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Changes in some physiological and osmotic parameters of several pistachio genotypes under drought stress

Farooq Fahimi Khoyerdi, Mohammad Hossein Shamshiri, Ahmad Estaji*

Department of Horticultural Sciences, Faculty of Agriculture, Vali-E-Asr University of Rafsanjan, Iran

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ABSTRACT

Drought is one of the main adverse environmental factors, obviously has an impact on plant growth and development. Many adaptive strategies have been developed in plants for dealing with water stress, among which osmoregulation is one of the most important factors of drought-tolerance in plants. The objective of this study was to evaluate the effect of drought stress on the physiological and osmotic parameters of several pistachio genotypes. A completely randomized design was applied with a factorial arrangement of two factors: drought (0, −0.5, −1 and −2 MPa) and genotypes (Abareqi, Badami–Rize–Zarand, Qazvini, and Sarakhs) at four replications. The results indicated that drought stress reduced shoot and root dry weight (RDW), leaf area, leaf relative water content (RWC), total chlorophyll (TChl), carotenoids, total soluble proteins (TSP) and increased proline, glycine betaine, total soluble carbohydrate (TSC), sucrose, phenol, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content in roots and leaves of four genotypes. The genotypes have differently responded to water stress. The highest and lowest of above parameters in the roots and leaves of pistachio seedlings under drought condition were found in Badami–Rize–Zarand and Abareqi genotypes, respectively. According to the results, Badami–Rize–Zarand was presented more resistance to drought stress as accumulated more osmolytes and produced better water relations and dry weights under drought stress.

1. Introduction

One critical abiotic stress that affects plant physiology and development is water deficiency (Gueta-Dahan et al., 1997). A continuous shortfall and irregular distribution of precipitation coupled with higher evapotranspiration demand leads to agricultural drought (Mishra and Cherkauer, 2010). Plant growth and development, due to substantial reductions in crop growth rate and biomass accumulation, severely affected by drought. The main subsequences of water stress in crop plants are reduced rate of cell division and expansion, leaf size, stem elongation, root proliferation, disturbed stomatal oscillations, plant water and nutrient uptake and water use efficiency (Li et al., 2009). The mechanisms (morphological, physiological and biochemical) of drought tolerance in plants can be divided into three categories; (i) escape; (ii) avoiding; and (iii) drought tolerance, which inhibit or mitigate the harmful effects of drought stress.

To evaluate plant water status, the leaf water potential (Ψ_w), RWC and chlorophyll content are necessary parameters generally used to study plant physiological responses to drought stress (Silva et al., 2010). Silva et al., 2009 Silva et al. (2009) reported that these parameters decrease in most plants under water deficit.

Accumulation of low molecular weight organic solutes compounds, such as osmoregulators is one of the mechanism that many plant species used to reduce the negative effects of water stress. This process is recognized as osmoregulation and considered as an important tolerance mechanism, which lets the retention of cellular turgor and favors the absorption of water (Chaves et al., 2003). It is well known that the biosynthesis and accumulation of nontoxic molecules of low-molecular weight in the vacuole and cytosol, such as inorganic ions, soluble sugars, amino acids, proline, glycine–betaine and others compounds seem to contribute to membrane stability (Javadi et al., 2008). The accumulation of osmoregulators in plants has been widely reported as a response to drought (Farooq et al., 2009; Marcinska et al., 2013) and salinity (Shamshiri and Fattahi, 2014).

Reduced water activity in the plant tissues under drought stress induces reactive oxygen species (ROS) including hydrogen per-

Abbreviations: RWC, relative water content; TChl, total chlorophyll; TSP, total soluble proteins; TSC, total soluble carbohydrate; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; RDW, root dry weight; SDW, shoot dry weight.

* Corresponding author.
E-mail address: estaji1366@gmail.com (A. Estaji).

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oxide, superoxide radicals, singlet oxygen and hydroxyl radicals accumulation by altering cells metabolism, which leads to oxidative stress and structural damages (Sircelj et al., 2007). H_2O_2 is the most stable radical in stress conditions, which it is able to quickly penetrate through the cell membrane (Del-Rio et al., 1992). H_2O_2 and superoxide radicals can also form hydroxyl radicals that can damage proteins, lipids and DNA and finally accelerate senescence of leaves (Reddy et al., 2004; Upadhyaya et al., 2007). It has recently been demonstrated that H_2O_2 is an integral component of cell signaling cascades (Vranova et al., 2002).

Rafsanjan is one of the major pistachio production and exportation areas in Iran and all around the world where water deficit and inefficient irrigation methods are the main limiting factors of pistachio production (Bagheri et al., 2011a). As pistachio cultivation requires the use of rootstock because grafting is the only form of vegetative propagation, use of drought-resistant rootstocks could be the main strategy for sustainable production under this condition (Blum, 1996). Gijon et al. (2010) reported that rootstock had significant effect on dry weight of leaf in different pistachio rootstock under drought condition. Furthermore, rootstock can affect nutrient uptake (Bagheri et al., 2012), transpiration rate, internal CO2 concentration, leaf temperature, RWC (Fotouhi Ghazvini et al., 2007), growth indices and proline content (Bagheri et al., 2011b) under water deficit status. It has been reported that similar to pistachio, rootstock type in apple (Alizadeh et al., 2011), almond (Karimi et al., 2013), and grape (Jogaiah et al., 2014) has also a significant influence on drought resistance. Although several workers have investigated the effects of drought stress on growth and chemical composition of pistachios, most of them have focused on three rootstocks of Qazvini, Badami–Rize–Zarand, and Sarakhs (Avanzato and Vassallo, 2008) and information on relative drought tolerance of the other local pistachio rootstocks is still scarce. However, as drought stress is approaching to critical point in Rafsanjan region, screening for tolerance to drought is vitally important to exploit new drought-tolerant rootstocks.

In this study, we used Abareqi as a local, less-known pistachio rootstocks and also Qazvini, Sarakhs and Badami–Rize–Zarand as the most populated ones to evaluate and compare them based on physiological and biochemical parameters.

2. Material and methods

2.1. Plant material and growth conditions

Four pistachio genotypes were used: Abareqi, Badami–Rize–Zarand, Qazvini, Sarakhs, (*Pistacia vera* L.). The seeds of these genotypes were pre-germinated in 9 cm glass petri dishes with two layers of Whatman No. 1 filter paper. After a week, five germinated seeds were selected and sown in a plastic pot (30 cm upper diameter, 20 cm lower diameter, and 25 cm high) containing perlite as a replication. The number of seedlings per pot was reduced to 3 within 21 days of germination. Seedlings were grown under greenhouse conditions with relative humidity of 50 ± 10%, temperature of 25/15 °C (day/night) and a 16/8 h photoperiod. During this period, seedlings were irrigated by a Hoagland solution containing: 5 ml/L KNO_3, 5 ml/L $Ca(NO_3)_2$, 2 ml/L $MgSO_4$, 1 ml/L KH_2PO_4, 1 g/L $MnCl_2$, 1 g/L $ZnSO_4$, 1 g/L $CuSO_4$, 1 g/L Na_2MoO_4, 2 g/L Fe-EDDHA, and 1 g/L H_3BO_3. The nutrient solution (pH 6.5 ± 0.1) was renewed every 3 days and the substrate was partially rinsed with distilled water to avoid nutrient accumulation. The water-stress treatments were started 70 days after transplanting and maintained for 90 days. The PEG treatment (drought stress) was applied along with Hoagland solution at four levels (0, −0.5, −1 and −2 MPa). The method of Michel and Kaufmann (1973) was used to determine levels of drought stress in nutrient solutions. Sampling for all parameters was done at the end of the dry period.

2.2. Shoot and root dry weight and leaf area

At the end of experiment, plants were pulled out from the soil and divided to shoot and root and then were oven dried for 72 h at 70 °C to determine dry weight. The leaf area meter (CI-202, USA) was used to measure plant leaf area before drying.

2.3. Determination of leaf relative water content

Leaf discs were obtained from expanded leaves of each pot in the morning. The leaf discs were weighed immediately to obtain the fresh weight (FW), and submerged in distilled water for 4 h at 4 °C in dark condition and then weighed to prepare turgor weight (TW). The leaves were dried in a forced-air oven at 70 °C for 24 h, and the dry weight (DW) was recorded. The RWC of samples was calculated using the following equation (Bastam et al., 2012):

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

2.4. Total chlorophyll and carotenoids

TChl and carotenoid contents were determined according to Lichtenthaler and Chlorophylls (1987). At the end of the experiment, 3 fully extended leaves from each pot (one leaf from each plant) were collected and wrapped in aluminum foil to avoid degradation of pigments by light. The extract was prepared from fresh leaves (1 g) by grinding in a cold mortar and pestle together with 10 ml of 80% aqueous acetone. After filtering, absorbance of centrifuged extracts was measured at 470, 646 and 663 nm using a spectrophotometer (U-2000, Hitachi Instruments, Tokyo, Japan).

2.5. Osmotic regulating compounds

2.5.1. Proline and total soluble carbohydrate content

To determine the free-proline concentration, leaves were homogenized in 5 ml of ethanol at 95%. The insoluble fraction of the extract was washed with 5 ml of ethanol at 70%. The extract was centrifuged at 3500 rpm for 10 min and the supernatant was preserved at 4 °C for the proline determination. An aliquot of this supernatant was taken and, after adding reactive ninhydrin acid reagent (ninhydrin, phosphoric acid 6 M, glacial acetic acid and glacial acetic acid at 99%), was placed in a bath at 100 °C. After 45 min, the tubes were cooled and absorbance was determined at 520 nm was determined. Proline concentration was calculated with a standard curve and expressed as $\mu g\, g^{-1}$ fresh mass (Paquin and Lechasseur, 1979).

In order to determine TSC, 0.1 ml of the extract prepared in ethanol (alcoholic extracts made for proline) was mixed with antron (200 mg antron plus 100 ml of 72% sulphuric acid). The tubes were put in a bath at 100 °C for 10 min. Then they were cooled and absorbance was recorded at 625 nm. The concentration of soluble sugars was calculated using the standard curve and the results were expressed as $mg\, g^{-1}$ fresh mass.

2.5.2. Sucrose content

Van handel's (1968) method was used to measure sucrose content. Fresh leaf tissue (0.5 g) was ground with 5 ml of 80% ethanol and then extracted three times with 5 ml of 70% ethanol. After centrifugation (3500 × *g* for 10 min), 0.2 ml of the supernatants were mixed with 0.1 ml 30% KOH and heated at 100 °C for 10 min. after cooling (in room temperature) 3 ml antron (150 mg antron and 100 ml 70% sulfuric acid) was added. After 10 min, the samples were