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Alleviating salt stress in almond rootstocks using of humic acid

Elnaz Hatami*, Ali Akbar Shokouhian, Ali Reza Ghanbari, Lotf Ali Naseri

Department of Horticulture, Faculty of Agriculture, University of Mohaghegh-E-Ardabili, Ardabil, Iran

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ABSTRACT

Salinity is one of the most important factors that reduces the growth and production of plants in arid and semi-arid regions. In our study, a pot experiment has been conducted in a factorial based on a complete randomized block design with 3 replications at the Faculty of Agriculture, University of Urmia, Iran, in 2016. In this study after applying the humic acid for two-months in four levels: (A0): control (A1): 2.5, (A2): 5 and (A3): 7 kg ha^{-1}, salinity was applied at four levels: (B0): control, (B1): 60, (B2): 120 and (B3): 180 mM NaCl, for two months on 3 almond rootstocks: (C0): Sangi almond seedling, (C1): GF677 and (C2): GN15. Results showed that increasing the salinity increased the leaf soluble proteins synthesis and CAT and POX activity up to 60 mM NaCl, but reduced them at higher levels. Also, electrolyte leakage increased from control to 180 mM NaCl. Using humic acid, by contributing to the absorption of essential nutrients such as N and K, increases the soluble proteins and enzymes synthesis more, leading to reduction in the electrolyte leakage. So, the highest and lowest protein and enzymes synthesis were related to 60 mM NaCL, 7 kg ha^{-1} humic acid and 180 mM NaCL, control treatment of HA, respectively. The highest and lowest electrolyte leakage was related to 180 mM NaCL, 2.5 kg ha^{-1} humic acid and control treatment of salinity, 7 kg ha^{-1} humic acid. Finally, GF677 with the highest protein and enzyme synthesis and the lowest electrolyte leakage was better and Sangi seedling and GN15 were placed in the next positions, respectively.

1. Introduction

Almond (*Prunus dulcis* Mill.) is one of the oldest and most important dry fruits in the world and belongs to the *Rosacea* family, and its main homeland is attributed to the Middle East, especially Iran (Ladizinsky, 1999). According to the FAO (2008), Iran by producing 110,000 tons has the 5 t h rank among the top 5 almond producers in the world, after the United States, Spain, Syria and Italy. About 10 million hectares of agricultural lands under irrigation face the problem of salinity annually, which this problem limits the yield of 40 million hectares of these lands (Manaf and Zayed, 2015). Most of the stone fruit trees, including almonds, are susceptible to salinity stress, and their yield decreases in salinity higher than 1.5 ds m^{-1} (Ottman and Byrne, 1988). Synthesizing the proteins with compatibility properties and antioxidant effects (Ashraf and Harris, 2004) and increasing the hydrolytic enzymes activity such as SOD, APX, CAT, POX, etc. (Sorkheh et al., 2012) are the main plants mechanism for coping with osmotic stress. Measuring the amount of electrolyte leakage as a simple, repeatable, fast and inexpensive method is a suitable physiological indicator for assessing the membrane damage caused by environmental stresses (Bajji et al., 2001; Al Busaidi and Farag, 2015). Since the salinity tolerance in glycophyte plants depends on the roots ability to prevent the toxic ions transfer to the aerial parts, the role of rootstocks in identifying the trees behavior is an important issue (Grattan and Grieve, 1999). The use of pronus inter specific hybrids such as GF677, GF557, Titan, Hansen, GN15 or Garnem (*P.amygdalus* cv. Garfi and *P.persica* cv. Nemared), Cadaman (*P.persica* * *P.daviana*) and etc. is very useful in salinity and drought tolerance (Leifert and Casselles, 2001; Felipe, 2009; Dejampour et al., 2012). However, botanists need faster and more complete methods to cope with intense environmental stresses (Parvaiz and Satyawati, 2008). Using humic substances is one of these options that increases plant resistance to environmental stresses through increasing metabolism (Banks and Percival, 2014).

Humic substances which are the components of humus contain a wide variety of molecular components such as polysaccharides, fatty acids, polypeptides, lignins, etc- and they are brownish black in color. They play a vital role in soil fertility and plant nutrition such as, by increasing the production of adenosine triphosphate (ATP) within plant cells and increasing the permeability, resulting in an increased transport of various mineral nutrients to sites of metabolic need (Russo and Berlyn, 1990; Cimrin et al., 2010; Turan et al., 2011), increasing the chlorophyll content by increasing of Fe^{+} absorption, specialty in alkaline soils (Delfine et al., 2005), increasing the photosynthesis by increasing of Rubisco enzyme activity (Russo and Berlyn, 1990; Delfine

* Corresponding author.
E-mail address: e.hatami2010@gmail.com (E. Hatami).

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et al., 2005; Cimrin et al., 2010), increasing the enzyme synthesis and an increase in the protein contents of the leaves by increasing the N absorption (MacCarthy et al., 1990; Russo and Berlyn, 1990; Delfine et al., 2005), degradation or inactivation of toxic substances due to the chelation exchange reaction (MacCarthy et al., 1990), and changing the soil physical, chemical, and biological structure (Russo and Berlyn, 1990; Parandian and Samavat, 2012). Application of humic substances to saline soils results in reduction in the concentration of sodium salts which is not correlated with a leaching of the salt, yet it may be correlated with improving the root growth and accumulation of Na^+ in the root and less Na^+ transduction to plant aerial parts (Cimrin et al., 2010). Humic substances can be subdivided into three major fractions: (1) Humin, (2) Humic acids (HAs), and (3) Fulvic acids (FAs) (Mosley and Mosley, 1998), and 65–70% of humic substances are formed of humic acid and fulvic acid (Parandian and Samavat, 2012). The size of fulvic acid particles are smaller than humic acids, and because of this they can readily enter plant roots, stems, and leaves. Also, by binding to water molecules, they can reduce the amount of evapotranspiration and transpiration, and help to maintain water inside the plant (Bronick and Lai, 2005). Humic acids comprise a mixture of aliphatic (carbon chains) and aromatic (carbon rings) organic acids which are soluble in water under alkaline conditions. On average, 35% of the humic acid (HA) molecules are aromatic, while the remaining components are in the form of aliphatic molecules (Asgharzade and Babaeian, 2012). The biological activity of humic acid is related to its chemical structure and active groups (Russo and Berlyn, 1990). Humic acid as a macromolecule and one of the main branches of humic substances (Canellas et al., 2017) increases soil water-holding capacity via high water absorption groups (Fahramand et al., 2014). This substance was recognized as a hormone like in the early 1900s (Dell'Agnola and Nardi, 1987). Different mineral elements are bound to humic acid molecules, as a result, humic acids function as an important ion exchange and metal complexing (chelating) systems (Asgharzade and Babaeian, 2012) which release these elements at time of need of the plant (Russo and Berlyn, 1990).

Using humic acid reduced the petal membranes peroxidation of *Polianthes tuberose* and increased the total protein content by increasing the elements absorption, especially Ca and N (Amani Beni et al., 2013). Humic acid increased the proteins amount in banana tissue culture media, consequently improving root development and POX activity. This was related to activity of humic acid, which was the hormone like (Fernandez et al., 2013). Humic acid caused the plant to absorb different nutrient elements, especially K^+ by increasing the soil CEC in kiwi trees under salinity. K^+ as a main element for enzymes synthesis increases the water and nutrition absorption and improves photosynthesis by helping the stomata to open more (Mahmoudi et al., 2014). Humic acid reduced the sodium adsorption ratio (SAR) in pistachio cv. Akbari, under salinity (Javanshah and Aminian-Nasab, 2016).

This report presents the beneficial effects of humic acid on the plants salinity resistance and points out whether the soil application of this biological treatment can play an important role in reducing the effects of salinity stress on almond rootstocks. The measured factors included leaf soluble proteins, electrolyte leakage and catalase and peroxidase enzymes activity.

2. Materials and methods

2.1. Plant material and treatments

This research was carried out in the research farm of Faculty of Agriculture, University of Urmia, Iran, in 2016. Almond rootstocks, two years old, with id tags, healthy, and with the same growth ability, were provided by the seedling production institute of "Rooyan Pajoohesh-e-Azarbaijan", located on Urmia-Tabriz road. In March 2015, they were removed from polyethylene pots and transformed into 7 kg plastic pots containing equal amounts of surface soil and peat moss and each pot was considered as a repeat. The used soil texture was sandy loamy with pH = 6.8, EC = 1.63 ds m^{-1}, 379 ppm of K, 71 ppm of P, 0.17% of total N and 1.41% of C. The experimental treatments were concluded: Humic Acid (HA) in four levels: (A0): control (A1): 2.5, (A2): 5 and (A3): 7 kg ha^{-1} and salinity in four levels: (B0): control, (B1): 60, (B2): 120 and (B3): 180 mM NaCl, that were applied on almond rootstocks including: (C0): Sangi almond seedling, (C1): GF677 and (C2): GN15. From the 5th of May and for 2 months, humic acid (Sigma Aldrich, USA), prepared from Sahab Shimi Pasargad Co., has been applied once a week with irrigation water and soil application. The pots were irrigated with water containing various NaCl levels every 2 days, from the 5th of July and for 60 days. Some solution was removed from the bottom of the pot at each irrigation with saline water. The plant roots were thoroughly washed with ordinary water to minimize EC and pH changes due to salt accumulation in the planting bed each week. At the beginning of the experiment, in order to prevent the occurrence of sudden stress on plants, salinity stress increased by increasing the amount of 25 mM NaCl daily (Fisarakis et al., 2001).

2.2. Measurements

2.2.1. Leaf soluble proteins

The Bradford method (1976) was used to assay the amount of leaf soluble proteins. For this purpose, 0.5 g of fresh leaf was homogenized in 25.6 ml of extraction buffer solution (121.4 g Tris [1,3-dichloro-2-propyl-phosphate] dissolved in 1 L of distilled water, and pH set on 6.8) and kept for 24 h. After this time, the leaves were thoroughly chopped, and homogenate was centrifuged at 6000 rpm for 20 min (PRP JENUS TDLSO-2B Centrifuge). 0.1 ml of supernatant obtained was separated into another test tube and 5 ml of bio red agent was added to it and the absorbance was measured at 595 nm with a spectrophotometer (Unico 2100 UV). To determine the leaf soluble proteins of almond rootstocks, a standard curve was made using pure bovine serum albumin (BSA) and was expressed in units of μg gr FW^{-1}.

2.2.2. Electrolyte leakage

Salinity tolerance of almond rootstocks was assayed by measuring leakage of electrolytes of the youngest mature leaf membranes as described by Zhao et al. (1992). Ten leaf discs (0.5 cm diameter) from almonds were placed in individual glass tubes contains distilled water, and the electrical conductivity was measured after a short time using conductance meter (MARTINI EC/TDS/Temp wp, Italy) at 25 °C (EC_0). The tubes were incubated in a refrigerator at 4 °C, and the electrical conductivity of the samples was re-measured after 24 h (EC_1). The samples were placed in an autoclave for 15 min at 120 °C, and their electrical conductivity was recorded after cooling at room temperature (EC_2). Finally, the electrolyte leakage was measured via statement below:

$$RP(\%)=[(EC_1-EC_0)/(EC_2-EC_0)]\times 100 \quad (1)$$

2.2.3. Enzymes activity

To measure enzymes activity, leaf protein extract was made by 0.8 M potassium chloride and 0.5 M potassium phosphate buffer with pH = 7. 0.1 g of fresh weight tissue with 10 ml of the solution was crushed, and the slurry was centrifuged at 4000 rpm for 20 min at 4 °C. The supernatant, which contained enzyme activity, was used as the enzyme source for experiment.

The POX activity was assayed as Mac-Adam et al. (1992). The substrate mixture contained 100 ml of 50 mM potassium phosphate (pH = 7), 90 μL of 1% guaiacol as the substrate, and 90 μl of H_2O_2 0.3%, as the hydrogen donor. The reaction cuvette contained 2.87 ml substrate mixture, 20 μl of enzyme extract and 0.03 ml treatment solution, in total volume of 3 ml. Finally, this enzyme activity was determined at 25 °C with a spectrophotometer at 470 nm.

The CAT activity was assayed as Chance and Maehly (1995).