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# Determination of Heat and Drought Tolerant Lines in Segregating Populations Produced by Interspecific Crosses in Eggplant

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**ABSTRACT**

Nowadays abiotic stresses originated by climate change are one of the main factors causing reductions to the agricultural production. Heat and drought are the most prominent abiotic stress factors affecting both eggplant production worldwide and food security. Although eggplant is known as more tolerant to these stresses compared to other vegetables and solanaceous crops, its quality and yield suffers from severe stress conditions. In this research, 256 $F_2$ plants developed from the interspecific cross between the wild relative of *Solanum insanum* L. and the pure line (BATEM-TDC47) from Batı Akdeniz Agricultural Research Institute, (BATEM) eggplant gene pool were used as plant materials. Seedlings at 3-4 true leaves stage bred in three-liter pots filled with a 1:1 mixture of peat and perlite were subjected to drought stress test to this end, a 75% deficit irrigation was applied to the plants, while control plants were irrigated with the required amount to recover the 100% of ETp as appropriate management strategy. The stress symptoms of plants were determined by morphological and chemical analyses. Plant heights were measured on the 25th day of the experiment and visual evaluation stress symptoms was observed according to the 0-5 scale. Morphological observations, MDA (malondialdehyde) and proline content of selected plants were performed to confirm their tolerance levels to heat and drought. Following the drought test, 100 $F_2$ lines, which were selected as drought tolerant, were transferred to the greenhouse for determination of heat tolerant individuals.

***Keywords**:* Abiotic stress, drought, eggplant, heat, MDA, proline

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## Introduction

Eggplant (*Solanum melongena* L.) is a crop species belonging to the family of Solanaceae but unlike the other solanaceous crops tomato, potato and pepper it has an old world origin as it is originated from both Southeast Asia and India (Barchi et al., 2022). It is widely grown in Southern and Southeast Asia, where great part of the world's population is located so and is included in the International Treaty on Plant Genetic Resources for Food and Agriculture's list of the most important 35 food crops substantial for global food security (Fowler et al., 2003). The most important factors limiting eggplant production are biotic and abiotic stresses (Rotino et al., 2014). For a sufficient yield and good quality of fruits, eggplant needs to be irrigated regularly, with an optimal temperature ranging between 22 and 30°C (Li et al., 2011).

Nowadays climate change is affecting the many parts of the world. Extreme weather events like floods after heavy rains, heat waves or low temperature shocks, increased soil salinity and prolonged water scarcity (drought) are the main results of the climate change, and are the cause of great losses in agricultural production and fruit quality in recent years worldwide (Wakchaure et al., 2020). Due to climate change and global warming, crops started to encounter more and more frequently drought and heat stresses during their vegetation and harvesting period in arid as also in semiarid regions especially with Mediterranean climate (Fahad et al., 2017). Although results of previous studies suggested that eggplant has tolerance to the principal abiotic stresses (including drought, heat, salinity) compared to the other vegetables (Behboudian, 1977; Sarker et al., 2004; Díaz-Pérez and Eaton, 2015), it has been demonstrated in recent research that it suffers from extreme water stress deficit in terms of fruit quality and yield (Plazas et al., 2019; Wakchaure et al., 2020: Singh et al., 2021; Toppino et al., 2021). According to Karam et al., (2011) water deficiency application increasing from 20 to 40% of the optimal watering amount causes a yield decrease of nearly 60% with respect to the control; moreover, high temperatures above 38°C can seriously inhibit the growth of seedling, flower development, and eventually impact the fruit quality and yield of eggplant (Zong et al., 2018). Eggplant is among the top five vegetables constituting the diet of people living in drought-affected regions of the world (Wakchaure et al., 2020), as a consequence yield loss due to abiotic stresses could severely affect the daily diet and food supply chain in these regions. Adapting eggplant production to altered climatic conditions requires the development of tolerant cultivars. Crop wild relatives of eggplant display a wide genetic diversity and some of them possess tolerance traits against the principal biotic or abiotic stresses (Knapp et al., 2013; Fita et al., 2015; Plazas et al., 2019). Therefore, interspecific hybridization and introgression of useful traits from allied species into the eggplant genetic background may play a prominent role in increasing heat and drought tolerance of this crop species (Kouassi et al., 2016; Plazas et al., 2016). Evaluation of the response of segregating populations derived from interspecific hybridization to heat and drought stresses and the selection of best performing progenies would reveal as a useful tool for the development of breeding lines with improved tolerance to stress (Espanani et al., 2019).

In this study, an interspecific hybridization was performed between *Solanum melongena* L. and *Solanum insanum* L. and the response of the $F_2$ segregating population to heat and drought was assessed aimed at the selection of best performing lines and development of tolerant lines to stressed conditions.

## Materials and Methods

In the present study, 256 individual $F_2$ seedlings derived from the selfing of the interspecific $F_1$ hybrid between the *S. melongena* L. BATEM-TDC47 pure line (sensitive parent) and the crop wild relative *S. insanum* L. (tolerant parent) were used as a plant material. The 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). The *S. insanum* L. (Coded as MM510 by INRAE) accession employed as donor of tolerance trait was provided from INRAE, France. In addition, 60 seedlings of the $F_1$ hybrid plus 60 seedlings of each parent line were tested for drought stress in a trial planned according to randomized block design with three replications to be compared with the $F_2$ individuals.

Seeds were sown in March 2021 and germinated in viols containing mixed peat moss and perlite (1:1) medium. Seedlings were equally watered with Hoagland solution (Hoagland and Arnon, 1950) until the 2nd-3rd true leaves stage and then they were transferred to the pots. Two seedlings were planted in each pot and they were normally watered with Hoagland solution until proper root development for two weeks. The application of the water deficit treatment started on April 30, 2021; the entire $F_2$ population and 10 plants for each of the three replications of $F_1$ and parent plants were subjected to stress by applying 75% deficit water compared to the control. To determine the water amount to be supplied in both control and stressed irrigation, all pots from control group were weighed daily and then the control group was watered up to full recover the ETp difference (the amount of weight lost each day due to evapotranspiration); a media performed on this value was considered as 100% control supply therefore deficit water supply was calculated as the 25% of the average control value and applied to the drought stressed group (Kıran et al., 2019).

On the 25th day of the drought application all plant heights were measured with a ruler and all $F_2$ plants were singularly evaluated according to the modified 0-5 visual scale already used by Banik et al., (2016) and Sseremba et al., (2018) where 0: No symptoms (control plants), 1: slow growth, 2: 25% yellowing and curling, 3: 26-50% yellowing and curling, dropping leaves, 4: 51-75% wilting and curling, drying, 5: more than 75% wilting and curling, dried plants. According to this scale, the 100 $F_2$ plants displaying "slow growth" (Scale = 1) were selected as "tolerant to drought" and transferred to the greenhouse for the heat tolerance study and further morphologic characterization.

Before the transfer, leaf samples were taken from each drought tolerant $F_2$ plant together with the $F_1$ and parents' both control and stressed plants to analyse the malondialdehyde (MDA) and proline leaf content. MDA was analysed according to Luts et al., (1996), while proline content was evaluated according to the Bates et al., (1973) method. Temperature and humidity were recorded during both the pot and greenhouse experiments and are presented in Figure 1 and 3.

Temperature and humidity ranged between 15-45°C and 23-65% respectively during the drought experiment in pots. While average temperature was measured as 36°C, average humidity was measured as 76%. Figure 2, shows general view from the compartment in which the experiment was established and the responses of $F_2$ population at the 25th day of drought application.

Selected drought tolerant plants were transferred to the greenhouse where their tolerance to heat was evaluated using the morphologic descriptors modified from Boyaci et al., (2015) and detailed in Table 1.

Greenhouse climatic conditions were also recorded and shown in Figure 3. In greenhouse, temperature ranged between 25-49 °C and the average temperature was recorded as 36 °C humidity ranged between, 13 - 99% and the average humidity was assessed as 76%. While the $F_2$ best performing plants were morphologically characterized in greenhouse for heat stress, they were also selfed to generate the $F_3$ generation. Characterized plants were self-pollinated by hand individually in greenhouse conditions to obtain $F_3$ progenies.

## Results and Discussion

In terms of plant height, differences were observed between the drought-stressed groups of tolerant (*S. insanum* L.), sensitive (BATEM-TDC47) parents, and $F_1$ hybrid plants measured on 25th day of the drought experiment with respect to their corresponding control group (Table 2). The average plant height of the drought stressed group of *S. insanum* L. plants was 13.4 cm, while the average plant height of its control group was 17.6 cm. Drought stress accounted for 25.6% variation between the two groups. The average plant height of the sensitive genotype BATEM-TDC47 was 17.3 cm in the drought stressed group while 24.2 cm in the control group, with variation between the two groups of 28.5%. In $F_1$ plants, the mean values ranged 19.4 cm in the control group, while being 15.9 cm in the stressed group and the change rate was 18%. Simultaneously, wide variation was observed also among the $F_2$ population in terms of plant heights under drought effect.

Similarly, Semida et al., (2021) reported that water deficit in eggplant significantly affected and reduced plant height, stem diameter and number of leaves, as well as Fita et al., (2015) reported that plant height and fresh weight were the most distinctive morphological characters to determine drought tolerance in eggplant.

In the current study, morphologic damage level of the plants from $F_2$ population was observed on the 25th day of the drought experiment using a 0-5 scale. Hundred plants showed least damage due to drought stress and got scale value 1. Being drought tolerant according to Kiran et al ( 2015) these plants were transferred to green house for further evaluation under heat stress. Additionally, in terms of the scale evaluation, 150 $F_2$ showed symptoms as 25% yellowing and curling on plants and got "2" from the scale, being therefore noted as sensitive. Six among the 256 $F_2$ plants got "3" as a scale value as they showed symptoms 25%-50% yellowing and curling on plants and dropping leaves and were also noted as sensitive. In addition, studies on eggplant (Sseremba et al., 2018) potatoes (Banik et al., 2016), melon (Kusvuran, 2010), pea (Ajayi et al., 2018), and kiwifruit (Zhong et al., 2018) revealed that the 0-5 scale is an effective tool in determining to drought tolerance.

Leaf samples harvested from 50 among the 100 tolerant $F_2$ plants which also displayed desired morphological features were then analyzed for MDA and proline accumulations. MDA is the most frequently used biomarker of oxidative stress in plants, while proline is an amino acid which protects the plants from various stresses. An increase of proline levels under drought is related to a higher degree of adaptability to the plants to the stress; on the contrary an increase of the MDA values is linked to a higher sensitiveness. While the MDA values ranged between 9.87-15.97 nmol/g FW, proline content was determined between 2.40 – 16.70 µmol $g^{-1}$ FW and Figure 4 showed the results of $F_2$ plants. MDA and proline values detected in Parents and $F_1$ plants' both under control and drought tested conditions are also presented in Table 3.

In this study, both MDA and proline amounts of $F_1$ and parents showed increases under the effect of drought with respect to their respective control plants (Table 3). However, as expected, the sensitive parent showed high MDA and low proline variation under drought stress with respect to the tolerant parent. Previously, Kıran et al., (2015) reported that there is an effective direct correlation between the scale value describing the level of visual symptoms and the levels of MDA, so that under drought stress conditions MDA levels display the lowest level of variation as the scale value decreases. Likewise previous studies conducted on beans (Kandemir et al., 2018) and tomatoes (Yekbun and Kabay, 2017), reported that MDA content increases

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according to the degree of damage to the cell membrane in plants subjected to stress therefore, as the MDA content increases, the range scale increases as the drought tolerance of plant decreases.

In a study on tomatoes, it was determined that dehydrated plants accumulate osmolytes such as proline in their leaves and protect themselves from the stress accordingly (Noori et al., 2018). In another panel study aiming at determining the drought effect among different cultivar of rice, which is a semi-aquatic species requiring consistent irrigation prolonged during all season to grow, it was established that the higher levels of proline were accumulated in those cultivars which also displayed the higher tolerance to water deficiency (Lum et al., 2014).

A panel of 100 $F_2$ plants were selected as drought tolerant according to their overall good performance regarding plant height, symptoms scale value, MDA and proline content, and were therefore transferred to the greenhouse to be kept during the summer period, where their heat tolerance was assessed under normal irrigation conditions. For this purpose, the fruiting capacity of each plant was examined and the genotypes that could not set fruit under high temperature conditions were recorded as more sensitive to heat. Faiz et al., (2020) studied on heat tolerance of 4 local eggplant genotypes and they reported that, under high temperature (45°C) stress different eggplant genotypes performed physiologically and bio-chemically different.

The results of phenotypic characterization with regard of many qualitative traits are detailed in Table 4. Average plant height, fruit length, fruit width and fruit weight were measured as 106.4 cm, 14.0 cm, 6.8 cm and 55 g respectively.

Among the selected lines, differences were observed for all traits related to plant architecture, leaf prickles, leaf hairiness and fruit characteristics. The growth habit of the plants was neither upright nor widespread, the number of lobes in the leaf was average, and the plants were usually slightly spiny and hairy. Anthocyanin distribution in plant and leaves were also noted as mostly absent or low. The fruits were of various sizes, preferentially round but also oval or elongated (Table 5).

The morphologic parameters revealed a wide range of phenotypic combination in the $F_2$ population; Similarly, a wide segregation and variability, is usually expected in a segregant population obtained from a cross between cultivated eggplant and its wild relatives as reported in previous studies (Prohens et al., 2013; Frary et al., 2014; Boyaci, 2020). Interspecific hybridization is a strategy which extremely helps to transfer many useful features from wild species to cultivated eggplant, leading to increase the genetic diversity of the latter (Kouassi et al., 2016; Plazas et al., 2019). Meanwhile, due to the wide variability that usually is generated in a progeny when two different species are combined, before taking part in any breeding program the best performing progenies selected for a desired trait need to be submitted to a deep phenotypic characterization to better assess their combination ability and avoid selection of also negative traits (Gaufichon et al., 2010). With this study, the $F_2$ plants determined as heat and drought tolerant were also morphologically characterized with a sufficient number of descriptors created for eggplant, and only the progenies of the plants with the best traits combination will be considered for future employment in breeding programs to develop new eggplant breeding lines with improved adaptability to stress.

## Conclusions

This research is aiming to develop heat and drought tolerant lines in eggplant. In this study, 100 $F_2$ lines selected as the most drought and heat tolerant were morphologically characterized, selfed and progressed to the $F_3$ generation. Drought tests and selfing of inbred lines in next generations will be continued to provide their durability and to be fixed at homozygous level, so that new eggplant hybrids with improved features as heat and drought tolerance will be developed in the future. In this respect, this study, which deals with the beginning of breeding studies for heat and drought stress, can be suggested as a model for breeding programs not only for eggplant but also for other species.

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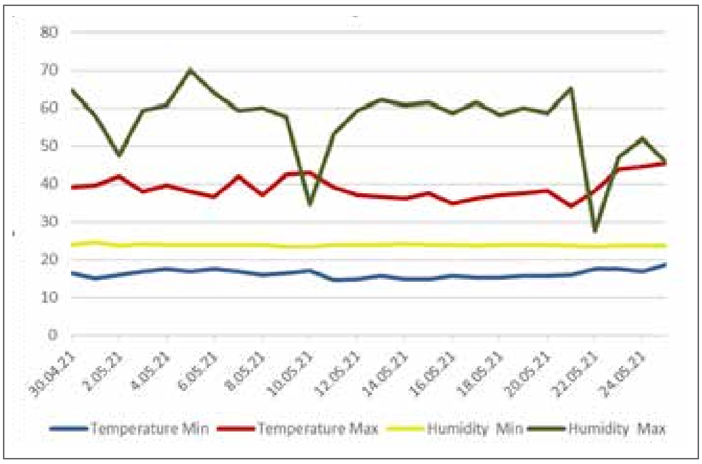


Figure 1. Temperature (°C) and humidity (%) recorded during the drought stress pot experiment.


Figure 2. A- General view from the compartment in which the drought experiment was established, B- The responses of $F_2$ population on the 25th day of drought application. (Original)

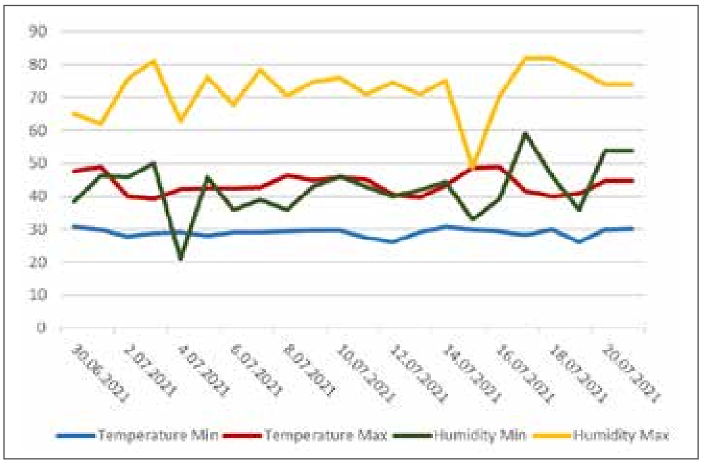


Figure 3. Temperature (°C) and humidity (%) recorded during the heat stress period.

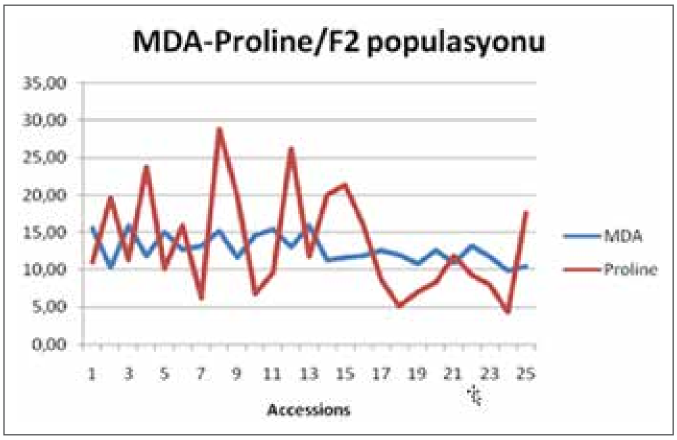


Figure 4. Graphic shows MDA (nmol/g FW) and proline (µmol $g^{-1}$ FW) alteration in selected $F_2$ plants.

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Table 1. The phenotypic descriptors and observation methods used in the study.

| No | Descriptors | Explanation |
|---|---|---|
| 1 | Growth habit | 1= very upright, 3= upright, 5= intermediate, 7= prostrate |
| 2 | Leaf blade lobs | 1= very weak, 3= weak, 5= intermediate, 7=strong, 9= very strong |
| 3 | Anthocyanin distribution in plant | 1=absent, 3= low, 5= intermediate, 7= high |
| 4 | Anthocyanin distribution in leaves | 1=absent, 3= low, 5= intermediate, 7= high |
| 5 | Leaf prickliness | 1= None, 3=Very few (1-2), 5= Few (3-5), 7= Intermediate (6-10), 9=Many (11-20), 11= Very many (>20) |
| 6 | Leaf hairiness | 1=absent, 3= low, 5= intermediate, 7= high |
| 7 | Number of flowers/ inflorescence | number |
| 8 | Fruit load | 1= very low, 3= low, 5= intermediate, 7=high, 9= very high |
| 9 | Leaf blade width | Measured in cm with ruler (Average of the best 3 leaves for each plant) |
| 10 | Leaf blade length | Measured in cm with ruler (average of the best 3 leaves for each plant.) |
| 11 | Total plant height | Measured in cm as the distance from the soil surface to the tip. |
| 12 | Varietal type | 1=long, 3=oval, 5=round, 7=striped, |
| 13 | Predominant fruit colour | 1=dark green, 3=green, 5=lilac, 7=dark lilac, 9=purple, 11=dark purple, 13=black, |
| 14 | Secondary fruit colour | 1=dark green, 3=green, 5=lilac, 7=dark lilac, 9=purple, 11=dark purple, 13=black |
| 15 | Fruit glossiness | 1=opaque, 3=intermediate, 5=bright peel colour |
| 16 | Fruit curvature | 1=round, 3=no curvature, 5=slightly curved, 7=curved, 9=S shaped, 11= U shaped |
| 17 | Presence of grooves | 1=absent, 3=few, 5=intermediate, 7=many |
| 18 | Calyx fruit coverage | 1= less than 10%, 3=10-20%, 5=21-30%, 7=31-40%, 9=41-49%, 11=50% and more |
| 19 | Fruit firmness | 1=spongy, 3=intermediate, 5= tight |
| 20 | Fruit weight | Measured in g (average of 2-3 fruits from each plant) |
| 21 | Fruit length | Measured in cm (average of 2-3 fruits from each plant) |
| 22 | Fruit maximum diameter | Measured in cm (average of 2-3 fruits from each plant) |
| 23 | Fruit length/breadth ratio | Calculated |
| 24 | Peduncle length | Measured in cm (average of 2-3 fruits from each plant) |
| 25 | Fruit calyx prickliness | 1= none, 3= very few (1-2), 5= few (3-5), 7= intermediate (6-10), 9= many (11-20), 11= very many (>20) |
| 26 | Petiole length | Measured in cm |
| 27 | Fruit end button size | 1=small, 3=intermediate, 5= large |
| 28 | Presence of chlorophyll on the pistil scar | 1=absent, 3= present |
| 29 | Fruit color distribution | 1=uniform, 3=mottled, 5=netted, 7=striped |

Table 2. The effect of 75% water deficit on the plant height applied during the seedling period.

| | *S. insanum* | | TDC47 | | TDC47 × *S. insanum* | | $F_2$** |
|---|---|---|---|---|---|---|---|
| | **Control** | **75% WD** | **Control** | **75% WD** | **Control** | **75% WD** | **75% WD** |
| Shortest plant height (cm) | 12.0 | 10.0 | 18.5 | 14.0 | 15.0 | 11.0 | 10.0 |
| Longest plant height (cm) | 22.0 | 19.0 | 28.0 | 20.0 | 23.0 | 18.0 | 24.0 |
| Average plant height (cm) | 17.6 | 13.4 | 24.2 | 17.3 | 19.4 | 15.9 | 19.4 |
| Standard deviation | 3.1 | 2.7 | 3.1 | 2.2 | 2.8 | 1.9 | 3.1 |
| % Variation | 25.6% | | 28.5% | | 18.0% | | - |

WD: Water deficit **:max., min. and average values of 256 $F_2$ individual seedlings

Table 3. MDA (nmol/g FW) and proline (µmol $g^{-1}$ FW) amounts showing different degrees of increases under drought compared to the control plants.

| | *S. insanum* | | TDC47 | | TDC47 × *S. insanum* | |
|---|---|---|---|---|---|---|
| | **Control** | **Application** | **Control** | **Application** | **Control** | **Application** |
| MDA (nmol/g FW) | 11.1 | 12.5 | 7.5 | 9.0 | 14.1 | 15.6 |
| % Variation | 12.75% | | 19.20% | | 10.81% | |
| Proline (µmol $g^{-1}$ FW) | 17.6 | 13.4 | 24.2 | 17.3 | 19.4 | 15.9 |
| % Variation | 20% | | 3.7% | | 25% | |

Table 4. Data of some phenotypic traits scored among the 100 $F_2$ plants determined as drought tolerant.

| **Trait** | **Minimum** | **Maximum** | **Average** | **Standard Deviation** |
|---|---|---|---|---|
| Plant height (cm) | 50.0 | 142.0 | 106.4 | 15.6 |
| Leaf blade length (cm) | 11.7 | 26.3 | 20.1 | 2.4 |
| Leaf blade width (cm) | 7.0 | 17.0 | 11.8 | 1.9 |
| Petiole Length (cm) | 3.0 | 15.5 | 7.3 | 1.5 |
| Fruit length (cm) | 3.0 | 14.0 | 8.4 | 1.7 |
| Fruit width (mm) | 28.0 | 68.0 | 48.9 | 6.8 |
| Fruit weight (g) | 26.0 | 140.0 | 55.0 | 20.9 |
| Fruit length/breadth ratio (cm) | 1.0 | 2.8 | 1.7 | 0.3 |
| Peduncle length (cm) | 3.0 | 15.5 | 7.3 | 1.1 |

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Table 5. Some phenotypic characteristics of 100 lines selected as drought tolerant.

| No | Descriptors | Results (*the numbers show how many plants are in which feature) |
|---|---|---|
| 1 | Growth habit | 2- very upright, 42- upright, 50- intermediate, 7- prostrate |
| 2 | Leaf blade lobs | 1-very weak, 12- weak, 67- intermediate, 20- strong |
| 3 | Anthocyanin distribution in plant | 30- absent, 65- low 5- intermediate |
| 4 | Anthocyanin distribution in leaves | 68- absent, 30- low. 2- intermediate |
| 5 | Leaf prickliness | 37- none, 14- very few. 35- few, 14- intermediate |
| 6 | Leaf hairiness | 2- absent, 51- low, 46- intermediate, 2- high |
| 7 | Number of flowers/inflorescence | 2- 1/2, 78- 1/3, 20- 3/4 |
| 8 | Fruit load | 10- low, 56- intermediate, 24- high, 10- very high |
| 9 | Varietal type | 12- long, 20- oval, 68- round |
| 10 | Predominant fruit color | 15- dark green, 9- green, 19- lilac, 15- dark lilac, 22-purple, 20-dark purple |
| 11 | Secondary fruit color | 3- dark green, 52- green, 6- lilac, 5- dark lilac, 18- purple, 16- dark purple |
| 12 | Fruit glossiness | 30- opaque, 46- intermediate, 24- bright peel color |
| 13 | Fruit curvature | 1- round, 66- no curvature, 33-slightly curvature |
| 14 | Presence of grooves | 100- absent |
| 15 | Calyx fruit coverage | 20- less than 10%, 55- 10-20%, 24-21-30%, 1-31-40% |
| 16 | Fruit calyx prickliness | 36- none, 7-Very few (<3), 33-Few (~5), 24-Intermediate |
| 17 | Fruit firmness | 2- spongy, 43- intermediate, 55- tight |
| 18 | Fruit end button size | 25- small, 36-intermediate, 39- large |
| 19 | Presence of chlorophyll on the pistil scar | 70- absent, 30- present |
| 20 | Fruit color distribution | 52- uniform, 28- mottled, 1- netted, 19- striped |

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