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Male pistachio seedlings exhibit more efficient protective mechanisms than females under salinity stress

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ABSTRACT

Salinity is one of the most important environmental stresses which affect most growth and physiological aspects of pistachio trees. In the present study, the effects of salinity and sexuality on physiological parameters and macro-nutrient concentration of shoot and root of pistachio (*Pistacia vera* cv. 'Badami-Riz-e-Zarand') were evaluated. The salinity levels were 0, 60, and 120 mM, and the seedlings were male and female. The results revealed that when the salinity level was 120 mM, Na concentration in the root, Ca concentration in the root and carotenoide in the leaves of male seedlings increased more than the female ones. The rate of the increase in Na concentration in the root, Ca concentration in the root and carotenoide in the leaves of male seedlings compared to control was 40.91%, 37.50% and 37% respectively while there were 21.78%, 21% and 28.5% in female seedlings. Furthermore, the male seedlings had higher proline in the leaf compared to the female seedlings. Although K concentration in the shoot was greater in the male seedlings than in the female, salinity reduced it in the shoot and the root. We found that male seedlings, in comparison with the female seedlings, exhibit better resistance to salinity.

1. Introduction

Pistachio is one of the most important horticultural crops in Iran. The majority of the pistachio-producing regions are located in arid and semi-arid areas with saline conditions. Therefore, selecting resistant genotypes as rootstock plays a primary role in increasing yield efficiency of this important nut crop. Although pistachio trees are classified as salt-tolerant, research has demonstrated that the growth rates of pistachio trees decline by increasing sodium chloride (NaCl) concentration in soil (Sepaskhah and Maftoun, 1988; Picchioni et al., 1990; Karimi et al., 2011; Karimi et al., 2012; Karimi and Maleki Kuhbanani, 2015). Karimi et al. (2011) studied the response of four pistachio rootstocks (Badami-e-Zarand A, Badami-e-Zarand B, Ghazvini and Sarakhs) to salinity stress and reported that all of the rootstocks, which were examined, limited the Na transportation to shoot tissue upto 15 ds m^{-1} and retained it in the roots; however, this ability was lower in the Sarakhs rootstock. In another research, Karimi et al. (2012) investigated the effect of salinity in a number of wild pistachio rootstocks, including *P. atlantica*, *P. atlantica* subsp. *kurdica*, *P. atlantica* subsp. *mutica*, and *P. atlantica* subsp. *cabulica*. They reported that by increasing salinity, the concentration of Na and K rises in the shoot of all rootstocks, especially *P. atlantica* subsp. *kurdica* rootstock. Thus far dioecious plants have provided an appropriate opportunity to investigate the sex-related differences in the field of energy consumption and reproduction ability (Suleman et al., 2011). It has been reported that the levels of tolerance to salinity and drought are differed in female and male plants (Bohlenius et al., 2006; Zhao et al., 2009; Zhang et al., 2012; Jiang et al., 2012; Karimi and Nasrolahpour-Moghadam, 2016). Karimi and Nasrolahpour-Moghadam (2016) studied the effects of three salinity levels (0, 60, and 120 mM) on growth indices and eco-physiological parameters of male and female seedlings of pistachio cv. Badami-Riz-e-Zarand, and reported that salinity reduced chlorophyll index, crown diameter, number of leaves, height of stem and shoot, and fresh and dry weight in both males and females; however, compared with salinity-stressed females, salinity-stressed males showed higher values of F_v/F_m, crown diameter, number of leaves, and stem height. In another study, Correia and Diaz-Barradas (2000) analyzed the difference between male and female trees of *Pistacia lentiscus* L. under laboratory and farm conditions. They found that under the laboratory conditions, the assimilation speed of CO_2 and the stomatal conductance in all plants were equal, whereas they were lower in female plants under the environmental farm stresses than those in the male ones. Moreover, Chen et al. (2010a,b) investigated the response of male and female plants of the *Populous cathayana* to

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the salinity stress, and reported that the leaf surface decreasing and dry matter accumulation in the female plants was 30% more than that in the male ones. In addition, they indicated that male plants had higher growth, gas exchange, and osmotic adjustment capacity than the females and lower accumulation of reactive oxygen species. Xu et al. (2008) appraised the sex-related differences of *Populous cathatyana* to salinity stress, discovering that the leaf-relative water content decreasing in male plants was higher than that in the female ones. In their study conducted on two populations of sea-buckthorn (*Hippophaer hamnoides* L.), Li et al. (2004) stated that in salinity stress, male plants had more dry matter accumulation, root to shoot ratio, and water-use efficiency compared to the female plants, while the special leaf area (SLA) in female plants was higher than that of the males. In another research, by studying the sex-related differences of *Populous yummnensis* compatibility to the dry and salinity stresses, Chen et al. (2010a,b) demonstrated that female plants had higher growth and gas exchange and larger reduction in osmotic adjustment capacity than the males, showing a higher accumulation of reactive oxygen species. Furthermore, they reported that female plants were more sensitive and vulnerable to the salinity and drought stresses than the males, and that the compatibility responses of both genders were different.

The plants resistance to the salinity stress with different mechanisms, such as the uniform distribution of toxic salt ions in the cell vacuoles, aggregation of the stabilizer ions in to that stabilize osmosis in the cytoplasm, reduction of the absorption of Cl or Na by the root and lack of transmission of Cl or Na to the shoot parts. The degree of plant resistance to salinity stress is changeable among different species and even among cultivar and the rootstock of a species. As rootstock has an important role in creating resistance to the salinity stress, identification, and selection of the rootstocks that are resistant to salinity has a deserved importance in the breeding programs (Karimi and Maleki Kuhbanani, 2015).

Pistacia vera L. is a type of dioecious plant commercially propagated by budding on the seedling rootstocks. The Badami-Riz-e-Zarand cultivar is employed as the rootstock in most pistachio-planting areas of Iran due to its relatively high resistance against the unsuitable environmental conditions such as saline soil and low quality of irrigation water (Karimi et al., 2011). At the present moment, pistachio seedlings, not considering their sexuality, are used as the rootstock. So far, no morphological index that can divide males and females with high certainty has been reported. In their study, by using two-dimensional electrophoresis, Xiong et al. (2013) found a total of 10 protein spots which were differentially expressed in leaf and stem between male and female plants in *Pistacia chinensis*. They pointed out that phosphoglycerate kinase (PGK) existed in high abundance in stem phloem in the female plants, but was almost not detected in the male ones; additionally, they found that ascorbate peroxidase (APX) and two temperature-induced lipocalin (TIL) species were highly abundant in the stem of male plants, while their abundance was much lower in the female ones. Hormoza et al. (1994) applied the RAPD marker to determine the sexuality of pistachio and tested 700 oligonucleotide primers, among which only did the OPO-08 primer produce the 945 bp bond in the female plants which the male plants lacked. Despite the fact that our previous study on pistachio seedlings proved that the sexuality of the seedlings can affect the resistance to salinity stress based on growth indices and eco-physiological parameters, thus far no research has been conducted on pistachio evaluating the sex-related differences in mineral composition of shoot and root under salinity stress. Therefore, the objective of this research was to study the physiological parameters in male and female pistachio seedlings under salinity stress in order to obtain an understanding of sex-specific responses of pistachio to salinity stress and related protective mechanisms.

2. Material and methods

2.1. Sexuality identification

A total number of 67 germinated nuts were randomly provided from a population of one hundred pistachios of *Pistacia vera* L. (Badami-riz-e-Zarand) from Iranian Pistachio Research Institute. The seeds were disinfected for 10 min in Chlorax solution (%10) and anti-fungal (1:1000). Each seed was planted in a pot containing soil and sand (4:1) with a pH of 7.83 and electrical conductivity (EC_e) of 0.86 dS m^{-1} and was kept in a greenhouse for seven months. The leaf samples of each seedling were collected for DNA extraction based on Cetyltrimeyhyl ammonium bromide (CTAB) method (Kafkas et al., 2001 Karimi and Sadeghi-Seresht, 2016). A 10-nucleotide primer, PO08945, from Sinaclone Co. was employed for the polymerase reaction. The electrophoresis results of PCR showed that a 945-bp allel existed in 33 female genotypes that was not observed in 34 male genotypes. Hence, male and female seedlings were divided based on the absence or the presence of the 945-bp band in electrophoresis results of PCR products (Hormoza et al., 1994; Karimi and Nasrolahpour-Moghadam, 2016).

2.2. Treatments and experimental design

After dividing the male and female seedlings, the salinity stress was imposed on the irrigation water. This experiment was conducted as factorial in the framework completely randomized design (CRD) with two factors, including genotype at two types of male and female, water irrigation salinity in three levels of 0 (control), 60, and 120 mM of sodium chloride, calcium chloride, and magnesium chloride combination with ratios of 1:2:3. Each treatment had 3 replications and each replication included 2–4 pots as samples. The number of plants in each treatment was 9–12. The seedlings were exposed to salinity for two months. The pots were irrigated with 500 ml of irrigation water every 5 days. In addition, 30% leaching were considered in order to avoid the accumulation of salt in the soil pots.

2.3. Extraction and determination of chlorophyll and carotenoides

One gram of fresh needles was ground with 20 ml of 80% acetone and was centrifuged at 5000 rpm for 5 min, and the supernatant was transferred to a 100-ml volumetric flask. After extraction, the supernatant was diluted to the final volume of 100 ml by 80% acetone. The absorbance was measured at 663, 645, 652, and 480 nm, using a spectronic spectrophotometer and the chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents were calculated (Arnon, 1949).

2.4. Extraction and determination of proline and soluble sugar

One gram of the leaf samples was grounded in liquid nitrogen, was mixed with 5 ml of ethanol, and was centrifuged for 10 min at 3500 rpm. Subsequently, the supernatant was decanted to new falcon. The concentration of proline was determined, using reagent Nine Hydrin solution (1.25 g Nine Hydrin mixed with 30 ml of acetic acid glacial and 20 ml of phosphoric acid 6 M). One milliliter of extract was mixed with 9 ml of distilled water and 5 ml of reagent Nine Hydrin solution. After wards, the samples were incubated at 65 °C for 45 min in water bath. After cooling, the absorbance was measured at 515 nm by a Spectronic spectrophotometer (Paquin and Lechasseur, 1979; Karimi et al., 2014). The total soluble sugar content of leaf was measured according to Irigoyen et al. (1992).