

## Determination of low temperature tolerances of some tomato genotypes

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### Abstract

Low temperature is one of the most important environmental stress factors. In this study, 9 of pure lines at the F<sub>9</sub> stage which belonged to Batı Akdeniz Agricultural Research Institute (BATEM) were used as a plant material to determine the lines tolerant to low temperatures. Low temperature applications were carried out in vegetative and generative stages. *Solanum hirsutum* (LA 1777) and 2 commercial varieties; (Cigdem F<sub>1</sub> and Anıt F<sub>1</sub>) used as tolerant genotypes (Control). The tomato seeds were germinated and grown in a mixture of peat and perlite (1:1 v/v). When the seedlings reached two true leaf stages, they were taken to the temperature, humidity and light controlled growth chamber for low temperature applications in vegetative test. At the vegetative test, plants were exposed to the 5±1°C, at light intensity 350 µmol m<sup>-2</sup> sec<sup>-1</sup> for five days. In the generative tests, the genotypes were selfed in the growth chamber with 5±1°C and 180 µmol m<sup>-2</sup> sec<sup>-1</sup> of light intensity and taken to the greenhouse for fruit set after three days. As a result, it was determined that genotypes 5 and 6 were found as tolerant to the low temperatures in terms of dry matter production and seed amount both in vegetative and generative stages.

**Keywords:** Abiotic stress; Cold tolerance; Chilling tolerance, Wild genotypes

### Bazı domates genotiplerinin düşük sıcaklığa toleranslarının belirlenmesi

#### Öz

Düşük sıcaklık en önemli çevresel stres faktörlerinden biridir. Bu çalışmada, Batı Akdeniz Tarımsal Araştırma Enstitüsüne (BATEM) ait F<sub>9</sub> kademesindeki 9 adet saf hat kullanılmıştır. Düşük sıcaklık uygulamaları vejetatif ve generatif dönemlerde gerçekleştirilmiştir. *Solanum hirsutum* (LA 1777) ve iki adet ticari domates çeşidi (Cigdem F<sub>1</sub> ve Anıt F<sub>1</sub>) tolerant genotipler olarak kullanılmıştır. Domates tohumları 1:1 oranında torf ve perlit karışımında çimlendirilmiştir. Fideler iki gerçek yaprak aşamasına ulaştığında vejetatif testleme için sıcaklık, nem ve ışık kontrollü bitki büyüme odasına alınmıştır. Vejetatif testlemede bitkiler beş gün boyunca 350 µmol m<sup>-2</sup> sec<sup>-1</sup> ışık yoğunluğunda 5±1°C'ye maruz bırakılmıştır. Generatif testlemede, genotipler 180 µmol m<sup>-2</sup> sn<sup>-1</sup> ışık yoğunluğunda 5±1°C'de kendilenmiş ve üç gün sonra meyve tutumu için seraya alınmıştır. Sonuç olarak, 5 ve 6 numaralı genotiplerin hem vejetatif hem de generatif dönemlerde kuru madde üretimi ve tohum miktarı açısından düşük sıcaklıklara toleranslı olduğu bulunmuştur.

**Anahtar Kelimeler:** Abiyotik stres; Soğuk toleransı; Düşük sıcaklık; Yabani genotipler

### 1. Introduction

Tomato (*Solanum lycopersicum* L.) is a single-year plant which belongs to the family Solanaceae. Gene center is South and Central America and is known to spread from America to Europe from there to the whole world. 100 g of tomato contains, 21 mg vitamin C, 1700 IU vitamin A, 0.10 mg vitamin B1 and 0.55 mg vitamin B6. Vitamin C is known antioxidant properties of vitamins and tomato is one of the important sources of this vitamin. Its production and trade, constitutes one of the most important

product group of fresh vegetables in Turkey as well (Gulistan, 2006). In terms of the production amount, Turkey, ranked fourth after China, India and the United States. Major abiotic stresses (drought, salt and high temperature) adversely affect growth and productivity and threaten food security Bita and Gerats (2013). The plants respond and survive at physiological and biochemical levels with molecular and cellular levels under stress conditions (Sanghera et al., 2011). The most studied abiotic stress factor is low temperature stress (Gokmen, 2006).

Cold stress is an important environmental factor limiting the agricultural productivity of plants grown in hilly areas. Many species of tropical or subtropical origin are injured or die due to the low temperatures. Tomato is one of the most affected plant by this problem. Tomato genotypes have shown important variation against low temperature stress (Koroleva et al., 2000). Low temperature stress usually occurs at temperatures below 15°C and it causes growth disorders and decrease of yield in serious dimensions due to the severity of stress in plants. Especially tomatoes which were grown under greenhouse conditions in winter season, shows yield loss in serious dimensions caused by low temperature stress. These problems can occur from germination to the fruit formation stage (Gokmen, 2006).

Under low heat conditions (the minimum temperatures below 10-12°C at nights), the number of pollens decrease and deformations happen in flower organs and sex cells thus such developmental disorders greatly reduce the yield (Dasgan et al., 1994). In the generative period of tomatoes, the time that spent at low temperatures is also very important as much as how much cold stress a potential cold temperature develops. Even when marginally low temperatures are applied (8/16°C) the rate of pollens and pollen liveliness of tomato plants is affected dramatically quick and this suggests that the tolerance of genotype differs from another genotype. For example, whereas pollens decreased by 60% in the 227/1 genotype as a result of 4 days low temperature stress, the LM 513 genotype stayed stable (Ozturk et al., 2006). In addition to high viability and germination ability of pollens for fruit or seed production in a plant genotype, the amount of pollen produced in anthers should be high and the whole of the pollen formed in anthers can not reach the toes or even if it does, it has been indicated that there is no suitable environment for germination here (Keles, 2007). The wilting and dryness caused by cold stress in sensitive plants, it is the result of the loss of the hydraulic conductivity of the roots. In addition to this, plants lose their turgor at low temperatures, and malfunction of stomatal control, causes further increasing water loss. The second, the photo-oxidative damage occurring on the leaves when the cold and the direct sunlight are together (Aroca et al., 2001; Hutchison and Groom,

2000; Venema et al., 2000). Photooxidative damage means that, low or high temperature, which limits crop production, cell destruction caused by environmental stress factors such as drought, it is mainly catalyzed by toxic O<sub>2</sub> derivatives (Inze and Van Montagu, 1995; Foyer et al., 1997). In recent years both in classical breeding studies and in molecular and physiological research, the development of new genotypes that are tolerant to low temperature stress and the identification of role-playing mechanisms are increasingly emphasized (Truco et al., 2000; Fellner and Shawney, 2001). Ma et al. (2015) recently pointed out that a protein (COLD1) is required for cold stress sensing in rice and for tolerance of cooling (0-15°C) in rice subpopulations of Nipponbare. Lately, chilling-responsive microRNAs have been detected in several plant species. Even so, little is known about the miRNAs in the model plant tomato. Also 'LA1777' (*Solanum habrochaites*) has been shown to survive chilling stress due to its diverse characteristics (Cao et al., 2014). Among the tomato varieties, the number of varieties suited to the winter season stage cultivation is very small.

The aim of this study is to determine the low temperature performance of the genotypes in the BATEM tomatoes gene pool. Also; thanks to the genotypes to be determined by the study; development of varieties suitable for winter season breeding, getting more and better quality products from unit area, tolerance to other abiotic stress factors, reduction in heating costs, the genotypes to be determined will contribute to the emergence of new and superior hybrids carrying several qualities together.

## 2. Material and Methods

This project was conducted at Bati Akdeniz Agricultural Research Institute (BATEM) Antalya/Turkey, in 2018 autumn season. In the low temperature tests were used nine pure tomato lines developed in BATEM and three tolerant genotypes (*Solanum hirsutum* (LA1777), Cigdem F<sub>1</sub>, Anit F<sub>1</sub>). *Solanum hirsutum* (LA1777) is a wild genotype. Cigdem F<sub>1</sub> and Anit F<sub>1</sub> are suitable for late autumn and single plantation. Vegetative and generative testing methods were used to determine tolerant genotypes.

## 2.1. Vegetative testing

The method used by Kantar (2016) was optimized. The seeds of the materials were planted in 2 sets for the greenhouse and controlled (temperature, humidity and light) plant growing room. 10 plants from each genotypes were used in low temperature applications and trial was planned with 3 replications. The seedlings were irrigated with the modified nutrient solution (Gokmen, 2006) until the 2 leaf stage. When the seedlings arrived at the 2 true leaf stage, they were taken to the temperature, humidity and light controlled plant growing room. They were treated with cold for 5 days. Day/night temperature values were  $5\pm 1^{\circ}\text{C}$  in there. The genotypes in the control series were kept in the greenhouse with day and night temperatures  $28/18\pm 1^{\circ}\text{C}$ . During the test, the light intensity was maintained at  $350\ \mu\text{mol m}^{-2}\ \text{sec}^{-1}$ .

Low temperature stress applied and every plant in the trials in which the control plants were involved were determined g ( $\pm 0.1$ ) fresh weights by weighing on a precision scale and the dry weight was recorded as g after 72 hours of drying at  $65^{\circ}\text{C}$  sterilizer.  $\text{TI}_{\text{VG}}$  was calculated according to dry matter produce of genotypes cold appliciated-non appliciated (control) and analyzed statistically (Öztürk, 2006).

$$\text{TI}_{\text{VG}} = (\text{Application} / \text{Control}) * 100$$

The vegetative growth cold tolerance index ( $\text{TI}_{\text{VG}}$ ) were calculated by determining the dry matter production in the genotypes applied at the end of the vegetative test and the genotypes found in the control series.

## 2.2. Generative testing

After, reached the stage of 2 true leaves, tomato seedlings were transplanted to the 3 liters pots, filled with the mixture of peat and perlite (1:1 v/v). This stage was planned with as five replicates. Two commercial varieties 'Cigdem F<sub>1</sub> and Anit F<sub>1</sub>' with wild *S. hirsutum* (LA 1777) were used as controls. The plants were grown in the greenhouse till the anthesis stage, then they were taken to the growth chamber.

The genotypes were selfed in the growth chamber at  $5\pm 1^{\circ}\text{C}$  and  $180\ \mu\text{mol m}^{-2}\ \text{sec}^{-1}$  of

light intensity and three days later they were taken to the greenhouse for fruit set. Generative growth cold tolerance index ( $\text{TI}_{\text{GG}}$ ) was determined according to amounts seed of genotypes cold appliciated, non-appliciated (control) and analyzed statistically. Statistical analysis of the cold tolerance index of the genotypes performed by vegetative and generative tests was done by using the Jump program.

## 3. Results and Discussion

### 3.1. Vegetative testing

Vegetative growth cold tolerance index ( $\text{TI}_{\text{VG}}$ ) was given in Figure 1. As it is seen in Figure 1, ( $\text{TI}_{\text{VG}}$ ) dry matter production of genotypes in vegetative testing stage varied between 15% and 40%. The highest  $\text{TI}_{\text{VG}}$  for dry matter production was found in genotypes 5 and 6 as 40%.  $\text{TI}_{\text{VG}}$  values which were related to the dry matter production of the genotypes were subjected to the statistical analyzes, it was found that genotypes 5 and 6 took the first place according to the controls.

In the study conducted by Gokmen (2006), stated that dry matter production of some genotypes shows significant differences depending on the duration of and the degree low temperature. Different tomato genotypes were exposed to low temperature ( $2^{\circ}\text{C}$ ) at different periods of time and on 8. day researchers stated that the cold tolerance index ( $\text{TI}_{\text{VG}}$ ) of dry matter production of genotypes varied between 37% and 52%. Foolad and Lin (2001) reported that the dry matter production of the genotypes which were exposed to cold stress varied between 12% and 34%. In our study, the cold tolerance index ( $\text{TI}_{\text{VG}}$ ) of the dry matter production of tomato genotypes exposed to  $5\pm 1^{\circ}\text{C}$  cold for 5 days varied between 15% and 40%. According to these results, the tomato genotypes used in our study showed different cold tolerance index during 5 days at  $5\pm 1^{\circ}\text{C}$ , for this reason, it can be said our results compatible with the literature.

### 3.2. Generative testing

The seed ratios and generative growth cold tolerance index ( $\text{TI}_{\text{GG}}$ ) of the genotypes are given in Figure 2.

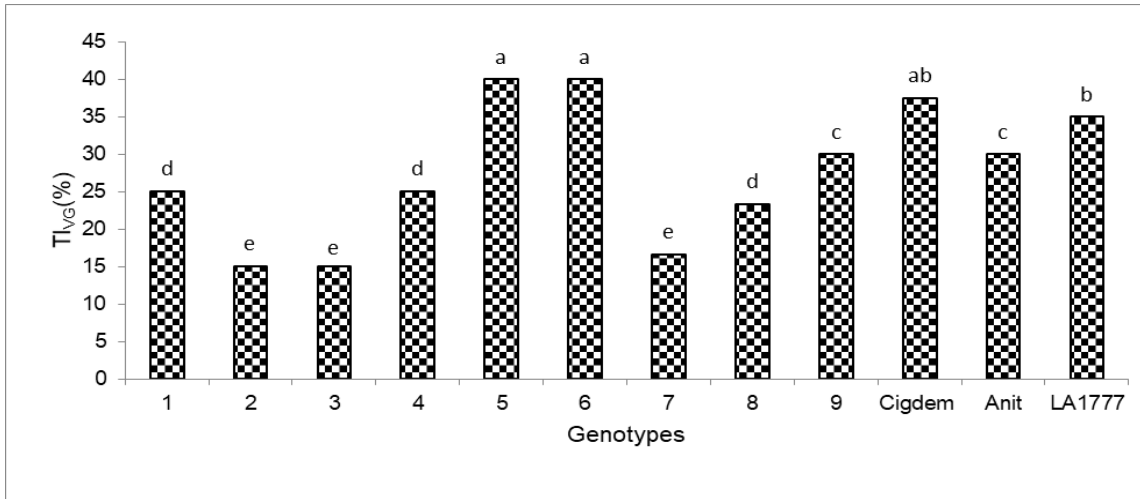


Figure 1. Cold tolerance index(TI<sub>VG</sub>) of dry matter production of genotypes

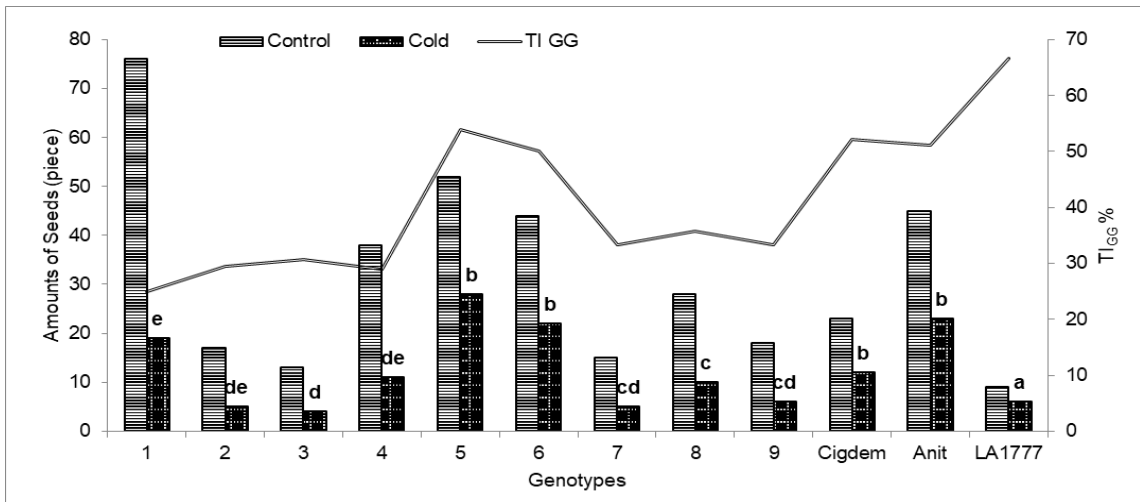


Figure 2. Cold tolerance index (TI<sub>GG</sub>) of genotypes seed amount rates

When the seed numbers obtained from the control treatment were considered, it was understood that cold application caused the decrease almost 50%. In some genotypes, the number of seeds decreased by 80%. The wild genotype ranked first in the tolerance index (TI<sub>GG</sub>) of seed quantities. On the other hand genotypes 5 and 6 were in the same group with control commercial varieties. As it is seen in Figure 2 generative growth tolerance index (TI<sub>GG</sub>) were investigated with respect to seed amounts rates of cold applied and non-applied genotypes. While the cold activity was found to be 67% in the highest wild genotype, numbers 5 and 6 genotype, commercial varieties Anit F<sub>1</sub> and Cigdem F<sub>1</sub> were found to be 54%, 50%, 51% and 52%, respectively. Venema et al. (2008), reported that low-temperature tolerant tomato rootstocks are not yet available. For this

purpose, the soil temperatures and air temperatures with optimum 15/25°C in different combinations were determined the rootstock performance. As a result, *S. habrochaites* LA1777 appeared to be a valuable genotype in order to increase the low temperature tolerance of commercial tomato rootstocks. The genotype of LA1777 was found to be tolerant in the results obtained from our study.

As it is seen in Figure 3, the correlation between dry matter cold tolerance index (TI<sub>VG</sub>) that we used to identify tolerant genotypes at low temperatures and seed amount rates was found  $r = 0.77$ . Tomato fruit is one of the most consumed species among the vegetables. Environmental factors have important effects on plant development. Jouyban et al. (2013) reported that low temperatures (1-10°C) lead to

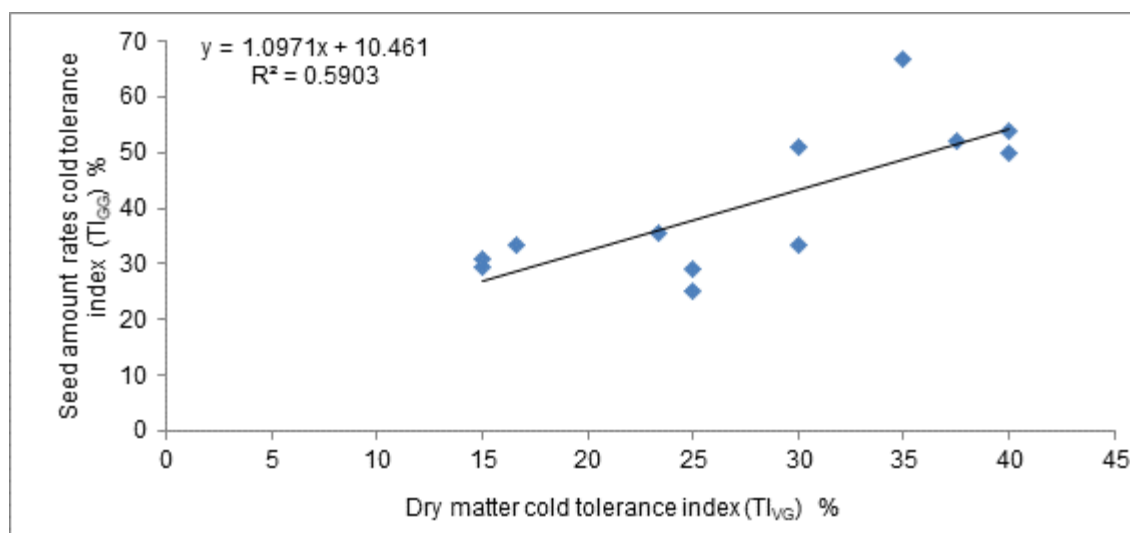


Figure 3. Relationship between dry matter cold tolerance index(TI<sub>VG</sub>) and seed amounts rates cold tolerance index(TI<sub>GG</sub>)

significant physiological changes on tropic and subtropic vegetable species and susceptible plants exposed to low temperature cause deterioration in the physiological process like water regime, nutrient uptake, photosynthesis and respiration. One of the plant species most affected by low temperature is the tomato.

Identification of new genotypes with low temperature tolerance and physiological characterization of tolerance will increase the speed of selection and breeding work. Therefore, this study was conducted to find new genotypes tolerant to low temperatures. In this study, 9 commercial tomatoes and 2 commercial varieties and one wild tomato genotype were used. In identifying cold stress tolerant lines, plant dry matter production in vegetative testing, and in the generative test, cold tolerance index was calculated by considering the seed amount rates. In vegetative and generative testing, while the cold tolerance index (TI<sub>VG</sub>) of genotypes 5 and 6 were ranked first in the dry matter production according to the cold tolerance index (TI<sub>GG</sub>) of the tomato varieties used in the winter season cultivation on the market, they were found to be in the same group in terms of seed quantity. Many studies have been done to identify cold stress tolerant genotypes. Different criteria such as leaf cell damage, chlorophyll content, dry matter production, antioxidative content in genotypes have been investigated. Cold tolerance of tomato genotypes do not seem to

be sufficient to explain changes in antioxidative systems alone. Therefore, determining the cold activity of tomatoes may also play a role in different plant characteristics such as osmotic potential, root hydraulic conductivity, photosynthetic activity (Gokmen, 2006). In our study, determining cold stress tolerance of the used genotypes together with dry matter production and the seed amount rates were taken into account in the generative stage, it is seen that the correlations of these two criteria have a value of 77%.

#### 4. Conclusion

According to these results, the tolerant genotypes 5 and 6 have shown the same performance as the tomato varieties which are most preferred by producers in the market for winter season cultivation and these genotypes can be used to develop cold tolerant varieties.

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