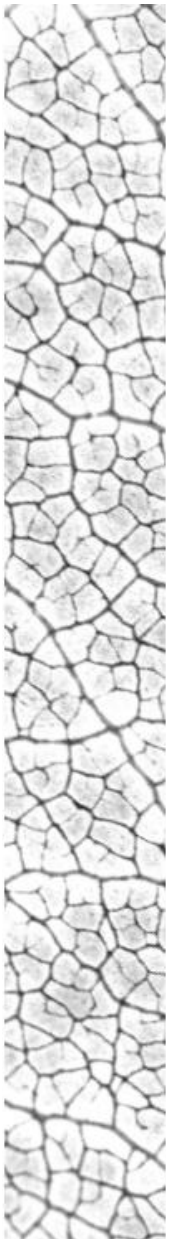

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The impact of NaCl on different genotypes of tomato (*Solanum Lycopersicon* Mil) on germination, some physiology characteristics and gene expression

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Abstract
Four common landraces of tomato were investigated for the response in some physiological parameter and variation in gene expression. The results of the present study found that salinity stress is negatively affect germination percentage and ranged from 60% to 80% and the effect of salinity levels was found to be related to the exposure time to salinity treatment and the genotypic of tomato landraces. similar negative effect of salinity was reported the length of radical length. The amount of chlorophyll was highly influenced by salinity and was ranged between about 26 to 40 and was found to be largely dependent on genotypic characteristics of tomato landraces and the time of exposure to salinity. Moreover, it was found that the increase in salinity level significantly impacted the stomatal conductance of varied genotype of tomatos. Additionally, germination rate and germination rate were both affected by salinity with varied degrees. Based on the results (chlorophyll content, stomatal conductance and germination ability) presented in this study it was found that the landrace (111) and landrace (975) were both salt tolerant and the landrace of Quaresma (111) is highly sensitive to salinity.

Keywords: salinity, Jordan, tomato, photosynthesis, stomatal conductance, germination

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INTRODUCTION

There are several varieties of salt-tolerant plants, and these plants have become a major hope in desert and non-arable land cultivation through either directly benefits and cultivation, as human-consuming crops as animal feed, or indirectly benefit from them and indirectly(Othman et al., 2006; Al-Tawaha and Al-Ghzawi,2013; Abu Obaid et al. 2017; Abu Obaid et al. 2018; Al-Tawaha et al. 2018).

Soil salinity is an important determinant limiting fruit production and agricultural biomass in various location worldwide and it limited the economy and the utilization of the influenced landscape. Salinity common hit the arid and semiarid lands such as Jordan (in which the rainfall is not sufficient to leak the accumulated minerals out of the zones of the plant. Increased in soil saline can also resulted in watered lands when polluted water is utilized for watering (Mullens and Luhring 1996). The shortages of water reservoir in Jordan has led local breeder for utilization of polluted H_2O for watering. Agriculturally important plant varies in their potentiality of growing under such saline soil and lead to the concentration of high saline in their varied tissues and organs of plants. High level of saline soil lead to high osmotic stress and/or can potentially lead to minerals deleterious effects (Odat 2018; Seyednejhad et al, 2020) which results inhibiting in the water root content and solutes utilization by crops (Benzarti et al. 2014; Ahmadinejad and Talebi Trai, 2019). Growing tomato in salt mixture is considered an efficient methodology that vigorously limit the habitat for the estimation of the adaptability of the tomatoes to salinity stress. Using such methodology reduces interferences and the confounding factors induced by the habitat conditions relative control approach (Meyer et al., 1989). Salinity in soil can impair nutrient and minerals utilization by antagonistic impacts

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with essential nutrients (Shibli, 1993). The biomass and growth of tomato is highly decreased by increasing salinity levels (Snapp et al 1994). The effects of salinity on tomato to salinity is different in correspondence to varieties and genotypes (Mitchell et al. 1991). Knight et al. (1992) indicated that the length of the root was decrease to about 55% upon exposure to saline conditions of 4 days. *Asr1* and *Asr2* genes are prominent genes in tomato as well as in several plants' species. These two genes were found to be strongly regulated under stress factors such as salinity and drought. Additionally, the genes also control the transcript of transporter in grape and is responsible for the storage of in sugar and amino acid maize and potato. The significate impact of salinity on the crops can result from the low quality to soil conditions that impact seed germination and some physiological characteristics. The main aim of this investigation was to study the root length, germination ability and some physiological characteristics of four different genotype of tomato.

MATERIALS AND METHODS

Four genotypes of local Jordanian tomato (*Solanum Lycopersicon* Mill), were used in this investigation: of Jordan (111; Kharja and 975, 973; Rhaba), and one commercially used on Quaresma; Syngenta Company). Initially germinated seed were placed into the PVC pots of 40 cm in height and 37 cm in diameter, these were watered with H_2O every second day until zero time of experiment. After about 15 days the second seedling stage were treatment first with salinity using different concentration of NaCl solutions (0, 50, 100 and 200 mM) using randomized complete block design (RCBD) by four control replicates under greenhouse setting. 20 of sanitized seeds were vortexed after washing for twenty minutes on the shaker and then placed at a temperature of about 50 °C. Studied tomato genotypes were put on plates which was lined filtration papers, and were watered with (11 ml) of DW or NaCl mixture under varied level of concentration (50, 100, 200 mM), Germination characteristics and their calculation (Number of germinated seeds (#G) Radical root length (RL), the germination percentage (G%), germination speed (GS) and germination rate (GR)) were measured at 4, 6 and 8 days after placed in the a chamber at 25°C. Afterward sixty of seeds of every genotype were watered with DW and then were resettled into pots which using a ratio of (1:1) Peatmoss and perlite and were watered with H_2Owater once every two days for 2 weeks (zero time). At the zero time at the second week tomato were treated with distilled water and/or salt solutions (NaCl) of different concentration at three variable time (zero time, 4 and 6 days), and different physiological characteristics were estimated such as total chlorophyll content using SPAD meter (MINOLAT, Japan) and stomata conductivity using Porometer (AP4, Delta-T Device, Cambridge, UK). RNA isolation and the real time PCR for both Asr1 and Asr2 under various concentration of salt was conducted following the protocol described in

RESULTS AND DISCUSSIONS

The impact of NaCl on germination root growth

Genetic diversity within crops represent a unique opportunity for deciphering the underling process of salt adaptability being utilized for providing local breeding confronting the issue of high soil salinity (Osmond et al. 1987). The finding in this work revealed in general variation response to different genotypes of tomato cultivars used in Jordan. Generally, the finding indicated that increased levels of NaCl salinity (50 mM, 100 mM, 200 mM) are largely impact the percentage of germination (P value < 0.05, Fig. 1). Nonetheless, the highest level of NaCl (200 mM) produced the minimum germination percentage at all time period used. Furthermore, the concentration of 100 mM NaCl level produced germination about 31% at day 4, whereas the lower level (50 mM) has resulted a ranged of germination of about 51% and about 85%. With regard to the effect of NaCl on the rate of germination the results indicated impact of different degree. The rate was revealed to be inhibited by the higher levels of salinity at all time period, the rate was ranged between (6-8) days followed by (4-6) days and the least degree was found between (4-8) days, particularly at 200 mM (Figure 2). Additionally, the highest time of germination of tomato genotype as affected salinity was reported at 4 day. Variation in time of germination at NaCl levels was approximately (0-21.39). (Figure 2). Additional results revealed inhibitory influence of of NaCl on the radical root length at all time of exposure to NaCl. Figure (3) depicts lengthening of radical root length between different genotypes of studied tomatos upon exposure to various levels of NaCl at all times used. The highest length of the radical was reported at 5.44 cm at zero mM during day 8, while 200 mM of NaCl level inhibited the completed growth of root (figure 3). Our results agree with several studies that that root system is the first to be in contact and influence by stress conditions (Kafi and Rahimi 2011). Our results, therefore, indicated that the content of water in root tissue are significantly affect by the varied concentration of salinity of NaCl and to the time exposed to salt. Moreover, the results showed that the high concentrations of NaCl lead to inhibition of the radicle length in all genotypes studied most likely because radical roots are direct in touch with the habitat of plant and with their H_2O and micronutrients captivated from it and affected by soluble salts in the root zone (Garg et al. 1993). Additionally, the results indicated that there is a negatively and differential response to salinity level of NaCl. The germination of four landraces of tomato was significantly decreased by different salt concentrations. Generally in agreement with various studies (Munns and Tester 2008) our results revealed that the salinity impact was significant when salinity concentration increased, probably due to decrease in

germination process and increasing in germination time that need it to complete the process germination and most likely because of decreasing in seeds moisture level that prevent or decrease the possibility of breaking seed dormancy stage by inhibition of many enzymes under salinity stress (Kazemi et al. 2014; Mozafariyan et al. 2013; Mirzaie, A. 2018. 2018).

Effect of salinity on physiological characteristics of (*Solanum Lycopersicon* Mill*)*

NaCl salt in soil is also responsible for may alternation for the biochemical characteristics of tomato that directly impact growth and yield. We found that the conductivity ability of stomata is high influence by salinity of NACl (Figure 4). In this study the results revealed that the highest conductivity of stomata was at intermediary level of NaCl (50, 100 mM) and it ranges from 0.053 cm/s to 0.18 cm/s relative to control treatment. With regards to whole chlorophyll content it considers a vital determinant of photosynthesis. Figure (4) indicates a high influence and the inverse association between salinity and the content of chlorophyll under all concentration for salt and at all times periods used. Namely, the findings showed that the genotypes (Quaresma) is the most affected among all studied genotypes at day 4 (39.73, 38.10, 34.70, 33.83 and 39.90). Moreover, our results indicated that increased salinity on the four studied landraces tomato, have significant impact on the conductivity stomata with is probably related to influencing ABA signal pathway that lead to loss of water by guard cell under habitat stress (REFERERE). Moreover, our results indicated that the total content of chlorophyll in greatly inhibited by salinity probably because salinity can lead to the synthesis of key enzyme and/or destruction of many pigments and/or alteration of chloroplast microstructure (Suo et al. 2017)

Effect of salinity on gene expression of *Asr*1 and *Asr*2 genes under salinity stress

β-actin gene is a common gene that employed as a control gen because it stable transcription and regulation at all plant tissues and under stress conditions. (Coker and Davies, 2003). In this study, the level of transcript of *Asr*1 and *Asr* 2 genes was investigated for all genotypes varied levels of salinity and at all time of exposure to salinity stress. Results revealed a varied expression levels among the studied genotypes. Under 100 mM of NaCl at day 4, the the expression of *Asr1* genotype 111 was found to be around (13 fold) and genotype 973 about (0.9 fold). Especially for the genotype of Quaresma (21.122 fold) it showed the highest expression recorded at the highs level of salinity. In general, there was increase of gene transcript after day 6 and day 4 with the higher increase in fold variation all genotype but 973 genotypes with highest recorded at about (16) at 200 mM (Table 1). (Table 2) showed the expression level fo the gene *Asr2* which was found to be increased at day 4 (1.61-fold, 33.43-fold), and 111 and (Quaresma) genotypes was at 50 mM, and the expression 973 genotype was found to be (18-fold, 28 fold) at 100 mM and finally the genotype 975 recorded the highest expression level at 200 mM (Table 2). We found that the two genes Asr1 and Asr2 are both influence by all levels of salinity (Table). It is commonly accepted that the environmental stimulus, such as salinity was increasing the Abscisic acid level can induced Asr genes to expression (Golan et al. 2014). The increase in the expression of these genes is believed that they lead to increase to adaptably to salinity level (Shkolnik and Bar-Zvi 2008).

CONCLUSION

Soil NaCl has large impact of the ability of tomato germination and the root growth of different landraces. The degree of impact of salinity is highly dependent on the level of (NaCl) and to their exposure time. The transcription of Asr genes (*Asr1*, *Asr2*) is highly influenced by salt, whereas the variation in expression level different landrace was found to be genotypic dependent.

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