the statistical analyses revealed a highly significant medium effect ( $P < 2 \times 10^{-16}$  and  $F = 13636.36$ ) and variety effect ( $P < 2 \times 10^{-16}$  and  $F = 168.73$ ), as well. In fact, for each variety, more the salt concentration increases, lower the final germination rate. However, *Ganila* and *Xewel* tolerated salinity up to [NaCl 70 mM], with a final germination rate of 65% while the other varieties recorded rates of 55% (*Mongal*) and 50% (*Lady Nema* and *Rodeo*), respectively. At [NaCl 105 mM], all varieties registered a significant decrease in their final germination rate. *Lady Nema* and *Mongal* recorded a 65% reduction

**Table 2.** Final germination rate (%) of 5 tomato seed varieties incubated under different NaCl treatments.

Varieties	Final Germination Rate (%)			
	[NaCl] (mM)			
	0	35	70	105
<i>Ganila</i>	100a	75.5b	65c	30d
<i>Lady Nema</i>	100a	75b	50c	35d
<i>Mongal</i>	100a	80b	55c	35d
<i>Rodeo</i>	87.7a	75b	50c	25d
<i>Xewel</i>	100a	75b	65c	30d

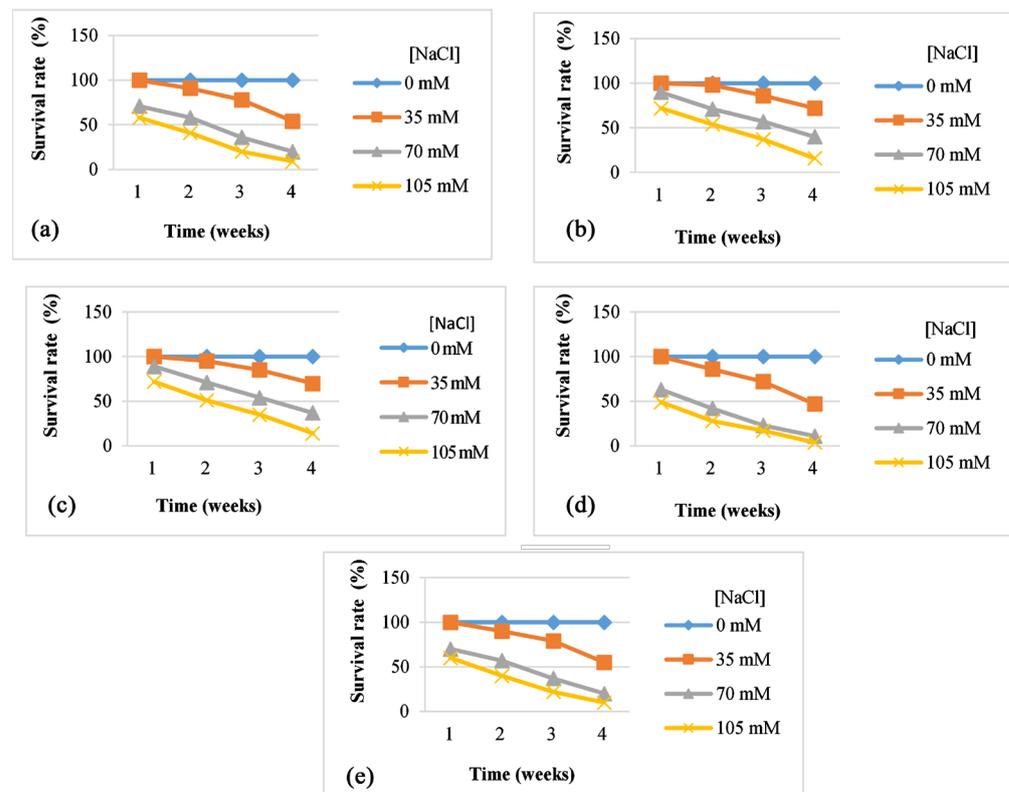
In lines, values followed by the same letter are not significantly different according to the Student-Newman-Keuls's test ( $P > 0.05$ ).

in their final germination rate, *Ganila* and *Xewel* 70% and, finally, *Rodeo* 71%. However, the *Lady Nema* and *Mongal* varieties recorded the best final germination rate (35%) in [105 mM of NaCl]. The *Rodeo* variety appeared to be more sensitive to the presence of salt in the culture medium with a drastic decrease, because its final germination rate fell from 87.7% in [NaCl 0 mM] to 25% in [NaCl 105 mM] while the *Lady Nema* and *Mongal* varieties are found to be the most tolerant, with germination rates reduction from 100% to 35%.

### 3.2. Effect of NaCl on *in Vitro* Early Vegetative Growth of Seedlings

#### 3.2.1. Survival Rate

**Figure 1** summarizes the evolution of the survival rates of vitroplants of five tomato varieties grown under increasing concentrations of NaCl: [0.35, 70 and 105 mM]. The survival rate of vitroplants gradually decreases over time for all varieties in the presence of salt, whereas it remains 100% constant for the control plants incubated in the culture medium devoid of salt. The survival rate of vitroplants in all varieties is inversely proportional to the salt concentrations of the culture media. At the end of the first week, in all varieties, vitroplants mortality is already observed with the two highest concentrations. However, the survival rate is greater at [NaCl 70 mM] than at [NaCl 105 mM]. The *Lady Nema* and *Mongal* varieties recorded a decrease of 28% in survival rate, depending on NaCl concentrations, followed by the *Xewel* (40%), *Ganila* (42%) and *Rodeo* (51%)

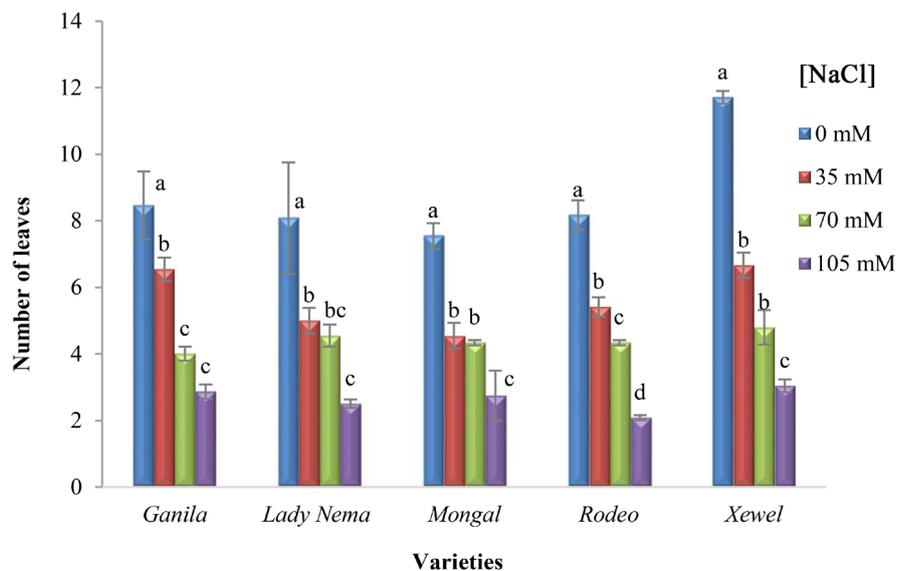


**Figure 1.** Evolution of vitroplants survival rates of five tomato varieties incubated under different NaCl treatments for 30 days. *Ganila* (a), *Lady Nema* (b), *Mongal* (c), *Rodeo* (d) and *Xewel* (e) varieties.

varieties. By the second week, the survival rate drops slightly in all varieties in the presence of [NaCl 35 mM]. The *Lady Nema* variety recorded the highest survival rate at [NaCl 35, 70 and 105 mM], with 98%, 71% and 54%, respectively, followed by the *Mongal* (95%, 71% and 51%), *Ganila* (91%, 58% and 41%), *Xewel* (90%, 57% and 40%) and *Rodeo* (86%, 42% and 28%) varieties. Only *Lady Nema* and *Mongal* varieties recorded a reduction rate less than 50%. By the third and fourth weeks, there is no change in the ranking of varieties with respect to their survival rate relative to salt concentrations. At the end of the fourth week, there is a significant decrease in the survival rate of 28%, 56% and 78%, respectively, at [NaCl 35, 70 and 105 mM] for the *Lady Nema* variety, which is more tolerant to the presence of salt. Under the same salinity conditions, it is followed by the *Mongal* (30%, 58% and 81%), *Xewel* (45%, 71% and 83%), *Ganila* (46%, 72% and 84%) and *Rodeo* (53%, 83% and 92%) varieties. However, taking into account [NaCl 0 and 105 mM], an 84% decrease in the survival rate of vitrop-ants is observed for the *Lady Nema* variety. The other varieties recorded a decrease, under the same conditions, of 86% (*Mongal*), 90% (*Xewel*), 91% (*Ganila*) and 96% (*Rodeo*).

### 3.2.2. Influence of Saline Stresses on the Average Number of Leaves

The increasing concentrations of NaCl provoked a significant decrease ( $P = 7.36 \times 10^{-05}$  and  $F = 4.845$ ) in the average number of leaves of the plants of the different varieties (Figure 2) after 30 days of culture. The statistical analyses revealed a highly significant medium effect ( $P < 2 \times 10^{-16}$  and  $F = 183.389$ ) and variety effect ( $P = 0.000531$  and  $F = 6.244$ ). Therefore, the decrease in the average number of leaves with increasing NaCl concentrations is significant in all varieties at [NaCl 35 mM]. However, the decrease is more marked in the *Xewel*

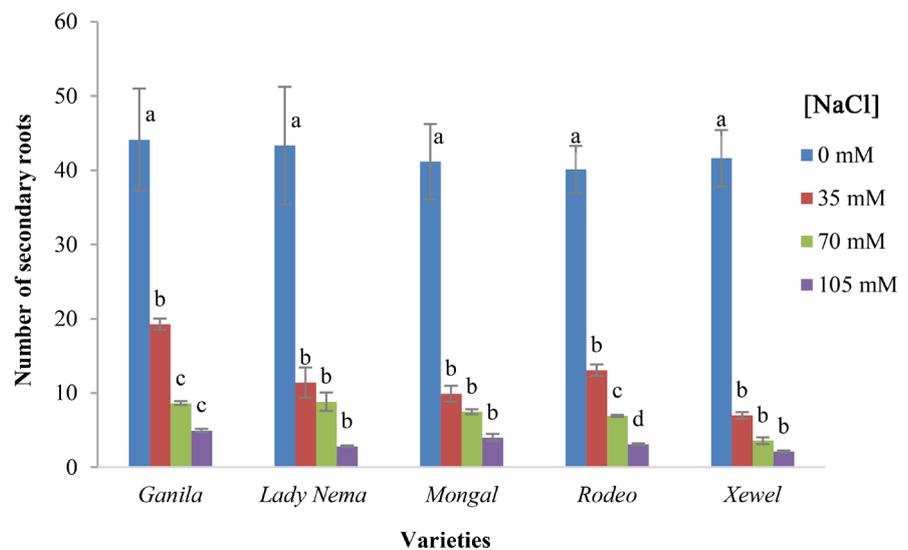


**Figure 2.** Effects of NaCl treatments on the number of leaves of five tomato (*Solanum lycopersicum* L.) varieties. For each variety, columns followed by the same letter do not differ significantly at 5% level.

variety with 43.1%, while it is less important in the *Ganila* variety with 22.66%. At [NaCl 70 mM], the reduction is still greater in the *Xewel* variety (59.04%), while the *Mongal* variety revealed the lowest reduction (42.53%). At the highest concentration (105 mM), the *Rodeo* variety was the most affected with a 74.46% reduction in the average number of leaves, while the *Mongal* variety still recorded the lowest reduction (63.56%). The variety *Xewel* recorded the highest average number of leaves with 11.71, 6.66, 4.80 and 3.04 leaves, respectively for [NaCl 0, 35, 70 and 105 mM]. The *Mongal* variety recorded the lowest average number of leaves (7.55 leaves) at the control concentration [NaCl 0 mM] and 4.55 leaves in the presence of [NaCl 35 mM] while the *Rodeo* variety obtained the lowest average number of leaves (2.09) at the highest applied concentration [NaCl 105 mM].

### 3.2.3. Influence of Saline Stresses on the Number of Secondary Roots

A significant reduction in the average number of secondary roots of vitroplants of different varieties is obtained with increasing concentrations of NaCl (Figure 3;  $P = 0.0452$  and  $F = 2.334$ ). The analysis of variance also revealed a highly significant medium effect ( $P < 2 \times 10^{-16}$  and  $F = 551.589$ ) and variety effect ( $P = 0.00552$  and  $F = 4.295$ ). This decrease in the average number of secondary roots compared to the control is very high at [NaCl 35 mM] with 56.37% in the *Ganila* variety, 67.39% in the *Rodeo* variety, 73.66% in the *Lady Nema* variety, 75.9% in the *Mongal* variety and 83.18% in the *Xewel* variety. At [NaCl 70 mM], the decrease in the average number of secondary roots is more marked in *Xewel* (91.35%), while it is lower in *Lady Nema* (79.61%) and *Ganila* (80.43%). At [NaCl 105 mM], the more sensitive *Xewel* variety recorded a decrease of 94.83% while the less sensitive *Ganila* variety recorded a decrease of 88.78%. The *Xewel* variety appeared more sensitive to the different saline constraints applied, whereas



**Figure 3.** Effect of increasing NaCl concentrations on the number of secondary roots of five tomato (*Solanum lycopersicum* L.) varieties. For each variety, columns followed by the same letter do not differ significantly at 5% level.

the *Ganila* variety is more tolerant. Indeed, the *Xewel* variety obtained 42, 7, 4 and 3 secondary roots and the *Ganila* variety 44, 19, 9 and 5 secondary roots at [NaCl 0, 35, 70 and 105 mM] respectively.

### 3.2.4. Impact of Salinity on the Length of the Aerial and Root Parts

The different NaCl concentrations have a significant effect ( $P < 2 \times 10^{-16}$  and  $F = 34$ ) on the length of the aerial part (LAP) of the different varieties (Table 3). In fact, a decrease in the height of the plants inversely correlated with the increase in NaCl concentrations from [35 mM] was observed. The *Lady Nema* variety is the most tolerant to saline stress because it records a decrease of 29.01% at [NaCl 35 mM], but this decrease is very marked (71.78%) at [NaCl 105 mM]. For this variety, lengths of 13.44, 9.54, 5.98 and 3.79 cm were also recorded at the respective concentrations of [NaCl 0, 35, 70 and 105 mM]. The *Mongal* variety appeared to be more sensitive, with a decrease in average stem height of 81.30% at [NaCl 105 mM]. In fact, 13.16, 5.05, 3.23 and 2.46 cm lengths are recorded for

**Table 3.** Mean comparison of shoot and root part lengths of vitroplants submitted to NaCl treatments for the five tomato (*Solanum lycopersicum* L.) varieties by Student-Newman-Keuls's test at the 5% threshold.

Varieties	[NaCl] (mM)	LAP (cm)	LPR (cm)	TLP (cm)	(LAP/TLP)/100	(LPR/TLP)/100	Reduction Rate LAP (%)	Reduction Rate LPR (%)
<i>Ganila</i>	0	13.14a	6.42a	19.56a	67.18	32.82	–	–
	35	9.23b	3.79b	13.02b	70.87	29.13	–29.76	–40.92
	70	4.55c	2.92c	7.47c	60.89	39.11	–65.40	–54.52
	105	3.13d	2.54d	5.67d	55.15	44.85	–76.20	–60.39
<i>Lady Nema</i>	0	13.44a	6.28a	19.72a	68.17	31.83	–	–
	35	9.54b	4.36b	13.90b	68.64	31.36	–29.01	–30.54
	70	5.98c	3.75c	9.73c	61.44	38.56	–55.52	–40.21
	105	3.79d	2.96d	6.75d	56.19	43.81	–71.78	–52.89
<i>Mongal</i>	0	13.16a	6.28a	19.44a	67.68	32.32	–	–
	35	5.05b	3.92b	8.97b	56.28	43.72	–61.64	–37.61
	70	3.23c	3.17c	6.40c	50.47	49.53	–75.45	–49.55
	105	2.46d	2.29d	4.76d	51.79	48.21	–81.28	–63.50
<i>Rodeo</i>	0	12.96a	5.97a	18.93a	68.46	31.54	–	–
	35	7.42b	4.17b	11.58b	64.03	35.97	–42.76	–30.20
	70	5.12c	2.79c	7.92c	64.72	35.28	–60.46	–53.22
	105	3.94d	2.21d	6.15d	64.07	35.93	–69.59	–62.98
<i>Xewel</i>	0	13.92a	6.25a	20.17a	69.03	30.97	–	–
	35	4.52b	2.83b	7.36b	61.49	38.51	–67.51	–54.65
	70	4.19b	2.84b	7.02b	59.61	40.39	–69.93	–54.59
	105	3.13c	2.23b	5.36c	58.34	41.66	–77.54	–64.25

For each variety, values in the same column followed by the same letter do not differ significantly at 5% level. LAP: length of Aerial Part; LPR: length of Plant Root; TLP: Total Length of Plant.

the aerial part at the respective concentrations of [NaCl 0, 35, 70 and 105 mM].

The average length of the taproot (LPR) decreases significantly under salt stress conditions ( $P < 3.11 \times 10^{-05}$  and  $F = 5.271$ ) as the different salt concentrations increase (**Table 3**). Indeed, there is a reduction of more than 50% of this length for all varieties. The *Xewel* variety appears to be more sensitive, with a reduction of 54.65% at [NaCl 35 mM]. However, in the presence of [NaCl 70 mM], this reduction regresses slightly to reach 54.59%. At [NaCl 105 mM], the *Xewel* variety recorded a reduction of 64.25%. Likewise, for vitroplants of this variety, taproot lengths of 6.25, 2.83, 2.84 and 2.23 cm are obtained at the respective concentrations of [NaCl 0, 35, 70 and 105 mM]. The *Lady Nema* variety appeared to be more salt tolerant, with a reduction of 30.54%, 40.21% and 52.89% at the respective concentrations of [NaCl 35, 70 and 105 mM]. This variety obtained taproot lengths of 6.28, 4.36, 3.75 and 2.96 cm at the respective concentrations of [NaCl 0, 35, 70 and 105 mM].

The length of the aerial part is greater than that of the root part for all varieties regardless of the applied saline stress. The importance of the development of the aerial part to the detriment of the root part is more marked in the *Rodeo* variety where the length of the aerial part almost triples that of the root part at different NaCl concentrations. For the other varieties, the length of the aerial part is twice or slightly longer than that of the root part at [NaCl 35 mM]. However, with the increase of salt concentrations, this difference decreases sharply to less than 17%. This difference is less marked in the *Mongal* variety, with less than 1% at [NaCl 70 mM] and less than 4% at [NaCl 105 mM].

### 3.3. Biomass Determination: Effects of Salt Stress on the Fresh and Dry Weights of the Aerial and Root Parts

**Table 4** reveals the effects of increasing NaCl concentrations on the fresh and dry aerial and root weight plants of the five tomato varieties. These increasing NaCl concentrations have a significant effect on the fresh and dry weights of the aerial parts (fresh weight:  $P = 5.59 \times 10^{-06}$  and  $F = 6.167$ ; dry weight:  $P = 9.28 \times 10^{-14}$  and  $F = 21.553$ ) and the fresh and dry weights of the root parts (fresh weight:  $P = 0.00126$  and  $F = 3.541$ ; dry weight:  $P = 0.002428$  and  $F = 3.258$ ).

Concerning the fresh aerial weight, it is significantly reduced in each variety by the NaCl concentrations. The *Xewel* variety is the most sensitive with a reduction of 91.61% at [NaCl 105 mM]. In fact, its weight went from 0.584 g to 0.049 g. The *Rodeo* variety is the least affected by salt. However, its weight increased from 0.369 g at [NaCl 0 mM] to 0.0623 g at [NaCl 105 mM] *i.e.* a reduction of 83.12%.

With regard to root weight, all varieties are severely and significantly affected by salt. Indeed, the *Lady Nema* variety which appears more tolerant recorded a decrease of 90.72% because its weight increased from 0.251 g at [NaCl 0 mM] to 0.0233 g at [NaCl 105 mM]. The more sensitive *Mongal* variety recorded 0.234 g at [NaCl 0 mM] and 0.0273 g at [NaCl 105 mM] *i.e.* a decrease of 95.73%.

The dry weights of all varieties decrease sharply with increasing NaCl concentrations in the medium. Aerial dry weights are greater than root weights in the

**Table 4.** Mean Comparison of the aerial and root part fresh and dry weights of the vitroplants of the five tomato (*Solanum lycopersicum* L.) varieties by the Student-Newman-Keuls's test at the 5% threshold.

Varieties	[NaCl] (mM)	AFW (g)	ADW (g)	RFW (g)	RDW (g)	TDW (g)	(ADW/TDW)/100	(RDW/TDW)/100	Reduction Rate ADW (%)	Reduction Rate RDW (%)
<i>Ganila</i>	0	0.552a	0.148a	0.257a	0.050a	0.198a	74.823	25.177	–	–
	35	0.231b	0.019b	0.059b	0.009b	0.028b	67.188	32.812	–87.230	–81.466
	70	0.074c	0.016b	0.019b	0.007b	0.023b	68.813	31.187	–89.459	–85.803
	105	0.072c	0.013b	0.01b	0.005b	0.018b	71.312	28.688	–91.149	–89.418
<i>Lady Nema</i>	0	0.542a	0.152a	0.251a	0.050a	0.202a	75.397	24.603	–	–
	35	0.194b	0.016b	0.03b	0.004b	0.020b	80.325	19.675	–89.605	–92.198
	70	0.102bc	0.013bc	0.026b	0.003b	0.016b	80.982	19.018	–91.316	–93.750
	105	0.055c	0.010c	0.02b	0.002b	0.012b	81.452	18.548	–93.355	–95.363
<i>Mongal</i>	0	0.507a	0.137a	0.234a	0.047a	0.184a	74.578	25.422	–	–
	35	0.119b	0.014b	0.019c	0.009b	0.023b	60.870	39.130	–89.781	–80.728
	70	0.111b	0.013b	0.027b	0.008b	0.020b	63.054	36.946	–90.657	–83.940
	105	0.046b	0.009c	0.01d	0.006b	0.015b	59.215	40.785	–93.504	–86.874
<i>Rodeo</i>	0	0.369a	0.119a	0.208a	0.041a	0.160a	74.468	25.532	–	–
	35	0.160ab	0.019b	0.037b	0.007b	0.026b	74.422	25.578	–84.034	–83.995
	70	0.106c	0.016b	0.029c	0.005bc	0.021b	75.133	24.867	–86.975	–87.426
	105	0.062d	0.014b	0.01d	0.004c	0.018b	77.398	22.602	–88.403	–90.123
<i>Xewel</i>	0	0.584a	0.162a	0.216a	0.043a	0.205a	79.140	20.860	–	–
	35	0.073b	0.010b	0.028b	0.008b	0.018b	54.142	45.858	–93.827	–80.164
	70	0.053b	0.009b	0.030b	0.007b	0.016b	57.223	42.777	–94.302	–83.841
	105	0.049b	0.007b	0.01c	0.005b	0.013b	57.399	42.601	–95.451	–87.190

For each variety, the values on the same column followed by the same letter are not significantly different at the 5% threshold. AFW: aerial fresh weight; ADW: aerial dry weight; RFW: root fresh weight; RDW: root dry weight; TDW: total dry weight of the vitroplants.

five varieties studied under all stress conditions. The reduction rate of the root part is greater than that of the aerial part for *Lady Nema* and *Rodeo* varieties, whereas the opposite is observed in the other varieties.

Regarding the aerial dry weights, the *Rodeo* variety tolerates the presence of NaCl better than the other varieties, with a weight reduction of 84.034%, 86.975% and 88.403% at the respective concentrations of [35, 70 and 105 mM of NaCl]. For the same conditions, the more sensitive *Xewel* variety recorded reductions of 93.827%, 94.302% and 95.451%. The *Rodeo* variety recorded the highest dry weights at the concentrations of [35, 70 and 105 mM of NaCl] with 0.019, 0.016 and 0.014 g, respectively, while under the same conditions of saline constraints, the *Xewel* variety recorded the least significant weights at 0.010, 0.009 and 0.007 g.

Concerning dry root weights, the *Mongal* variety is the most tolerant of all with reductions of 80.728%, 83.940% and 86.874% at the respective concentra-

tions of [NaCl 35, 70 and 105 mM] while the *Lady Nema* variety, under the same conditions, records reductions of 92.198, 93.750 and 95.363. The *Mongal* variety obtained the highest root dry weights with 0.009, 0.008 and 0.006 g, respectively at the concentrations of [NaCl 35, 70 and 105 mM] unlike the *Lady Nema* variety, which recorded the lowest root dry weights, at 0.004, 0.003 and 0.002 g.

The weight of the aerial part of vitroplants in all varieties is greater than that of the root part. Indeed, from 35 to 105 mM of NaCl, the difference between these two weights is accentuated except for the *Mongal* variety where a slight improvement is noted at [NaCl 105 mM] compared to [NaCl 70 mM]. For the *Lady Nema* variety, the aerial dry weight represents more than 80% of the total dry weight of the vitroplants at all NaCl concentrations. For the *Xewel* variety, the proportion of aerial dry weight to the total dry weight of the vitroplants is the lowest of all (between 59% and 63%).

## 4. Discussion

### 4.1. *In Vitro* Seed Germination

This study showed that a saline constraint has a significant and depressive effect ( $p < 2 \times 10^{-16}$ ) on the final germination rate of the different tomato varieties studied, alike the research carried out by [34]. Germination is the first physiological stage affected by salinity, it is thus an essential phase for early identification of varieties tolerant or not to salinity. Indeed, the germination process is very critical for plant establishment and growth specifically in the presence of adverse environmental constraints [35] such as a salinity stress. For this purpose, five different genotypes of hybrid F1 tomato seeds were screened *in vitro*, in the presence of increasing concentrations of NaCl [0, 35, 70, 105 mM], at the germination stage, in order to discriminate the salt-tolerant varieties versus salt stress sensitive ones. As the concentration of NaCl introduced into the *in vitro* culture medium increases, the final germination rate decreases drastically and significantly, whatever the variety tested. The varietal response to stress is diverse and variable, hence the identification of tolerant varieties and susceptible ones. The *Rodeo*, *Ganila* and *Xewel* varieties are the most sensitive because they were strongly impacted by the presence of [NaCl 105 mM] in the medium with a significant reduction of 71.5%, 70% and 70% of the final germination rate, respectively. The more tolerant *Lady Nema* and *Mongal* varieties nevertheless recorded a significant 65% reduction in their final germination rate. Indeed, the lowest germination rates are obtained *in vitro* in culture media with a supplement of the highest concentration of [NaCl 105 mM]. At this high concentration, the final germination rates, the most significantly high, are obtained from seeds of the *Lady Nema* and *Mongal* varieties and corresponds to 30%. On the contrary, the lowest final germination rate is observed with the seeds of the *Rodeo* variety, at 25%, after 7 days of culture. Similarly, [36] obtained a decrease in the germination capacity of different species of tomatoes, 46% at [NaCl 190 mM] and 100% at [NaCl 265 mM]. The author [37] obtained a 70% reduction in germination in

two tomatoes species. Our study reveals a reduction of more than 60% in the final germination rate at [NaCl 105 mM], while [38] obtained in her work a reduction of more than 70% at only [NaCl 100 mM]. The authors [39] obtained similar results with 40% of germination at 4.97 g·L<sup>-1</sup> of NaCl with the *Mongal* variety and 20% with the *Campbell* 33 variety. Multiple disturbances on the metabolism, growth and development of plants at the cellular, molecular, biochemical and physiological levels are observed when the plant is confronted with high concentrations of NaCl [15] [40] [41]. The decrease in the germination percentage is due either to an increase in the external osmotic pressure [42] which affects the water absorption by the seeds [43], or to an accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions in the cells of the embryo. The difficulty of water absorption affects the elongation of the radicle but germination can take place with low water potential thanks to certain growth regulators [44]. The seeds did not have time to put in place mechanisms allowing them to tolerate the presence of salt and thus absorb the optimal quantity of water and manage the excess of Na<sup>+</sup> and Cl<sup>-</sup> ions.

At low concentration of NaCl, such as 35 mM, seeds of all tomato varieties germinate: a slight decrease in the final germination rate is observed globally, but the lowest rate reached 75% for the *Rodeo* and *Xewel* varieties. For a concentration of 70 mM of NaCl, the final germination rates are reduced by half. They reached 50% for *Lady Nema* and *Rodeo* varieties. In addition to reducing the germination rate for sensitive cultivars, salt stress also delays germination and slows its speed. The decrease observed may be due to the alteration of enzymes and hormones contained in the seeds [45] or to a problem of seed hydration due to a high osmotic potential, which inhibits the emergence of the radicle off husks [46]. It would have been beneficial to introduce the increasing concentrations of salt gradually into the culture media to prevent rapid osmotic stress and ionic toxicity of the NaCl as recommended by [47].

#### **4.2. Effects of NaCl on *in Vitro* Early Vegetative Growth of Vitroplants**

In terms of growth and development parameters, the response of vitroplants to salt stress of the five tomato varieties varies depending on the parameter. According to the results obtained in this study, salt stress causes a delay in plant growth. The salt stress induced a reduction of more than 70% in the size of the young plants. This decrease is more marked in the *Xewel* variety. The authors [48] obtained a reduction in plant height of 26% in the presence of [NaCl 70 mM]. The reduction in plant growth can be explained by the fact that NaCl causes an increase in the osmotic pressure of the culture medium which prevents the absorption of water by the root system [49]. The reference [50] showed that salinity affects the plant growth at different stages of its life cycle by increasing the osmotic pressure of the soil solution, favoring the accumulation of certain ions in toxic concentrations in the plant tissues and by altering its mineral nutrition. Thus, the roots being in contact with high concentrations of NaCl lose water for the benefit of the culture medium, leading inside the plant to a decrease

in volume, elongation and cell divisions. Reduced growth may also result from increased abscisic acid concentration in the aerial part or reduced cytokinin concentrations [51].

Some varieties used in this study appeared to be sensitive to salt stress, with a reduction of more than 60% in the number of their leaves. However, [48] reported an 11% reduction in the number of leaves in tomato in the presence of [NaCl 70 mM]. In agreement with these results, previous studies had shown that tomato plants affected by salinity tend to reduce their number of leaves [52]. This reduction can be as much as 10%, which reduces photosynthesis [53]. The authors [54] explained this reduction in the number of leaves by a specific harmfulness of the Cl<sup>-</sup> ions accumulated at levels exceeding the compartmentalization capacity. According to [41], vegetative growth and, particularly leaf expansion, is severely inhibited by salt stress. The new leaves develop slowly and the senescence of the old ones accelerates. On the other hand, the toxicity of Na<sup>+</sup> ions on plants, in particular at the leaf scale can result in a significant decrease in the growth rate but also in the leaf area and the leaf elongation rate of seedlings, especially in grasses (wheat, rice, maize, etc.) and *Solanaceae* [55]. The reduction in growth rate is not linked specifically to the addition of NaCl but to a transient change in the plant-water relationship.

This study also revealed a decrease in the number of secondary roots. Similarly, [56] also observed a clear reduction in root volume under saline stress. These different observations are also cited by different authors as being one of the causes of the reduction in growth and vegetative productivity [57] [58], which is in accordance with our study. Thus, the presence of NaCl in the culture media causes a reduction in the fresh and dry aerial and root weights. In this experiment, the weight of the aerial parts is greater than that of the root parts; the plants favor the development of the leaves and stems to the detriment of the roots [49] [59].

The early events of plant adaptation to stress begin with the mechanisms of perception followed by signaling through the transduction of signals and messengers to activate various physiological and metabolic responses, including expression of stress response genes. The main cellular reactions developed by the plant in order to face and adapt to salt stress are inevitably preceded by a cascade of signaling and regulatory elements, which can take different pathways involving in particular that of calcium, abscisic acid (ABA), Mitogen-Activated Protein Kinases (MAPKinases), Salt Overly Sensitive” proteins (SOS) and ethylene [60].

A salt constraint provokes a depressive and significant effect on the agromorphological parameters studied. Indeed, seedlings under saline stress preferentially accumulate Na<sup>+</sup> (and Cl<sup>-</sup>) ions in the aerial parts of rice leaves, in particular in the leaf apoplast [61]. According to their results, salt stress causes a delay in plant growth. In fact, all the varieties are affected by salt with a significant reduction of more than 70% in the length of the aerial part while the reduction in the length of the root part is more than 50%. Salt affects aerial growth more than root growth in all varieties except for the *Rodeo* variety in which

these two parts are affected with the same intensity, because the rate of reduction of these two parts are almost identical. The *Mongal* variety is more sensitive to salt, with a 81.28% reduction in the length of the aerial part at [NaCl 105 mM] while the length of the underground part in the *Xewel* variety is more affected (64.25% reduction). The reference [48] obtained a 26% reduction in plant height in the presence of [NaCl 70 mM]. The reduction in the growth of vitroplants can be explained by the fact that NaCl causes an increase in the osmotic pressure of the medium, which prevents the absorption of water by the root system [49]. The authors [50] showed that salinity affects the growth of the plant at different stages of its development cycle by increasing the osmotic pressure of the soil solution, thus promoting the accumulation of certain ions in toxic concentrations in plant tissue and altering its mineral nutrition. Thus, the roots being in contact with high concentrations of NaCl lose water for the benefit of the culture medium. This leads to a decrease in volume, elongation and cell divisions. Besides, the osmotic effects of salt stress can also limit root growth and subsequently the possibilities of absorption of nutrients from the soil [15]. Indeed, the Na<sup>+</sup> and Cl<sup>-</sup> ions can interfere with transporters located on the plasma membrane of the roots, such as for example, the selective channels of K<sup>+</sup> [62].

Reduced growth may also result from increased abscisic acid concentration in the aerial part or reduced cytokinin concentrations [51]. Osmotic stress triggers the production of ABA in the roots, which is then transported to the leaves, causing the stomata to close. However, [63] and [64] explain this by a combination of the osmotic effect and the specific effect of Na<sup>+</sup> and Cl<sup>-</sup> ions. The accumulation of Na<sup>+</sup> ions in the plant limits the absorption of essential cations such as Ca<sup>2+</sup> and K<sup>+</sup> [16]. The K<sup>+</sup> ion, for example, is involved and required in the activation of more than 50 enzymes while Na<sup>+</sup> ions cannot provide the same function at the level of cellular metabolism. A nutritional imbalance and a reduction in plant growth occur when these essential ions (Ca<sup>2+</sup> and K<sup>+</sup>) become limiting [16] [65]. A competition occurs between the Na<sup>+</sup> - K<sup>+</sup> ions for their binding to enzymes and important proteins [66]. In addition, the K<sup>+</sup> ion is vital for the synthesis of certain proteins (inhibited by the presence of Na<sup>+</sup>, the osmoregulation of plant cells, the maintenance of cell turgor, the stimulation of photosynthesis, the binding of tRNA to ribosomes (Translation affected), *etc.*

### 4.3. Biomass Determination

Plants are not equal when it comes to salt stress. Some are sensitive while others are tolerant to salinity. This determines their biomass production in the presence of salt and governs their classification into four categories: True halophytes (*Salicornia sp.*, the mangrove, *Spartina sp.*, *Cakile maritima*), Facultative halophytes (*Plantago maritima*), Resistant non halophytes (*Hordeum sp.*), Glycophytes or halophobes (*Phaseolus vulgaris*, *Glycine max*, *Solanum sp.*) [67]. In all plant species, both halophytes and glycophytes, the salinity of the environment leads, above a certain threshold, to a reduction in the produced biomass. However, the degree of inhibition of growth depends on the genus, species, variety as

well as the stage of development of the plant and the nature of the organ being measured [68]. Likewise, in our experiments, the aerial dry weights are greater than those of the roots in the varieties examined. However, the reduction rate of these parts differs depending on the variety. In fact, the reduction rate of the root part is greater than that of the aerial part in *Lady Nema* and *Rodeo* varieties, while the reverse is noted in the other varieties. At the highest concentration [NaCl 105 mM], taking into account all the varieties, the reduction rates of the two parts do not differ too much, as they are between 88% and 95%. The authors [59] also observed a marked decrease in root volume under saline stress. Several authors have also observed a reduction in the growth of plants under saline stress [60] [61]. This is in agreement with our work. In this experiment, the average weight of the aerial parts is greater than that of the root parts and vitrop-lants reveal better development of leaves and stems to the detriment of the roots [49] [62]. However, the depressive effect of NaCl at high concentration is in general more marked in the aerial organs than in the root organs. This difference in sensitivity between absorption organs and photosynthetic organs is described as characteristic of glycophytes [69]. Indeed, the inhibition of root growth is generally less marked than that of the aerial parts for which an accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions can inhibit growth and become toxic to the plant [70]. This would be a strategy developed by the plant to accumulate resources and energy that allow it to fight against salt stress [71].

Salinity tolerance in plants has several aspects: detoxification, regulation and restoration of homeostasis, and growth control. Among the mechanisms of plant salt tolerance is the exclusion and compartmentalization of Na<sup>+</sup>. This is the most effective strategy for avoiding the toxicity of Na<sup>+</sup> at metabolic sites in the cytoplasm. Thus, in barley, tomato and tulip, it has been shown that Na<sup>+</sup>/H<sup>+</sup> activity at the root level increases in response to the presence of Na<sup>+</sup> ions [72] [73] [74]. In addition, overexpression of the *NHX1* gene promoter improves tolerance to salinity in *Arabidopsis* [75], tomato [76] and rice [77]. The regulation of ionic homeostasis involves the SOS (Salt Overly Sensitive) pathway. With regard to osmotic adjustment, an important component of salinity tolerance in plants, it is affected by the accumulation of Na<sup>+</sup> in the vacuole, which causes a local drop in the osmotic potential. Another strategy is developed by plants. It consists in synthesizing and accumulating osmoprotectors in the cell cytoplasm which do not inhibit important biochemical reactions. These osmoprotectors vary according to the species but the most widespread are simple or complex sugars (trehalose, raffinose, etc.), derivatives of tertiary amino acids (proline, glycine betaine, etc.) and polyols. The main properties of osmoprotectors are the preservation of the activity of enzymes in saline solutions [78], the stabilization of the protein structure and the detoxification of ROS (Reactive Oxygen Species) [79].

## 5. Conclusion

The reaction of plants to salt stress is through adaptive, morphological, anatom-

ical, structural and metabolic changes [68]. To determine the tolerance or sensitivity of tomato plants to salinity, experiments carried out *in vitro* allowed to examine the effects of increasing concentrations of [NaCl 0, 35, 70 and 105 mM] on seed germination and the growth of young plants from 5 varieties of tomato cultivated in Senegal. Under *in vitro* culture conditions, the study of the morphological response at the seedling stage showed that the five varieties of tomato (*Solanum lycopersicum* L.) exhibit different levels of sensitivity, with respect to the applied salt concentrations. The increasing concentrations of NaCl significantly affect the germination process, growth and development of plants of all tomato varieties. According to the *in vitro* experiments conducted, tomato seeds are able to germinate and develop under salt stress, even in the presence of the highest concentration of [NaCl 105 mM]. Taking into account all analyzes carried out, the tomato varieties can be classified according to their degree of tolerance to salinity:

- a first group formed by the vigorous and fairly tolerant *Lady Nema* and *Mongal* varieties;
- a second group made up of varieties *Ganila* and *Xewel*, slightly less vigorous, and therefore moderately sensitive;
- and, a third group composed by the *Rodeo* variety, which is not very vigorous and is sensitive to salinity.

Finally, these results will be of an important contribution to a better management of the cultivation of tomato varieties in semi-arid or arid zones where the quality of agro-ecological soils becomes unfavorable to this agronomic speculation because of the natural or the water irrigation-related salinization of lands.

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### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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