American Journal of Plant Sciences, 2021, 12, 1478-1502
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

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

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How to cite this paper: Sané, A.K., Diallo, B., Kane, A., Sagna, M., Sané, D. and Sy, M.O. (2021) *In Vitro* Germination and Early Vegetative Growth of Five Tomato (*Solanum lycopersicum* L.) Varieties under Water Stress Conditions. *American Journal of Plant Sciences*, **12**, 1478-1502.
https://doi.org/10.4236/ajps.2021.1210105

Received: August 31, 2021
Accepted: October 15, 2021
Published: October 18, 2021

Abstract

Water is the main limiting factor in the cultivation of tomato (*Solanum lycopersicum* L.) in Senegal. Thus, the selection of varieties tolerant to water stress would be an alternative solution for their production. *In vitro* germination, growth, total chlorophyll and proline levels were studied in five varieties of tomato subjected to increasing osmotic pressures (0, 5, 10 and 15 kPa) thanks to the PEG-8000 incorporated in an MS/2 medium for 30 days. A strong sensitivity to water stress for *in vitro* seed germination in the *Rodeo* variety (41%) is recorded at 5 kPa and maintained at 15 kPa (20.83%) while it was only noticed at 15 kPa in the other tomato varieties. The *Xewel* and *Lady Nema* varieties obtained the smallest reductions in the number of leaves of vitroplants, with 30.79% and 27.97% at 15 kPa, respectively, and the *Rodeo* variety recorded a reduction of 35.97%. From 5 kPa, the varieties record reductions in the number of secondary roots of more than 15%. The effect of osmotic pressures on decreasing the taproot height and length is not significant. The *Xewel* variety had the highest average fresh (0.483 g) and dry (0.082 g) weights of the aerial part at 15 kPa and the *Rodeo* variety had the lowest ones (0.308 g and 0.0501 g). The *Lady Nema* variety had the highest average fresh (0.171 g) and dry (0.039 g) root weights and the *Rodeo* variety had the lowest ones (0.086 and 0.020 g). The vitroplants of *Rodeo* variety recorded the highest decreases in total chlorophyll contents at all osmotic pressures and the lowest increase in proline content (53.37%) at 15 kPa. *A contrario*, the *Xewel* variety recorded the greatest increase in proline content (116.26%). Ultimately, the vitroplants of *Lady Nema* and *Xewel* varieties were more tolerant to water stress, the *Ganila* and *Mongal* varieties were moderately tolerant and the *Rodeo* variety was the most sensitive.

Keywords

Solanum lycopersicum, PolyEthylene Glycol, Water Stress, Germination, Growth, Tolerance, *In Vitro* Conditions

1. Introduction

The tomato (*Solanum lycopersicum* L.) is one of the very important vegetable crops in the world and, in particular, in Senegal. Globally, production and cultivated areas are constantly increasing [1] *i.e.* an increase of 5.08% and 2.55%, respectively, from 1960 to the present [2]. Despite the possibility of cultivating this species on a large scale, in Senegal tomato yields (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 water deficit of agricultural lands. Furthermore, the negative impacts of climate change are becoming more and more noticeable for the growth and development of plants, especially in semi-arid and arid areas [3]. They are characterized by annual rainfall between 300 and 600 mm of water per year for semi-arid zones, and between 100 and 300 mm of water per year for arid zones, whereas they hardly exceed 100 mm of water per year for desert areas [4]. It is estimated that around 40% of cultivated areas in the world are subject to drought [5]. Under these conditions, the physiology of plants is disturbed, and this is especially the case in tomatoes [6].

Water has a fundamental role in the development of plants as it participates in the metabolic and physiological activities by serving as a vector for nutrients. It also ensures the thermoregulation of plants by evapotranspiration and participates in photosynthesis as well as the permanent upright growth of plants thanks to the water pressure inside the cells. It is, thus, the first limiting factor for plant production [7] and for plant growth in agroecosystems and this production factor will become more and more restrictive due to global warming, especially in the Sahel areas. The consequences of water stress are essentially a decrease in growth as well as a reduction in photosynthetic activity, thus affecting the yield and causing the death of the plant if the stress persists. Water deficit also induces oxidative stress with the formation of free radicals. By their unstable nature, these active forms of oxygen are very harmful to cellular constituents, in particular, to membrane lipids [8]. The consequences of the water deficit can also be the reduction of gas exchanges, of the assimilation of CO_2 *via* the reduction of stomatal conductance following the closure of the stomata [9] as well as the lowering of cellular water potential and the quantity of water contained in cells [10]. Morphological changes such as abscission of leaves [10] and fruits have been observed following manifestations of stress. At the reproductive development level, the effects of water stress vary depending on the development phase of the plant, were been shown in tomatoes [11]. The survival of a plant under stressful conditions depends on its ability to perceive stress, generate and transmit the signal to different parts of the plant and to initiate a set of physiological and chemical modifications. In response to a water deficit in the soil, depending on its intensity, the plant sets up mechanisms to adapt and maintain a water status favorable to its development, *i.e.* the establishment of its organs and their growth as well as the establishment of a symbiotic interaction of the mycorrhizal type at the level of its recovery system. Thus, the plant promotes the development and activity of the root system to better explore soil and extract water from them [12].

In Senegal, during the last decades, drought and salinity have caused serious damage in arable areas making any agricultural activity difficult. This situation is at the root of many serious socio-economic problems. Indeed, the water deficit accompanied by a rise in salts considerably limits agricultural yields in the soils. This inevitably pushes local populations to abandon their land and to migrate from rural areas to urban centers. Indeed, the water deficit accompanied by a rise in salts considerably limits agricultural yields in the soils. It is, therefore necessary even imperative, to seek methods and cultivation practices which would aim to develop, in cultivated plants, in this case in tomatoes, mechanisms of resistance to water and salt stress, and, thus, to contribute to the restoration of agricultural activities and the rehabilitation of abandoned lands. This would promote the return and stabilization of rural populations. In order to achieve such a result, it would be strategic to be able to use tomato varieties that are more tolerant to water stress. In this context, this study aims at evaluating the effects of increasing osmotic pressures on the *in vitro* germination and the physiology of the growth of different varieties of tomato with a view to identify the underlying mechanisms of adaptation involved in young vitroplants subjected to water stress.

2. Materials & 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, with an 85% minimum germination rate and a varietal purity of 99%. They were stored at an average temperature of 4˚C ± 1˚C.

2.2. Culture Conditions

The basic culture medium used is that of Murashige and Skoog [13], the macro-elements of which were diluted by half (MS/2). To establish the water stress,

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

VARIETIES	ORIGIN	CHARACTERISTICS
Ganila	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 *Fusarium*, Resistance to TMV (Tobacco Mosaic Virus)
Lady Nema	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 *Fusarium*
Mongal	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), *Fusarium*, *Stemphylium spp*, *Ralsonia solacerum*, *Meloidogyne spp*.
Rodeo	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)
Xewel	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 *Fusarium*, Resistance to TMV (Tobacco Mosaic Virus)

different concentrations of polyethylene glycol (PEG-8000) were incorporated into the culture media: 0 - 9.915 - 17.211 and 23.267 $g \cdot L^{-1}$ corresponding, to the osmotic pressures of 0, 5, 10 and 15 kPa, respectively, according to Michel's equation [14]. The pH of the culture media was adjusted to 5.7 before solidification with agar at 9 $g \cdot L^{-1}$. The culture media, for each treatment described above, were distributed in culture tubes (25 × 150 mm), filled up with 20 mL each before sterilization by autoclaving at 110˚C for 20 minutes.

2.3. Seed Disinfection and Germination Screening

The seeds of each hybrid variety were surface-disinfected following the procedure adopted for the application of a saline stress on tomato seeds. The incubation conditions for the experiments in the culture chamber were also identical [15].

For each variety and each water stress treatment, a daily count of germinated seeds was performed and translated into cumulative germination percentage. The effect of the different osmotic pressures, applied to simulate water stress, 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 [16] [17] [18].

2.4. Agro-Morphological Parameters of Growth and Biomass Determinatio

The trials for the *in vitro* growth and development of tomato seedlings were conducted for 30 days. The criteria and methods for evaluating the agro-morphological parameters of growth as well as the determination of the fresh and dry biomasses of the aerial and root parts were carried out as for vitroplants submitted to salt stress [15].

The Susceptibility Index (SI) was used to classify varieties according to their ability to tolerate or not the water stress. This index was calculated from the aerial dry weights of the vitroplants and according to Slama's equation [19]:

$$SI = \left(\left(Ps - Pt\right)/Pt\right)*100$$

SI = Susceptibility Index, expressed as a %,

Ps = Aerial dry weight of vitroplants raised under water stress,

Pt = Aerial dry weight of control vitroplants.

The survival rate of vitroplants for each condition was also calculated and expressed as a percentage (%).

2.5. Biochemical Parameters

2.5.1. Measurement of Total Chlorophyll Contents

To assess the sensitivity or tolerance of the varieties to water deficiency, the average chlorophyll content was determined according to the method described by Makeen *et al.* [20]. So, 20 mg of fresh leaves from each sample were ground in 7 mL of a 80% acetone solution (80 mL of acetone + 20 mL of 2.5 mM sodium phosphate, pH = 7.8). The ground material was poured into a Falcon tube, centrifuged at 4500 g at 4˚C for 10 min and finally incubated at 4˚C in the dark for 24 h. After incubation, the supernatant was assayed by measuring the absorbance of chlorophyll with a spectrophotometer (Evolution 300 UV-VIS, accuracy: ±0.15 nm). The total chlorophyll contents were determined by measuring the absorbance of chlorophylls a and b compared to that of a control made with 80% acetone. The amount of total chlorophyll was calculated thanks to the Arnon's equation [21]:

$$C = \left[\left(20.2\left(A_{645}\right) + 8.02\left(A_{663}\right)\right)*V\right]/M$$

A_{663} = Absorbance of chlorophyll a; A_{645} = Absorbance of chlorophyll b; V = Extraction volume (mL); M = Mass of crushed fresh leaves (mg).

The amount of total chlorophyll (C) is expressed in milligrams per gram of fresh matter.

2.5.2. Proline Content Determination

To assess the water deficit tolerance levels of tomato varieties, the average proline amounts accumulated by vitroplants were determined by colorimetric method as described by Monneveux and Nemmar [22]. The extraction was carried out from a composite mixture of 100 mg of fresh leaf segments from three vitroplants per treatment. The concentration of proline was determined with a spectrophotometer (Evolution 300 UV-VIS, accuracy: ±0.15 nm) by measuring the optical density (OD) at λ = 520 nm. The proline contents were determined according to a calibration curve *i.e.* a standard calibration curve, prepared and constructed with a range of known and increasing proline concentrations:, from 0 to 800 µmoles (**Figure 1**).

2.6. Statistical Analysis

The overall experiment was set up as a standard randomized design with osmotic pressure chosen as the primary factor variable and tomato variety as the sub-factor variable. The collected data were subjected to a multiple means comparison and to a two-factor (Variety × osmotic pressure) analysis by the Student-Newman-Keuls test (SNK) for vitroplant growth under water stress. Analyzes were carried out according to a general linear model by the R-4.0.3 software using the "*Agricoleae*" package. The 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. *In Vitro* Germination of Tomato Seeds under Water Stress

3.1.1. Influence of Water Stress on the Final Rate of Seed Germination

Table 2 shows the effect, after 7 days, of increasing concentrations of PEG-8000 on the average final seed germination rate of the five tomato varieties. The mean final seed germination rates varied very significantly ($F = 196.5$; $P = 2 \times 10^{-16}$), with increasing osmotic pressure. The results of the analysis of variance reveal both a very significant medium effect ($F = 4589$; $P < 2 \times 10^{-16}$) and a variety effect ($F = 7834$; $P = 2 \times 10^{-16}$).

The average final germination rate decreased as the osmotic potential increases but the magnitude of this decrease varies among varieties. The osmotic pressure of 5 kPa has appeared as a sensitivity threshold from which significant germination losses have manifested in seedlings of the *Rodeo* variety, while this threshold is not reached for the other varieties.

The *Xewel* and *Lady Nema* varieties tolerated the highest osmotic pressure with a decrease in their average germination rate of 16.7% and 25%, respectively.

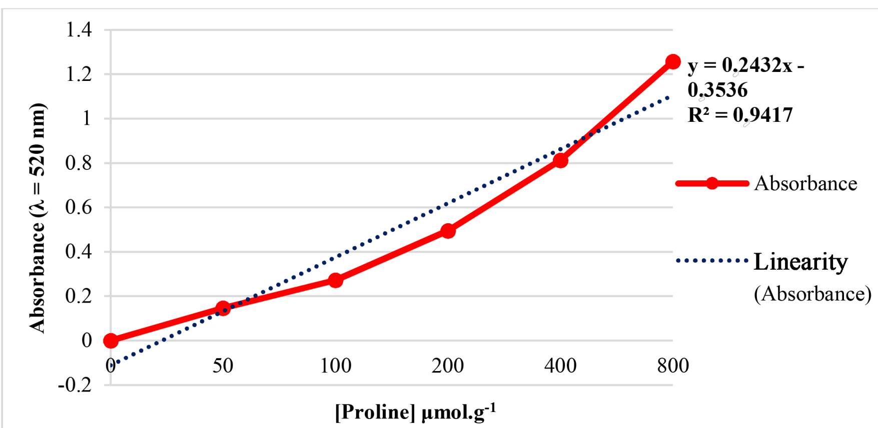

Figure 1. Standard calibration curve for proline constructed with a series of standard increasing concentrations [0, 50, 100, 200, 400 and 800 µmol·g^{-1}].

Table 2. Effect of increasing osmotic pressures on the final average seed germination rate of the five tomato varieties (*Solanum lycopersicum* L.).

Final Germination Rate (%)				
Varieties	Osmotic Pressure (kPa)			
	0	5	10	15
Ganila	100a	83.33b	79.17c	70.83d
Lady Nema	100a	87.5b	83.33c	75d
Mongal	100a	83.33b	79.17c	62.5d
Rodeo	**74.17a**	**41b**	**33.33c**	**20.83d**
Xewel	100a	95.87b	87.5c	83.33d

For each variety, the values followed by the same letter are not significantly different at the 5% level.

In contrast, the *Ganila* variety recorded a decrease of 29.2%, the *Mongal* variety 37.5% and the *Rodeo* variety 71.91%. The *Xewel* variety recorded the best average final seed germination rates at all PEG concentrations, namely 100%, 95.8%, 87.5% and 83.3%, at the respective osmotic pressures of, 0, 5, 10 and 15 kPa. On the other hand, the *Rodeo* variety recorded at these same pressures the lowest average germination rates of seeds, *i.e.* 74.17%, 41%, 33.33% and 20.83%.

3.1.2. Influence of Water Stress on Seed Germination Kinetics

Figure 2 displays the seed germination kinetics of the five varieties studied under the effect of water constraints. The germination rate increased over time to its maximum in all varieties.

At 0 kPa, more than 50% of the seeds germinated, 48 hours after sowing, except for the *Rodeo* variety (25%). The *Lady Nema* and *Xewel* varieties registered 100% germination rate on the 3rd day after sowing while the *Ganila* and *Mongal* varieties only reached 100% germination rate on the 4th day. The *Rodeo* variety recorded only a germination rate of 83.33% but, for other osmotic pressures applied, no variety reached 100% of germination rate, 7 days after sowing. The osmotic pressures lengthened the germination time of the seeds which resulted in a slow and gradual increase in the germination rates compared to the control ones for which the maximum germination rate is quickly reached. Indeed, at 5 kPa, the *Ganila* and *Lady Nema* varieties reach maximum germination after 5 days after sowing. This period is 6 days for the *Mongal* variety and 7 days for the *Rodeo* and *Xewel* varieties. At 10 and 15 kPa, the maximum germination rate is reached after 7 days in all varieties.

3.2. Influence of Water Stress on the Survival Rate of Vitroplants

The survival rate of vitroplants decreased significantly ($F = 91.18$; $P = 3.25 \times 10^{-04}$) in all varieties with increasing osmotic pressure and time (**Table 3**).

The *Xewel* and *Lady Nema* varieties recorded a slight decrease of 13% and 24% respectively, in their survival rates for vitroplants at 15 kPa on the first week. This decrease is accentuated over time to reach 39% in the *Xewel* variety

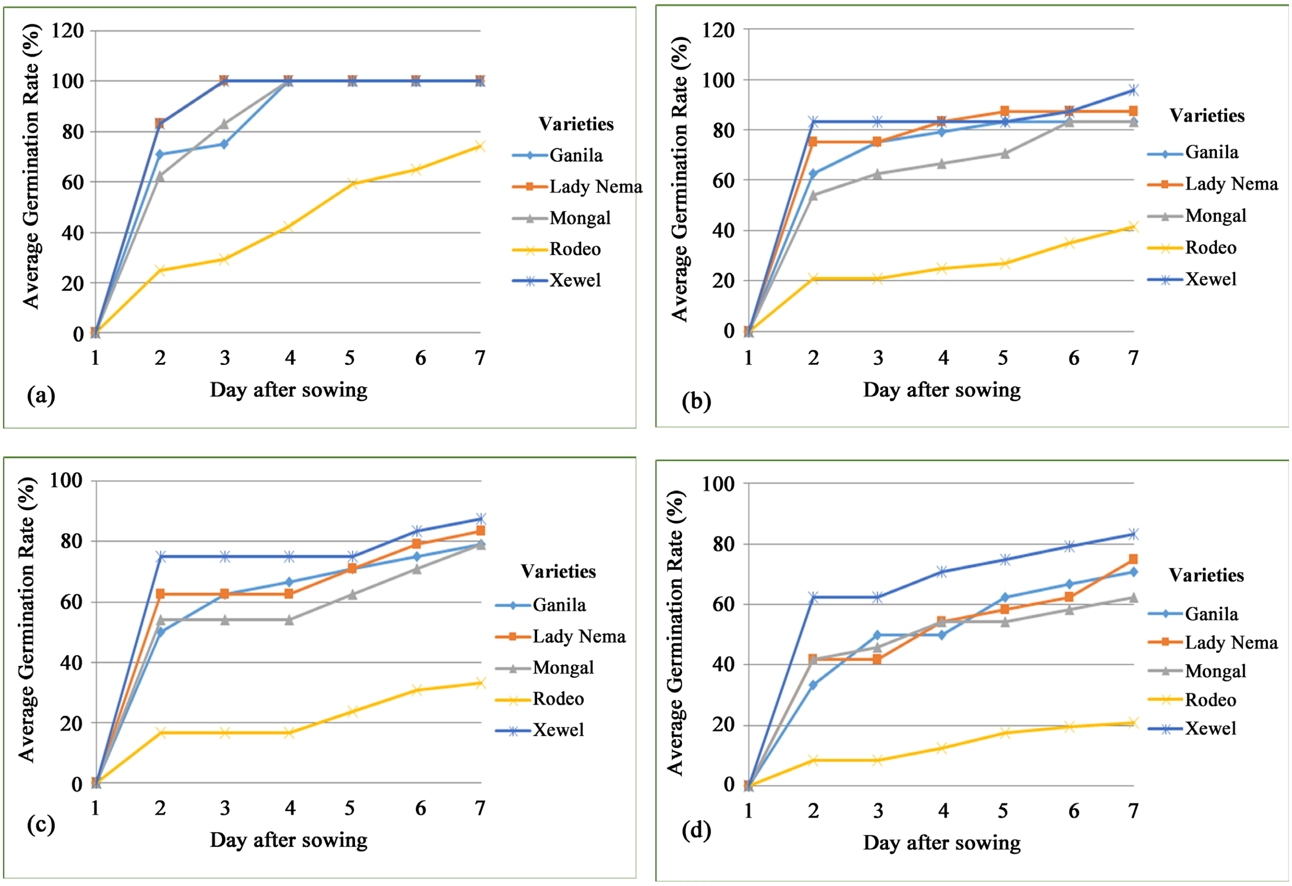

Figure 2. Effect of increasing osmotic pressures on the kinetics of the average seed germination rate of five tomato varieties (*Solanum lycopersicum* L.). (a) 0 kPa; (b) 5 kPa; (c) 10 kPa and (d) 15 kPa.

and 50% in the *Lady Nema* variety. Under the same conditions, the *Ganila* and *Mongal* varieties recorded a 45% decrease in the first week, while this decrease was more severe in the fourth week, *i.e.* 73% for *Ganil*a and 75% for *Monga*l. The most affected variety *Rodeo* recorded a decrease of 56% in the first week and 86% in the fourth week. Its first to fourth week vitroplant survival rate increased from 44% to 14% in presence of a 15 kPa osmotic pressure.

3.3. Growth and Development of Tomato Vitroplants under Water Constraint

3.3.1. Influence of Water Stress on the Average Number of Leaves

Figure 3 revealed that the average number of leaves of tomato vitroplants significantly decreased with increasing osmotic pressure. However, the analysis of variance did not reveal a significant difference in osmotic pressure x variety interaction: F = 0.189; P = 0.998). However, there is a significant environmental effect (F = 30.265; P = 2.22×10^{-10}) and an equally significant variety effect (F = 31.605; P = 6.72×10^{-12}). Thus, the average number of leaves of vitroplants decreases significantly with increasing osmotic pressure.

The vitroplants of *Lady Nema* and *Xewel* varieties tolerated well the pressure of 5 kPa, with a respective reduction in their average number of leaves of 5.17%

Table 3. Effect of increasing PEG-8000 concentrations on the survival rate of vitroplants of the five tomato varieties (*Solanum lycopersicum* L.).

Survival rate of vitroplants (%)					
Varieties	Weeks	Osmotic Pressure (kPa)			
		0	5	10	15
Ganila	1	100a	91a	79b	55c
	2	100a	83ab	67bc	43cd
	3	100a	72b	59c	32d
	4	100a	60bc	51cd	25d
Lady Nema	1	100a	100a	89a	76b
	2	100a	98a	80ab	63bc
	3	100a	89a	69bc	57c
	4	100a	77b	55c	50c
Mongal	1	100a	92a	78b	55c
	2	100a	84ab	68b	44cd
	3	100a	72b	59c	32d
	4	100a	61bc	52c	27d
Rodeo	1	100a	87a	66b	44cd
	2	100a	79b	60bc	35d
	3	100a	65b	49cd	24de
	4	100a	53c	38d	14e
Xewel	1	100a	100a	95a	87ab
	2	100a	100a	85ab	80ab
	3	100a	95a	76bc	72bc
	4	100a	89a	67bc	61c

For each variety, the values followed by the same letter are not significantly different from each other at the 5% level.

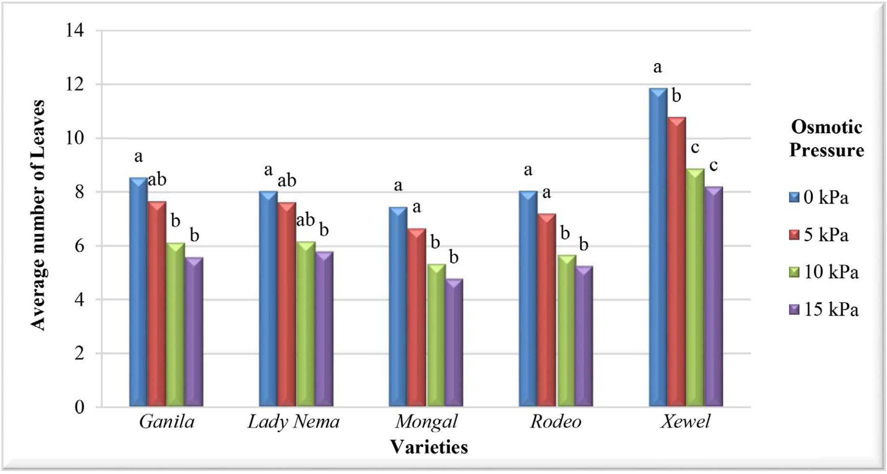

Figure 3. Effect of increasing concentrations of PEG-8000 on the average number of leaves of vitroplants of five tomato varieties (*Solanum lycopersicum* L.). For each variety, the values followed by the same letter are not significantly different from each other at the 5% level.

and 8.99% while the other varieties registered a reduction of more than 10%. However, the varieties were all roughly affected to the highest osmotic pressure (15 kPa), with reductions of 27.97% for the *Lady Nema* variety, 30.79% for the *Xewel* variety, 34.63% for the *Ganila* variety, 34.72% for the *Rodeo* variety and 35.97% for the *Mongal* variety. The *Lady Nema* and *Mongal* varieties were the least affected. The *Xewel* variety recorded the highest average number of leaves of vitroplants namely: 11.85 - 10.78 - 8.87 and 8.20 leaves while the *Rodeo* variety recorded the lowest, with 8.05 - 7.21 - 5.67 and 5.25 leaves at respective osmotic pressures of 0, 5, 10 and 15 kPa.

3.3.2. Influence of Water Stress on the Average Number of Secondary Roots

The average number of secondary roots recorded in vitroplants of the five varieties subjected to increasing water constraints is presented in **Figure 4**. Analysis of the variance of the effect of increasing PEG concentrations on the average number of secondary roots in vitroplants of the varieties did not reveal a significant difference in the osmotic pressure *versus* variety interaction ($F = 0.241$; $P = 0.9945$). However, this analysis of variance revealed a very significant medium effect ($F = 56.646$; $P = 1.85 \times 10^{-14}$) and a relatively insignificant variety effect ($F = 0.496$; $P < 0.001$). Consequently, the number of secondary roots of vitroplants of varieties decreases with increasing osmotic pressure of the medium.

From 5 kPa, a reduction of 15 to 24% was observed. This decrease was accentuated with 15 kPa, of which 40% was observed in the *Ganila* variety, 44% in the *Lady Nema* variety, 47% in the *Mongal* variety, 48% in the *Rodeo* variety and 52% in the *Xewel* variety. For this last one, its average number of secondary roots drops from 39.54 (0 kPa) to 30.63 (5 kPa), then 21.5 (10 kPa), and finally, to 19.17 (15 kPa), *i.e.* a decrease of more than half. Conversely, the *Ganila* variety registered 43.33 - 34.44 - 28.96 and 25.84 secondary roots at the respective

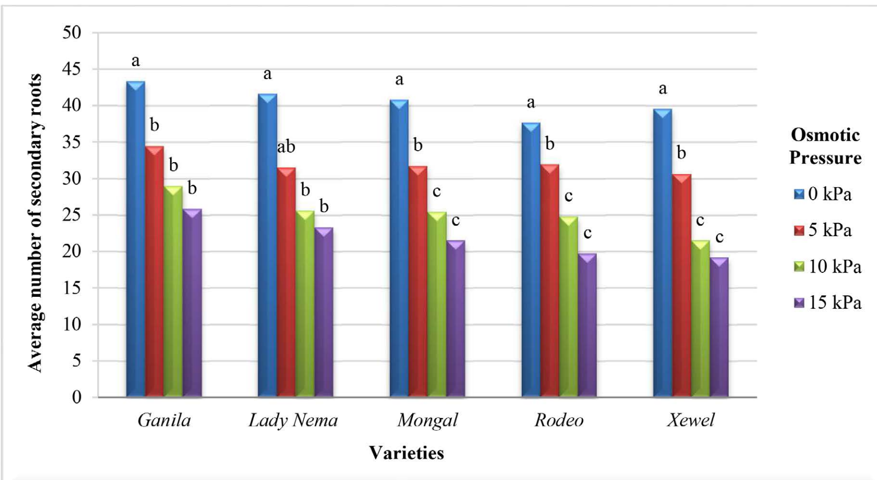

Figure 4. Effect of increasing concentrations of PEG-8000 on the average number of secondary roots of vitroplants of five tomato varieties (*Solanum lycopersicum* L.). For each variety, the values followed by the same letter are not significantly different from each other at the 5% level.

osmotic pressures of 0, 5, 10 and 15 kPa.

3.3.3. Influence of Water Stress on the Average Height of the Aerial and Root Parts

The increasing osmotic pressures did not have a significant effect (osmotic pressure x variety interaction: F = 0.447; P = 0.933) on the average stem height of vitroplants of the five tomato varieties even if this height decreased with increasing osmotic pressures (Table 4). However, there is a significant variety effect (F = 11.072; P = 3.86 × 10^{-06}) and a very significant environment effect (F = 19.119; P = 7.53 × 10^{-08}). All vitroplants of the varieties except the *Rodeo* one (16.44% decrease) tolerated the different PEG concentrations, with a maximum decrease in average stem height of 12% at 15 kPa. As for the length of the root part of the vitroplants of the five tomato varieties, the analysis of variance does not show a significant effect of the variety x osmotic pressure interaction (F = 0.579; P =

Table 4. Comparison of the means of the length of the aerial and root parts of vitroplants as a function of the PEG concentration of the five varieties of tomato by the Student-Newman-Keuls test at the 5% threshold.

Varieties	OP (kPa)	APL (cm)	RPL (cm)	TLV (cm)	(APL/TLV) × 100	(RPL/TLV) × 100	Reduction Rate APL (%)	Reduction Rate RPL (%)
Ganila	0	12.96a	6.14a	19.10a	67.86	32.14	-	-
	5	12.49a	5.46ab	17.95b	69.58	30.42	3.63	11.07
	10	11.92a	5.14ab	17.06b	69.87	30.13	8.02	16.29
	15	11.39a	4.36b	15.74c	72.33	27.67	12.11	28.99
Lady Nema	0	13.32a	6.17a	19.49a	68.33	31.67	-	-
	5	12.94a	5.72ab	18.67ab	69.34	30.66	2.85	7.29
	10	12.56a	5.25b	17.81b	70.51	29.49	5.71	14.91
	15	12.01a	4.61c	16.61c	72.27	27.73	9.83	25.28
Mongal	0	13.06a	6.21a	19.27a	67.76	32.24	-	-
	5	12.70ab	5.57b	18.27b	69.53	30.47	2.76	10.31
	10	12.40bc	5.14c	17.53c	70.70	29.30	5.05	17.23
	15	11.98c	4.53d	16.51d	72.55	27.45	8.27	27.05
Rodeo	0	12.84a	5.84a	18.68a	68.73	31.27	-	-
	5	12.36b	4.61b	16.97b	72.82	27.18	3.74	21.06
	10	11.23c	4.21bc	15.44c	72.72	27.28	12.54	27.91
	15	10.73d	3.72c	14.45c	74.26	25.74	16.43	36.30
Xewel	0	13.77a	6.11a	19.88a	69.27	30.73		
	5	13.47a	5.03b	18.5b	72.81	27.19	2.18	17.68
	10	13.1ab	4.7b	17.8bc	73.60	26.40	4.87	23.08
	15	12.57b	4.55b	17.12c	73.42	26.58	8.71	25.53

For each variety, the values on the same column followed by the same letter are not significantly different at the 5% level. OP: Osmotic Pressure, APL: Aerial Part Length, RPL: Root Part Length, TLV: Total Length of Vitroplant.

0.846). However, it reveals a variety effect ($F = 8.278$; $P = 5.81 \times 10^{-05}$) and a medium effect ($F = 49.285$; $P = 1.68 \times 10^{-13}$) both very significant. The results obtained and presented in **Table 4** displayed that the increasing concentrations of PEG induce a reduction in the average length of the root part in all vitroplants of the five varieties. This decrease is more marked at 15 kPa in the *Rodeo* variety (36%) than in the other varieties (less than 30%). At 5 kPa, vitroplants of the *Xewel* variety which had the longest taproot at 0 kPa (6.11 cm) recorded the fourth most important average length (5.03 cm) while vitroplants of *Lady Nema* which had at 0 kPa (6.17 cm) and held the third longest taproot is found at 5.72 cm. This same *Lady Nema* variety recorded the longest taproot of vitroplants at osmotic pressures of 10 and 15 kPa, with 5.25 and 4.61 cm, respectively.

Analysis of variance did not reveal a significant effect of the strain x osmotic pressure interaction ($F = 0.219$; $P = 0.099$) on the total length of vitroplants (**Table 4**). However, it revealed a variety effect ($F = 6.147$; $P = 2.19 \times 10^{-06}$) and a medium effect ($F = 18.937$; $P = 1.01 \times 10^{-14}$) very significant. However, the increasing concentrations of PEG induce a reduction in the total mean length of the vitroplants in all five varieties. All the varieties have opted for a better development of the aerial part compared to the root part. Thus, the aerial part represents more than 65% of the total length of the vitroplants at all osmotic pressures. With the *Rodeo* variety, at 15 kPa, the length of the aerial part represents 74.26% of the total length of the vitroplant. This proportion is lower in the *Lady Nema* variety with 72.27%.

3.3.4. Influence of Water Stress on the Average Fresh and Dry Weights of the Aerial and Root Parts

The different increasing concentrations of PEG induced a non-significant drop (osmotic pressure × variety interaction) in the average fresh and dry weights of the aerial part of the vitroplants ($P > 0.05$) (**Table 5**). However, there is a variety effect and a medium effect which are very significant ($P < 0.05$). Average fresh and dry weights decreased with increasing water stress caused by increasing concentrations of PEG in the growing medium. For the average fresh weights, the varieties performed relatively well at all osmotic pressures with a slight decrease in their average weights. At 15 kPa, the vitroplants of the *Rodeo* variety recorded a decrease of 6.67% while the other varieties recorded decreases of 13.24% (*Xewel*), 15.33% (*Lady Nema*), 15.47% (*Ganila*) and 18,7% (*Mongal*). With regard to the average dry weights, from 5 kPa, a weight reduction of more than 15% in all varieties was noticed. However, At 15 kPa, all the varieties see their weight reduced by more than half except the *Xewel* variety (49.20%).

The average fresh and dry weights of the root part of the vitroplants of the five tomato varieties studied are also reported in **Table 5**. A non-significant decrease in the average fresh and dry root weights of vitroplants was observed, with the increase in osmotic pressure ($P > 0.05$). However, there is a variety effect and a medium effect which were very significant ($P < 0.05$).

Regarding the average fresh and dry root weights of vitroplants, the *Lady Nema*

Table 5. Comparison of the means of the fresh and dry weights of the aerial and root parts of the vitroplants as a function of the PEG concentration of the five varieties of tomato by the Student-Newman-Keuls test at the 5% threshold.

Varieties	OP (kPa)	AFW (g)	ADW (g)	RFW (g)	RDW (g)	TDW (g)	(ADW/TDW) × 100	(RDW/TDW) × 100	Reduction Rate ADW (%)	Reduction Rate RDW (%)
Ganila	0	0.527a	0.146a	0.238a	0.049a	0.195a	75	25		
	5	0.513a	0.108b	0.198a	0.041a	0.149b	72	28	26	17
	10	0.486a	0.080c	0.184a	0.038a	0.118c	68	32	45	23
	15	0.446a	0.071c	0.170a	0.039a	0.110c	65	35	51	22
Lady Nema	0	0.531a	0.151a	0.237a	0.049a	0.201a	75	25		
	5	0.515a	0.123b	0.202a	0.042a	0.165b	74	26	19	14
	10	0.499a	0.088c	0.181a	0.038a	0.126c	70	30	42	23
	15	0.449a	0.076c	0.171a	0.039a	0.115c	66	34	50	20
Mongal	0	0.465a	0.136a	0.214a	0.047a	0.183a	74	26		
	5	0.426a	0.096b	0.149b	0.031a	0.127b	76	24	29	34
	10	0.407a	0.068c	0.135b	0.028a	0.096c	70	30	50	39
	15	0.378a	0.061c	0.108b	0.025a	0.086c	71	29	55	47
Rodeo	0	0.330a	0.118a	0.185a	0.040a	0.158a	75	25		
	5	0.323a	0.076b	0.127b	0.026b	0.103b	74	26	35	34
	10	0.317a	0.055c	0.113b	0.024bc	0.079c	70	30	53	41
	15	0.308a	0.051c	0.086c	0.020c	0.070c	72	28	57	51
Xewel	0	0.557a	0.161a	0.201a	0.042a	0.203a	79	21		
	5	0.540a	0.136b	0.142b	0.030b	0.166b	82	18	16	29
	10	0.503a	0.102c	0.126b	0.027b	0.129c	79	21	37	36
	15	0.483a	0.082c	0.098b	0.023b	0.104c	78	22	49	46

For each variety, the values on the same column followed by the same letter are not significantly different at the 5% level. OP: Osmotic Pressure, AFW: Aerial Fresh Weight, ADW: Aerial Dry Weight, RFW: Root Fresh Weight, RDW: Root Dry Weight, TDW: Total Dry Weight.

and *Ganila* recorded respective reductions of 28.12% and 28.85% while those of other varieties were more than 49%. At 10 kPa, the vitroplants of the *Ganila* and *Lady Nema* varieties recorded a decrease of root dry weight of 23% and the other varieties a decrease of more than 35%. However, at 15 kPa, the *Lady Nema* and *Ganila* varieties recorded a slight increase (2.6%) in their root dry weight. The *Xewel* and *Mongal* varieties recorded respectively 46.27% and 47.23% decrease while the Rodeo variety recorded the largest decrease (50.68%).

The analysis of variance showed that the increasing concentrations of PEG did not induce a significant response on the osmotic pressure × variety interaction ($F = 0.763$; $P = 0.108$) of the total mean dry weights of the vitroplants (Table 5). Average total dry weights decrease with increasing water stress induced by increasing PEG concentrations. However, a variety effect ($F = 31.901$; $P = 1.02 \times 10^{-13}$) and a medium effect ($F = 134.432$; $P < 2 \times 10^{-16}$) were detected.

All varieties have a better weight of the aerial parts compared to the weight of the root parts. Thus, the weight of the aerial parts represents more than 65% of the total weight of vitroplants in all varieties. However, the weight of the aerial parts decreased more sharply compared to the weight of the root parts, with the increase in osmotic pressure.

3.4. Classification of Varieties by the Water Stress Sensitivity Index

Table 6 groups together the indices of sensitivity to water deficit. Analysis of variance did not reveal a significant difference in the osmotic pressure x variety interaction (F = 0.693; P = 0.69477). However, a significant difference is noted with a variety effect (F = 5.104; P = 0.00294) and a medium effect (F = 42.870; P = 1.6×10^{-09}).

The osmotic pressures, whatever the variety considered, led to more or less significant weight losses compared to the respective control ones. The indices increased with the increase in osmotic pressure in all varieties except for the *Rodeo* variety in which an increase was observed at 10 kPa, but this index decreased slightly at 15 kPa. The values of the calculated water deficit sensitivity indices were more discriminating at osmotic pressures of 5 and 10 kPa. For these treatments, the varieties can be classified in three groups as it was obtained with the Fisher's test:

- the first group (a) more tolerant consists of the *Xewel* variety which, at osmotic pressures of 5 and 10 kPa, only lost, respectively, 15.61% and 36.82% of its morphogenetic capacities to develop and produce aboveground biomass;
- the second group (ab), moderately tolerant, is formed by the varieties *Lady Nema*, *Ganila* and *Mongal* which have lost, respectively, at 5 kPa, 18.72%, 25.27% and 28.97% and at 10 kPa, 41.79%, 44.82% and 49.99% of their capacity to produce biomass;
- finally, the third, more sensitive group (b) is made up of the *Rodeo* variety which was more sensitive to water stress because it loses, at 5 and 10 kPa, respectively, 35% and 61.39% of its capacity to develop and produce an aboveground biomass.

Table 6. Variation in the water stress sensitivity index of vitroplants of the five tomato varieties (*Solanum lycopersicum* L.).

	Osmotic Pressure (kPa)		
Varieties	**5**	**10**	**15**
Ganila	−25.27a	−44.82b	−50.39b
Lady Nema	−18.72a	−41.79b	−49.49b
Mongal	−28.97a	−49.99b	−54.64b
Rodeo	−35.00a	−61.39b	−53.27ab
Xewel	−15.61a	−36.82b	−49.10b

For each variety, the values followed by the same letter are not significantly different from each other at the 5% level.

3.5. Influence of Water Stress on the Average Total Chlorophyll Contents of Vitroplants

The different increasing concentrations of PEG induced a non-significant drop ($F = 0.671$; $P = 0.75$) in the average total chlorophyll content of the vitroplants of the five tomato varieties concerning the osmotic pressure x variety interaction (Figure 5). However, the analysis of variance revealed a significant medium effect ($F = 255.266$; $P < 2 \times 10^{-16}$) and a variety effect ($F = 200.340$; $P < 2 \times 10^{-16}$). At 5 and 10 kPa, the *Lady Nema* variety recorded the smallest reduction in its average total chlorophyll content (respectively 9.56% and 20.85%) compared to the other (respectively +15% and +25%). However, at the highest osmotic pressure (15 kPa), the *Rodeo* variety recorded a reduction of more than half (52.95%) of its average total chlorophyll content while the *Lady Nema* variety, recorded a 33.55% reduction. The other varieties recorded reductions of 38 to 46%. The *Xewel* variety recorded the highest average total chlorophyll contents, with 5.6 - 4.74 - 4.18 and 3.47 $mg \cdot g^{-1}$ of fresh matter, respectively at 0, 5, 10 and 15 kPa, while the *Rodeo* variety recorded the lowest average contents (3.58 - 2.95 - 2.26 and 1.68 $mg \cdot g^{-1}$ of fresh matter).

3.6. Influence of Water Stress on the Average Proline Content of Vitroplants

The average proline contents vitroplants of the five tomato varieties subjected to water stress are presented in Figure 6. Analysis of the variance of the effect of increasing PEG concentrations on the averages proline contents of vitroplants revealed a significant difference ($F = 106$; $P < 2 \times 10^{-16}$). The analysis of variance also revealed a very significant variety effect ($F = 1490$; $P < 2 \times 10^{-16}$) and a medium effect ($F = 2664$; $P < 2 \times 10^{-16}$). Indeed, the average proline content of varieties increased significantly with increasing osmotic pressure. However, the

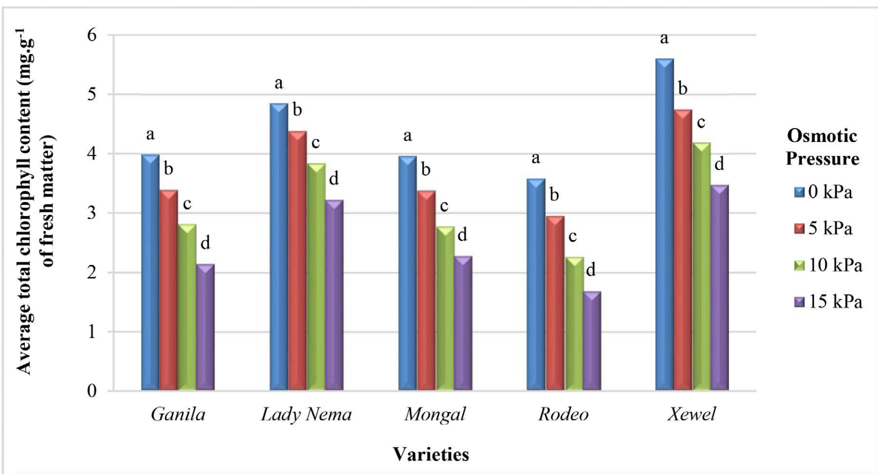

Figure 5. Effect of increasing PEG-8000 concentrations on the average total chlorophyll content of vitroplants of five tomato varieties (*Solanum lycopersicum* L.). For each variety, the values followed by the same letter are not significantly different from each other at the 5% level.

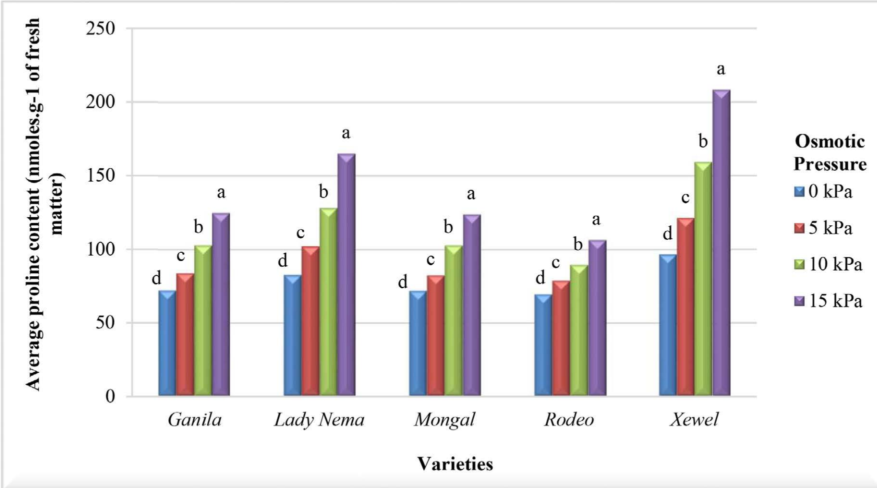

Figure 6. Effect of increasing PEG-8000 concentrations on proline accumulation *in vitro*plants of five tomato varieties (*Solanum lycopersicum* L.). For each variety, the values followed by the same letter are not significantly different from each other at the 5% level.

intensity of this increase is not the same at 15 kPa because it is greater in the varieties *Xewel* (116.26%), and *Lady Nema* (100.41%), followed by the varieties *Ganila* and *Mongal* (73%) and, finally, the *Rodeo* variety (53.37%). The *Xewel* variety records the highest proline contents at osmotic pressures of 0, 5, 10 and 15 kPa, with 96.33 - 121 - 159 and 208.33 nmoles.g^{-1} of fresh material, respectively. Under these same osmotic pressure conditions, the *Rodeo* variety recorded the lowest accumulations of proline, with 69.33 - 78.33 - 89 and 106.33 nmoles·g^{-1} of fresh material.

4. Discussion

4.1. *In Vitro* Germination and Germination Kinetics of Seeds

Water stress is one of the most important abiotic factors limiting seeds germination, plant growth and yield [23]. Polyethylene glycol (PEG) is considered to be a superior chemical for inducing water stress [24]. PEG molecules are inert, non-ionic, virtually impermeable chains and have been used frequently to induce water stress.

Germination is the first stage in the development cycle of plants to produce a new generation [15] [17] [18]. The ability of seeds to initiate and accomplish this biological process, *i.e.* their germination capacity, is therefore a determining characteristic for plant production and a key development process in the plant life cycle [25]. Thus, this physiological parameter can be used as a selection criterion for tolerance to water stress since a variety tolerant to abiotic stress gives a reasonable rate of germination. To this end, five tomato varieties were tested *in vitro* for their tolerance to water stress at the germination stage, with increasing osmotic pressures (0, 5, 10 and 15 kPa) in order to discriminate the varieties according to their tolerance or sensitivity. The results obtained in this study made it possible to show that the increasing osmotic pressures significantly reduce the final germination rate of the seeds of the five varieties studied ($P = 2 \times 10^{-16}$). Taken individually, the behavior of varieties in the face of water constraints is different. In fact, the seeds of the more tolerant *Xewel* and *Lady Nema* varieties recorded a respective decrease in their average final germination rate of 16.7% and 25% in the presence of 15 kPa of osmotic pressure in the culture media. They also record, at the same osmotic pressure, the best germination kinetics, reaching respectively 76% and 53% of their final germination rate on the second day after sowing. The seeds of the moderately tolerant *Ganila* and *Mongal* varieties recorded a decrease of 29.2% and 37.5%, respectively. They reached, on the second day after sowing, 24.5% and 66% respectively of their final germination rate. The seeds of the *Rodeo* variety, more sensitive, have a 71.91% decrease, and an average germination kinetics (40% of its maximum germination rate), on the second day after sowing. [26] obtained better germination rates (greater than 50%) in about twenty tomato accessions at -0.5 MPa. [27] reported a slight decrease in the final germination rate in tomatoes with 12% PEG-6000, however, with 22% PEG, they did not observe germination. Three types of behavior, for tomato seeds germination compared to the control, were obtained: improvement, reduction or indifference in the presence of 4% PEG-6000 depending on the variety [28]. It should be noted that a slight water deficit improves the germination capacity of seeds. The morphological as well as physiological behavior of a given plant depends on the species or variety, duration and severity of the drought as well as the time of its application [29]. Thus, seeds subjected to very high water constraints, could not absorb sufficient quantities of water and oxygen which would allow the growth of the embryo. The delay in germination, caused by the increasing concentrations of the PEG medium, would result from a difficulty in hydration of the seeds as a result of a high osmotic potential and this can be explained by the time required for the seed to set up mechanisms allowing it to adjust its internal osmotic pressure [30]. This would affect the process of radicle elongation.

4.2. Effect of Osmotic Pressures on *in Vitro* Growth of Vitroplants

In this study, all the varieties tested appeared sensitive to water stress with a reduction in the number of leaves of vitroplants of 27% to 36%. The *Lady Nema* (28% reduction) and *Mongal* (31% reduction) varieties appear to be the least sensitive while the *Rodeo* variety (36% reduction) is the most sensitive. [31] observed a reduction in the number of leaves in tomatoes from 13% to 23% after stopping watering from the macroscopic appearance of the first inflorescence. [12] got a 13.5% decrease in the number of leaves in tomato. [32] obtained, with 4% of PEG-6000, reductions in the number of leaves, of up to 30%, in several tomato genotypes but also a 21% increase in the number of leaves in another genotype. Plants respond to water deficit by morphological, physiological and metabolic modifications. The water deficit causes a delay in plant growth which results in a reduction in the number of leaves. An early senescence of the leaves, in limiting water conditions, could also explain the decrease in the number of leaves [33]. The decrease in leaf area, under a limiting water regime, is an adaptive mechanism in plants aimed at limiting their leaf transpiration when water conditions become unfavorable [34].

The length of the stem is also a varietal characteristic strongly influenced by the effects of the environment. Water stress, during the young seedling stage, can inhibit the development of the coleoptile of grasses [35]. Our work made it possible to show that the osmotic pressures induced a drop in the height of the stem of the varieties tested. However, this decrease is not significant in *Ganila* and *Lady Nema* varieties. All varieties appeared to tolerate increasing PEG concentrations in the medium with an 8.3% to 16.4% reduction in mean stem height of vitro plants at 15 kPa. However, *in vitro* plants of the *Rodeo* variety appeared to be more sensitive, with the greatest reduction in height (16.4%). Reductions of 2% to 22% in several tomato genotypes in the presence of 4% PEG-6000 were also reported [32]. The reduction in height growth of the plant under water stress is well documented in several species such as tomato [36]. However, a slight increase in the height of the stem may be noted in some species as observed by [28] in five tomato genotypes. The water deficit most often leads to a delay in plant growth which results in a reduction in the height of the stem and a shortening of the internodes. It seems also_that water stress reduces plant growth by reducing the division and enlargement of cells which leads to decreased plant growth [37].

The length and number of roots is an important adaptation criterion for drought tolerance. In fact, varieties that develop a strong root system can pump water to considerable depths which allows them to tolerate certain dry periods. The length of the root part is also affected by increasing osmotic pressures. Indeed, a reduction in the average length of the taproot of vitroplants is observed in all varieties. However, this decrease is more marked *in vitro*plants of the *Rodeo* variety (36%) than in others (25% to 29%), at 15 kPa. The number of secondary roots is considerably reduced, *i.e.* 40% for vitroplants of the *Ganila* variety and 52% for the *Xewel* variety. Under stressful conditions, the uptake of water by the plant is directly related to the degree of development of the root system. The relationship between the degree of development of the root system and drought tolerance has been proven in several species._A reduction in the number of secondary roots in tomato by 13.6% in the presence of PEG was reported by [38]. A reduction in the length of the taproot in tomatoes under water stress had reported by [39]. PEG-induced decline in taproot growth is reported by [32]. The latter have achieved reductions of up to 49% in tomatoes. A negative effect on taproot growth under water stress in a tomato genotype was reported by [28]. However, they observed stimulation of the lengthwise development of the taproot in nine other genotypes. Taproot length in several tomato genotypes was significantly reduced from 10% to 100%, with 14% PEG-6000 [40]. However, with 4% PEG-6000, they observed a slight increase of 1.45% in the length of the taproot. Water stress severely affects, at the root level, meristematic activities as well as cell elongation; this results in a reduction in the development of the root system [41]. The roots are generally the first affected under water stress. Better development of the root system increases the plant's water absorption capacity because there is a positive relationship between root length and tolerance to water deficit [42].

Better development of the aerial part compared to the root part of vitroplants was observed in the varieties of the study. Thus, the length of the aerial part represents between 67% and 74% of the total length of the vitroplant. At the highest osmotic pressure, in the *Rodeo* variety which appeared to be more sensitive, the length of the taproot represents 25.74% of the total length of the vitroplant while in the more tolerant *Lady Nema* variety, this represents 27.73%. Similar results are obtained in tomato by [39].

4.3. Effect of Osmotic Pressures on Vitroplant Biomass

The different increasing concentrations of PEG also induced a significant drop in the fresh and dry weights of the aerial parts and of the root parts of vitroplants in all the varieties studied. Thus, air fresh weights of vitroplants have decreased from 7% (*Rodeo*) to 19% (*Mongal*) while the decrease in fresh root weights varies from 28% (*Lady Nema*) to 54% (*Rodeo*). Regarding the aerial dry weights, reductions from 49% (*Xewel*) to 57% (*Rodeo*) are observed at 15 kPa while those concerning dry root weights vary from 20% (*Lady Nema*) to 51% (*Rodeo*). The average air dry weights represent between 65% (*Ganila*) and 78% (*Xewel*) of the total dry weight of the vitroplants at the highest osmotic pressure. Indeed, the aerial part is much more developed than the root part in all varieties. A reduction in air fresh weight in tomatoes from 28% (Tom-143) to 63% (Tom-163) under moderate water stress is obtained by [38]. A significant reduction of 71% to 99% in the fresh weight of the aerial and root part of tomato vitroplants in the presence of 15% PEG-6000 was recorded by [40]. Genetic material, which grows better in a stressed environment, may have developed a drought tolerance mechanism and these plants may have the ability to maintain homeostasis under stress conditions. Nevertheless, [43] recorded better development of the root part compared to the aerial part which is a criterion of drought tolerance, allowing better use of available water.

4.4. Effects of Osmotic Pressures on Total Chlorophylland Proline Contents

Chlorophyll is one of the major chloroplast components of photosynthesis, and the relative chlorophyll content has a positive relationship with the rate of photosynthesis [44]. All vitroplants of varieties had their mean total chlorophyll content decreased with increasing osmotic pressure. At the highest osmotic pressure (15 kPa), the *Rodeo* variety recorded a reduction of more than half (52.95%) of its average total chlorophyll content while the *Lady Nema* variety, more tolerant, recorded a 33.55% reduction. A decrease in the net rate of photosynthesis in tomato at osmotic pressures of −0.5 and −1.22 Mpa was observed by [45]. A decrease in chlorophyll content in tomatoes under severe water stress was also observed by [46]. The decrease in chlorophyll content under water stress is mainly the result of damage to chloroplasts caused by active oxygen species [47]. Likewise, decreasing chlorophyll levels tend to reduce photosynthetic rates, causing plants to be damaged under stressful conditions. Decrease in chlorophyll under water stress usually occurs due to damage to chloroplasts caused by oxidative blasts or due to altered ratios of lipid complexes or high activity of chlorophyllase which degrades chlorophyll and damages receptors light [48]. According to [49], the lowering of chlorophyll contents is due either to degradation of proteins or to inhibition of their synthesis. A decrease in the transpiration of the plant following the closure of its stomata in response to water deficit can be seen as a positive response, thus saving water.

Average proline levels significantly increased ($F = 106$; $P < 2 \times 10^{-16}$) with increasing osmotic pressures. Indeed, at 15 kPa, a very significant increase is noted in the vitroplants varieties *Xewel* (116.26%) and *Lady Nema* (100.41%), an average increase in the varieties *Ganila* and *Mongal* (73%) and in the variety *Rodeo* (53.37%). The accumulation of proline has been demonstrated in many species and in different stressful situations such as osmotic, water, and thermal stress. Aydoner *et al.* [32] reported increases in proline in tomatoes from 65% to 529% under water stress conditions. An increase in proline content of 124% is observed by [38]. Ghorbanli *et al.* [46] recorded also, in tomatoes under severe water stress, an increase in proline content of 500%. Proline, accumulated in the cytoplasm, can play a key role in the osmotic adjustment of the vacuole [50]. The difference in rate of accumulation with stress levels applied in this study reflects the responses of varieties to increased stress levels. This suggests that the accumulation of proline concentrations is more a consequence of stress than a mechanism of adaptation for some varieties [51]. However, some authors believe that the accumulated amounts could be linked to the level of stress tolerance [52]. The plant, under water stress, synthesizes and accumulates osmolytes and proline is the first to be synthesized in these stressed plants [44]. The accumulated proline could play a role of osmoticum. The varieties that have a low reduction in chlorophyll are those that have accumulated a greater amount of proline. These results suggest the existence of a likely connection between the biosynthetic pathways of chlorophyll pigments and proline. However, [53] have reported competition between these two compounds on their common precursor, glutamate; the variety which accumulates more proline is also the one which experiences the greatest decrease in its chlorophyll pigment content and *vice versa*. According to [54], an alteration in protein biosynthesis would be, in part, at the origin of the accumulated proline. Stewart *et al.* [54] believe that the accumulation of proline, induced by stress, may be the result of inhibition of its oxidation. [55], working on tomatoes under water stress conditions, suggests that the accumulation of proline is due either to an induction or activation of the enzyme involved in the biosynthesis of proline, or to a lowering of its oxidation to glutamate and an improvement in the "turnover" (*i.e.* renewal) of proteins. These facts have shown that proline is an effective organic substance, not only in its function as an osmolyte, but also in cell stabilization. A high proline content, under water stress, maintains the existence of the plant with a good level of cellular water [46]. This, prevents damage to plants caused by drought conditions. Proline acting as an osmolyte allows carbon and nitrogen storage and stabilizes macromolecules, proteins and cell membranes of vitroplants [56]. The most susceptible varieties are those which have recorded the highest mortality rates. Thus, the mechanisms they put in place did not allow vitroplants to tolerate water stress during the 30 days of the experiment.

5. Conclusion

Under *in vitro* culture conditions, the study of the seed germination, the morpho-physiological and biochemical response of vitroplants in the five varieties of tomato showed different levels of sensitivity with respect to the concentrations of PEG applied. At the end of this study, it emerged that the water deficiency induced, affected the vitroplants in all aspects. However, tomato seeds are able *in vitro* to germinate and grow under water stress. Based on the results and taking into account all analyses carried out, the tomato varieties tested can be classified according to their degree of tolerance to water stress:

- A first group, made up of vitroplants of the *Lady Nema* and *Xewel* varieties, are vigorous and fairly tolerant;
- A second group, made up of vitroplants of the *Ganila* and *Mongal* varieties, are slightly less vigorous, and therefore moderately sensitive;
- A third group, comprising the vitroplants of the *Rodeo* variety, are not very vigorous and, therefore, sensitive.

Acknowledgements

The authors are grateful to Prof Oumar KA for proofreading the manuscript in English version and to Dr Seyni SANE for his help in statistical analysis.

Conflicts of Interest

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

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