*HortiS (2022) 39(2):56-62*
*http://doi.org/10.16882/HortiS.1122901*

RESEARCH PAPER

HORTICULTURAL STUDIES

# The Performance of Some Tomato Pure Lines under Cold Stress in the Vegetative and Generative Stage

**Akın TEPE[1] Volkan GÖZEN[1] Aylin KABAŞ[2] Volkan TOPÇU[1] Orçun ÇINAR[1]**

[1] Batı Akdeniz Agricultural Research Institute, 07100, Antalya, Türkiye
[2] Akdeniz University, Manavgat Vocational School, 07600, Antalya, Türkiye

**Article History**
Received 18 April 2022
Accepted 28 May 2022
First Online 05 June 2022

**Corresponding Author**
E-mail: akin.tepe@tarimorman.gov.tr

**Keywords**
Cold stress
Electrolyte leakage
Malondialdehyde
Pollen germination
Tomato

## Abstract

Low temperature stress decreases yield and quality of tomato in greenhouse conditions. For successful tomato cultivation under the cold stress, cultivars performances are extremely important both vegetative and reproductive growth stage. In this study, 20 tomato pure lines and 3 commercial cultivars (Cigdem $F_1$, Anit $F_1$ and Bestona $F_1$) and also *Solanum hirsutum* (LA 1777) known as tolerant genotypes were evaluated at vegetative and reproductive stage. The studies were conducted under both the cold stress in growth chamber and the optimal temperature condition (control) in the greenhouse. They were evaluated by measuring malondialdehyde (MDA), electrolyte leakage (EL) and dry matter yield (DM) at vegetative stage. The results showed that EL rate and MDA content increased while DM decreased under the cold stress when compared with leaves of plants grown at optimal temperature. In reproductive stage, pollen viability and pollen germination were evaluated under both cold stress and control conditions for all genotypes. All the sensitive genotypes exhibited low pollen viability and pollen germination. Consequently, three pure lines were identified with low-temperature tolerant in vegetative and reproductive growth stage.

## 1. Introduction

Tomato is one of the most economically important vegetable crops after potatoes (Ronga et al., 2018). Türkiye ranks fourth in world tomato production after China, India and the USA. World tomato production is 180.7 million tons, 7% of which is produced in Türkiye. (FAO, 2021).

Both chilling (<20°C) stress and freezing (<0°C) stress are called low temperature stress (Ma et al., 2018; Ражаметов et al., 2020). Low temperature stress is one of the most abiotic stress factors that reduce the productivity of crops (Duan et al., 2012), affected about 24.6% of the entire area of the world's land (Peel et al., 2007). Cultivated tomato is a cold sensitive crop; crop growth and development are severely damaged below 12°C (Elizondo and Oyanedel, 2010; Ronga et al., 2018). Duration of exposure is also important as well as temperature for the severity of damage (Elizondo and Oyanedel, 2010; Barrero-Gil et al., 2016). Both of them adversely affect growth and productivity in tomato plants depending on the severity of the low temperature stress (Gökmen, 2006; Atayee and Noori, 2020; Ражаметов et al., 2020). When the tomato plants are exposed to low temperatures, injury symptoms begins initially at the vegetative growth stage. The most noticeable injury could be observed in the vegetative stage such as stunted seedlings, leaf-hypocotyl wilting, leaf chlorosis and local necrosis (death of tissue) (Cao et al., 2015; Atayee and Noori, 2020). On the other hand, low temperature stress at the reproductive stage of plants causes poor pollen viability, weak fruit set,

poor fruit quality, which result in loss of crop yield. Further, dry matter production (DM) is also widely used parameter to select tolerant plants in the cold stress studies (Foolad et al., 2000; Foolad and Lin, 2001; Gökmen, 2006). Likewise, cold stress gives rise to membrane damage and increases electrolyte leakage. Electrolyte leakage (EL) and lipid peroxidation (LPO) are an important indicator of plant membrane damage level under the cold stress (Duan et al., 2012; Malekzadeh et al., 2014). Most of the previous investigations focused only on individual stages. A few studies that included more than one stage, however, evaluated only a few genotypes, thereby there is no specific conclusions could be guide about the cold stress at different stages. Therefore, the aim of this study is to determine the tolerant pure lines in tomato during vegetative and reproductive growth under the cold stress conditions.

## 2. Materials and Methods

### 2.1. Plant materials, growing and stress conditions

In this current study, cold stress studies were conducted with 20 tomato genotypes belonging to Batı Akdeniz Agricultural Research Institute (BATEM) tomato gene pool (over $F_6$ generation) and three commercial varieties (Cigdem, Anit and Bestona $F_1$) wild type LA 1777 (*S. hirsutum)* were used as control genotypes. The experiment was governed five replication and each replication consisted of five seedlings per replicate at the early seedling stage, in the greenhouse. Seedlings at 2-3 true leaf stage were grown at the optimal temperature and then transferred to plastic pot. After transplanting the seedlings, they were irrigated with Hoagland nutrient solution and grown up to 3-4 true leaf stage at the optimal temperature. Seedlings of genotypes were exposed to chilling treatment at 5±1°C with a 12 h photoperiod (day/night) and light intesity (200 µmol $m^{-2}$ $sec^{-1}$) for 5 days in growth chamber and grown at the optimal temperature (control) in the greenhouse. For analysis, samples were collected from the third-fourth leaf in the seedling at both cold stress (T) and control condition (CC) in the study.

### 2.2. Vegetative growth stage

#### *2.2.1. Measurement of malondialdehyde (MDA)*

The MDA content was determined by the reaction of thiobarbituric acid (TBA), as described by Sayyari (2012). 1 g of leaf samples were taken and added 10 ml of 0.1% trichloroacetic acid (TCA), and then centrifuged at 15000 rpm for 5 minutes, further 4 ml of 0.5% thiobarbituric acid (TBA) was added. The mixture obtained kept in the bath at 95°C hot water for 30 minutes and then quickly cooled in an ice bath. Afterwards, absorbance values were read at A532 and A600 nm in the spectrophotometer. The values obtained were calculated with Lipid peroxidation = (A532-A600) x extract volume (ml) / (155mM / cm x sample amount (mg).

#### *2.2.2. Measurement of electrolyte leakage (EL)*

The structure and function of cell membranes are damaged under the cold stress. Thus, EL increase in chilling stress. EL was used to evaluate membrane permeability. EL was measured using an electrical conductivity meter, according to the methods by Lutts et al. (1996). In the laboratory, 10 discs of 1 cm diameter taken from tomato leaf samples were washed with pure water and placed in brown glass bottles. After adding 20 ml of pure water to the samples, they were shaken for 24 hours and the $EC_1$ values were read by pouring the solutions into tubes. The same samples were autoclaved at 120°C for 20 minutes and their $EC_2$ values were read after the samples reached room temperature. The EL calculated as $EC_1$ / $EC_2$ × 100 formula was used to calculate the cell membrane damage of the samples.

#### *2.2.3. Measurement of dry matter yield (DM)*

Seedling of the genotypes were individually harvested for shoot (leaf+stem) both control and chilling treatments. Genotype's shoot were dried in an oven at 65°C for 72 h and weighed (± 0.1 g) and dry weight (DM) of individual plants determined (Gökmen, 2006). For each genotype, vegetative growth was defined as ratio of dry weight (DW) under cold stress to under control condition.

### 2.3. Reproductive stage

After sowing the seeds of the materials, the seedlings that reached the stage of 2-3 true leaves were transplanted to be used in reproductive testing with 5 replications, one plant per pot. Plants were grown at in the greenhouse until the flowering stage and then they were taken to growth chamber 24 h before anthesis after 72 h exposed to cold stress in growth chamber, samples of pollen were collected from each replication of all genotypes. Pollen viability and germination percentages were determined both under cold stress and under control condition in genotypes flowering in pots.

#### *2.3.1. Pollen viability*

Viability levels of flower powders were tested with 2,3,5 Triphenyl Tetrazolium Chlorid (TTC), as described by Boyaci et al. (2009). In plants grown in the control greenhouse and growth chamber, the pollens of the flowers that bloom in the first cluster were taken in 3 replications 3 readings were made in each repetition.

*2.3.2. Pollen germination ability*

The method was used to determine the pollen germination ability (Boyacı et al., 2009). The pollens derived from tomato genotypes flowering in the cold room and control greenhouse were planted in 1% agar + 12% sugar + 300 ppm $H_3BO_3$ + 300 ppm Ca $(NO_3)_2$ germination medium and kept at 25°C for 20 hours then counted under the light microscope. In the pollen germination test, two petri dishes were prepared for each genotype, and four randomly selected areas were counted and pollen germination percentages were determined.

*2.3.3. Cold tolerance index*

Cold tolerance index (%) for all traits was calculated as under cold stress (T) and as the percentage of the control condition (CC) for all traits. To determine the cold tolerance genotypes, cold tolerance index (TI) was calculated according to Funatsuki et al. (2005) as follows:

$$TI = \left[\frac{T}{CC}\right] \times 100$$

where; TI: Cold tolerance index; T: Trait value in cold stress condition; CC: Trait value in control condition.

Genotypes whose TI values were close to 100 or 1 were considered tolerant in all traits.

**2.4. Statistical analysis**

The genotypes means and standart deviations were analyzed for samples within each replicate for all parameters. All statistical analyses were performed with JUMP (version 8.0). Cold tolerance index (TI) among the genotypes in each parameter were subjected to the analyses of variance (ANOVA) and compared among genotypes using LSD multiple range tests at the P<0.05 level. The data shown are means values ± TI for all parameters. Levels of significance are represented by at P<0.05 (*), P<0.01 (**) and P<0.001 (**).

**3. Results and Discussion**

**3.1. Vegetative stage**

*3.1.1. Measurement of electrolyte leakage (EL)*

The amount of EL in tomato seedlings increased under cold stress that we looked for lower EL ratio among the genotypes. (Table 1). To determine the cold tolerance genotypes, we used tolerance index ($TI_{EL}$) with the electrolyte leakage (EL) ratio. The data statistical anaysis showed that the ratio of EL was significantly increased (Table 1; P<0.001) treated genotypes under the cold stress.

Genotypes were selected as tolerant with the least diffrence of $TI_{EL}$ values in the applied genotypes compared to the control group $TI_{EL}$ values. Moreover, the value of $TI_{EL}$ measured in G5, G8 and G11 were much lower than in all other cultivars. For $TI_{EL}$ can be showed that G5, G8 and G11 were highly tolerant genotypes.

*3.1.2. Measurement of dry matter yield (DM)*

While the growth of all genotypes decreased in response to cold stress, there was significant genotypic variations. $TI_{DM}$ showed significant (p<0.01) differences between genotypes. Genotypes No.8 (G8) grew as fast as commercial hybrid-1 (CV-1) and commercial hybrid-2 (CV-2) under cold stress conditions. Besides, the value of TI G8 (0.88) and G1 (0.87) showed greater plant vigour as high as commercial varieties among the genotypes (Table 1).

*3.1.3. Measurement of malondialdehyde (MDA)*

The concentration of MDA was used an indicator of lipid peroxidation (LPO) in plant cells and increases in chilling stress. Therefore, we observed for tolerant genotypes which had lowest MDA content. Furthermore, there were significant (P<0.001) differences among the genotypes MDA tolerance index ($TI_{MDA}$). $TI_{MDA}$ of the genotypes ranged between 1.21 (highly tolerant) to 1.76 (highly sensitive) with a mean of 1.53. Consequently, for $TI_{MDA}$ indicated that not only WT (wild type) but also G8 and G5 were tolerant genotypes both under control and cold stress (Table 1).

Levitt (1980) defined the electrolyte leakage (EL) method as one of the most reliable protocols for evaluating the chilling and freezing tolerance in plants. To measure membrane injury, electrolyte leakage (EL) is an important indicator of membrane damage. Therefore, we evaluated EL analysis in twenty genotypes with one wild type (WT- LA 1777) and three commercial cultivars (CV) under cold stress. G5 and G8 were more cold tolerance (CT) than the other genotypes except for WT and CV under cold stress. Among the genotypes, differences and CTI can be suitable indicator to select the tolerant and sensitive genotype. Similar finding reported Cao et al. (2015) after the cold treatment, exhibited lowest level of EL among the genotypes, these lines found higher cold tolerance than others. In a similar study, Ma et al. (2018), stated that EL cell membrane permeability increased with cold stress in tomato. Xia et al. (2018), they exposed tomato genotypes to cold stress at 4°C for 3 days. They found that in wild types mutant types and also in transgenic tomato genotypes EC increased with cold in all genotypes. Zhao et al. (2009), has found that chilling susceptibility tomato cultivars had higher differences the coefficient between chilling injury and electrolyte leakage under the cold stress. The

Table 1. Means of electrolyte leakage (EL), dry matter yield (DM) and malondialdehyde (MDA) value under the control condition (CC) and cold stress(T), differences (%) and cold tolerance index (TI) for all tomato genotypes.

| Genotypes | Electrolyte leakage (EL) | | | | Dry matter yield (DM) | | | | Malondialdehyde (MDA) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CC | T | Diff.(%) | TI | CC | T | Diff.(%) | TI | CC | T | Diff.(%) | TI |
| G1 | 37±2.3 | 46±1.5 | 24.3 | 1.24 | 3.0±0.5 | 2.6±0.4 | -13.3 | 0.87 | 56±3 | 91±4 | 62.5 | 1.63 |
| G2 | 47±2.1 | 60±2.1 | 27.7 | 1.28 | 2.0±0.6 | 1.5±0.5 | -25.0 | 0.75 | 52±3 | 79±5 | 51.9 | 1.52 |
| G3 | 41±1.0 | 57±2.0 | 39.0 | 1.39 | 2.0±0.4 | 1.6±0.4 | -20.0 | 0.8 | 49±4 | 80±3 | 63.3 | 1.63 |
| G4 | 53±2.1 | 69±2.1 | 30.2 | 1.30 | 3.0±0.7 | 2.3±0.6 | -23.3 | 0.77 | 47±4 | 69±5 | 46.8 | 1.47 |
| G5 | 52±2.5 | 62±2.5 | 19.2 | 1.19 | 3.0±0.6 | 2.5±0.5 | -16.7 | 0.83 | 42±3 | 55±5 | 31.0 | 1.31 |
| G6 | 46±1.5 | 64±1.7 | 39.1 | 1.39 | 3.0±0.6 | 2.3±0.2 | -23.3 | 0.77 | 47±2 | 72±4 | 53.2 | 1.53 |
| G7 | 50±2.0 | 67±2.5 | 34.0 | 1.26 | 4.0±0.6 | 3.1±0.4 | -22.5 | 0.78 | 48±5 | 64±4 | 33.3 | 1.33 |
| G8 | 43±2.5 | 52±2.1 | 20.9 | 1.21 | 4.0±0.8 | 3.5±0.5 | -12.5 | 0.88 | 43±4 | 55±3 | 27.9 | 1.28 |
| G9 | 47±1.5 | 59±2.3 | 25.5 | 1.26 | 2.0±0.6 | 1.4±0.7 | -30.0 | 0.7 | 53±3 | 86±4 | 62.3 | 1.62 |
| G10 | 48±2.1 | 61±2.1 | 27.1 | 1.27 | 3.0±0.6 | 2.2±0.4 | -26.7 | 0.73 | 54±2 | 86±4 | 59.3 | 1.59 |
| G11 | 48±2.1 | 59±1.7 | 22.9 | 1.23 | 2.3±0.8 | 1.8±0.4 | -21.7 | 0.78 | 63±2 | 96±3 | 52.4 | 1.52 |
| G12 | 42±2.3 | 58±2.1 | 38.1 | 1.38 | 3.1±0.7 | 2.3±0.4 | -25.8 | 0.74 | 55±3 | 88±5 | 60.0 | 1.6 |
| G13 | 46±2.1 | 68±2.5 | 47.8 | 1.48 | 3.5±0.6 | 2.3±0.6 | -34.3 | 0.66 | 46±3 | 81±4 | 76.1 | 1.76 |
| G14 | 50±1.7 | 69±2.6 | 38.0 | 1.38 | 2.6±0.7 | 1.9±0.6 | -26.9 | 0.73 | 55±4 | 96±3 | 74.5 | 1.75 |
| G15 | 41±2.1 | 52±1.5 | 26.8 | 1.27 | 2.9±0.6 | 2.4±0.4 | -17.2 | 0.83 | 54±2 | 79±3 | 46.3 | 1.59 |
| G16 | 38±2.5 | 52±2.1 | 36.8 | 1.37 | 3.3±0.8 | 2.5±0.6 | -24.2 | 0.76 | 51±2 | 77±4 | 51.0 | 1.55 |
| G17 | 37±2.0 | 51±2.6 | 37.8 | 1.38 | 4.2±0.5 | 3.1±0.3 | -26.2 | 0.74 | 58±3 | 89±4 | 53.4 | 1.53 |
| G18 | 48±2.5 | 62±2.5 | 29.2 | 1.29 | 2.9±0.8 | 2.1±0.5 | -27.6 | 0.72 | 44±2 | 71±3 | 61.4 | 1.61 |
| G19 | 49±2.3 | 61±1.5 | 24.5 | 1.24 | 3.6±0.5 | 2.7±0.3 | -25.0 | 0.75 | 51±3 | 80±3 | 56.9 | 1.57 |
| G20 | 49±1.5 | 66±2.3 | 34.7 | 1.35 | 2.7±0.8 | 2.0±0.5 | -25.9 | 0.74 | 59±2 | 91±4 | 54.2 | 1.54 |
| WT | 45±1.5 | 59±2.0 | 31.1 | 1.31 | 3.0±0.9 | 2.6±0.6 | -13.3 | 0.87 | 34±2 | 41±2 | 20.6 | 1.21 |
| Com-1 | 43±2.1 | 53±2.5 | 23.3 | 1.26 | 2.0±0.5 | 1.8±0.3 | -10.0 | 0.9 | 45±2 | 69±4 | 53.3 | 1.53 |
| Com-2 | 42±2.5 | 51±2.6 | 21.4 | 1.28 | 4.0±0.6 | 3.5±0.4 | -12.5 | 0.88 | 48±3 | 70±3 | 45.8 | 1.46 |
| Com-3 | 40±2.6 | 53±2.1 | 32.5 | 1.33 | 3.4±0.7 | 2.8±0.6 | -17.6 | 0.82 | 42±2 | 67±3 | 59.5 | 1.6 |
| Means | 45.1 | 58.8 | 30.5 | 1.31 | 3.0 | 2.4 | -21.7 | 0.78 | 49.8 | 76.3 | 52.4 | 1.5 |
| Significance | ***[a] | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |

[a] Levels of significance are represented by at ***$P$<0.001.

results of this study correspond to Caffagni et al. (2014), examined EL analysis of fourteen tomato genotypes at different temperatures (5, 3, and 1°C) and at five time points (2, 4, 8, 24, and 72 h). They reported that differences in cold tolerance between the accessions were the most apparent when the plants were exposed to 1°C for 24 h; the EL values ranged between 26.4 and 71.0 %.

Dry matter yields are one of the most important parameters in response to cold stress. Percentage of growth ($TI_{DW}$) both under nonstress (TIc) and stress condition ($TI_S$) is reliable indicator of stress tolerance (Foolad and Lin, 2001). When the cold tolerance index of the dry matter yields ($TI_{DM}$) of the genotypes were examined, it was determined that the G1 and G8 could be tolerant to cold stress. Dry matter yields of genotypes have decreased as a result of cold stress. Foolad and Lin (2000), reached similar conclusion when they evaluated the tomato accessions for DM and TI under control conditions and cold stress. Furthermore, they also determined that there was a positive correlation ($r$=0.68, $P$ < 0.01) between under cold stress and vegetative growth tolerance index. Similarly, Foolad and Lin (2001) evaluated the genetic control of cold tolerance (CT) in tomato *L. esculentum* breeding lines. They determined that there was a significant correlation between DM under cold stress (DMs) and TI among the lines. Similarly, Foolad and Lin (2001) measured plant vigour via germination tolerance index ($TI_G$) and vegetative growth index ($TI_{VG}$) under cold stress. When they compared both of them, they found that $TI_G$ and $TI_{VG}$ were good indicators of relative CT, but they may not be used alone good selection for cold tolerance breeding. Gökmen (2006) points out that different low temperature and duration applications can show significant differences in dry matter production in some genotypes depending on the time spent at low temperature and the degree of low temperature in tomato genotypes. Liu et al. (2018) reported that in their study on photoscent rates at different irrigation levels under low temperature conditions, dry matter yields decreased with cold application. The findings obtained in this study were consistent with these results. Considering the changing environmental

factors in dry matter yields, it shows that the genotypes with the highest dry matter yield can be low temperature tolerant.

Lipid peroxidation (MDA) is evaluated in studies of plant mechanisms and aceppted as an indicator in various stresses like as cold stress. Thus we evaluated with MDA content of the genotypes at vegetative growth stage. Compared with MDA content of tomato genotypes, it was found that the differences of MDA content increased in all genotypes because of cold treatment. But WT and G8 had lower difference rates of MDA content and smaller rising cold TI than the other genotypes. Duan et al. (2012), studied the contribution of thylakoid ascorbate peroxidase (tAPX) to protect the plant under cold stress in wild type (WT) and transgenic plants. They found that the lower level of MDA was measured in transgenic plants compared with WT plants after 12h cold treatment. And they also suggested that MDA and EL are good markers of the oxidative stress suffered by plants. Malekzadeh et al. (2014) used different concentration of Gamma-aminobutyric acid (GABA) in tomato seedling under cold stress. They found that under cold stress there was an increase in MDA content in tomato seedling. They suggested that applying GABA can protect tomato seedlings against cold stress. Similarly, Xia et al. (2017) in their study, They found that MDA content was lower transgenic (DWF:OX2) genotypes than in wild-type as well as mutant type. Similarly, Li et al. (2015) compared grafted and ungrafted plants under cold stress. They detected that MDA content was increased in both ungrafted and grafted plants in the first 24 h after treatment. Liu et al. (2018) reported that cold applications increased lipid peroxidation rates in their study on photosensitive rates at different irrigation levels under low temperature conditions.

Findings obtained were consistent with these results. It should be taken into account that genotypes with the least change in MDA ratio at low temperatures may be tolerant, but fluctuate according to genotypes.

### 3.2. Reproductive stage

#### *3.2.1. Pollen viability*

Due to cold stress, all genotypes pollen viability rates decreased. Statistically significant differences ($p < 0.01$) were found among group of within the tested genotypes. Pollen viability of the G8 and WT were higher than the others (Table 2).

Table 2. Means of pollen viability (PV) and pollen germination (PG) value under the control condition (CC) and cold stress (T), differences (%) and tolerance index (TI) for all tomato genotypes.

| Genotypes | Pollen viability (PV) | | | | Pollen germination (PG) | | | |
|---|---|---|---|---|---|---|---|---|
| | CC | T | Diff. (%) | TI | CC | T | Diff. (%) | TI |
| G1 | 91±5 | 79±7 | -13.2 | 87.0 | 86±3 | 71±4 | -17.4 | 82.7 |
| G2 | 86±3 | 67±7 | -22.1 | 78.0 | 90±3 | 70±8 | -22.2 | 78.3 |
| G3 | 93±4 | 77±6 | -17.2 | 83.0 | 83±4 | 62±5 | -25.3 | 75.3 |
| G4 | 89±4 | 70±4 | -21.3 | 79.0 | 91±3 | 73±6 | -19.8 | 80.3 |
| G5 | 91±6 | 69±8 | -24.2 | 76.0 | 91±4 | 66±6 | -27.5 | 76.3 |
| G6 | 86±5 | 76±6 | -11.6 | 88.0 | 89±2 | 68±6 | -23.6 | 76.3 |
| G7 | 91±4 | 70±7 | -23.1 | 77.0 | 89±3 | 67±8 | -24.7 | 75.3 |
| G8 | 89±6 | 80±5 | -10.1 | 90.0 | 93±4 | 75±6 | -19.4 | 81.3 |
| G9 | 94±4 | 75±6 | -20.2 | 80.0 | 89±4 | 69±5 | -22.5 | 78.3 |
| G10 | 92±4 | 73±7 | -20.7 | 79.0 | 92±2 | 76±5 | -17.4 | 83.0 |
| G11 | 90±4 | 75±5 | -16.7 | 83.0 | 95±3 | 78±7 | -17.9 | 82.3 |
| G12 | 93±2 | 74±6 | -20.4 | 80.0 | 89±4 | 69±6 | -22.5 | 78.3 |
| G13 | 92±4 | 70±5 | -23.9 | 76.0 | 91±5 | 69±5 | -24.2 | 76.3 |
| G14 | 95±6 | 77±7 | -18.9 | 81.0 | 88±4 | 69±6 | -21.6 | 78.3 |
| G15 | 85±5 | 61±7 | -28.2 | 72.0 | 90±3 | 65±6 | -27.8 | 72.0 |
| G16 | 89±3 | 69±8 | -22.5 | 78.0 | 93±3 | 66±5 | -29.0 | 71.0 |
| G17 | 90±3 | 65±5 | -27.8 | 72.0 | 90±4 | 71±5 | -21.1 | 79.3 |
| G18 | 91±3 | 75±4 | -17.6 | 82.0 | 93±4 | 70±6 | -24.7 | 75.3 |
| G19 | 89±4 | 63±5 | -29.2 | 71.0 | 92±3 | 65±6 | -29.3 | 71.3 |
| G20 | 88±5 | 65±7 | -26.1 | 74.0 | 88±4 | 67±7 | -23.9 | 76.0 |
| WT | 94±2 | 86±4 | -8.5 | 91.5 | 95±2 | 84±4 | -11.6 | 88.3 |
| Com-1 | 94±4 | 83±3 | -11.7 | 88.3 | 95±3 | 82±4 | -13.7 | 85.7 |
| Com-2 | 92±6 | 82±3 | -10.9 | 89.1 | 96±2 | 81±4 | -15.6 | 83.7 |
| Com-3 | 93±3 | 76±5 | -18.3 | 82 | 92±3 | 79±5 | -14.1 | 86.3 |
| Means | 90.7 | 73.2 | -19.4 | 80.7 | 90.8 | 71.3 | -21.5 | 78.8 |
| Significance | **[a] | ** | ** | ** | *** | *** | *** | *** |

[a] Level of significance are represented by at **$P<0.01$ and ***$P<0.001$.

*3.2.2. Pollen germination*

In all genotypes, pollen germination rates decreased during the cold stress. There were significant differences (P<0.001) among group of the genotypes, and so cold tolerance exists within the tested genotypes. Pollen germination of the WT and CV was higher than the others and they evaluated in the same group. On the other hand, G1, G11, G10 and G8 found much higher than the others (Table 2).

The pollen viability is the most important criteria at reproductive stage under the cold stress. When the plants were exposed to low temperature, pollen damage happened. Therefore it caused poor pollen viability. Our results indicated that there was decrease in polen viability under cold stress. Among the genoypes, two genotypes (G8 and G1) were classified high pollen viability under cold stress condition. Likewise, higher pollen viability rate were also detected in WT and commercial varieties (Com-1 and Com-2). Similar results were obtained by Picken (1984) who found that poor pollen viability was recorded at low temperatures. Domínguez et al. (2005) who investigated five populations pollen performance at low temperature. They found that there were no differences in pollen viability among the populations except for NNNC that showed a higher mean percentage of viability. Similar results were obtained by Maisonneuve et al. (1986) and Zamir and Gadish (1987).

Pollen germination is severely reduced at temperatures below 10°C. To assess pollen germination rate, whole genotypes screened under cold stress at reproductive stage. As expected, the genotypes showed lower percentage of pollen viability and percentage of pollen germination under cold stress. In our study, wild type (LA1227) and all the commercial varieties exhibited higher means of pollen germination than the other genotypes. Nevertheless, genotypes No. G11, G10, G8 and G1 showed better response among the genotypes except for WT and commercial varieties. Moreover, these genotypes had higher cold tolerance index than others. It was observed that pollen germination of all genotypes was affected by low temperature applications. Keleş (2006) also reported that the germination percentage may be effective in distinguishing genotypes from each other in pepper. The data we obtained in tomato were consistent with these results. It is thought that the germination percentage may be effective in distinguishing genotypes from each other. Some authors reported that low temperatures affected pollen viability and germination of sensitive genotypes compared to tolerant genotypes. Zamir and Gadish (1987), Mulcahy et al. (1996) and Domínguez et al. (2005) conducted their experiment in segregating populations by using pollen selection. Researchers also stated that pollen selection may determine both vegetative and reproductive stage to tolerance for cold stress.

As a result of all these evaluations, it indicates that genotypes determined as tolerants can not only create more dry matter under cold stress, but also show high fertilization and fruit set with a high rate of live pollen and germination.

## 4. Conclusion

As a result, cold stress tolerance of twenty genotypes was evaluated with different physiological parameters during vegetative growth and reproduction. These parameters (EL, MDA, DM, PV and PG) have been succesfully used to screen cold stress in tomatoes. Furthermore, results of all these parameters indicated that when breeding for improved tolerant genotypes, both stages are necessary for selecting cold tolerant genotypes in tomato. Besides, cold tolerance index could be effectively used for evaluating cold tolerance in tomatoes. G1, G5 and G8 genotypes could be tolerant to cold stress as shown by physiological parameters indicators EL, MDA, DM at vegetative groth stage and also PV and PG at reproductive stage among the genotypes. These genotypes will be valuable for breeding programs as sources of cold stress tolerance.

## Acknowledgements

Funding support was provided by Republic of Türkiye Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (Project no: BBAD/16/A09/P01/01).

### References

Atayee, A.R., & Noori, M.S. (2020). Alleviation of cold stress in vegetable crops. *Journal of Scientific Agriculture*, 4:38-44.

Barrero-Gil, J., Huertas, R., Rambla, J.L., Granell, A., & Salinas, J. (2016). Tomato plants increase their tolerance to low temperature in a chilling acclimation process entailing comprehensive transcriptional and metabolic adjustments. *Plant, Cell & Environment*, 39:2303-2318.

Boyacı, H.F., Oğuz A., Ünlü, M., Eren, A., Topçu, V., Erkal, S. (2009). Relationship between some parameters of vegetative and generative developments of parthenocarp and non-parthenocarp eggplants (*Solanum melongena* L.). *Derim,* 26:28-39.

Cao, X., Jiang, F., Wang, X., Zang, Y., Wu, Z. (2015). Comprehensive evaluation and screening for chilling-tolerance in tomato lines at the seedling stage. *Euphytica*, 205:569-584.

Caffagni, A., Pecchioni, N., Francia, E., Pagani, D., Milc, J. (2014). Candidate gene expression profiling in two contrasting tomato cultivars under chilling stress. *Biologia Plantarum*, 58:283-295.

Domínguez, E., Cuartero, J., & Fernández-Muñoz, R. (2005). Breeding tomato for pollen tolerance to low temperatures by gametophytic selection. *Euphytica*, 142:253-263.

Duan, M., Feng, H.L., Wang, L.Y., Li, D., & Meng, Q.W. (2012). Overexpression of thylakoidal ascorbate

peroxidase shows enhanced resistance to cold stressin tomato. *Journal of Plant Physiology*, 169:867-877.

Elizondo, R., & Oyanedel, E. (2010). Field testing of tomato chilling tolerance under varying light and temperature conditions. *Chilean Journal of Agricultural Research*, 70:552-558.

FAO, (2021). Food and Agriculture Organization (FAO). FAO Statistical Databases (FAOSTAT): Crops and livestock products. Available at: http://www.fao.org/faostat/en/#data/QC (Accessed: 02 July 2021).

Foolad, M.R., & Lin, G.Y. (2000). Relationship between cold tolerance during seed germination and vegetative growth in tomato: germplasm evaluation. *Journal of the American Society for Horticultural Science*, 125:679-683.

Foolad, M.R., Lin, G.Y. (2001). Genetic analysis of cold tolerance during vegetative growth in tomato, Lycopersicon esculentum Mill. *Euphytica*, 122:105-111.

Funatsuki, H., Kawaguchi, K., Matsuba, S., Sato, Y., & Ishimoto, M. (2005). Mapping of QTL associated with chilling tolerance during reproductive growth in soybean. *Theoretical and Applied Genetics*, 111:851-861.

Gökmen, Ö.Ö. (2006). Investigation of Low Temparature Stress in tomato in Respect to Antioxidative Mechanisms. MSc Thesis, Çukurova University, Adana (in Turkish).

Keleş, D., (2007). Characterization of Different Pepper Genotypes and Low Temperature Tolerance. PhD Thesis, Çukurova University, Adana (in Turkish).

Levitt, J. (1980). Responses of Plants to Environmental Stress, Volume 1: Chilling, Freezing, and High Temperature Stresses. Academic Press.

Li, Y., Tian, X., Wei, M., Shi, Q., Yang, F., & Wang, X. (2015). Mechanisms of tolerance differences in cucumber seedlings grafted on rootstocks with different tolerance to low temperature and weak light stresses. *Turkish Journal of Botany*, 39:606-614.

Liu, G., Du, Q., Jiao, X., & Li, J. (2018). Irrigation at the level of evapotranspiration aids growth recovery and photosynthesis rate in tomato grown under chilling stress. *Acta Physiologiae Plantarum*, 40:1-11.

Lutts, S., Kinnet, J.M., & Bouharmont, J., (1996). NaCl induced senescence in leaves of rice cultivars differing in salinity resistance. *Annual Botany,* 78:389-398.

Ma, X., Chen, C., Yang, M., Dong, X., Lv, W., & Meng, Q. (2018). Cold-regulated protein (SlCOR413IM1) confers cold stresstolerance in tomato plants. *Plant Physiology and Biochemistry*, 124:29-39.

Maisonneuve, B., Hogenboom, N.G., & Den Nijs, A.P.M. (1986). Pollen selection in breeding tomato (*Lycopersicon esculentum* Mill.) for adaptation to low temperature. *Euphytica*, 35:983-992.

Malekzadeh, P., Khara, J., & Heydari, R. (2014). Alleviating effects of exogenous Gamma-aminobutiric acid on tomato seedling under chilling stress. *Physiology and Molecular Biology of Plants*, 20:133-137.

Mulcahy, D.L., Sari-Gorla, M., & Mulcahy, G.B. (1996). Pollen selection-past, present and future. *Sexual Plant Reproduction*, 9:353-356.

Ражаметов, Ш.Н., Янг, Ы., Чо, М.Ч., Чэ, С.Ё., & Жеонг, Х.Б. (2020). Screening of pepper (*Capsicum L*.) seedlings tolerance to low temperature. *Аграрный научный журнал*, 11:78-82.

Peel, M.C., Finlayson, B.L., & McMahon, T.A. (2007). Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences*, 11:1633-1644.

Picken, A.J.F. (1984). A review of pollination and fruit set in the tomato (*Lycopersicon esculentum* Mill.). *Journal of Horticultural Science*, 59: -13.

Ronga, D., Rizza, F., Badeck, F.W., Milc, J., Laviano, L., Montevecchi, G., Pecchioni, N., & Francia, E. (2018). Physiological responses to chilling in cultivars of processing tomato released and cultivated over the past decades in Southern Europe. *Scientia Horticulturae*, 231:118-125.

Sayyari, M., (2012). Improving chilling resistance of cucumber seedlings by salicylic acid. *American-Eurasian Journal of Agricultural & Environmental Sciences,* 12: 04-209.

Xia, X.J., Fang, P.P., Guo, X., Qian, X.J., Zhou, J., Shi, K., Yu, J.Q. (2018). Brassinosteroid-mediated apoplastic $H_2O_2$-glutaredoxin 12/14 cascade regulates antioxidant capacity in response to chilling in tomato. *Plant, Cell & Environment*, 41:1052-1064.

Zamir, D., & Gadish, I. (1987). Pollen selection for low temperature adaptation in tomato. *Theoretical and Applied Genetics*, 74:545-548.

Zhao, D.Y., Shen, L., Fan, B., Liu, K.L., Yu, M.M., Zheng, Y., Ding, Y., Sheng, J.P. (2009). Physiological and genetic properties of tomato fruits from 2 cultivars differing in chilling tolerance at cold storage. *Journal of Food Science*, 74:C348-C352.