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Responses of interspecific hybrid eggplant f4 inbred lines to drought and heat stress

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Abstract

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Introduction

Eggplant (*Solanum melongena* L.) as an old-world plant has been known and consumed for thousands of years in China and India where it is originally cultivated and spread around the globe. It is produced nearly 59 million tonnes on 1.9 million ha areas yearly in the World. Türkiye annually produces around 835 thousand tonnes on 17 thousand ha areas. With this production amount, it ranks fourth after China, India, and Egypt whose production annually 37, 12 and 1 million tonnes respectively. Considering the yield values obtained per hectare, Turkey ranks first among these four countries (Anonymous, 2023). Eggplant is a valuable healthy vegetable with its high fibre content, bioactive compounds (Sharma and Kaushnik, 2021) and strong antioxidant capacity (Bouhajeb et al., 2020). However, while global production has been increased steadily, slower scientific development achieved on eggplant compared to the other *Solanaceae* crops.

Eggplant is grown in and near the tropic and sub-tropic zones where climate change effects

can be seen dramatically in agricultural production. As a vegetable, it is considered has tolerance to some abiotic stresses compared to others. However, significant reductions in yield and marketable fruit quality were observed under stress conditions that exceeded the

tolerance level. Eggplant has wild relatives which are known as tolerant to stresses. As a

part of the comprehensive project this research includes drought-tolerant line

development from the F4 population obtained by interspecific crossing between Solanum

incanum L., a wild relative of eggplant naturally distributed in the Sub-Saharan zone, and a

pure line with superior characteristics developed in BATEM. Qualified 50 inbred lines were

selected as drought tolerant among F3 progenies in previous studies. In this experiment, 12

plants from each 50 genotypes at F4 level were exposed to drought stress, which was

created by 25% watering of full irrigation. On the 25th day of treatment, tolerant plants

were distinguished by using 0-5 scale. From each genotype, 4 plants were selected as

drought tolerant, and they were transferred to greenhouse to identify their heat tolerance.

Among the drought-tolerant group 42 F4 lines were found as heat tolerant.

Average yield is a fraction of maximum or record yield obtained under optimal cultivation conditions. Decreases in the yield are mostly caused by abiotic stresses affecting the agricultural production in the fields, such as drought, heat, salt, wind, flooding, etc. (Fita et al., 2015). Drought is the most stressful factor that limiting the crop productivity. Osmotic stress reduces some traits such as plant height, and leaf size (Nakanwagi et al., 2018). Prolonged drought periods can cause losses of yield at first, then even abandonment of agricultural lands in time. Today, with the effect of climate change, arid and semi-arid regions, which are almost 50% of the earth's land surface, are in danger of more frequent and more intense drought periods (IPCC, 2014). For this reason, it is urgent to enhance crop productivity by developing crop adaptation.

High temperature is another important environmental stress causing a heavy reduction in the yield of eggplant especially in the Mediterranean region. Eggplant optimum growth and development temperature ranges from 22 to 30 °C. Today, under the effect of global warming, temperatures in subtropical and tropical regions where eggplant is mostly grown, are observed as often above 35 °C. Although eggplant is one of the heat-loving vegetables, when the temperature rises above 35 °C, it may show defects (Wu et al., 2020). High temperature causes heat injuries in eggplant, including restricted growth, reduced productivity, and harmed quality (Li et al., 2011). Heat stress leads to abnormal flower development, low pollen viability, bud drop, reduced fruit set (Hazra et al., 2007; Santhiya et al., 2019). Lack of rain accompanying with high temperatures are contributing to the spreading of desertification therefore, it is substantial to develop new strategies for improving agricultural production (Fita et al., 2015). Adaptation success of crops to drought and heat depends on effective and combined use of modern methods and traditional breeding tools.

Drought and heat stresses beneath changing conditions affect eggplant like climatic other vegetables. Varieties used in the production are generally sensitive to abiotic stress conditions. However, tolerant local germplasm of the countries (Faiz et al., 2020) and crop wild relatives of eggplant are gaining interest in new breeding programmes (Plazas et al., 2016; Prohens et al., 2017; Afful et al., 2018; Kouassi et al., 2021). Interspecific hybridization of cultivated local heirlooms with the related species is an important step in improving tolerance to abiotic factors. Because many CWRs can thrive in harsh environmental conditions like semi-desertic or desertic areas, they represent a valuable tool for improving drought and heat tolerance in tolerant variety development studies (Knapp et al., 2013; Davidar et al., 2015; Kaushik et al., 2016; Vorontsova and Knapp, 2016).

Because eggplant production generally spreads near the tropic and subtropic climate zone regions, it is expected to tolerate drought and extreme heat. Therefore, the development of drought and heattolerant varieties are a major eggplant breeding objective (Gramazio et al., 2018; Prohens et al., 2017). Although the genetic diversity of cultivated eggplant has limited, it can be expanded by interspecific hybridization which ensure a significant source of variation (Daunay and Hazra, 2012; Plazas et al., 2016; Rakha et al., 2020). Some of these eggplant wild relatives, which have tolerance to abiotic and biotic stresses also used in rootstock development studies (Gisbert et al., 2011; Sabatino et al., 2018; Rakha et al., 2020). However, in some cases, grafting incompatibility may emerge as a problem in eggplant production made by using grafted plants (Krommydas et al., 2018). The development of tolerant cultivars offers several advantages and low-cost solutions for the producers.

This study, which is a part of the breeding project on the development of drought-tolerant lines in eggplant, it was aimed to determine the heat tolerance levels of selected plants of F4 inbred lines after subjected to drought stress.

Material and Method

Plant material

This study, consisted of 800 plants of 50 F4 inbred lines were used as a plant material. These plants were provided by interspecific hybridization between *S. incanum* L., which is a crop wild relative naturally distributed in Sub-Saharan zone of Africa, and a pure line "BATEM-TDC47" was developed in Bati Akdeniz Agricultural Research Institute under the project "Development of Qualified Genitors (Halfway Material) for Eggplant Breeding Programs and Seed Technology" (Project number: TAGEM/BBAD/10/A09/P01/12). In this study, plants belong to parents were also tested against drought in the same method as the F4 population.

Method

This study is a part of a comprehensive project "Development of Tolerant Lines to the Salt and Drought Stress by Interspecific Hybridization in Eggplant" (Project number: TAGEM/BBAD/B/20/A1/P1/1476). In this project, wild relative *S. incanum* L. and pure line "BATEM-TDC47" were crossed in 2020. Following years resulting 256 plants of F3 progenies were drought tested and 50 tolerant individuals were selected, and self-bred thus, F4 population was generated. In this study, 20 seeds of each of these tolerant 50 F4 progenies and parents were sown on 01.03.2022. Seedlings with 2-3 true leaves were transferred to the 3-liter pots filled with peat moss and perlite (1:1) on 21.04.2022. All seedlings were irrigated normally for two weeks after transfer. When the seedlings reach 3-4 true leaves stage rooting was completed and drought treatment was started on 10.05.2022. The 12 plants from each 50 genotypes were drought treated and 4 plants used as control. While the group of control plants were fully irrigated (100%), drought tested plants watered with 25% of full irrigation. Drought treatment continued 25 days and irrigation was done considering control plants situation. On the 25th day all plants were evaluated using 0-5 visual scale (Kuşvuran et al., 2012; Kiran et al., 2016) where 0: No symptoms (control plants), 1: slow growth (compared to control plants) 2: start of wilting on lower leaves, 3: curling and wilting of upper leaves, 4: severe wilting, yellowing of leaves and drying of leaf margins, 5: wilting of whole plant and drying of lower leaves.

On the 25th day of the treatment 4 of 12 each 50 F4 plants showing "slow growth" (Scale = 1) were selected as "tolerant to drought" by using 0-5 scale. Selected drought tolerant 200 plants transferred to the greenhouse for the heat tolerance study. Temperature and humidity values were recorded during both drought and heat stress experiments, which were conducted in pots and in greenhouse respectively, by hobo data logger (Figure 1 and 2).











Figure 2. Temperature and air humidity records during heat experiment in greenhouse.

All drought tolerant plants selected by their performance in pots were transferred to the greenhouse. Heat sensitive plants were defined observing their fruit setting performance. While their heat tolerance was evaluated in greenhouse, each drought and heat tolerant plant was selfed to generate the F5 generation.

Results and Discussion

Previous studies on eggplant proved that tolerance to abiotic stresses can be improved by interspecific hybridisation studies (Daunay and Hazra, 2012; Plazas et al., 2016; Rakha et al., 2020). Therefore, this study consists of developing drought and heat tolerant eggplant lines from F4 population, which was generated interspecific hybridization between the S. incanum L. and an eggplant valuable pure line BATEM TDC-47. Due to its location, Türkiye is under the effect of different climatic conditions and varieties that has already used in production need to develop their adaptation skills against the climate change. Because of interspecific hybridization enhances the hybrid vigour, it is a common concept that has been extensively applied for improvement of adaptation traits to changed climatic conditions in breeding (Sseremba et al., 2018).

Recent studies showed that *S. incanum* L. as a wild relative from primary genepool could provide fertile hybrids with eggplant (Knapp et al., 2013; Davidar et al., 2015; Plazas et al., 2016). In this study, hybridization between the cultivated eggplant and wild relative *S. incanum* L. which is known as drought tolerant (Gramazio et al., 2017) was achieved successfully (Figure 3). Improving crop adaptation to abiotic stresses through interspecific hybridisation can result in new cultivars with better resilience (Prohens et al., 2017; Gramazio et al., 2018). Rotino et al. (2014) has been described many tolerance sources in eggplant wild relatives to abiotic stresses but conducted studies transferring these skills to the cultivated eggplant have to develop (Toppino et al., 2008; Liu et al., 2015).



Figure 3. An eggplant pure line Batem TDÇ-47, *S. incanum* L. and their F4 progeny.

Along with the drought treatment in pots temperatures were recorded as minimum 18.5 °C, maximum 49.4 °C, and average 36.1 °C. Humidity was also recorded as minimum 30.9%maximum 98% and average 83.6% (Figure 1). Eggplant optimum growth and development temperature ranges from 22 to 30 °C. Average temperature was recorded during the drought treatment as 36.1 °C in the study. It was clear that the created environment for the drought experiment for this study was successful. In addition to this, irrigation with 25% of the full watering provided drought conditions to seedling in the pots filled with peat moss and perlite (Figure 4).



Figure 4. From left to right: BATEM TDÇ-47, BATEM TDÇ-47 × *S. incanum* and *S. incanum* plants in A: normally irrigated control conditions, B: drought treated conditions.

After hybridisation, population was tested against drought stress. During drought treatment, tested plants were selected in terms of their growing capacity under stress conditions. While selection was making among the drought treated individuals, beside drought tolerance capacity, spineless, hairless, and strong individuals were preferred for the desired line development. Because it is known that, under some stress conditions plants can give responses by increasing their spine and hair however, these characters undesirable attributes in cultivars. The cross ability among the S. melongena genotypes with their wild relatives showed wide range of variations in morphological characters. Definition of character traits of breeding objects is a fundamental step for the efficient utilization of them in breeding studies (Prohens et al., 2013; Kaushik et al., 2016).

During drought treatment in pots, obtained results showed that F4 progenies have better tolerance under drought and heat stress (Figure 5). As shown in figure 5, leaves of parents' plants have shrunk more than F4 plants. In a study, eggplant, its close wild relatives *S. insanum* and *S. incanum* and their interspecific hybrids with *S. melongena* were subjected to drought for 18 days. Their results which were support our results, showed that the hybrid between *S. melongena* and *S. incanum* displayed a better response than the other hybrids and even its parents (González-Orenga et al., 2023).



Figure 5. Control plants (right), drought treated plants (left): A- BATEM TDÇ-47, B- *S. incanum* L., C- BATEM TDÇ-47 × *S. incanum* L.

On the 25th day of the treatment 4 of 12 each 50 F4 plants showing "slow growth" (Scale = 1) were selected as "tolerant to drought" by using 0-5 scale. Selected drought tolerant 200 plants transferred to the greenhouse for the heat tolerance study. Greenhouse temperatures were recorded as maximum 61 °C, minimum 27.1 °C and average 38.8 °C and air humidity was also recorded as minimum 26%, maximum 98.9 %and average 70.3 % for 2022 summer season (Figure 2).

Previous studies reported that *S. incanum* is highly tolerant to drought and to some fungal diseases (Knapp et al., 2013; Plazas et al., 2016), but its tolerance to other abiotic stresses have not been performed so far

(Gramazio et al., 2019). In this study, drought tested F4 lines developed with introgressed genetic material from the wild species S. incanum, were also subjected to heat stress. Heat stress leads to abnormal flower development, low pollen viability, bud drop, reduced fruit set (Hazra et al., 2007; Santhiya et al., 2019). Because of pollen and ovaries can be damaged above the optimum growing temperature and this led to embryo abortion, flower and fruit shedding, heat sensitive plants were defined observing their fruit setting performance in this study. Some of heat tolerance performance of some F4 individuals are presented in figure 6. According to this, in terms of fruit setting capacity, picture A and B represents relatively heat tolerant plants compared to the picture C. Because it was observed that, end of the season eggplant in picture C has only one fruit.



Figure 6. Plants under heat stress in greenhouse, A and B: heat tolerant, C: heat intermediate tolerant.

Beside this, total 32 individual plants from eight genotypes could not set any fruit. These plants were determined as drought tolerant but their performance under heat found insufficient and they were categorized as heat sensitive in the study. Beside this, 18 genotypes were found heat intermediate tolerant and the other 24 genotypes were found heat tolerant. <u>Ansari et al. (2011)</u> reported that when the day and night temperature exceeds 35 °C there are dramatic decreases in fruit set and temperatures above and low of the limits also causes decrease in fruit weight.

Conclusion

Climate change induced drought and heat stress have already influenced many parts of the world. Especially, Mediterranean region most effected of these abiotic stresses firstly. Therefore, it is urgent to develop varieties that tolerant to extreme weather conditions. Crop wild relatives and landraces which has tolerance to abiotic stresses are valuable sources could be used in breeding programs. This study is a part of the comprehensive project aimed to improve tolerant eggplant lines to the drought and heat stresses using interspecific hybridization technique. Previously, hybridisation between eggplant and crop wild relative was produced successfully and F3 population were tested against drought conditions. Derived 50 genotypes from F3 population selfed and F4 population was generated. In this study, these 50 genotypes (800 plants) were tested under drought conditions, 200 plants were selected as drought tolerant. These 200 plants responses under high heat stress tested in greenhouse and 32 plants from 8 genotypes defined as heat sensitive in terms of their fruit set capacity.

Further research in progressing years heat and drought tolerant pure breeding lines with desired characteristics will be developed by using these inbred lines. Produced lines could also be used in rootstock development studies.

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Author Contribution

Conceptualization: ŞŞE, Investigation: EC and HFB, Methodology: HFB, SK, Field work: EC and HFB, Writing -original draft: EC, Writing -review and editing: ŞŞE, SK, HFB, EC.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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