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Morphological and Physiological Responses of Tomato Varieties Under Saline Environment

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Abstract: *The Crops in dry and semi-arid locations are frequently subjected to negative environmental variables such as high soil salinity. An experiment was carried out to investigate the response of tomato to salinity, a variety that has received little attention. The impacts on gas exchange parameters, relative water content (Rwc), leaf area, and total chlorophyll and phenol levels were all studied. Salt stress was administered with four treatments were tested: 0 mM NaCl (Control), 75 mM; 145 mM and 200 mM. . The results showed that the salt stress and salinity treatments had a considerable influence on the examined parameters, with the effects being much more pronounced in all treatments especially in 200 mM salt treatment. Different modes of adaptation to saline stress were demonstrated by Tomato. The adaptations under this stress are mostly morphological (by decreasing leaf area), physiological (reduction in net CO₂ assimilation rate, stomatal conductance and transpiration, and Rwc), and biochemical (decrease of chlorophyll content). As a result, phenol buildup was increased in Tomato leaves as a common defensive strategy. These characteristics enabled tomato to be classified as a salinity-tolerant cultivar.*

Keywords: *Tomato, salinity, Gas Exchange, phenol, retention water content, chlorophyll, leaf number, branche number*

I. INTRODUCTION

Salinity is a significant abiotic element that limits plant development and fruit yield (Parada and Das, 2006) [1]. Excessive salt concentrations currently impact around 77 million hectares (5 percent). (Sheng et al., 2008) [2]. Because of the low quality of irrigation water, this Fig continues to rise from year to year. (Villa-Castorena et al., 2003) [3]. Algeria is one of the countries faced by a water deficit (especially when it comes to water quality). Faced with this challenge, the country is obligated to find a solution by calculating the actual water needs of various cultures and exploring the option of using salt water for irrigation. However, it has been established that salts harm approximately 30% of irrigated areas to varying degrees. (Hachicha, 2007) [4]. Salt-affected soils span around 1.5 million hectares, or about 10% of the country's total area. Several studies have found that salinity causes morphological, physiological, and biochemical alterations in a wide variety of crops. (Ashraf and Foolad, 2007) [5]. Tolerance to various pressures varies by species, variety, and even ecotype. (Ullah et al., 2008) [6]. Plant antioxidant systems, both enzymatic and non-enzymatic, are activated in response to stress. (e.g., catalase, glutathione reductase, several peroxidases) and non-enzymatic (vitamins C and E, carotenoids, flavonoids, and other phenolic compounds, etc.) (Apel and Hirt, 2004) [7]. These occurrences have been reported in agricultural and horticultural crops such as tomato. (Juan et al., 2005) [8]. The most common abiotic stress response is the buildup of osmolytes, which is a well-known phenomena observed in all plants. (Munns and Tester, 2008) [9]. We identified glycine betaine and proline as two of the most common solutes accumulated in plants in response to abiotic stress, and numerous studies have been conducted on these molecules, particularly in tomato plants. (Kahlaoui et al., 2013) [10]. It was discovered in an earlier work. (Bacha et al., 2015) [11] Tomatoes plants acquired substantial levels of the osmoprotectants glycine betaine and proline as protective mechanisms to acclimatize to abiotic stress when exposed to varying salinity concentrations. Aside from that, several additional substances were discovered to accumulate in response to abiotic stress; among these are phenolic compounds, which play an important role as potent antioxidants. (Petridis et al., 2012) [12]. Tomatoes are one of the most important vegetables in the human diet, and they can be either fresh or cooked. Its cultivation is vital to the Algerian agricultural economy. Several research have been conducted to study the impact of salt on tomato crops. (Sholi, 2012) [13]. Because the reaction to salt stress varies according to genetics, (Maggio et al., 2004) [14], There has been an increase in interest in examining the reaction of novel cultivars to salt stress in order to assess their salinity tolerance and choose the most resistant ones. Only few studies have been carried out in investigating the response of this cultivar to salt stress (Knight et al., 1992) [15]. This study was carried out in the same setting with the ultimate goal of analyzing the response and adaptation to salt stress of tomatoes cultivars Aicha, Marmande ,Henz1573 and Cerise. Modifications to critical physiological parameters such as gas exchange parameters, water use efficiency, leaf area, leaf number, height stem and chlorophyll content, as well as stress metabolites, particularly total phenols, will be studied.

II. MATERIALS AND METHODS

A. Preparation Des Plants

The sowing of tomato seeds was carried out in the nursery and then the transplanting of the seedlings was carried out in a pot in a greenhouse Experimental protocol Location of the test The trial was conducted at the Faculty of Natural and Life Sciences in Tiaret; under semi controlled conditions (plastic greenhouse). The plant material used The varieties of tomatoes tested in this test are :

Marmande, Heinz 1573 Cerise and Aicha. Experimental device The test shall be conducted in plastic cylinders (P.V.C) with a diameter of 10 cm and a height of 100 cm, each filled with a substrate composed of a mixture of well-decomposed organic matter, sand and earth according to the respective proportions of 1.8.1. This device has four blocks; each block consists of 12 cylinders with four varieties of tomato repeated with three repetitions. three saline concentrations are applied with 0, 75, 145 and 200 mM of NaCl respectively. For the control lot is irrigated with tap water (not salted). The experiment was conducted according to a random experimental device with four treatments. Sowing is carried out in the nursery on March, 2013 at the rate of two seeds per pot at a depth of 1 cm. Transplanting is carried out one month after sowing at the rate of one plant per cylinder. The seedlings are watered with a nutrient solution until the six-leaf stage when saline treatment has begun. Phytosanitary treatment Before the start of the experiment, the substrate used for the experiment was treated with an insecticide (karate) and a fungicide (Anvil5Sc). Irrigation Every 48 hours' irrigation is maintained at capacity in the field by the contribution of 250 ml of water for all lots; to calculate the watering dose, the weight of the pot is subtracted after twenty-four hours of wiping from the weight of the pot saturated with water. From transplanting until the application of saline stress, irrigation is provided with a nutrient solution, diluted in distilled water at a rate of 2g/l. This solution contains balanced nutrients to strengthen the vigor of plants, stimulate rooting, improve the quality of crops and help the plant recover quickly in the event of a climatic accident. Methods and measurements carried out The measurements were carried out one month after the application of the saline solution and concerned the morphological parameters; physiological of the aerial part.

B. Morphological Parameters

- 1) *Stem Height*: The length of the stem is measured using a tape measure (cm), from the soil surface to the end.
- 2) *Number of leaves/plant*: The leaves formed for each plant were counted.
- 3) *Number of branches/Plant*: The branches formed for each plant were counted.
- 4) *Leaf area/plant (cm²)*: The measurement of the leaf area was carried out at the end of the experiment using a planimeter
- 5) *Dry weight of leaves and stems/plant (g)*: Dry above-ground biomass was determined using a precision balance. The fresh weight was directly determined after the recovery of the organ. The dry peas were obtained after the passage of the organs 48 hours in the oven at 80 ° C.

C. Physiological parameters

- 1) *Relative moisture content (Rwc)*: The relative water content was determined using the method of Sangakkara et al. (1996) [16]. The excised sheets at the base are immediately weighed, this is the initial weight (Pi) they are then introduced into test tubes containing distilled water and placed at a temperature of 4 ° C for 24 hours away from light. The leaves are removed, delicately wiped by a blotting paper and weighed again, it is the weight in full turgidity (Ppt), the dry weight of the leaves (Ps) is determined by passing through the oven at a temperature of 80 ° C for 48 hours. The relative moisture content is determined by the

Next formula: $Rwc (\%) = \frac{P_i - P_s}{P_{pt} - P_s} \times 100$

- 2) *Total Chlorophyll Content*: The method used is that of Lichtenthaler and Welburn (1983) modified by Porra (2002) [17], it consists in grinding a portion 100 mg of sheet in a porcelain mortar in 8ml of acetone diluted to 80%. The grind is filtered into a test tube using Watman Paper No. 22. Then the volume of the tube is completed to 10 ml by adding diluted acetone. The absorbances were read by the spectrophotometer at wavelengths 645 nm and 663 nm. The absorbances were converted according to the authors in order to quantify the content of chlorophyll a, chlorophyll b and total chlorophyll in 1

Ug/g MF, using the following formulas:

$$Chl a = (12.21 \times 663nm) - (2.81 \times 645nm)$$

$$Chl b = (20.13 \times 645 nm) - (5.03 \times 663 nm)$$

$$Chl t = Chl a + Chl b$$

- 3) *Gas Exchange Parameters:* The net CO₂ assimilation rate (A_{CO2}), stomatal conductance (gs), and transpiration (E) were measured with an infra-red gas analyser WALZ HCM 1000 (Walz, Effeltrich, Germany). This physiological measurement was carried out within 2 h across solar noon (i.e., between 11:00 and 13:00).
- 4) *Total Phenol Content:* Total phenols were extracted in a solution of methanol (90%) and were quantified colorimetrically according to the method described by Velioglu et al. (1998) [18]. The Folin–Ciocalteu reagent was added to a suitable aliquot of the leaf extracts, and the absorption of the solution at 765 nm was measured. Values are given as mg of gallic acid per gram of D.W.
- 5) *Statistical Analysis:* Two-factor analysis of variance (ANOVA2) at the 5% threshold is used to process the results Obtained using Excel software version 2016.

III. RESULT AND DISCUSSION

A. Morphological parameters

- 1) *Stem Height:* The plant height varied significantly ($p \leq 0.01$) by different level of salinity stress at different Days After Transplanting (DAT) of tomato (Fig 1). Salinity significantly reduced the plant height of tomato at different concentrations and the reduction was quite incremental with the increase of NaCl concentrations. The natural plant height increased with increasing age but decreased with increasing salinity in tomato. Similar results were also recorded by Mohammad et al. (1998) [19] in tomato, Jafari et al. (2009) [20] and Nawaz et al. (2010) [21] in sorghum, Milne et al. (2012) [22] in lettuce and Ewase et al. (2013) [23] in coriander. The reduction of plant height may be due to inhibitory behavior of salt stress on cell division and cell expansion (Hernandez et al., 2003) [24]. Fig 8 demonstrates that the presence of salt in the environment had a negative impact on the heighta of the stressed plants. When stressed plants are compared to controls, all kinds show a drop in plant height. It should be observed that at the level of severe saline stress 200mM, the length of the stem is affected by a decrease in this parameter. Indeed, we note that all cultivars have a substantial rate of decrease when compared to the controls. Including The Aicha variety has a length of 27.43 cm and a rate of 30 percent decline in comparison to the control, followed by the Cultivar Marmande, which has a rate of 29 percent decrease in comparison to the control and a length of 31.4 cm. However, as compared to the controls, the variety Heinz1573 has a smaller decrease of 14 percent and a stem growth of 33.5 cm, followed by the variety Cerise with a value of 33.36cm and a rate of decrease of 26 percent. A level of the lot of 145mM causes a reduction in length, but it is less significant than cultivars of 200mM. Indeed we display a rate of decrease the greatest 23% in Aicha with 30.13 cm followed by intermediate values that are close to each other it is the Marmande and Heinz1573 with values respectively of 21% compared to the control (31cm) and 20% (31.23cm). While the Cerise variety displays the small value and a low rate with 15% (38.46cm) so it is less sensitive to stress at this concentration. We note that at 70mM saline stress, the varieties are less susceptible to this concentration; there is a drop, although it is small. Indeed, the reduction rate is nearly the same for all three types, Marmande, Aicha, and Cerise, with 11 percent, 10%, and 10%, respectively, in contrast to the control. The variety, Heinz1573, had the lowest rate of 8% when compared to the control.

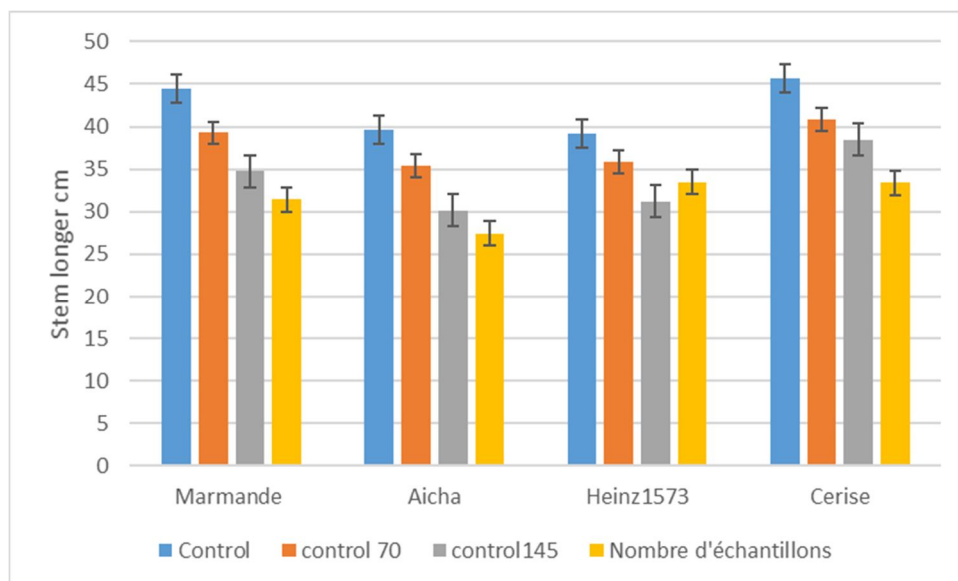


Fig 1: Stem length.

2) *Number of leaves/plant*: The number of leaves is the very important character for the growth and development of plants because the leaf is the main photosynthetic organ. Salinity adversely affected the production of the number of leaves/plant in the tomato. The results of this experiment showed that different salt concentrations have a significant effect on the number of tomato leaves/plant at different concentrations. The number of leaves/plants decreased with the increase in saline stress. A similar observation was also observed by Ewase et al. (2013) [23] who reported that the number of leaves or plants decreased with increasing concentration of NaCl in coriander. Islam (2004), Jafari et al. (2009) [20], Saberi et al. (2011) [25] also obtained a reduced number of leaves/plant under salinity stress. In this study, it was found that, the number of leaves/plant was gradually increased with increasing age with calcium supplementation with salt. Thus, these results suggest that the application of calcium increases the number of leaves by reducing the effect of salt. This fact was supported by Tzortzakis (2010) [26] in leafy vegetables, Lolaei (2012) [27] in tomatoes. This is stated in Fig 2, which shows that the leaves under stress has been negatively influenced by the presence of salt in the environment. A decrease in the number of leaves is noted for all varieties studied in stressed plants compared to controls. It should be noted that tomatoes at the level of severe saline stress 200mM, the number is affected by a significant decrease, Indeed we note that all cultivars have a considerable rate of decrease compared to controls, notably the Marmande variety where it displays a rate of decrease of 38% with a number of 11 leaves compared to the control, followed by the cultivar Heinz 1573 with a rate of decrease of 34% compared to the control and an average number of 14. At the nverse, the Cerise variety records a smaller decrease compared to the controls which is 24% and a leaf number of an average of 19.66 followed by the Aicha variety with a value of 17.33 and a decrease rate of 25%. At the level of the lot of 145mM we notice a reduction in the number of leaves but less important than the tomatoes of 200mM. It is to be noted that the rate of decrease the greatest is 15% in Aicha with a myenne of 19.66 followed by intermediate values that are identical to each other it is the Marmande and Cherry with a rate of 16% compared to the control, the first recorded the value of 19.66 and the second 21.66. While the Heinz 1573 variety displays a higher rate with 23% and an average 16.33 leaves. We notice at the level of saline stress at 70mM that the varieties are less sensitive to this concentration, certainly there is a decrease but it is slight. Indeed the cherry is less sensitive records the lowest rate compared to the control and other plants with a percentage of 6% and a number of an average of 24.33 leaves. The variety, Heinz1573 and Marmande have intermediate rates of 10% (with an average of 19 leaves) and 11% (with an average of 16 leaves) respectively.

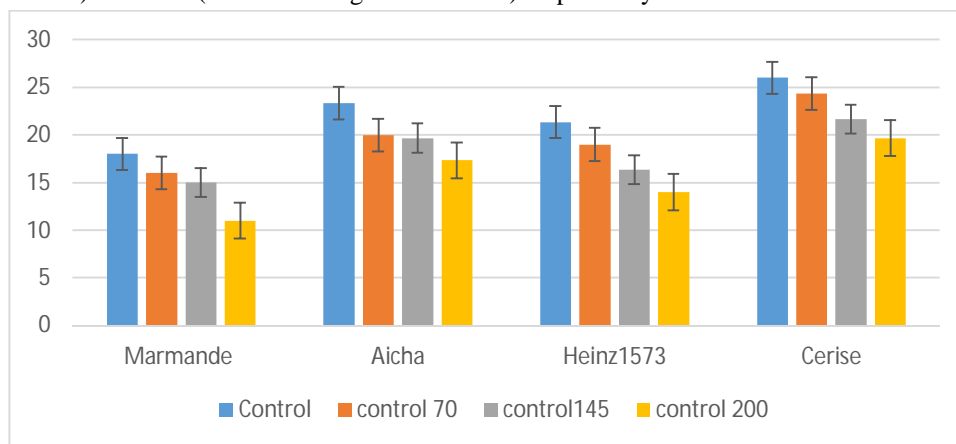


Fig. 2 Number of leaves

3) *Number of branches/Plant*: The number of tomato branches/plant was significantly affected by the different salinity levels at 70, 145 and 200 (Fig 3). Uddin et al. (2005) [28] also found that the number of branches decreased with increased salinity in *Brassica* species. A similar observation was also found in rice where the number of tillers decreased in response to salinity, which was reported by Mortazainezhad et al. (2006) [29]." At the level of severe saline stress 200mM, the number is affected by a significant decrease. Indeed, we note that all cultivars have a significant rate of decrease compared to controls, including the Marmande variety, which shows a greater rate of decrease of 70% with a number of an average of 6.33 leaves compared to the control, followed by the cultivar Aicha, which shows a rate of decrease of 34% with an average number of 10.66 branches. On the other hand, the Cherry variety has a smaller reduction compared to controls, with a decrease rate of 13% and an average number of leaves of 11, followed by the Heinz1573 variety, which has a value of 8.66 branches on average and a decrease rate of 29%.

The number of branches at the level of 145mM has decreased, It should be noted that the lowest rate of decline is 2% recorded by the Cerise variety with an average of 12.33 branches followed by registered intermediate values, at Aicha and Hanz1573 with respectively a rate of 16% (An average of 13.66 branches) and 18% (An average of 10 branches) compared to the control, . The Marmande variety has a higher rate of 31% and an average of 7.33 leaves.

At the level of the saline concentration of 70mM, the varieties are less sensitive to this concentration; and show a slight decrease. Indeed, the Hanz1573 is less sensitive, recording the lowest rate compared to the control plant with a percentage of 8% and an average of 11.33 branches. The Cherry and Aicha varieties have intermediate rates of 10% (with an average of 11.33 branches) and 11% (with an average of 14.66 branches). Respectivement.la Marmande recorded the highest rate with 15% and an average value of around 9 branches per plant.

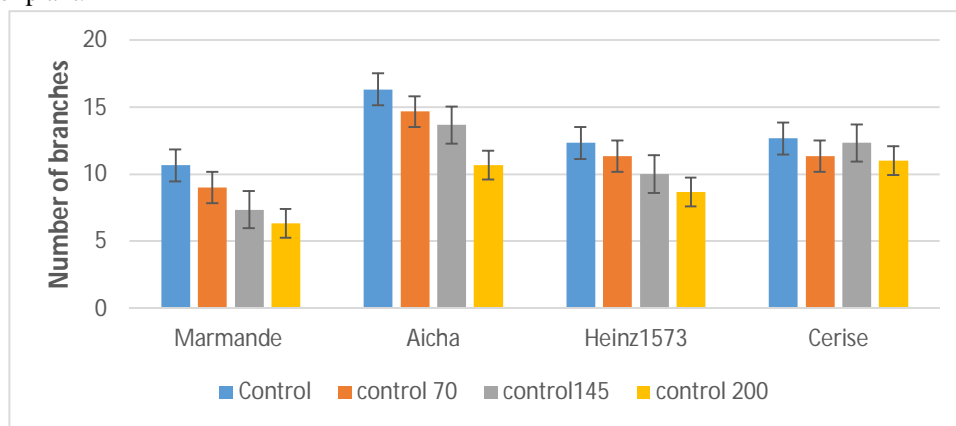


Fig. 3 Number of branches.

4) *Leaf area/plant*: Leaf area/plant was significantly influenced by different salinity levels (Fig 4). Leaf area decreased with increasing concentration of salinity in tomato. Similar result was also reported by Sixto et al. (2005) [30], Munns and Tester (2008) [9] and Saberi et al. (2011) [25]. According to Hernandez et al. (2003) [24] salt stress inhibited the cell division and cell expansion, consequently leaf expansion and as a result leaf area is reduced. Fig4 illustrates that saline stress affects the leaf surface in all tomato lines when compared to the control. The Fig indicates that the more severe the salt stress develops in tomato seedlings, the less leaf area there is. When stressed plants are compared to controls, their leaf area decreases. Furthermore, at a concentration of 200mM, the largest value is found in Marmande, with a leaf area of 16.79 cm², and the lowest in Cerise, with a value of 12.11 cm². The leaf area decreases from 28.35 cm² in the Marmande line (control) to 18.88 cm² at the lot 145mM level, which is the highest Fig reported. In this concentration, the Cerise line reported a tiny value of 13.23 cm². The value of the leaf area gradually drops to 24.14 cm² in Marmande seedlings treated with 70mM because it is the most essential value. Unlike the Cerise variety, which has a tiny surface area of 15.69 cm², the other henz1573 and Aicha types have intermediate surface measurements of 19.98 cm² and 17.43 cm² respectively. The analysis of variance (ANOVA 2) performed on the leaf area demonstrates a highly significant difference for the lined factor, as well as the concentration factor

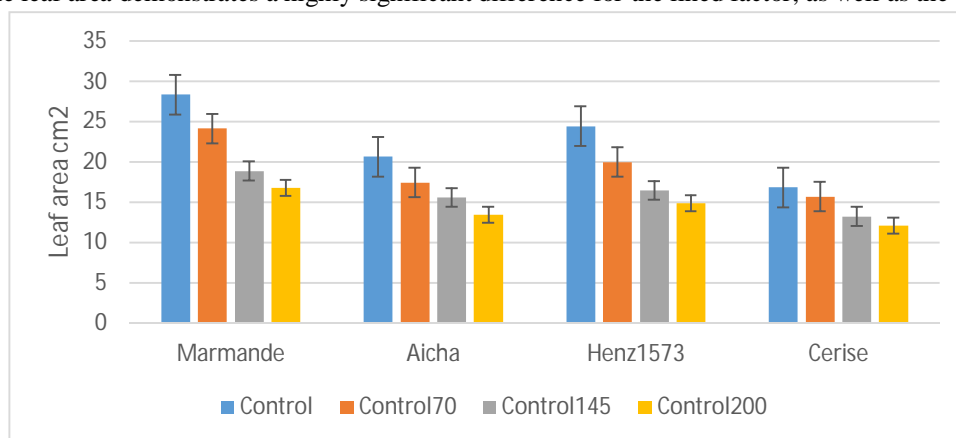


Fig .4 Leaf area.

5) *Aerial dry Matter*: NaCl induced changes in dry matter production in different parts of tomato plant. There was significant effect on leaves and stems dry weight with the different levels of salinity (Fig 5). It was found that, leaves and stems dry weight decreased with the increasing salinity level. The present results were in line with those of Akhtar and Hussain (2009) [31], who reported decline in dry weights of shoots under high salinity stress. Decreased leaves dry weight does not seem to be due to a reduction in leaves number (Cruz and Cuatreno, 1990) [32] but due to a reduction in leaf area which can be reduced proportionately more than the stems dry weight (Van Ieperen, 1996) [33]. Fig.5 shows that dry above-ground biomass is affected in all of the lines investigated. For control circumstances, there is a reduction in all lines. Figure shows that the highest dry above-ground biomass is recorded in stressed plant levels with 70mM, specifically the Marmande and Aicha lines with 5.31g and 4.99g respectively, compared to controls. The lowest values are recorded by Heinz1573 and Cerise with 3.61g and 4.79g respectively. For plants subjected to a 150mM stress, values ranging from 4.69g at the level of Marmande stressed to 4.01g at the level of Aicha stressed have been recorded. Heinz1573g with 2.30g has the smallest value, followed by Cerise with 3.74g. The application of a more severe stress of 200mM results in a significant decrease in dry matter at the line level, with the Heinz1753 variety recording the smallest amount (1.37g), followed by the Cerise variety (2.58 g). While the Marmande variety has the highest value (4.58 g), Aicha has the lowest value (3.27 g). The analysis of variance data revealed a significant difference ($P < 0.05$) between tomato lines treated to varied saline concentrations and this parameter.

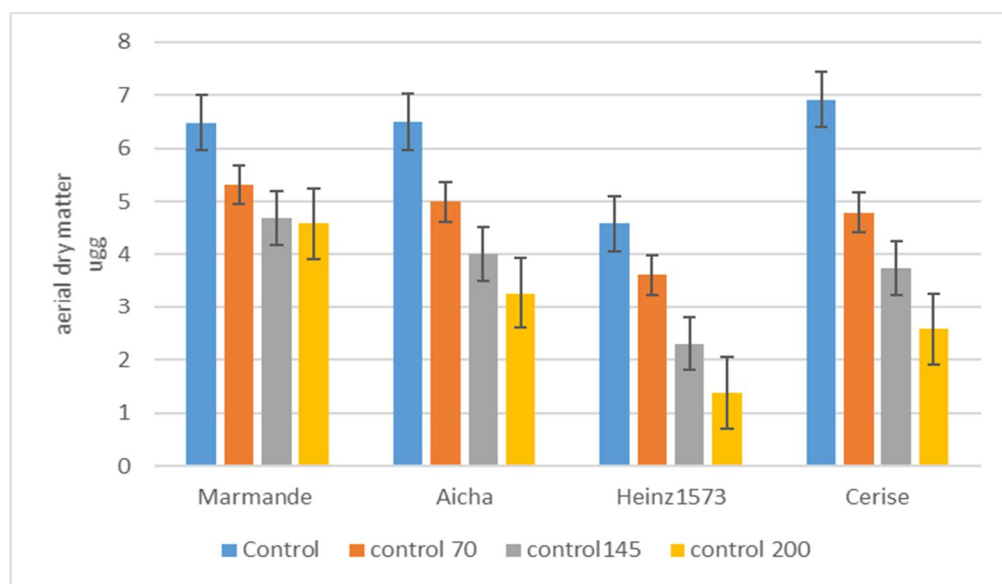


Fig. 5 Aerial dry matter.

B. Physiological Parameters

1) *Relative Water conTent (Rwc)*: The relative water content of the tomato stressed with salts combined, it indicates that the turgor of leaves is inversely proportional to the concentration of combined salts (Fig6). We notice that the relative water contents of the stressed plants are low compared to the control, or the contents are high with values more or less close where the Marmande and Aicha record respectively (78.74% and 78.42%) and these contents decrease as the concentration of the medium increases. This decrease is noticed on all cultivars. Indeed, at 70 mM, the relative water content is 63.63%, displayed by the Heinz1573 genotype while at 200 mM, it is 51.80%. The statistical analysis shows that salinity acts very significantly on turgor. According to the results obtained, it seems that La Marmande has a higher tolerance to salinity followed by Aicha compared to other tomato plants, given the maintenance of greater turgor in the first species and therefore better osmotic regulation. Our results also showed that the more stress intensifies, the lower the relative water content (TRE or RWC). These results are in line with the work of [34] having indicated that in *Atriplex halimus* L. stressed with NaCl and seawater (50% and 100%) the TRE decreases; Albouchi A., Bejaoui Z., and Hedi El Aouni M., 2003 [35] in *Casuarina glauca*, Mefti M., Abdelguerfi A., and Chebouti A., 2000 [36] in *Ykhlef A. alfalfa*, 2001 [37] Adjab and M., 2002 [38] in wheat. Similarly, Albouchi and Al, 2003 [39] show a decrease in relative water content and a significant reduction in total biomass production.

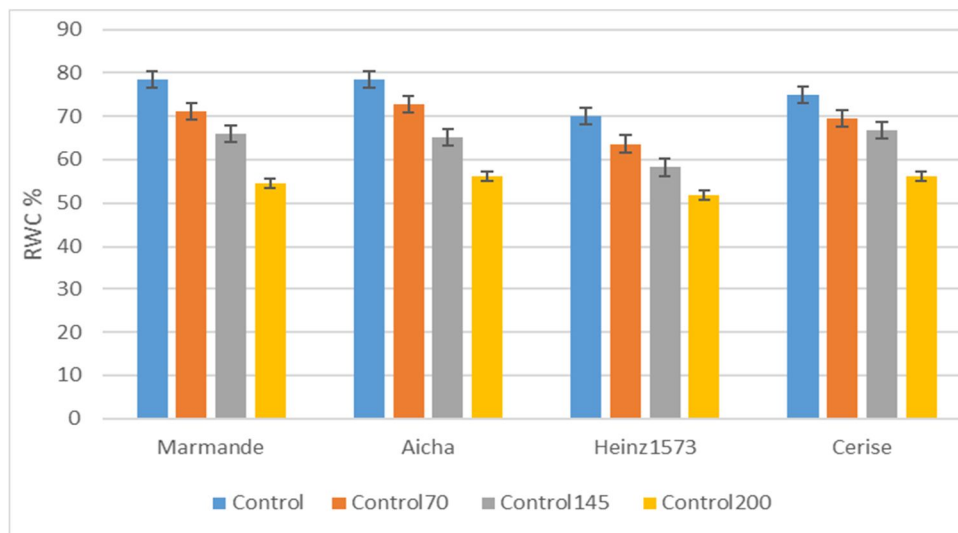


Fig. 6 Relative water content.

2) *Leaf Chlorophyll Total Content*: There was a clear effect of salinity on the leaf chlorophyll content of tomato plant (Fig7). The chlorophyll content in tomato leaves decreased with increasing salinity levels. From the results, it was found that the high levels of salinity (200mM) induced a significant decrease in the total chlorophyll content as compared to control plants. these results were also supported by Naher (2014) [40]. Salinity reduced the total chlorophyll content in leaves which was also supported by Netondo et al. (2004) [41] and Amini and Ehsanpour (2006) [42]. The total chlorophyll content is the sum of the two preceding values. In the absence of saline stress, Fig 7 reveals that Aicha and Marmande are the types with the highest total chlorophyll content. Cerise and henz1573, on the other hand, produce less chlorophyll under normal conditions. When these plants are treated to 70mM of moderate stress, the percentage reduction compared to the control ranges from 14 to,23,20, and 21 in the plants Marmande, Aicha, Heinz1573, and Cerise, respectively. At the maximum degree of saline stress, 145mM, we see a drop of 49 percent and 47 percent observed by Aicha and Heinz1573, respectively. The other two kinds, on the other hand, show the same decline rate of 40%. In the presence of severe stress at 200mM, Cerise and Aicha maintain a relatively high chlorophyll content compared to the others, with 41.62 ug/g MF and 44.89ug/g MF, respectively, with a decline rate of 52% and 63%. Marmande and Heinz1573, on the other hand, exhibit a 68 percent and 61 percent reduction in comparison to the control, respectively. On the one hand, we discovered that the overall chlorophyll content declines as the level of stress increases.

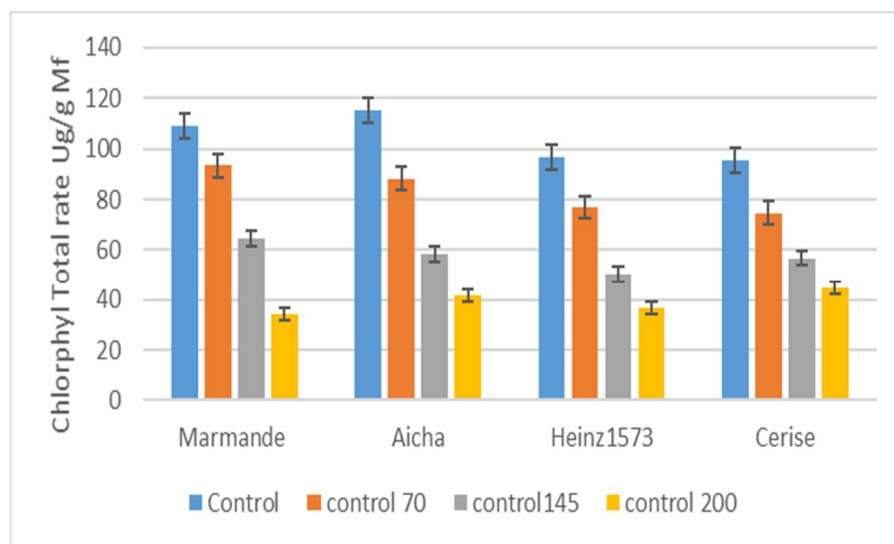


Fig. 7 Total Chlorophyll rate A+ B.

3) Gas Exchange

a) **Stomatal Conductance:** Statistical analysis exhibited significant differences among treatment and genotypes regarding stomatal conductance. Which means that each genotype performed differently under stress and normal conditions. There was non-significant relation for the interaction between genotypes and different salt stress levels. Which implies that at interaction that there was no variation existed. Overall results indicated that due to salinity level increased, plant decreased. Fig. 8 indicated the interaction of the stomatal conductance means between genotypes and different salt stress levels. There was significant relationship among genotypes under different salt stress levels. The stomatal conductance at the treatment level of 200mM is important, where Marmande reaches 72% with $0.033 \text{ H}_2\text{O m}^{-2} \text{ s}^{-1}$ and the lowest rate recorded by the Heinz1573 variety which displays a percentage of 37 with a value of $0.083 \text{ H}_2\text{O m}^{-2} \text{ s}^{-1}$. Cherry and Aicha has intermediate rates. At the level of salinity treatment 145 mM, the highest rate of stomatal conductance was obtained in Marmande with 48%, with an average of $0.06 \text{ H}_2\text{O m}^{-2} \text{ s}^{-1}$ and the lowest was recorded in the Cerise line with 22% with a conductance of $0.13 \text{ H}_2\text{O m}^{-2} \text{ s}^{-1}$ which is the highest value. The other cultivars have intermediate values. For the 70 mM concentration, the smallest reduction in conductance occurred in the Cerise line with 10% and the highest rate recorded by the Marmande variety with 24%.

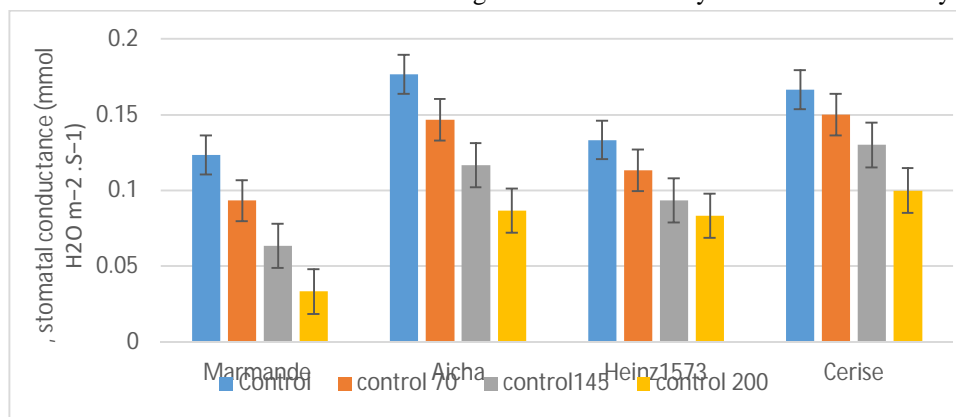


Fig. 8 Stomatal conductance.

b) **Transpiration Rate:** The analysis showed significant differences between treatment and genotypes with regard to sweating rate. This means that the Xgenotypes worked differently under stress and normal conditions. Fig 9 revealed that sweating at the treatment level of 200mM, in Marmande reaches a reduction rate compared to temoins which is 60% with $2.16 \text{ mmol H}_2\text{O m}^{-2} \text{ S}^{-1}$. On the other hand, the lowest rate is displayed by the Cherry line with a value of 49% and an average of $3.59 \text{ mmol H}_2\text{O m}^{-2} \text{ S}^{-1}$. Heinz1573 and Aicha have intermediate rates. At the level of the batch treated at 145 mM, it is noted that the highest rate of transpiration was recorded by Marmande with 50%, with an average of $2.72 \text{ mmol H}_2\text{O m}^{-2} \text{ S}^{-1}$ and the lowest rate displayed in the Cherry cultivar with 40% and a transpiration of $4.26 \text{ mmol H}_2\text{O m}^{-2} \text{ S}^{-1}$, the other lineages record intermediate values. Regarding saline stress at 70 mM, the greatest rate of reduction is observed in the Marmande plant with 42% with a value of $3.15 \text{ mmol H}_2\text{O m}^{-2} \text{ S}^{-1}$ and the lowest rate displayed by the Cherry variety with 32% with an average transpiration of $4.8 \text{ mmol H}_2\text{O m}^{-2} \text{ S}^{-1}$.

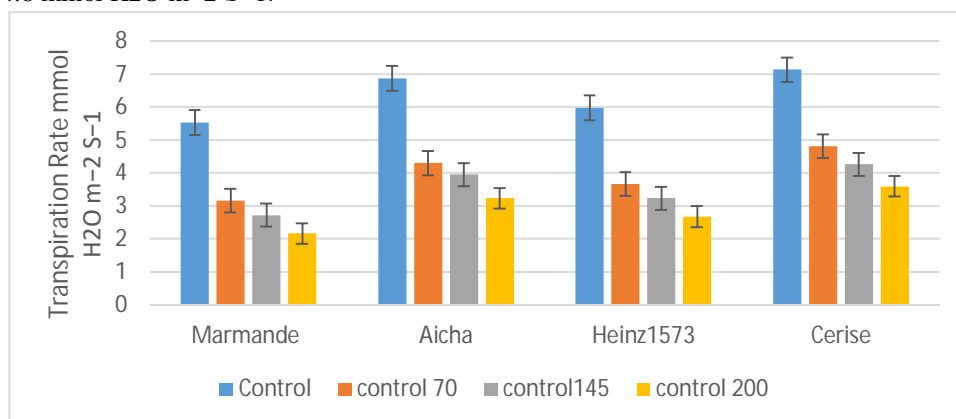


Fig. 9 Transpiration rate.

c) *CO₂ Gas Exchange*: The analysis showed significant differences between treatment and genotypes with regard to CO₂ gas exchange. This means that the genotypes worked differently under stress and normal conditions. There was a non-significant relationship for the interaction between genotypes and different levels of saline stress. This implies that at the interaction, there was no variation. Changes in CO₂ gas exchanges show that all genotypes studied respond negatively to saline stress. However, this response varies depending on the intensity of the stress and the variety in question (Fig 10). When stress is moderate (70 mM), all cultivars experience a decrease in this CO₂ gas exchange. Tomato varieties Marmande, Aicha, Heinz1573 and Cerise showed a significant decrease compared to their control and showed percentage reductions of 32, 23, 25 and 23 respectively (Fig 10). At the stress level at 145 mM, these varieties also show a reduction in this gas exchange where the greatest value is recorded by the cultivar Aicha with 8.63 which represents a reduction compared to the control of 32% and the lowest value is observed in Marmande n 4.74 $\mu\text{mol CO}_2\text{ m}^{-2}\text{ s}^{-1}$ with a rate of 48%, the other Heinz1573 and Cerise plants recorded intermediate values of 7.31 ($\mu\text{mol CO}_2\text{ m}^{-2}\text{ s}^{-1}$ (36%)) and 8.36 $\mu\text{mol CO}_2\text{ m}^{-2}\text{ s}^{-1}$ (35%) respectively. When the stress is severe (200mM), this mechanism is even more affected, especially in the case of Marmande whose percentage reduction is 74% compared to that of the control. The statistical study shows that there are significant differences between the varieties studied ($p < 0.05$) and shows that in case of severe stress, the Cerise genotype displays a lower percentage of 44% compared to controls with 7.16 $\mu\text{mol CO}_2\text{ m}^{-2}\text{ s}^{-1}$ followed by Heinz1573 and Aicha with a 48% reduction both with the following values 5.89 $\mu\text{mol CO}_2\text{ m}^{-2}\text{ s}^{-1}$ and 6.6 $\mu\text{mol CO}_2\text{ m}^{-2}\text{ s}^{-1}$. Photosynthesis is the most fundamental and complex physiological process that all green plants undergo, as it significantly affects plant growth. Since the mechanism of photosynthesis involves various components, including photosynthetic pigments and photosystems, the electron transport system, and CO₂ reduction pathways, any damage at any level caused by stress can reduce the overall photosynthetic capacity of a green plant (Ashraf and Harris, 2013) [43]. These results are in agreement with those of Lycoskoufis et al. (2005) [44], Niu et al. (2010) [45] and Cheng-Jin et al. (2011) [46] who found a significant decrease in gas exchange parameters under saline stress. In fact, the rapid stomatal response of stressed plants may represent a mechanism of salinity tolerance (Jones, 1974) [47]. Salinity may limit net photosynthesis and stomatal conductance, either due to a limitation in CO₂ supply resulting from the partial closure of the stomata (stomatal function), or by modifying the biochemical mechanism of CO₂ fixation (not a stomatal function), or by both procedures (Chaves et al., 2003) [48]. Cell membranes have been reported to be highly sensitive to stress (Tayefi-Nasrabadi et al., 2011) [49]. Therefore, there is evidence that the accumulation of high concentrations of Na⁺ and Cl⁻ in chloroplasts under salinity stress damages thylakoid membranes (Omoto et al., 2010) [50].

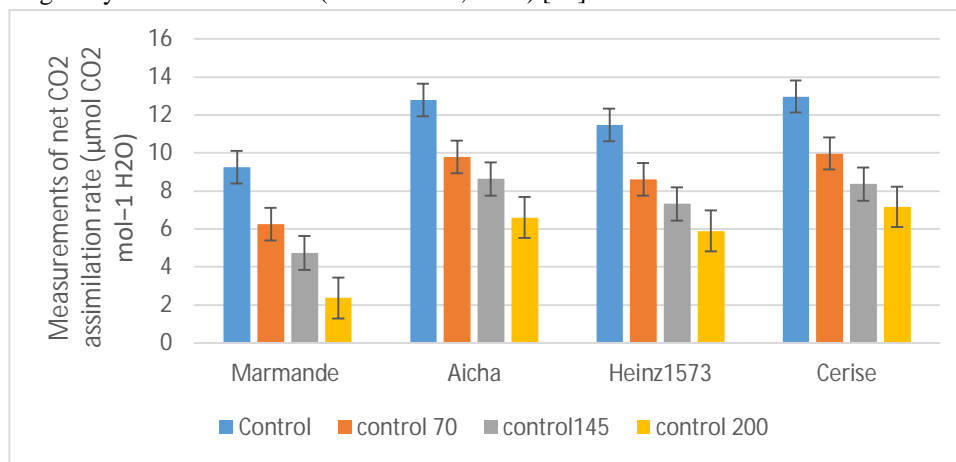


Fig. 10 Gas exchange CO₂.

d) *Response of Phenols to salt-stress Treatments*

In response to abiotic stress, plants have developed a wide variety of highly sophisticated and effective mechanisms to detect, respond to, and adapt to a wide range of environmental changes. A common defense mechanism activated in plants exposed to stressful conditions is the production and accumulation of phenolic compounds. The antioxidant property of plant phenolic compounds, the metabolic pathways of their biosynthesis and the enzymes involved are well documented in the most important plant species (Balasundram et al., 2006) [51]. Some enzymes involved in phenolic metabolism such as polyphenol oxidase (PPO) and peroxidase (POD) generally react actively to the presence of stress in the plant (Lotfi et al., 2010) [52].

The Analysis (Fig 11) revealed that salt had a significant effect on phenol production, and that the accumulation of this product increased in the leaves of all four tomato genotypes when subjected to saline stress.

Fig 11 illustrates the average results obtained for the phenol content generated in the leaves of the four tomato lines treated at varying saline concentrations. Phenol content increases in stressed plants compared to controls, with the highest levels observed in severely stressed batches (2000 mM). The Marmande line had the highest concentration at 4.8 mg eq. GA g⁻¹ DW, which represents an increase of 48% compared to control. the Aichaa tomato displayed the lowest amount of phenol under saline stress with 4.38 mg eq. GA g⁻¹ DW, which represents a rate of increase of 49%, while tomato henz1573 and Cherry showed intermediate accumulation with values of 4.57 mg eq. GA g⁻¹ DW (a lower rate of increase with 41%) and 4.66 mg eq. GA g⁻¹ DW (an increase rate of 49%).

At the stressed batch level of 145 mM, the percentage of phenol accumulation was higher in Marmande (35%) with an amount of 3.72 mg eq. GA g⁻¹ DW and the lowest amount recorded in Aicha with 3.22 mg eq. GA g⁻¹ DW (33%). Henz1573 and cherry accumulate an amount of phenol intermediate depending on the genotype, with values of 3.37 mg eq. GA g⁻¹ DW (27%) and 3.7 mg/ml (37%).

We note that in the saline concentration of 70mM, the Henz1573 and Cherry variety displays the same significant amount of phenol, of the order of 2.78 mg eq. GA g⁻¹ DW, which is 21% and 25% higher than the control, respectively. The Aicha cultivar, on the other hand, has the lowest amount observed, which is 2.1 mg eq. GA g⁻¹ DW with an increase rate compared to control of 18%. The Marmande variety scored an average of 2.21 mg eq. GA g⁻¹ DW (17%). Our results are consistent with Al Hassan et al. (2015) [53] who observed a significant increase in phenolic compounds in stressed tomato leaves under moderate and high salt concentrations in cherry tomato (*S. lycopersicum* L. cv. cerasiforme). Increasing the levels of antioxidant phenolic compounds in the leaves can be considered part of the induced response to cope with salinity-induced oxidative stress. Thus, salt-stressed plants could represent potential sources of polyphenols, increasing the concentration of polyphenols in tissues, which is a problem directly related to human health since these compounds are known to be bioactive compounds (Jemai et al., 2008) [54]. In fact, an optimal polyphenol content would be achieved using stress-tolerant species (De Abreu and Mazzafera, 2005) [55].

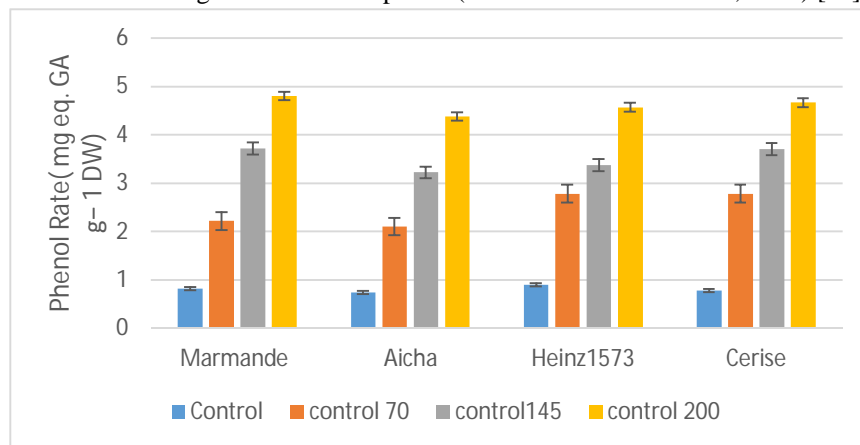


Fig. 11 Phenol Rate

IV. CONCLUSION

The mechanism of adaptation to saline stress results in morphological, physiological, and biochemical responses. Our results also showed that the salt induced limitation of the photosynthetic activity was mostly attributed to stomatal limitation.

In this study, tomato plants adapted to salt stress by reducing leaf area, stomatal conductance, and minimizing water loss by transpiration. These led to greater reduction in photosynthetic rate, especially in salin concentration of 200 mM salt treatment, and a decrease of the content of the major photosynthetic pigment. Besides, Tomato has the ability to increase the efficiency of water use, which is of vital importance in terms of water saving and which represents a strategy to improve the performance of crops in arid conditions. In consequence phenol accumulation was stimulated in the leaves of Tomato as a common defensive mechanism. These facts could be one of the strategies used by these plants to tolerate the severe conditions imposed by soils with a high salt concentration in arid lands. Though some other yet unidentified factors could also be involved in the survival of these plants under continuous salt stress.

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