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RESEARCH PAPER

Assessment Results of Salinity Stressed F_2 Population Originated from Interspecific Hybridization of Eggplant with Wild Relative *Solanum incanum* L.

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Abstract

Salinity, which is one of the major abiotic stresses, prevails in mostly arid and semiarid areas that is nearly 20% of the world's cultivated area. Excessive amounts of salt around the plant root zone are detrimental to vegetative growth and economic yield. Today salinization is still severely expanding and posing a great threat to the development of sustainable agriculture. Although eggplant (*Solanum melongena* L.) is considered moderately sensitive, soil salinity mitigates strictly the growth and yield. Eggplant has significant crop wild relatives (CWRs) which are thought to be more tolerant to abiotic stresses and it is substantial to exploit their potential against salinity in hybrid breeding studies. It has previously been proven that *Solanum incanum* L. has tolerance to salinity stress. This study aimed to improve salinity-tolerant pure eggplant lines. Therefore, the acquired F_2 population from interspecific hybridization between the pure line (BATEM-TDC47) with distinctive features from BATEM eggplant gene pool and *S. incanum* L., were subjected to salinity stress at 150 mM NaCl level with its parents and F_1 plants. On the 12^{th} day after the last salt treatment, the plants were evaluated using a 0-5 visual scale. Among the 256 stressed plants, 50 F_2 individuals were determined to be salt tolerant. Additionally, some of their morphological and physiological features, such as shoot length, stem diameter, number of leaves, anthocyanin presence, prickliness, malondialdehyde (MDA), and proline levels, were studied and compared to the controls of their parent and F_1 plants. Results showed that shoot length and stem diameter decreased dramatically under salt stress. According to the analysis, the average MDA and proline levels of the F_2 population were identified as 10.9 μ mol g^{-1} FW and 8.4 μ mol g^{-1} FW, respectively. The distinguished 50 F_2 plants that showed salinity tolerance were transferred to the greenhouse and self-pollinated to produce the F_3 generation.

1. Introduction

In order to supply the food demand of the world's constantly expanding population, the total yield per unit area should be increased. However, agricultural production is gradually decreasing due to the negative effects of various factors such as climate change (Hemathilake and Gunathilake, 2022). Climate change is a multifaceted system that affects biotic and abiotic components on the world (Chaudhry and Sidhu, 2022). Drought, salinity, and heat are the main abiotic stresses that cause reduced growth and crop yield (Giordano et al., 2021).

Salinization occurs due to the accumulation of salt ions (mostly Na^+ and Cl^-) in soil and it is expressed with electrical conductivity (EC). When soil EC reaches 4 dS m^{-1} (equivalent to 40 mM NaCl), the yield of most crops is significantly reduced (Munns and Tester, 2008). In addition, excessive amounts of Na^+ inhibit water conductivity, soil porosity, and aeration (Singh et al., 2018).

Salinity is one of the alarming abiotic stresses and it threatens the sustainable agriculture. Harmful impacts are expected to spread to nearly 50% of total agricultural land by 2050 (Singh et al., 2018; Kumar et al., 2020). Vegetables are more sensitive to saline conditions when compared to the other crops (Chinnusamy et al., 2005). Due to salinity, reduced nutrient mobilization causes poor plant growth and decreases in yield. The negative effects of salt stress on plants are mitigated by some external applications such as, arbuscular mycorrhizal fungi (Porcel et al., 2012; Hanci et al, 2014; Evelin et al., 2019), plant growth promoting bacteria (Etesami and Noori, 2019) and some seed priming methods (Ibrahim, 2016; Johnson and Puthur, 2021). However, the use of these techniques has not yet become practical. Therefore, the development of salt-tolerant crop varieties remains important and necessary to achieve economic yields in affected areas.

High salt concentration near the plant root zone damages to vegetative growth and economic production in all crops. Plants respond to salt stress in a number of ways in terms of morphological, physiological, and biochemical ways. Mainly, 100-200 mM NaCl concentration in soil can limit growth or lead to plant mortality (Tang et al., 2015; Brenes et al., 2020a). Salt stress escalates the toxicity of some ions such as Na^+ and Cl^-, which causes water stress and prevents nutrient uptake in plants (Mbarki et al., 2018). Increasing salinity causes an increase in osmotic pressure, reducing the water uptake or even preventing it completely (Chen and Jiang, 2010). This leads to a reduction in leaf growth, reducing the amount of photosynthesis during plant development, and ultimately limiting plant growth under salinity.

Reactive oxygen species (ROS) are generated in abundance under abiotic stress conditions (Sharma et al., 2019). Anthocyanins, a type of flavonoid, have antioxidant functions. Anthocyanins usually associated with increased stress tolerance in plants and they are activated in response to abiotic stresses (Li and Ahammed, 2023). Unbalanced ROS activity results in cell damage (Nouman et al., 2014). Anthocyanins as an antioxidant play a vital role in ROS scavenging and reducing oxidative stress (Cheah et al., 2015; Khan et al., 2020) and proline is a kind of amino acid and the most abundant endogenous osmolyte act as a protector of the plants against various stresses. It's accumulation increases under various abiotic stresses including salinity (Slama et al., 2015). Malondialdehyde (MDA), which is one of the final products of lipid peroxidation, is employed as a marker of oxidative stress. Assessment of MDA, is used as a method in determining degree of susceptibility of plants by researchers (Singh et al., 2014) and increase of MDA concentration in leaves indicate increased susceptibility.

Eggplant belongs to Solanaceae family and cultivated mostly around the South East Asia, Middle East and Mediterranean countries. It is known as moderately sensitive to abiotic stresses (Unlukara et al., 2010; Díaz-Pérez and Eaton, 2015; Brenes et al., 2020b). Environmental changes in recent years lead to an enthusiasm for crop wild relatives (CWRs) in agronomically important crop breeding programs (Knapp et al., 2013). Moreover, the inclusion of crop wild relatives in breeding programs may broaden the genetic base of the germplasm. Although, eggplant has many crop wild relatives (CRWs) that are believed to be more tolerant to abiotic stresses and it is essential to exploit their potential against salinity through interspecific hybridization studies. Cultivated eggplant can be crossed successfully with several wild relatives (Plazas et al., 2016). *Solanum incanum* L. which was defined as cross-compatible with *Solanum melongena* L., has desirable properties in development of cultivar eggplant breeding schemes (Gramazio et al., 2016). After interspecific hybridization, backcrossing with *S. melongena* for introgression breeding can cause addition of some traits of wild relatives into the eggplant gene pool (Kouassi et al., 2016; Garcia-Fortea et al., 2019; Brenes et al., 2020a).

The aim of this study was to improve ideal and high potential salt tolerant eggplant pure lines. Therefore, F_2 population derived from interspecific cross between inbred eggplant line (BATEM-TDC47) having desirable features and *S. incanum* L. was evaluated under the dose of 150 mM NaCl based salinity stress.

2. Material and Methods

2.1. Plant material

A total of 256 F_2 seedlings derived from a segregating population from an interspecific hybridization between the inbred line BATEM-TDC47 (sensitive parent) and the crop wild relative *Solanum incanum* L. (tolerant parent) were used as plant material. The inbred line "BATEM-TDC47" is distinguished with its agro morphological features and high marketable capacity. Tolerant parent *S. incanum* L. was provided by INRAE (French National Research Institute for Agriculture, Food and Environment) France. In addition, 36 seedlings from the F_1 hybrid and 36 seedlings from each parent were kept as negative and positive controls under non-saline and saline stress conditions.

2.2. Method

Seeds were sown on 13 August 2021 and seedlings were grown in trays containing a 1:1 mixture of peat moss and perlite. They were evenly watered with Hoagland solution (Hoagland and Arnon, 1950) until they reached the stage of 2-3 true leaves. They were transferred to 1-liter pots containing a 1:1 mixture of peat moss and perlite on

September 13, 2021, 31 days after sowing (DAS). Following transplantation, all plants were irrigated equally with Hoagland solution (Hoagland and Arnon, 1950) for 2 weeks to ensure proper root development. When seedlings reached to 4-5 true leaves stage, the experiment was initiated using 0 mM NaCl (only the control group of F_1 and parent seedlings) and 150 mM NaCl doses. In this study dose of 150 mM NaCl was applied to the 256 F_2 seedlings to distinguish salt-tolerant ones. Considering the previous studies (Akinci et al., 2004; Assaha et al., 2013; Hannachi and van Labeke, 2018; Brenes et al., 2020a; Alkhatib et al., 2021), the dose of 150 mM NaCl was used to define salt-tolerant plants. This amount did not kill the plants completely but enabled them to determine of tolerance in a short time. Before applying salt stress, a sufficient volume of 50 mM NaCl solution was prepared using distilled water and NaCl. When the seedlings reached the stage of 4-5 true leaves, pod treatment was initiated on September 28, 2021, with a concentration of 50 mM NaCl per day. This was followed by two consecutive days of 50 mM NaCl treatment until September 30, 2021, when a final concentration of 150 mM NaCl was reached. At the same time, a trial was set with parents and F_1 seedlings as the negative and positive control under non-saline and salt-stressed conditions. The positive control group was treated with 150 mM NaCl, similar to the F_2 seedlings. Meanwhile, the negative control group consisted of an equal number of F_1 and parent seedlings, which were kept in non-saline conditions and irrigated with salt-free water. A few days after the last treatment, damage symptoms began to develop on salt-treated plants. During this stage, irrigation was done manually when required. Although the salt-treated plants didn't require much watering, control plants were irrigated with regular water as needed. According to our earlier research from the ongoing project titled "Development of Tolerant Inbred Lines to Salt and Drought Stresses in Eggplant through Interspecific Hybridization" (Project number: TAGEM/BBAD/B/20/A1/P1/1476), and considering previous studies on eggplant (Assaha et al., 2013; Alkhatib et al., 2021), observations were made on the 12th day after the last salt treatment. On the 12th day, symptoms' severity was assessed using a 0-5 visual damage scale as suggested by Kıran et al. (2016), 0 indicating no effect, 1 indicating local yellowing and curling of leaves with slow growth, 2 indicating necrosis and chlorosis on 25% of the leaf, 3 indicating necrotic spots on the leaves and defoliation by 25-50%, 4 indicating necrosis by 50-75% and death of several plants, and 5 indicating severe necrosis on leaves by 75-100% and/or predominant plant deaths. After this, all plants were phenotypically observed on the same day. While shoot length and stem diameter were measured using a ruler and digital caliper, final leaf numbers were recorded for each plant, and observations for anthocyanin presence and prickliness features were conducted visually. Subsequently, leaf samples were collected from the selected 50 salt-tolerant F_2 seedlings, as well as from the F_1 and parent plants, for MDA and proline analysis. For leaf sampling, the top third vigorous leaf of each plant was collected, wrapped individually in aluminum foil, and stored in a freezer at -20°C until laboratory analysis. The MDA level was determined according to the method outlined by Lutts et al. (1996), while the proline content was evaluated using the method described by Bates et al. (1973). After the observations, 50 F_2 plants that scored "0" on the 0-5 scale, indicating the highest level of salt tolerance, were transferred to the greenhouse on October 12, 2021, for the inbreeding of tolerant individuals. Each 50 F_2 plants was self-pollinated to produce fruits for seeds of the F_3 generation. After fruits ripening 65-70 days later, mature fruits were harvested. Following, seeds were separated from fruit flesh, washed and dried under controlled room conditions at +25ºC. They were then stored at +4ºC with 5% humidity until further studies.

2.3. Electrical conductivity of the substrate

At the end of the salt treatment, after removing the plants from the pots, the remaining substrate was dried and a soil/water (1:5) suspension was prepared in deionized water and stirred well for an hour at a shaker. Electrical conductivity (EC) was measured using a portable conductivity meter (model mw - 302, Milwaukee Instruments, USA) and expressed in dS m^{-1}.

2.4. Data analysis

For the evaluation of MDA, proline, stem diameter, leaf number and shoot length data, percent rate of change was calculated using the following equation.

$$Percentage\ change = \left[\frac{Control - Salt\ treatment}{Control}\right] \times 100$$

The percent rate of change was based on the data collected from salt stressed and control plants. This equation didn't employ on F_2 population, because F_2 population was tested without control group. Each F_2 individual was treated as a separate breeding line in the study. The extent of the salt stress effect on F_1 and parents were based on the percent rate of change ratios. When evaluating morphological data, it was hypothesized that a lower percent rate of change indicated a higher tolerance level to salt stress.

3. Results and Discussion

3.1. Analysis of morphological parameters

In this study, plants in early growth stage were subjected to test under salt effect. At the end of the experiment, the electrical conductivity (EC) of the substrate was determined as 0.65 dS m^{-1} for the control and 7.90 dS m^{-1} for the salt-treated samples**.** This proved that, salt stress was created successfully around the plant root zone. The sensitive plants exposed to 150 mM NaCl displayed symptoms such as necrosis, chlorosis and defoliation (Figure 1) increasing progressively after the salt treatment.

The effects of salinity stress on shoot length, stem diameter and leaf number of 256 F_2 segregating population and negative/positive control seedlings were investigated and presented in Table 1. According to the findings, while shoot length and stem diameter decreased dramatically, number of leaves per plant was not affected much however decreases on leaf size under salt effect could be visually observed during the experiment. Moreover, it was estimated that, if the stress condition would be prolonged number of leaves could be affected negatively and decreased. It was clear from the study, salt stress induced reduction of all growth parameters in plants (Table 1). However, this reduction in growth was greater in *S. melongena* than in *S. incanum* L. (Figure 2) whereas F_1 and F_2 plants showed heterosis.

The rate of change ratios were calculated for negative and positive control group using equation 1 and presented as figure (Figure 3). Shoot length, stem diameter and leaf number values were reduced under salt effect compared to their control plants (for F_1 and parents). Additionally, change ratios of BATEM TDC47 is defined as higher than the *S. incanum* L. that means inbred line BATEM TDC47 was more affected from salt stress. This result proved that, even in early growth stage, genotypes could be differentiated with their tolerance level using basic morphological measurements.

A total of 256 seedlings from F_2 segregating population were classified using 0-5 visual scale (Kıran et al., 2016), considering damages caused by salt stress and presented in Table 2 and Figure 4. According to data collected on the 12th day (unpublished project data; Assaha et al., 2013; Alkhatib et al., 2021) after final salt application, 50 seedlings responded well to the salt stress, showed "no effect" and scored as "0" (Table 2). Brenes et al. (2020a) suggested that the survival percentage is one of the most effective criteria and generally used to assessing of the salt tolerance degree of tested plants.

Seedlings from F_2 segregating population examined in this study were classified into six groups based on 0-5 scale. According to scoring, 50 seedlings from group "0" with desirable features were defined as salt tolerant. Thus, 50 seedlings amongst the 256 F_2 individuals were selected to develop new salt tolerant pure lines.

Anthocyanin and prickliness of the genotypes were also observed (Table 3). While selection was making among the salt treated individuals, beside salt tolerance capacity, spineless, hairless and strong individuals were preferred for the desired line

Figure 1. F_2 seedlings responded to 150 mM NaCl stress as visual deterioration.

Table 1. Minimum, maximum, and average values of shoot length, stem diameter, and leaf numbers of *S. incanum* L., BATEM TDC47, BATEM TDC47 × *S. incanum* L., and F_2 population under 150 mM salt stress on the 12th day of salt application.

Accessions	Shoot length (cm)			Stem diameter (mm)			Leaf number		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
S. incanum L.	5.5±0.2	8.0±0.5	7.3±0.9	1.8±0.1	2.4±0.1	2.1±0.2	4.0±0.5	6.0±0.5	5.0±0.7
BATEM TDC47	8.0±0.5	11.0±0.3	9.6±0.9	2.1±0.1	2.9±0.1	2.6±0.2	5.0±0.4	7.0±0.5	6.2±0.5
F_1	7.0±1.0	14.0±1.3	10.3±1.8	2.0±0.2	4.6±0.5	2.8±0.5	3.0±0.5	6.0±0.4	5.0±0.7
F_2	9.0±1.3	15.0±0.9	10.8±1.9	2.0±0.2	3.7±0.3	2.8±0.3	3.0±0.3	6.0±0.2	5.0±0.5

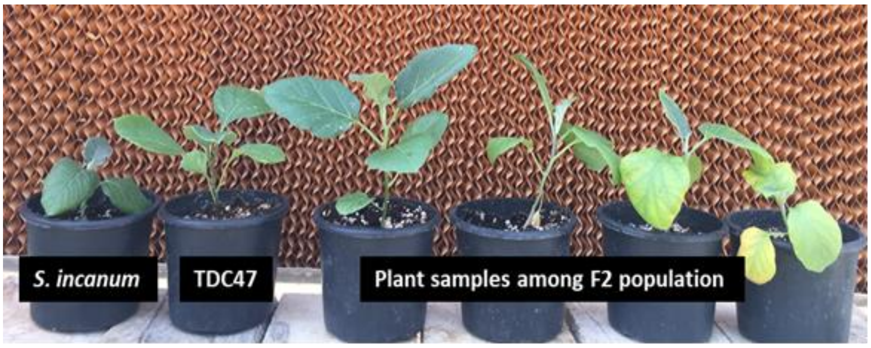

Figure 2. Parents and F_2 segregating population in pots under salt stress effect.

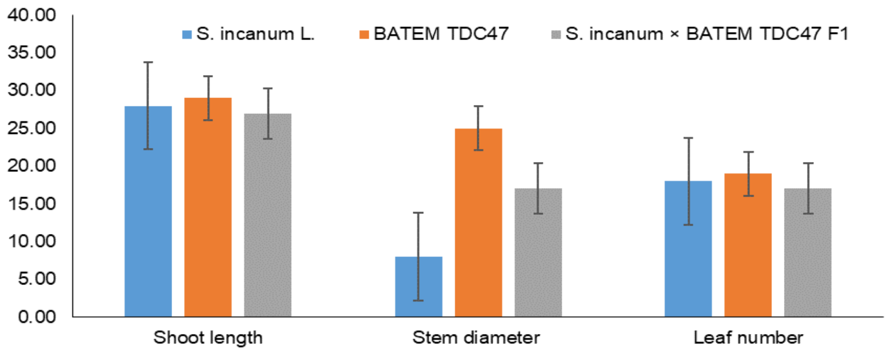

Figure 3. Rate of change (%) of *S. incanum* L., BATEM TDC47 and BATEM TDC47 × *S. incanum* L. hybrid seedlings in terms of shoot length, stem diameter and leaf number.

Table 2. Classifying of F_2 segregating population using 0-5 visual scale.

Scale	Description	Number of F_2 plant
0	No effect	50
1	Local yellowing and curling of leaves, slow growth	88
2	Necrosis and chlorosis in 25% of the leaf	62
3	Necrotic spots on the leaves and defoliation by 25-50%	36
4	Necrosis by 50-75% and death of several plants	18
5	Formation of severe necrosis in leaves by 75-100% and/or predominant deaths in plants	2

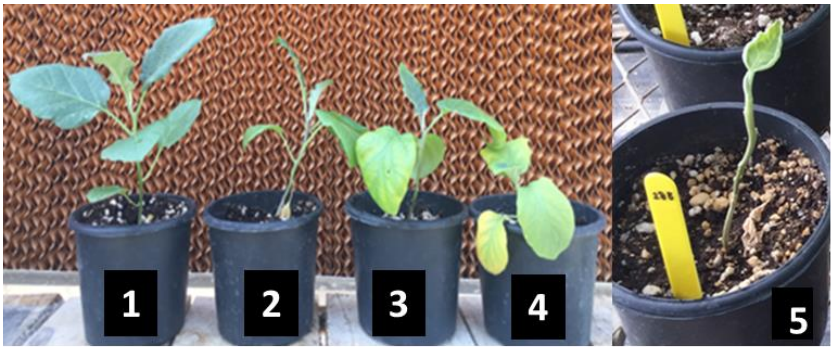

Figure 4. Samples from F_2 population, from left to the right, Pot 1: No effect (0), Pot 2: Local yellowing and curling of leaves, slow growth (1), Pot 3: Necrosis and chlorosis in 25% of the leaf (2), Pot 4: Necrosis by 50-75% and death of several plants (3), Pot 5: Necrosis by 50-75% and death of several plants (5).

Table 3. Anthocyanin and prickliness features of the salt stressed BATEM TDC47, *S. incanum*, BATEM TDC47 × *S. incanum* L. F_1 seedlings, and F_2 segregating population.

Accessions	Anthocyanin	Prickliness
BATEM TDÇ47 (Control)	+	-
BATEM TDÇ47 (Salt treated)	+	-
S. incanum (Control)	-	+
S. incanum (Salt treated)	-	+
F_1 (Control)	+	+
F_1 (Salt treated)	+	+
F_2 (Salt treated)	145 of 256 F_2 plants show anthocyanin presence	-

Table 4. Mean malondialdehyde (MDA) and proline values of *S. incanum* L., BATEM TDC47 and BATEM TDC47 × *S. incanum* L. F_1 and selected 50 F_2 seedlings as salt tolerant under salt stress.

Accessions	MDA (μ mol g^{-1} FW)			Proline (μ mol g^{-1} FW)		
	Minimum	Maximum	Average	Minimum	Maximum	Average
S. incanum L.	6.2±0.8	8.2±0.5	7.3±0.7	10.9±1.3	14.9±1.6	12.9±1.8
BATEM TDC47	7.8±0.7	9.7±0.5	8.7±0.6	7.3±1.0	9.7±1.0	8.8±1.0
F_1	15.3±0.5	17.3±0.9	16.0±0.7	6.3±1.1	10.1±1.5	8.1±1.3
F_2	2.1±1.7	21.4±3.0	10.9±4.9	4.2±0.9	12.1±1.2	8.4±1.6

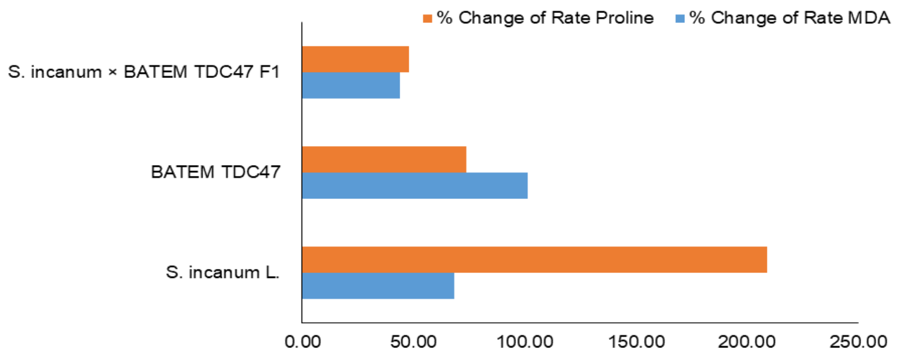

Figure 5. Rate of change (%) of *S. incanum*, BATEM TDC47 and BATEM TDC47 × *S. incanum* L. hybrid seedlings in terms of MDA and proline accumulation.

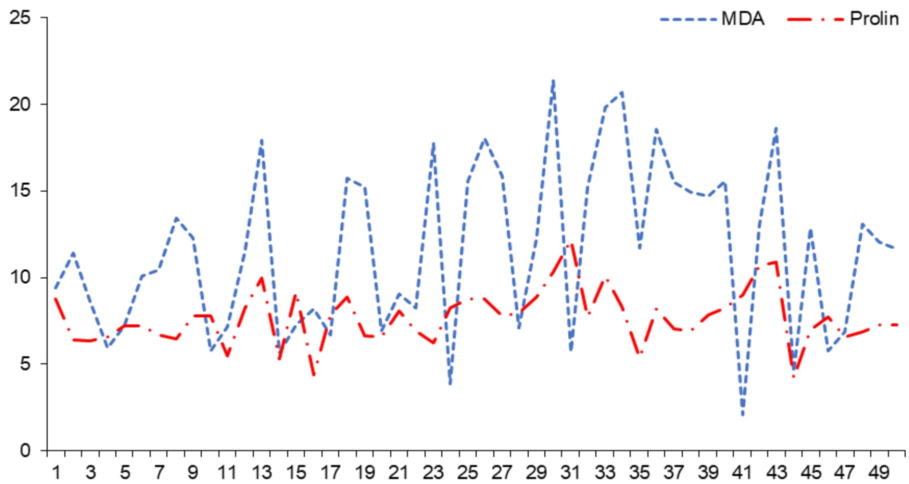

Figure 6. Graphic shows MDA and proline alteration in selected 50 F_2 plants.

development. Because it is known that these characters are undesirable for cultivars. While the tolerant parent lacks anthocyanin but possesses spines, the sensitive parent exhibits anthocyanin but lacks spines. F_1 seedlings have both anthocyanin and spine on body parts. A total of 145 F_2 plants show anthocyanin presence but no prickliness (Table 3).

3.2. Analysis of biochemical parameters

In the research, considering the visual scale results, MDA and proline amounts of selected 50 F_2 plants were analyzed together with F_1 and parent leaf samples and presented in Table 4. As an indicator to lipid peroxidation, MDA increased in all samples under salt stress. Segregating population's MDA level ranked between 2.1- 21.4 μ mol g^{-1} FW. Salt stress resulted in increases of proline accumulation thus, F_2 population's proline amount ranked between 4.2-12.1 μ mol g^{-1} FW.

Rate of change ratios were calculated for negative and positive control plants using equation 1 and presented as Figure 5. MDA and proline amounts showed increases in different degrees under salt effect compared to the control plants (for F_1 and parent plants). While the most increase of proline was obtained from *S. incanum* L., the most increase of MDA was obtained from BATEM TDC47. Changes in F_1 seedlings under salt, stayed between the parents' values. At the plant selection stage, these habits should be considered by the plant breeders.

In this study, considering the visual scale results, MDA and proline amounts of selected F_2 plants were analyzed together with F_1 and parent leaf samples. MDA and proline contents in F_2 plants subjected to the salt stress displayed the high levels of variation. While the highest MDA level was determined as 21.40 μ mol g^{-1} FW, the lowest MDA level was determined as 2.11 μ mol g^{-1} FW. Whereas, proline content was recorded in between 12.14 μ mol g^{-1} FW and 4.21 μ mol g^{-1} FW. Alterations in proline and MDA amounts among the selected 50 salt-tolerant individuals are presented as a graphic in Figure 6.

This study aimed to improve cultivar eggplant tolerance to salt stress. Although many research has been done to reveal resistance and tolerance of eggplant wild relatives to abiotic and biotic stresses (Rotino et al., 2014; Plazas et al., 2016; Gramazio et al., 2017; García-Fortea et al., 2019; Caliskan et al., 2023), efforts on transferring these skills to the cultivar eggplant were limited (Toppino et al., 2008; Liu et al., 2015). From the secondary gene pool, *S. incanum* L. is a close relative of eggplant, and its hybrids and backcrosses with cultivar eggplant are found mostly fertile by the researchers (Knapp et al., 2013; Kouassi et al., 2016; Plazas et al., 2016; Gramazio et al., 2017) and in agreement with previous studies, obtained seeds from the hybridization gave highly fertile individuals in this study. Although, drought tolerance of *S. incanum* L. has been described previously in many studies (Gramazio et al., 2017; Plazas et al., 2022; Cebeci et al., 2022; Cebeci et al., 2023), salt tolerance of this wild relative studied first in the present breeding project. Studies on salt tolerance levels of some commercial or local eggplant varieties (Akinci et al., 2004; Unlukara et al., 2010; Hannachi et al., 2014; Hannachi et al., 2018; Suarez et al., 2021) and some wild relatives, such as, *S. torvum* (Brenes et al., 2020a), *S. insanum* (Brenes et al., 2020b) were studied before however, responses of F_2 segregating population were observed first under the salt effect in this study. However, salt tolerance of *S. incanum* L. has been studied in molecular level and scientists have found unigenes related to the drought and salt tolerance in *S. incanum* L. (Gramazio et al., 2016).

In the present study, plant growth characteristics and biochemical parameters were assessed to determine responses of segregating population at seedling stage to 150 mM NaCl stress. Previous researches were reported that final plant performance heavily depends on seedling features under stress conditions (Bybordi and Tabatabaei, 2009). Because of disrupted a number of physiological mechanisms such as photosynthetic efficiency and water uptake (Evelin et al., 2019), plants respond first as retardation or stopping growth, yellowing on leaves and at the further stage, necrotic spots on leaves, loss of leaves and death of whole plant under salt stress. The effects of salinity on plant growth may vary depend on plant species and even on different genotypes of a species (Bhati et al., 2020).

Analysis of salt effects on growth parameters can be useful for the formation of stress tolerance scale when comparing different species, varieties or inbred lines (Al Hassan et al., 2016). The results indicated that *S. incanum* L. is a good candidate for improving salt tolerance in eggplant germplasm through breeding and introgression programs. Compared to the cultivated species, wild relatives of eggplant show higher stress tolerance, since they habitually found in arid/semiarid regions and in saline environments (Knapp et al., 2013; Ranil et al., 2016).

A total of 50 seedlings responded well to the salt stress, showed “no effect” and scored as “0” (Table 2). Brenes et al. (2020a) suggested that the survival percentage is one of the most effective criteria and generally used to assessing of the salt tolerance degree of tested plants. Different visual damage scales were used on different plants by researchers in previous studies such as Kıran et al. (2016) and Bhati et al. (2020) for eggplant, Kusvuran (2010) and Ekincialp (2019) for melon, Fidan and Ekincialp (2017) for bean and Shaheenuzzamn (2014) for chickpea.

Plant breeders are currently focusing their effort on genotype × environment interactions with variation in morpho-physiological variables to identify breeding methods to create more stress tolerant crops (Toppino et al., 2022). Plants under stress showed MDA content increases as stress increase. The more these increase the more plant sensitive. Previous studies on eggplant reported that, plants with high proline accumulation showed greater tolerance to stress conditions (Kıran et al., 2016; Plazas et al., 2019). Consistent with previous studies, in the present study proved that *S. incanum* L. as a crop wild relative has greater tolerance to salt than the susceptible parent.

It was stated that progress has been made in breeding in the study conducted on rice to improve salinity tolerance and that selection is important in early generations. The selection of early generation (F_2-F_5) breeding materials, especially in severe salinity conditions, according to good plant type and seedling tolerance, brings success in the development of salinity tolerance lines in the later generations (F_6-F_7) (Gregorio et al., 2002). In this study, 50 seedlings selected as having desirable attributes and tolerance in severe salinity conditions were transferred to the greenhouse to obtain the next generation.

4. Conclusion

This study aimed to contribute to development of new eggplant inbred lines and varieties tolerant to salt stress by understanding the salt tolerance level within this eggplant F_2 segregating population. In accordance with the purpose, qualified inbreed eggplant line (BATEM TDC47) and *Solanum incanum* L. were crossed successfully, F_2 segregating population was tested under salt stress conditions comparatively with parents and F_1 plants. Selected as salt tolerant 50 F_2 lines were transferred to the greenhouse for selfing and progressed to the F_3 level. Parameters that utilized in the study were found highly useful for the selection of salt tolerant individuals. Studies on the salinity tolerant inbreed line development are still processing under the project of "Development of Tolerant Inbred Lines to Salt and Drought Stresses in Eggplant through Interspecific Hybridization" (Project No: TAGEM/BBAD/B/20/A1/P1/1476)".

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References

Akinci, I. E., Akinci, S., Yilmaz, K., & Dikici, H. (2004). Response of eggplant varieties (*Solanum melongena*) to salinity in germination and seedling stages. *New Zealand Journal of Crop and Horticultural Science*, 32:193-200.

Al Hassan, M., Morosan, M., López-Gresa, M.P., Prohens, J., Vicente, O., & Boşcaiu, M. (2016). Salinity-induced variation in biochemical markers provides ınsight into the mechanisms of salt tolerance in common (*Phaseolus vulgaris*) and runner (*P. coccineus*) beans. *International Journal of Moleculer Science*,17:1582.

Alkhatib, R., Abdo, N., & Mheidat, M. (2021). Photosynthetic and ultrastructural properties of eggplant (*Solanum melongena*) under salinity stress. *Horticulturae*, *7*(7):181.

Assaha, D.V., Ueda, A., & Saneoka, H. (2013). Comparison of growth and mineral accumulation of two solanaceous species, *Solanum scabrum* Mill. (huckleberry) and *S. melongena* L. (eggplant), under salinity stress. *Soil Science and Plant Nutrition*, 59(6):912-920.

Bates, L.S., Waldren, R.P., & Teare, I.D. (1973). Rapiddetermination of free proline for water-stressstudies. *Plant and Soil*, 39(1):205-207.

Bhati, S., Gangopadhyay, K.K., Yadav, S.K., & Garg, G. (2020). Seedling growth and antioxidant activity of eggplant (*Solanum melongena* L.) genotypes under salt stress. *Vegetable Science*, 47(2):207-212.

Brenes, M., Solana, A., Boscaiu, M., Fita, A., Vicente, O., Calatayud, Á., & Plazas, M. (2020a). Physiological and biochemical responses to salt stress in cultivated eggplant (*Solanum melongena* L.) and in *S. insanum* L., a close wild relative. *Agronomy*, 10(5):651.

Brenes, M., Pérez, J., González-Orenga, S., Solana, A., Boscaiu, M., Prohens, J., & Vicente, O. (2020b). Comparative studies on the physiological and biochemical responses to salt stress of eggplant (*Solanum melongena*) and its rootstock *S. torvum*. *Agriculture*, 10(8):328.

Bybordi, A., & Tabatabaei, J. (2009). Effect of salinity stress on germination and seedling properties in canola cultivars (*Brassica napus* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 37(2):71-76.

Caliskan, S., Toppino, L., Boyaci, H.F., Rotino, G.L., & Cebeci, E. (2023). Assessment of new genetic resources to uncover potential nematode resistance traits for eggplant (*Solanum melongena*) improvement. *Phytoparasitica*, 1-14.

Cebeci, E., Boyaci, H.F., Kiran, S., & Ellialtioğlu, Ş.Ş., (2022). Selection of Drought tolerant individuals in segregating populations generated by interspecific cross in eggplant. *Bahçe*, 51:1:35-42.

Cebeci, E., Boyaci, H.F., Kiran, S., & Ellialtioğlu, Ş.Ş. (2023). Responses of interspecific hybrid eggplant f4 inbred lines to drought and heat stress. *Soil Studies*,12(2):70-76.

Chaudhry, S., & Sidhu, G.P.S. (2022). Climate change regulated abiotic stress mechanisms in plants: A comprehensive review. *Plant Cell Reports*, 41(1):1-31.

Cheah, B.H., Nadarajah, K., Divate, M.D., & Wickneswari, R. (2015). Identification of four functionally important microRNA families with contrasting differential expression profiles between drought-tolerant and susceptible rice leaf at vegetative stage. *BMC Genomics*, 16:1-18.

Chen, H., & Jiang, J.G. (2010). Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. *Environmental Reviews*, 18(NA):309-319.

Chinnusamy, V., Jagendorf. A., & Zhu. J.K. (2005). Understanding and improving salt tolerance in plants. *Crop Science*, 45(2):437-448.

Diaz-Perez, J.C., & Eaton, T.E. (2015). Eggplant (*Solanum melongena* L.) plant growth and fruit yield as affected by drip irrigation rate. *HortScience*, 50(11):1709-1714.

Ekincialp, A. (2019). The differences among melon genotypes and varieties under salt stress based on certain morphological and physiological properties-mixture modeling and principal component analysis (PCA). *Applied Ecology & Environmental Research*, 17(2):2965-2981.

Etesami, H., & Noori, F. (2019). Soil Salinity as a Challenge for Sustainable Agriculture and Bacterial-Mediated Alleviation of Salinity Stress in Crop Plants. In: Kumar, M., Etesami, H., Kumar, V. (eds) Saline Soil-based Agriculture by Halotolerant Microorganisms. Springer, Singapore.

Evelin, H., Devi, T.S., Gupta, S., & Kapoor, R. (2019). Mitigation of salinity stress in plants by arbuscular mycorrhizal symbiosis: Current understanding and new challenges. *Frontiers in Plant Science*, 10:470.

Fidan, E., & Ekincialp, A. (2017). Investigation of responses of some bean (*Phaseolus vulgaris* L.) genotypes to different levels of salt stress. *Yuzuncu Yıl University Journal of Agricultural Sciences*, 27(4):558-568.

García-Fortea, E., Gramazio, P., Vilanova, S., Fita, A., Mangino, G., Villanueva, G., Arrones, A., Knapp, S., Prohens, J., & Plazas, M. (2019). First successful backcrossing towards eggplant (*Solanum melongena*) of a New World species, the silverleaf nightshade (S. elaeagnifolium), and characterization of interspecific hybrids and backcrosses. *Scientia Horticulturea*, 246:563-573.

Giordano, M., Petropoulos, S.A., & Rouphael, Y. (2021). Response and defence mechanisms of vegetable crops against drought, heat and salinity stress. *Agriculture*, 11(5):463.

Gramazio, P., Blanca, J., Ziarsolo, P., Herraiz, F. J., Plazas, M., Prohens, J., & Vilanova, S. (2016). Transcriptome analysis and molecular marker discovery in *Solanum incanum* and *S. aethiopicum*, two close relatives of the brinjal eggplant (*Solanum melongena*) with interest for breeding. *BMC Genomics*, 17:300.

Gramazio, P., Prohens, J., Plazas, M., Mangino, G., Herraiz, F.J., & Vilanova, S. (2017). Development and genetic characterization of advanced backcross materials and an introgression line population of *Solanum incanum* in a *S. melongena* background. *Frontiers in Plant Science*, 8:1477.

Gregorio, G.B., Senadhira, D., Mendoza, R.D., Manigbas, N.L., Roxas, J.P., & Guerta, C.Q. (2002). Progress in breeding for salinity tolerance and associated abiotic stresses in rice. *Field Crops Research*, 76:91-101.

Hanci, F., Cebeci, E., & Polat, Z. (2014). The effects of *Trichoderma harzianum* on germination of onion (*Allium cepa* L.) seeds under salt stress conditions. *International Journal of Agricultural and Natural Sciences*, 7(1):45-48.

Hannachi, S., Van Labeke, M.C., & Mehouachi, T. (2014). Application of chlorophyll fluorescence to screen eggplant (*Solanum melongena* L.) cultivars for salt tolerance. *Photosynthetica*, 52:57-62.

Hannachi, S., & Van Labeke, M.C. (2018). Salt stress affects germination, seedling growth and physiological responses differentially in eggplant cultivars (*Solanum melongena* L.). *Scientia Horticulturae*, 228:56-65.

Hemathilake, D.M.K.S., & Gunathilake, D.M.C.C. (2022). Agricultural productivity and food supply to meet increased demands. (pp. 539-553). Academic Press.

Hoagland, D.R., & Arnon, D.I. (1950). The Water-Culture Method for Growing Plants without Soil. 2nd Edition, California Agricultural Experiment Station.

Ibrahim, E.A. (2016). Seed priming to alleviate salinity stress in germinating seeds. *Journal of Plant Physiology*, 192:38-46.

Johnson, R., & Puthur, J.T. (2021). Seed priming as a cost effective technique for developing plants with cross tolerance to salinity stress. *Plant Physiology and Biochemistry*, 162:247-257.

Kıran, S., Kusvuran, Ş., Ozkay, F., & Ellialtioğlu, Ş.Ş. (2016). The change of some morphological parameters in salt tolerant and salt sensitive genotypes under drought stress condition. *Journal of Agricultural Faculty of Mustafa Kemal University*, 21(2):130-138.

Kouassi, B., Prohens, J., Gramazio, P., Kouassi, A.B., Vilanova, S., Galán-Ávila, A., Herráiz, F.J., Kouassi, A., Seguí-Simarro, J.M., & Plazas, M. (2016). Development of backcross generations and new interspecific hybrid combinations for introgression breeding in eggplant (*Solanum melongena*). *Scientia Horticulturea*, 213:199-207.

Khan, A., Jalil, S., Cao, H., Tsago, Y., Sunusi, M., Chen, Z., & Jin, X. (2020). The purple leaf (pl6) mutation regulates leaf color by altering the anthocyanin and chlorophyll contents in rice. *Plants*, 9(11):1477.

Knapp, S., Vorontsova, M.S., & Prohens, J. (2013). Wild relatives of the eggplant (*Solanum melongena* L.: Solanaceae): New understanding of species names in a complex group. *PLoS One,* 8:e57039.

Kumar, A., Singh, S., Gaurav, A.K., Srivastava, S., & Verma, J.P. (2020). Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. *Frontiers in Microbiology*, 11:1216.

Kusvuran, S. (2010). Relationships between physiological mechanisms of tolerances to drought and salinity in melons. PhD Thesis, Çukurova University, Adana, Türkiye.

Li, Z., & Ahammed, G.J. (2023). Plant stress response and adaptation via anthocyanins: A review. *Plant Stress*, 100230.

Liu, J., Zheng, Z., Zhou, X., Feng, C., & Zhuang, Y. (2015). Improving the resistance of eggplant (*S. melongena*) to Verticillium wilt using wild species Solanum linnaeanum. *Euphytica*, 201:463-469.

Lutts, S., Kinet, J.M., & Bouharmont, J. (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Annals of Botany,* 78(3):389-398.

Mbarki, S., Sytar, O., Zivcak, M., Abdelly, C., Cerda, A., & Brestic, M. (2018). Anthocyanins of coloured wheat genotypes in specific response to salt stress. *Molecules*, 23(7):1518.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review Plant Biology,* 59:651-681.

Nouman, W., Basra, S.M.A., Yasmeen, A., Gull, T., Hussain, S.B., Zubair, M., & Gul, R. (2014). Seed priming improves the emergence potential, growth and antioxidant system of *Moringa oleifera* under saline conditions. *Plant Growth Regulation*, 73:267-278.

Plazas, M., Vilanova, S., Gramazio, P., Rodríguez-Burruezo, A., Fita, A., Herráiz, F.J., Prohens, J. (2016). Interspecific hybridization between eggplant and wild relatives from different genepools. *Journal of American Society Horticultural Science*, 141:34-44.

Plazas, M., Gonzalez-Orenga, S., Nguyen, H.T., Morar, I. M., Fita, A., Boscaiu, M., & Vicente, O. (2022). Growth and antioxidant responses triggered by water stress in wild relatives of eggplant. *Scientia Horticulturae*, 293:110685.

Porcel, R., Aroca, R., & Ruiz-Lozano, J.M. (2012). Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. *Agronomy for Sustainable Development*, 32:181-200.

Ranil, R.H.G., Prohens, J., Aubriot, X., Niran, H.M.L., Plazas, M., Fonseka, R.M., Vilanova, S., Fonseka, H.H., Gramazio, P., & Knapp, S. (2016). *Solanum insanum* L. (subgenus Leptostemonum Bitter, Solanaceae), the neglected wild progenitor of eggplant (*S. melongena* L.): A review of taxonomy, characteristics and uses aimed at its enhancement for improved eggplant breeding. *Genetic Resources Crop Evoluation,* 64:1707-1722.

Rotino, G.L., Sala, T., & Toppino, L. (2014). Eggplant. *Alien gene transfer in crop plants, volume 2: Achievements and impacts*, 381-409.

Singh, Z., Karthigesu, I.P., Singh, P., & Rupinder, K.A.U. R. (2014). Use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies: a review. *Iranian Journal of Public Health*, 43(Supple 3):7-16.

Singh, B.K., Trivedi, P., Singh, S., Macdonald, CA., & Verma, J.P. (2018). Emerging microbiome technologies for sustainable increase in farm productivity and environmental security. *Microbiology Australia*, 39(1):17-23.

Shaheenuzzamn, M. (2014). Screening of chickpea genotypes against salinity stress. *Bangladesh Journal of Agricultural Research*, 39(4), 605-619.

Sharma, A., Shahzad, B., Kumar, V., Kohli, S.K., Sidhu, G.P.S., Bali, A.S., & Zheng, B. (2019). Phytohormones regulate accumulation of osmolytes under abiotic stress. *Biomolecules*, 9(7):285.

Slama, I., Abdelly, C., Bouchereau, A., Flowers, T., & Savouré, A. (2015). Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Annals of Botany*, 115(3):433-447.

Suarez, D. L., Celis, N., Ferreira, J.F., Reynolds, T., & Sandhu, D. (2021). Linking genetic determinants with salinity tolerance and ion relationships in eggplant, tomato and pepper. *Scientific Reports*, 11(1):16298.

Tang, X., Mu, X., Shao, H., Wang, H., Brestic, M. (2015). Global plant-responding mechanisms to salt stress: Physiological and molecular levels and implications in biotechnology. *Critical Review Biotechnology*, 35:425-437.

Toppino, L., Valè, G., & Rotino, G.L. (2008). Inheritance of Fusarium wilt resistance introgressed from Solanum aethiopicum Gilo and Aculeatum groups into cultivated eggplant (*S. melongena*) and development of associated PCR-based markers. *Molecular Breeding*, 22:237-250.

Toppino, L., Barchi, L., Rotino, G.L. (2022). Next Generation Breeding for Abiotic Stress Resistance in Eggplant. In: Kole, C. (eds) Genomic Designing for Abiotic Stress Resistant Vegetable Crops. Springer, Cham. https://doi.org/10.1007/978-3-031-03964-5_4

Unlukara, A. Kurunç, A. Kesmez, G.D. Yurtseven, E. & Suarez, D.L. (2010). Effects of salinity on eggplant (*S. melongena* L.) growth and evapotranspiration. *Irrigation and Drainage*, 59:203–214.