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ADAPTATION OF TWO TOMATO VARIETIES (SOLANUM LYCOPERSICON L.) UNDER SALINITY STRESS

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Abstract

In order to study the adaptive behavior of two tomato varieties (Solanumlicopersicum L.) Safa and Assila subjected to salt stress with concentrations of (50, 100, 150 and 200 mM) in ($NaCl+CaCl_2$) during the growth phase, a morphological (number of leaves, stem length, stomatal density, length of the main root axis) and physio-biochemical (relative water content, chlorophyll pigments content, soluble sugars content) evaluation was conducted. Our results confirm the sensitivity of both varieties to applied salt stress; this sensitivity was manifested by a statistically significant decrease in: number of leaves, length of the stem and the contents of chlorophyll a and total chlorophyll. While, the soluble sugars content at the leaf level increased associated with an increase stomatal density on both ventral and dorsal epiderms. On the other hand, the ratio of root dry weight and shoot dry weight (RDW/SDW) was decreased with an elongation of the main root according to the different doses of salts.

Keywords: Salt stress, Solanumlicopersicum L, Morphology, Physiology, Adaptation

1. INTRODUCTION

Tomato (Solanumlycopersicon L.) is an important vegetable crops, particularly in tropical and Mediterranean countries, it is cultivated in more than 165 countries on an area of about 5.052 million ha (FAO 2022 [1]). It occupies the second place after the potato, both in production and consumption (Naïka et al. 2005 [2]). Tomato is cultivated in countries with dry and hot climates where saline soils cover large areas, which poses the problem of its adaptation to salinity for its extension in cultivated areas (Boumendjel et al. 2012 [3]). In Mediterranean climate regions, the problem of salinity is accentuated by accumulation of high concentrations of sodium in irrigation water (Cheeseman 2015 [4]). Its cultivation in Algeria occupies a prominent place in the agricultural economy. Nearly 49794 ha are devoted to its cultivation (market gardening and industrial), for an average production of 31 million quintals with average yields of 607 q/ha (MADR 2019 [5]).Tomato is often exposed to multiple environmental stresses, salinity significantly reduce crop growth and productivity in many parts of the globe (Tang et al. 2015 [6]). The problem is particularly acute in Mediterranean regions where the limitation of good quality water resources for irrigation associated with high evapotranspiration can be the cause of progressive soil salinization.

Excessive salt in the soil affects plant development at different levels (germination, growth, flowering and fruiting) to varying degrees leading eventually to decreases productivity (Benazzouk et al. 2018 [7]).

Responses of plants to salinity are complex and different mechanisms involve allowing the plant to survive (Tapia et al. 2016 [8]).

Many researchers have focused their work in particular on the selection of varieties adapted to these regions, either through genetic improvement, which remains, arguably, the most accessible means, or through an intensive study of the different mechanisms of adaptation (Singh et al. 2020 [9]). Improving salinity tolerance would be of great value to a moderately sensitive crop such as tomato when grown on soils that have salinity problems. In recent decades, considerable progress has been made in the search for salinity-tolerant plants through conventional breeding and selection techniques (Ashraf 2002 [10]). While most breeding procedures are based on differences in agronomic traits, which represent the combination of genetic and environmental effects on plant growth and incorporate physiological mechanisms conferring salinity tolerance (Ashraf 2004 [11]).

In this research we make a comparative study of the physiological, biochemical and morphological parameters of resistance of two varieties of tomatoes (Safa and Assila) to salt stress at different concentrations under controlled conditions.

2. MATERIALS AND METHODS

Plant material

Experiment consisted of two tomato varieties (Solanumlycopersicum L.) : Safa of Thaïndaise origin and Assila of Indian origin. The experiment was carried out in a greenhouse under controlled conditions and germination was cconducted in trays. After one month, the young seedlings were transplanted into P.V.C. cylinders of 60 cm length and 20 cm diameter with one seedling per cylinder. The growing medium was a mixture of sand, soil and organic matter.

Induction of salt stress

The experimental set-up includes five treatments: a control watered at field capacity with tap water until the beginning of the measurements. The other four treatment were watered with a salt solution composed of a combination of two salts NaCl+$CaCl_2$ (V/V) at four different concentrations 50, 100, 150 and 200 mM. The use of $CaCl_2$ in this composition is necessary because of the physiological role of calcium in plants. Moreover, NaCl associated with $CaCl_2$ produces a saline medium whereas the solution obtained with only NaCl is rather sodium. The stress was applied with saline irrigation on the 58th day of sowing and then repeated at a frequency of one application per three days for 15 days. In each treatment, the varieties were repeated 3 times. The growing medium was irrigated to the pot capacity by the daily contribution of a watering dose which is according to the daily evapotranspiration of each cylinder. From transplanting until the application of salt stress, irrigation was provided with tap water. Every week, the irrigation water was substituted by a commercial nutrient solution.

Aerial and underground morphology

Stomatal density

The stomatal density (SD) was obtained according to the method of (Dohman et al. 1991[12]) used by (Denden and Lemeur, 2000 [13]), whose principle is based on the obtaining of leaf prints by spreading a few drops of nail polish on the two epidermis of the superior (SDS) and inferior (SDI) leaf. The SD is the equivalent of the number of stomata per unit area (ns/ua).

Number of leaves (NL)

A count was made on the different leaf stages to know the number of leaves on each plant.

Stem length (SL)

Stem length was measured with a ruler from the point of separation to the plant apex. Roots were recovered and thoroughly washed with tap water to remove residues from the substrate to proceed with the identification of the following morphological characteristics:

- The length of the main axis of root (RL).
- The ratio of root dry matter to aerial dry matter (RDW/SDW).

Physiological and biochemical parameters

Relative Water Content (RWC)

Relative water content was determined according to the method of (Ladiges, 1975 [14]), used by (Clarke, 1982 [15]) according to the following formula:

$$RWC\ \% = (Wi - dw) / (Wt - dw) \times 100 \quad (1)$$

Wi : initial fresh weight; Wt: weight in full turgor; dw: dry weight.

Extraction of chlorophyll pigments

The content of chlorophyll a, chlorophyll b, carotenoids and total chlorophylls were determined according to the method of (Shabala and Lew, 2002 [16]) at the level of the penultimate leaf. To 1 gr of fresh sample, cut into small fragments, 10 ml of 95% acetone was added in test tubes, the whole was kept in the dark and at 4°C for 48 hours. The content of chlorophyll a, chlorophyll b and carotenoids were carried out using a UV spectrophotometer (type Pharmacia Biotech. NOVASPEC II) at optical densities of 662, 644 and 470 nm respectively, with a control solution of acetone at 95%. The concentrations of chlorophyll a, chlorophyll b, carotenoids and total chlorophylls were calculated by the following formulate:

$$Chl\ a = 9{,}784x\ OD\ (662) - 0{,}99x\ OD\ (644) \quad (2)$$

$$Chl\ b = 21{,}42x\ OD\ (644) - 4{,}65x\ OD\ (662) \quad (3)$$

$$Total\ chlorophyll = Chl\ a + chl\ b \quad (4)$$

$$C(x+c)=(1000OD\ (470)-1.90Chla–Chlb)/214 \quad (5)$$

OD: optical density; x: xanthophylls; c: carotenoids.

Soluble sugars

The method of Rekika, 1997 [17], was adopted for the quantification of (glucoses, fructose and sucrose). These sugars were extracted after maceration in a solvent capable of solubilizing them and blocking the enzymatic activity likely to degrade them. The principle of the reaction is based on the condensation of the degradation products of neutral oses by sulfuric acid: the latter, highly concentrated, transforms the oses at high temperature into furfural derivatives that give a blue-green coloration with anthrone, assayed by colorimetry at 585 nm,the concentration was expressed in µg/100mg Fresh weight.

Statistical treatment

A statistical study including an analysis of the variance of the GLM model and the calculation of correlations, are carried out by the Statistica software.

3. RESULTS AND DISCUSSIONS

Effect of salinity and genotypes on morphological parameters

Salinity significantly affected all studied samples included in Table 1 except RL. The genotype was not significant for all morphological tests except SD.

Salt stress increase significantly SDS from 21.5 ns/ua under control to 43.1 ns/ua under 100 mM treatment, associated with significant losses in LN, which decrease from 13 leaves under control to 10 leaves under150 and 200mM treatments.

In addition, salinity conditions increase significantly RL in two genotypes in comparison to control. RL increase from 36.2 cm under control to 48.5cm and 45cm under 150mM and 200mM treatments respectively (Table 1).

RDW/SDW of control plant of Assila genotype was higher than Safa genotype with 0.50±0.09 and 0.41± 0.01 respectively (Tab. 01). Salinity cause a decrease in RDW/SDW propotional to the severity of stress.

Likewise, salinity decrease SL from 37.5 cm under control to 23.65 cm under 200 mM. The interaction G x T was strongly significant on SDS (Table 1).

The both varieties show increases in stomatal density on both ventral and dorsal epidermis. According to Terashima et al. (2005) [18] , this increase in the SD on the ventral and dorsal side under salt stress would promote CO2 assimilation and reduce water loss: indeed, a high number of stomata can result in small and rapidly closing stomata. The closure of stomata is a mechanism that allows the reduction of water loss (MunnsetTester 2008 [19]).

Salt has a depressive effect on plant growth, especially on the height of the aerial part. Indeed a negative relationship was established between salinity and NL. Salt stress affects the morphogenesis of tomato and retards the formation of new leaves. Several studies working on the effect of salt stress in cultivated tomato have highlighted its effect on the growth of aerial parts (Singh et al. 2020 [9]).

Salinity had adverse effects not only on LN, but also on other morphological parameters such as SL.

Indeed, our results show that high salt doses exert a depressive effect on stem growth. These results are in agreement with those of Mani and Hannachi (2015) [20], who showed that the addition of NaCl in irrigation water has an inhibitory effect on SL especially at 200 Mm concentration. Similarly, Wang and Nil (2000) [21] consider growth reduction under salinity stress is caused by reduction in photosynthesis, that leads to reduction in expansion of tissues and disturbance in the mineral supply. The reasons for inhibition of tomato leaf expansion by salinity stress are due to inhibition of cell division, disturbance of waterbalance and closure of leaf stomata (Jaleel et al. 2008 [22]). The salinity of the soil leads to an elongation of the main root axis, a developed root system ensures a better absorption of water from the deeper areas. A similar response has been reported in other salt tolerant plants (Benmahioul et al. 2009 [23]).

Plants protect their aerial organs from the invasion of toxic ions (Na+ and Cl-) by retaining them in the roots. This response would therefore constitute a resistance mechanism of glycophytic plants under saline conditions (Acosta Motoset al. 2017 [24]).

The RDW/SDW ratio was affected by soil salinity. However, V1 shows significant RDW/SDW ratios compared to V2, the aerial part is less affected by salinity than the root system resulting in a decrease in RDW/SDW ratio. This decrease in ratio is often related to water stress following salt stress (Hsiao and Xu 2000 [25]).

Table 1. Effect of salinity and genotype on morphological parameters

G	SS	Shoot				Root	
		SD (ns/ua)			SL (cm)		
		SDSE	SDIE	LN		RL	RDW/SDW
V1	Control	21,5±1,5	39,5±2,5	13±0	37,5±0,5	36,2±2,6	0,41±0,01
	50Mm	40,5±5,5	64,5±5,5	11±0	25,85±0,95	41,1±0,9	0,31±0
	100Mm	43±1	56±3	11±0	25,35±0,15	41,75±2,25	0,32±0
	150Mm	38±1	52,5±1,5	10±1	24,9±1,30	48,5±7,5	0,35±0
	200Mm	37±0	63±0	10,5±0,5	23,65±0,35	45±2	0,29±0
V2	Control	21,5±0,5	32,5±1,5	11,5±0,5	34,7±2,30	36,75±2,75	0,50±0,09
	50Mm	36±0	54,5±0,5	11±0	25,1±0,10	43,55±1,95	0,30±0,03
	100Mm	36,5±0,5	45,5±0,5	11±0	24,95±0,15	35,75±0,25	0,30±0,01
	150Mm	21,5±1,5	39,5±2,5	10,5±0,5	24±1,20	41,05±1,95	0,30±0
	200Mm	34,5±1,5	50±1	10	24,65±0,75	44,6±1,6	0,27±0,02
ANOVA	G	0,001***	0,000***	0,283	0,258	0,281	0,941
	T	0,000***	0,000***	0,004**	0,000***	0,070	0,001***
	G*T	0,017**	0,712	0,239	0,500	0,440	0,308

*G: genotype; T: treatment; G X T : Genotype by treatment interaction.; SS: Salinity Stress ; SD: Stomatal Density ; SDSE: StomatalDensity Superior Epidermal ; SDIE: Stomatal Density Inferior Epidermal ; LN: Leaf Number ; SL: Stem Length ; RL: Root Length ; RDW: Root Dry Weight ; SDW: Shoot Dry Weight ; Each value was expressed as means ± Standard deviations for triplicate experiments with the probability signification : *p<0.05. ** p<0.01.*** p<0.001.*

Effect of salinity and genotypes onphysio-biochemical parameters

Significant genotypic differences existed for Chl-a andChl-t. Salinity significantly affected Chl a, Chl tot and Carot (Table 2). The combination of genotype and salinity most affected all growth traits under study except RWC, Chl-b and soluble sugar.

Chlorophyll content significantly decreased in two cultivars with increasing salinity. A decrease in Chl-a was noticed following salt stress intensity from 11.97µg/gFw in control to 3.15µg/gFw in 150mM. Chl-b decreased from 4.67µg/gFw in control to 2.52 µg/gFw in treatment of 200 mM.

Chl-t decrease from 16.64µg/gFw in control to 6.60 µg/gFw in 150mM (Table 2).

Carotenoids content was about 7.52µg/gFw in control which decreases to 2.83 µg/gFw in 200mM. In addition, RWC was higher under control compared with water stress. A decrease in RWC was from 79.94% in control to 64.72% in 200mM. Soluble sugar was higher under salinity compared to the control conditions, values increased from 21.92 µg/gFw in control to 33.54 µg/gFw in 200mM. The genotype by treatment interaction was not significant for all studied samples (Table 2).

Table 2. Effect of salinity and genotypes on physio-biochemical parameters

G	SS	RWC(%)	Chl a (µg/100mgFw)	Chl b (µg/100mgFw)	T Chl (µg/100mgFw)	Carot (µg/100mgFw)	soluble sugar (µg/100mgFw)
V1	Control	79,94±7,06	11,97±0,33	4,67±0,30	16,64±0,04	7,52±0,31	21,92±6,69
	50Mm	74,51±5,67	5,77±0,75	2,74±0,26	8,50±1,01	3,77±0,17	26,62±2
	100Mm	75,43±2,27	5,89±0,11	3,15±0,14	9,04±0,03	3,93±0,40	24,15±2,54
	150Mm	69,18±0,48	3,55±0,26	3,05±0,99	6,60±1,24	3,29±0,57	30,15±1,92
	200Mm	66,51±0,26	5,27±1,29	2,52±0,62	7,79±1,91	3,84±1,06	33,54±6,69
V2	Control	78,11±0,78	5,72±1,07	2,92±0,59	8,65±2,26	4,03±0,79	27,42±0,27
	50Mm	73,88±1,20	6,63±0,26	3,92±0,13	10,55±0,13	4,16±0,24	33,62±12,38
	100Mm	70,88±7,21	5,33±1,50	3,32±0,76	8,65±2,26	3,98±0,81	39,81±1,73
	150Mm	74,69±6,26	4,46	2,21	6,68	4,17	28,5±0,19
	200Mm	64,72±3,55	3,84±0,18	1,88±0,57	5,72±0,39	2,83±0,13	32,81±17,65
ANOVA	G	0,819	0,024*	0,291	0,035*	0,100	0,307
	T	0,119	0,001***	0,088	0,001**	0,012**	0,822
	G*T	0,832	0,005**	0,145	0,009**	0,019**	0,787

*G: Genotype; T: Treatment; G X T: Genotype by Treatment interaction. ; RWC: Rate Water Content; Chlorophyll a: Chl a; Chlorophyll b: Chl b; Total Chlorophyll: T Chl; Carot: Carotenoids;Fw : Fresh weight ; Each value was expressed as means ± Standard deviations for triplicate experiments with the probability signification : *p<0.05. **p<0.01.***p<0.001.*

RWC varies inversely with increasing salinity intensity, it decreases in both varieties. The plants stressed at 200mM represent the lowest averages compared to the other treatments. According to (SnoussietAbbad, 2012 [26]), salt stress affect on the plant by decreasing their water potential, causing ionic imbalance or perturbations in ionic homeostasis and it can cause ionic toxicities.

Salinity causes a decrease in chlorophyll pigment contents, namely: chlorophyll a, carotenoids and total chlorophyll. This decrease negatively influences the photosynthesis of stressed plants, and this is explained by the degradation of thylakoid membranes of chloroplasts and the alteration of the photosynthetic process (Doudech et al. 2008 [27]).

The level of chlorophyll and carotenoids in leaves generally decreases under salt stress conditions.

Similarly, leaf chlorophyll can be influenced by several factors leaf age and position, light, temperature, salinity and water availability (Hikosaka et al . 2006 [28]).

According to our results, both tomato varieties confronted salt stress by high accumulation of soluble sugars. These results synchronize with those of Ben Kaddour, (2014) [29].

Soluble sugars play a key role in protecting membranes from dehydration and reduce the aggregation of denatured proteins (Tapia et al . 2016 [8]).

The involvement of this osmoticum in the osmotic adjustment allows the maintenance of cell turgorat the highest level possible for low water potentials, thus maintaining the photosynthetic activity, membranes structure and growth (Bouzoubaa et al . 2001[30]).

Relation between salinity and morphological /physiological parameters

A positive correlation was found between salinity and SDSE (r=0.349**), SDIE (r=0.380**), RL(r=0.540***) and soluble sugar (r=0.253).A negative correlations were showed between salinity and LN (r=-0.730***), SL (r=-748***), RDW/SDW (r= -0.445**) and chlorophyll content (Chla, r=-0.645***; Chlb r= 0.587***; Carot, r=-0.550*** and TChl, r= -0.668***) (Table 3).

4. CONCLUSIONS

In conclusion, our study revealed the existence of a certain genotypic variability for the analyzed metabolites. These metabolites can therefore constitute potential criteria to characterize salinitytolerance in tomato. In general, it would seem that the impact of salinity on glycophytes would depend on the crop, the duration of exposure and the intensity of salt stress.

Table 3. Correlation coefficient between salinity and all studied traits

	SS	RWC	Chl a	Chl b	T Chl	Carot	SDS	SDI	LN	SL	RL	Soluble sugar
RWC	-0,643***	1,000										
Chl a	-0,645	0,562	1,000									
Chl b	-0,587	0,478	0,716	1,000								
Chl T	-0,668	0,572	0,978	0,847	1,000							
Carot	-0,550	0,549	0,934	0,725	0,930	1,000						
SDS	0,349	-0,442	-0,375	-0,167	-0,336	-0,485	1,000					
SDI	0,380	-0,404	-0,248	-0,131	-0,229	-0,342	0,849	1,000				
LN	-0,730	0,573	0,845	0,470	0,786	0,774	-0,415	-0,368	1,000			
SL	-0,748	0,530	0,682	0,527	0,678	0,653	-0,626	-0,575	0,711	1,000		
RL	0,540	-0,513	-0,492	-0,440	-0,508	-0,583	0,427	0,454	-0,445	-0,527	1,000	
Soluble sugar	0,253	-0,503	-0,254	0,012	-0,190	-0,181	0,132	0,108	-0,194	-0,359	0,097	1,000

*Parameters studied are: Rate water content (RWC), chlorophyll content (chlorophyll a (chl a), chlorophyll b (chl b), carotenoids (carot), total chlorophyll (tot chl), stomatal density (superior epidermal (SDS), inferior epidermal (SDI)), leaf number (LN), stem length (SL), root length (RL), root dry weight shoot dry weight ratio (RDW/SDW) and soluble sugar. Each value was expressed as means ± Standard deviations for triplicate experiments with the probability signification : *p<0.05. **p<0.01.***p<0.001.*

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