Scientia Horticulturae 232 (2018) 1–12

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

# Tolerance evaluation and clustering of fourteen tomato cultivars grown under mild and severe drought conditions

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ARTICLE INFO

*Keywords:*
Drought stress
Growth and physiological parameters
Hierarchical clustering analysis (HCA)
Principle component analysis (PCA)
Tomato

ABSTRACT

The growth and physiological parameters of fourteen tomato cultivars were measured during both mild and severe drought. The tomato cultivars showed decrease in growth traits, relative water content and pigments content under both drought treatments compared with the control. By contrast, proline accumulation, malondialdehyde and electrolyte leakage increased in the cultivars. The activity of superoxide dismutase was more correlated to drought stress than those of ascorbate peroxidase or catalase. The combined approach of principal component analysis, hierarchical clustering analysis and calculating mean tolerance value showed relative differences in drought tolerance among the cultivars. The studied cultivars were clustered into four groups. Four cultivars were placed in cluster I as the drought-tolerant group. Moderately tolerant cultivars were grouped into Cluster II. The clusters III and IV represented the sensitive and highly sensitive cultivars, respectively. Early orbana, Roma and Cal-j cultivars were indicated as the highly sensitive tomato cultivars. It was concluded that such an approach could be used as a useful tool to screen the other abiotic or even biotic stress tolerance in tomato.

## 1. Introduction

Tomato (*Solanum lycopersicum* L.) belongs to Solanaceae family, originated from Peru-Ecuador area, where its indigenous name was Tomati (Jenkins, 1948). It is an important vegetable crop which is extensively used as fresh or processed forms (Khan et al., 2015). Tomato is rich in vitamins A, B, C, and antioxidants as well as other bioactive components which has been shown to be preventive of some diseases, such as coronary heart disease (Rao, 2002), blood pressure (Engelhard et al., 2006) and several types of cancer, such as breast, prostate, lung and stomach cancers (Choi et al., 2014; Perveen et al., 2015). Tomato is a perennial species and could grow in a variety of climatic conditions; however it often prefers temperate zone. From a commercial viewpoint, the cultivation of the species was developed extensively to arid and semi-arid areas where water scarcity is the most limiting factor for the plant productivity (Fischer and Turner, 1978).

Plant growth and productivity are complex processes which depend on interactions among physiological, genetic and environmental factors. Plants, when subjected to environmental stresses, undergo alterations in their growth due to changes in cellular processes, including gene expression, protein activation and subsequently metabolite profiling (Shao et al., 2008). Drought stress is a major environmental constraint that affects many aspects of plant physiology, morphology and biochemistry (Srivastava and Srivastava, 2014). Drought currently causes major damages to plants mainly resulting from the alteration in water relations (Silva et al., 2009), and the formation of reactive oxygen species (ROS) in plants (Zgallaï et al., 2006; Koffler et al., 2014). Drought could also be detrimental by the induction of lipid peroxidation and protein degradation in plant tissues (Sairam and Saxena, 2000). Prolonged water deficiency could potentially hamper the plant growth and photosynthesis which eventually leads to a higher loss in biomass and yield (Shao et al., 2008).

Critical alterations have been demonstrated to be induced in antioxidant substrates and enzymes, ABA biosynthesis, stress responsive genes expression and stress metabolites during plant exposure to abiotic stresses, like drought (Shao et al., 2008; Koffler et al., 2014). Such

*Abbreviations:* ANOVA, analysis of variance; AsA, ascorbic acid; APX, ascorbate peroxidase; Car, carotenoid; CAT, catalase; Chl, chlorophyll; CRD, completely randomized design; DW, dry weight; EC, electrical conductivity; EL, electrolyte leakage; FW, fresh weight; HCA, hierarchical clustering analysis; MDA, malondialdehyde; NaOCl, sodium hypochlorite; PCA, principle component analysis; ROS, reactive oxygen species; RWC, relative water content; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloroacetic acid; TW, turgid weight

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https://doi.org/10.1016/j.scienta.2017.12.041
Received 22 August 2017; Received in revised form 16 December 2017; Accepted 20 December 2017

extensive changes might eventually lead to adaptive responses and acclimatization strategies. The osmotic adjustment is an adaptive mechanism involved in drought tolerance. In this manner, the biosynthesis of compatible solutes like polyols and proline permit the maintenance of turgor under water deficit (Bajji et al., 2001; Liu et al., 2011), which ensure the survival or continued growth of plants under moderate and severe drought (Sánchez-Rodríguez et al., 2010; Khan et al., 2015). Another strategy involved in plant adaptive response to drought is antioxidant defense system. the effective involvement of the adaptive systems, including antioxidant substrates (e.g. ascorbate, α-tocopherol, carotenoids), and antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) have been reported in many tolerant plant species including several tomato's cultivars (Zgallaï et al., 2006; Sánchez-Rodríguez et al., 2010; Choi et al., 2014). The adaptive responses to drought stress are demonstrated through alterations in plants phenotype and morphology due to changes in gene expression patterns. A large diversity in drought adaptive responses has been reported among different varieties of crops, indicating that some genotypes tolerate drought better than others. Therefore, from an economic viewpoint, effective screening techniques should be utilized to select the most tolerant genotypes, which are able to provide unwavering yield under limited water resources. Additionally, the study of such adaptive variations among the genotypes could advance our understanding of the plants response to the environmental stresses at molecular and physiological levels.

The present work investigated the degree of tolerance among different cultivars of *Solanum lycopersicum* L. under drought stress, and identifyied the most tolerant varieties. Physiological changes in pigments content, growth parameters, relative water content, membrane leakage, lipid peroxidation, proline content, and the activity of antioxidant enzymes in fourteen tomato cultivars were analyzed during moderate and severe drought stress conditions. The variations in physiological responses among the genotypes during drought stress condition, and their possible mechanisms were discussed.

## 2. Materials and methods

### 2.1. Plant materials and growth conditions

The seeds of fourteen tomato cultivars were obtained from different sources (Table 1). The seeds were surface sterilized with 2.5% (v/v) sodium hypochlorite (NaOCl) solution for 15 min, rinsed 3–5 times with sterile distilled water to remove NaOCl, and then cultivated in cellular seed trays containing peat moss (Klasmann-Deilmann GmbH, Geeste, Germany). the seed trays were transferred to a controlled growth chamber at 70% relative humidity under a photon flux density of 500–600 μmol $m^{-2} s^{-1}$ with a 14:10 h light/dark photoperiod. The day/night temperatures were adjusted at 26/18 °C. The five-week old seedlings were used in the experiments.

**Table 1**
Tomato cultivars used in this study.

| Cultivars | Symbol | Source | Supplier reference |
|---|---|---|---|
| Has2274 | HS | Turkey | Pakanbazr[A] |
| Khorram | KO | Iran | SPII[C] |
| Caribou | CR | Iran | ICRASN[d] |
| 111-Falat | F1 | Iran | Pakanbazr[A] |
| CH-Falate | FC | Iran | Pakanbazr[A] |
| Early Orbana | EO | Turkey | Pakanbazr[A] |
| Y-Falat | YF | Iran | Pakanbazr[A] |
| Rio Grande | RG | USA | pishgamansb[B] |
| Korall | KR | Hungary | pishgamansb[B] |
| BetterBoy | BB | USA | Pakanbazr[A] |
| Roma | RM | Italy | ICRASN[d] |
| Cal j | CJ | Hungary | Pakanbazr[A] |
| Punta Banda | PB | USA | Native Seeds/SEARCH, USA |
| Quine | QI | Iran | ICRASN[d] |

A and B: a local seed supply company, Iran.
C: Seed and Plant Improvement Institute, Karaj, Iran.
D: Center for Research of Agricultural Science and Natural Resources, Isfahan, Iran.

### 2.2. Experimental design

The mild and severe drought stress treatments started with withholding the water from five-week old plants (35 days after germination) for 4 and 8 days, respectively. The plant materials were harvested at 3 timeframes, including 0 (T0), 4 (T1) and 8 (T2) days during water stress. The daily irrigated plants (C1 and C2) were harvested at the same times as the control groups for T1 and T2, respectively. There were 20 replicates per cultivar in individual pots (10 cm in diameter), in a completely randomized design (CRD).

### 2.3. Growth parameters

Shoot height and root length were measured using a graduated ruler. Fresh shoot and root weights were determined immediately after excision. The excised shoots and roots were dried in an oven at 65 °C until constant weight (about 72 h) to record the dry weights (Amjad et al., 2014).

### 2.4. Pigments

Pigments including chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total carotenoid (Cx + c) were extracted from the leaf samples, and calculated by the method described by Lichtenthaler and Welburn (1994). Fresh leaf tissue (0.2 g) was homogenized in 5 ml of 80% acetone using a mortar and pestle. The homogenate was centrifuged at 13,000 rpm for 20 min. Ten ml of 80% acetone was added to the supernatant, and the absorbance was read in 663.2 nm, 646.8 nm and 470 nm. The pigments content was calculated using the following equations:

$$Chl\ a = 12.21A663 - 2.81A646$$

$$Chl\ b = 20.13A646 - 5.03A663$$

$$C\_(x + c) = (1000A470 - 3.27Car - 104Chl\ b)/229$$

The pigments content was expressed in mg/g dry weight. The Tolerance Index (TI) was calculated according to the equation derived by Köhl and Lösch (2004) as follows:

$$TI\ (\%) = (Total\ chlorophylls\ of\ stressed\ plants)/(Total\ chlorophylls\ of\ control\ plants) \times 100$$ (Di Cori et al., 2013).

### 2.5. Relative water content

Leaves with the same developmental stage were used for measuring the Relative Water Content (RWC). The fresh weight of leaf samples were weighed immediately after excision, then rinsed in water for 24 h, and the turgid leaves were weighted again to record the turgid weight (TW). The dry weight (DW) was recorded after drying the water-saturated leaves at 70 °C until the constant weight. The relative water content was calculated on a newly-expanded leaf detached from three plants per treatment using the following formula (Ings et al., 2013):

$$RWC\ (\%) = (FW - DW)/(TW - DW) \times 100$$

### 2.6. Proline determination

The proline content of leaves was determined according to the method of Bates et al. (1973) using L-proline as the standard. About 0.5 g leaf sample was homogenized in 10 ml of 3% aqueous