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The physiological responses of various pomegranate cultivars to drought stress and recovery in order to screen for drought tolerance

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ABSTRACT

In order to screen pomegranate cultivars for drought tolerance, few rapid, less expensive and reliable methods were used. Two-year-old pomegranate (*Punica granatum* L.) plants of various commercial cultivars namely 'Rabab-e-Neyriz' ('Rabab'), 'Shishe-cap-e-Ferdows' ('Shishecap'), 'Malas-e-Saveh' ('M-Saveh'), 'Malas-e-Yazdi' ('M-Yazdi'), and 'Ghojagh-e-Qom' ('Ghojagh') were grown in large containers filled with a mixture of leaf mould, sand, and soil (1:1:1, by volume) in greenhouse. The plants were subjected to 14-day drought stress by withholding irrigation, followed by re-watering for 7 days. Midday stem water potential (Ψ_{stem}), leaf relative water content (RWC), membrane stability index (MSI), leaf dry mass per area (LMA), rapid test for drought tolerance (DTI), gas exchange parameters including net photosynthesis (A_n), leaf scale transpiration (T_r), and stomatal conductance (g_s), and intrinsic water use efficiency (IWUE) were determined in well-watered and drought-stressed plants. All cultivars showed an ability to tolerate drought stress, but 'Ghojagh' exhibited more tolerance, with a higher RWC and Ψ_{stem} and a greater osmotic adjustment. 'Ghojagh' also revealed higher cell membrane stability and IWUE and a lower reduction in net CO_2 assimilation rate. This study found that 'M-Yazdi' was more vulnerable to severe water stress, and displayed the lowest degree of cell membrane stability as compared to the other examined cultivars and showed no recovery for RWC at the end of recovery period.

1. Introduction

Pomegranate (*Punica granatum* L.), from the family Punicaceae, is a popular fruit of tropical and subtropical regions that is native to the area stretching from Iran to the Himalayas in northern India (Fawole and Opara, 2013; Parvizi et al., 2016). It has been cultivated since ancient times and is mentioned in the Christian bibles and holy Quran (Rahimi et al., 2012). Drought is the most abiotic stress factor limiting plant growth and crop production in the world. Increase in fruit splitting or cracking and decrease in vegetative growth and yield is economically important losses in pomegranate orchards resulting from water stress. Drought tolerance is observed in almost all plant species, but its extent varies from species to species and even in cultivars of the same species (Jain et al., 2011). Breeders look for new sources of variations when attempting to find drought tolerant plants with optimal characteristics for high rates of photosynthesis and productivity, but tolerance to water stress is very complex, due to the intricate of the interactions between stress factors and various physiological, biochemical, and molecular response affecting plant growth (Jaleel et al., 2009; Giancarla et al., 2012). A better understanding of how drought stress affects the physiology of the plant helps in the creation and selection of new drought tolerant cultivars. Plants experience drought stress, either when the water supply to roots becomes difficult or when the transpiration rate becomes higher than the rate of water uptake. These two conditions often occur under arid and semi-arid areas (Gholami et al., 2012). Different levels of drought stress could be observed in a plant. A moderate loss of water often leads to stomatal reactions, limiting CO_2 assimilation, stomatal conductance, and transpiration. Severe water stress, on the other hand, occurs when most of the protoplasmic water is lost and often leads to a major disruption of the metabolism, enzymatic antioxidant activities and cell structure with more of an effect on cell enlargement than on cell division (Jain et al., 2011).

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Plants respond to drought stress and adapt to arid and semi-arid drought conditions by various molecular, physiological, biochemical, and morphological changes. Plants also adapt different types of life strategies to cope with soil water shortage, and resist drought stress. Two such strategies are drought escape (via completing plant life cycle before severe water deficit) and resistance mechanism (Levitt, 1980; Price et al., 2002; Verslues et al., 2006; Wang et al., 2012). Identifying physiological markers which could be assessed in a rapid, reliable and less expensive methods and can characterize cultivars in germplasm banks may help pomegranate breeding programs in obtaining cultivars that are more tolerant to drought conditions or that may even be used in the initial phases of a breeding program. Some rapid and less expensive markers that have been used by researchers for screening drought tolerant cultivars are stem water potential (Ψ_{stem}), relative water content (RWC), chlorophyll stability index, photosynthesis and gas exchange parameters, drought injury index, leaf dry mass per area, stomatal size and density, chlorophyll fluorescence, water use efficiency and a rapid test for drought tolerance using drop in the pH of leaf extract. (Torrecillas et al., 1996; Thakur, 2004; Clavel et al., 2005; Šircelj et al., 2007; Guerfel et al., 2009; Ghaderi et al., 2011; Yadollahi et al., 2011; Gholami et al., 2012).

Iran has the first rank for the number and diversity of pomegranate cultivars, cultivated area, producing and exporting of the fruit in the world (Parvizi et al., 2016). The pomegranate orchards have been recently damaged due to severe droughts and intensive reduction in groundwater resources in Iran. There are 760 genotypes of pomegranate in a collection in Yazd province in central Iran. The most important commercial cultivars of pomegranate in Iran are 'Rabab-e-Neyriz' ('Rabab'), 'Shishe-cap-e-Ferdows' ('Shishecap'), 'Malas-e-Saveh' ('M-Saveh'), 'Malas-e-Yazdi' ('M-Yazdi'), and 'Ghojagh-e-Qom' ('Ghojagh'), but there is no data about drought tolerance of these cultivars. To date, some studies have been conducted on pomegranate under water deficit conditions mainly focused on the water relations of leaves under different irrigation regimes (Intrigliolo et al., 2011a; Rodríguez et al., 2012), the use of water stress indicators for managing irrigation (Intrigliolo et al., 2011b; Galindo et al., 2013), and the evaluation of different irrigation regimes on growth and fruit quality and quantity (Khattab et al., 2011; Mellisho et al., 2012; Parvizi and Sepaskhah, 2015; Parvizi et al., 2014, 2016). To the best of our knowledge, however, this is the first research applying rapid, reliable and less expensive methods for screening various pomegranate cultivars for drought tolerance.

Therefore, the objective of this study was to screen various cultivars of pomegranate for water stress tolerance via rapid, reliable, and less expensive methods as well as to enhance our knowledge of physiological mechanism involved in response of young pomegranate plants to water stress and recovery.

2. Materials and methods

2.1. Plant material and drought stress applications

The experiment was carried out during 2014 growing season at the experimental greenhouse in the college of Agriculture, Shiraz University, Iran (latitude 29°56′N; longitude 52°02′E; 1810 m altitude). Two-year-old pomegranate plants of the cultivars 'Rabab', 'Shishecap', 'M-Saveh', 'M-Yazdi', and 'Ghojagh' were used. All cultivars were propagated by stem cuttings. They were grown under greenhouse conditions, in large containers (15 L) filled with a mixture of leaf mould, sand, and soil (1:1:1, by volume) and a gravel layer at the bottom. The field capacity of the soil used for potting was determined according to the protocol described by Richards (1949). Potted pomegranate trees were irrigated regularly for 4 months to field capacity level. The minimum and maximum temperatures during the experiment period were 16 and 36 °C, respectively, and mean relative humidity was approximately 55%. All of the pots were covered with jute bags in order to minimize temperatures inside of the containers. Before starting the main experiment a pre-experiment was done by 15 plants (three plants for each cultivar) to determine the level of drought period. In the pre-experiment by the values of stem water potential and symptoms of drought stress on the leaves of pomegranate plants, the times of moderate water stress (7 days of withholding irrigation) and severe water stress (14 days of withholding irrigation) were considered. At day 7 of water stress, the majority of leaves of all pomegranate cultivars were fresh and green but the stem water potential in all cultivars was obviously less than day 1. At day 14 of water stress, the majority of leaves became withered and discolored and the stem water potential in all cultivars was markedly less than day 7. For applying main experiment, by the end of July, 32 plants of each cultivar were divided into two uniform groups: Control plants (16 plants of each cultivar) and drought-stressed plants (16 plants of each cultivar). The first group (control plants) was watered daily to field capacity level during the whole experimental period, whereas the second group (drought-stressed plants) subjected to a water stress period by withholding irrigation for 14 days until the plants showed a strong loss of turgescence and majority of leaves became withered and discolored. The symptoms of drought stress (withering and discoloring) in the 'Ghojagh' was obviously less than those in the other cultivars. In drought-stressed plants, the top of the pots was covered with a plastic film in order to reduce evaporation from the soil surface and to decrease the rate of development of water stress. After this period of drought, stressed plants were re-watered to field capacity and followed by a recovery for 7 days. We collected leaf material for physiological analyses at different times of experiment (1, 7, 14, and 21 days).

2.2. Plant water status

Midday stem water potential (Ψ_{stem}) was measured with a pressure chamber (Soil Moisture Equip. Corp. Model 5100A, Santa Barbara, CA, USA). For this purpose, similar number and type of leaves were enclosed in a small plastic bag covered with aluminum foil for at least 2 h before measurements in the pressure chamber (Rodríguez et al., 2012). Ψ_{stem} measurement was performed between 11:00 am and 13:00 pm. Leaf relative water content (RWC) was calculated from gravimetric measurements as $(FW - DW)/(TW - DW) \times 100$, where FW is the leaf fresh weight, DW is the dry weight after oven-drying the leaves at 80 °C for 24 h, and TW is the turgid weight after re-hydrating the leaves at 4 °C.

2.3. Leaf dry mass per area ratio (LMA)

The leaf dry mass per area ratio (LMA) was determined as the ratio between their DM and leaf area (DW/leaf area) (Varone et al., 2012). Measurements were made at midday on clear days on fully irradiated youngest mature leaves.

2.4. Rapid test for drought tolerance (DTI)

Rapid test for drought tolerance (DTI) was done by extracting 500 mg leaves with 0.025 M EDTA disodium salt by boiling for 25 min in a water bath. After cooling, pH of the extract was recorded. Drop in pH of leaf extract indicated the drought tolerance (Thakur, 2004).