Scientia Horticulturae 225 (2017) 156–162

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Using controlled salt stress and β-aminobutyric acid signaling to decrease transplant failure

Soheil Karimi[a,*], Zahra Mirfattahi[a], Louise Ferguson[b], Vahid Tavallali[c]

[a] Department of Horticulture, College of Aburaihan, University of Tehran, Tehran, Iran
[b] Department of Plant Sciences, University of California Davis, CA, USA
[c] Department of Agriculture, Payame Noor University (PNU), Tehran, Iran

ARTICLE INFO

Keywords:
Hydrogen peroxide
Malondialdehyde
Pistachio
Proline
Stomata
Water loss rate

ABSTRACT

Root injuries, heat and desiccation after transplanting are abiotic stresses that may cause transplant failure. Stress signal transduction for preventing transplant failure due to root injuries and growing in hot dry conditions was evaluated using *Pistacia vera*, a species highly sensitive to transplanting, as the model plant. Pistachio seedlings were primed by exposure to salt stress using 100 mM $CaCl_2$ 100 mM KNO_3, 50 mM $CaCl_2$ + 50 mM KNO_3, and three foliar applications of 2.0 mM β-aminobutyric acid (BABA) for 7 days. After removing 30% of the root length, the plants were then grown under hot dry conditions for 60 days. Significant increases were observed in the plasma membrane thermostability and proline and malondialdehyde leaf concentrations after the priming treatments. The BABA and KNO_3 treatments increased leaf H_2O_2 content by 26% while the KNO_3 + $CaCl_2$ treatment increased it by 63%. Detached leaves of non-primed plants lost water more quickly than leaves of primed plants. The BABA and KNO_3 + $CaCl_2$ treatments were most effective at limiting water loss from detached leaves. Transplanting killed all the non-primed plants. The $CaCl_2$ treatment increased transplant survival by 42.9% and the KNO_3 + $CaCl_2$ treatment by 100%. The survival rate of BABA and KNO_3 primed plants was 57.1%. These results suggest controlled salt stress could be a simple, rapid and efficient method of for controlling transplant shock.

1. Introduction

New orchards and forests are planted with seedlings or clones raised in greenhouses. These greenhouse grown transplants, raised with optimal temperature, relative humidity, irrigation, and light, are susceptible to transplant shock when planted outdoors. The low evaporative demand in greenhouses produces young plants with poor stomatal function, thin epicuticular leaf wax, high stomatal density and low transpiration rates even when stomata are fully open (Bañon et al., 2004; Shinohara and Leskovar, 2014). When transplanted root injuries can limit the root signal transduction to leaves preventing the abscisic acid (ABA) biosynthesis and translocation that regulates stomata alleviating drought stress (Jakab et al., 2005). Root injuries combined with thigh evaporative demand at transplanting exacerbates this transplant shock.

Previous studies have demonstrated that inducing defense responses in plants improves their performance under abiotic stress. Arabidopsis studies have demonstrated that priming improves recovery from abiotic stresses (van Loon et al., 2006; Wu et al., 2010). However, applying this technique for reducing transplant shock has not been fully investigated. Previous studies focused on how water stress in the nursery induced morphological and physiological adaptations for priming transplants (Bañón et al., 2004; Villar-Salvador et al., 2004; Bañon et al., 2006). Bañon et al. (2006) reduced mortality of *Nerium oleander* transplants from 92% to 32% with 4 weeks of deficit irrigation and decreased humidity. The priming produced formation of dense, less ramified roots, smaller leaves, osmoregulation, and improved stomatal regulation. Liptay et al. (1998) demonstrated that controlling water availability enhanced stress tolerance enabling transplants to withstand transplant shock. However, prolonged water stress and low humidity are not treatments that can be applied without harming plant growth and quality. For this study, we hypothesized that imposing controlled salt stress and/or application of a stress signaling molecule, β-aminobutyric acid (BABA), can induce defense responses that make nursery plants more tolerant of transplant shock.

Salinity stress manifests as osmotic stress, ion toxicity, and oxidative

Abbreviations: ABA, abscisic acid; BABA, β-aminobutyric acid; DM, dry mass; FM, fresh mass; FML, fresh mass loss; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; MTI, membrane thermostability index; RWL, rate of water loss; RWC, relative water content; TCA, trichloroacetic acid; TM, turgid mass

* Corresponding author.
E-mail address: skarimi@ut.ac.ir (S. Karimi).

http://dx.doi.org/10.1016/j.scienta.2017.06.070
Received 24 December 2016; Received in revised form 28 June 2017; Accepted 30 June 2017
Available online 11 July 2017
0304-4238/

injury, ultimately disrupting membrane integrity, nutrient balance and plant metabolism. Plants have multiple mechanisms to deal with salt stress. Ashraf and Foolad (2007) and Karimi et al. (2009) reported salt stress induces organic and inorganic osmolyte formation in plants, enhancing drought and salinity tolerance. Salinity stress has also been reported to elevate ABA, triggering stomatal closure (Chaves et al., 2009). Enhanced biosynthesis of polyamines has also been demonstrated to regulate plant stress and defense responses to salt and drought conditions (Alcázar et al., 2010). Over-production of hydrogen peroxide (H_2O_2) under salt stress has been reported to activate antioxidative plant defenses and promote cell membrane stability (Uchida et al., 2002; Karimi et al., 2017). Similarly, Fornes et al. (2007) demonstrated saline irrigation water improved performance of multiple ornamental species under drought stress.

Unlike inducing systemic defenses in plants with salt stress, upregulation, or application of plant hormones such as ABA, ethylene and salicylic acid, and use of signaling molecules, are widely reported in the literature (Conrath et al., 2002; Zimmerli et al., 2008). BABA is a simple non-proteinogenic amino acid, which induces plant resistance to biotic stresses (Tavallali et al., 2008). Plants primed with BABA have been demonstrated to respond more quickly and strongly to biotic (Ton and Mauch-Mani, 2004) and abiotic stresses (Wu et al., 2010). BABA has been shown to up-regulate many stress-responsive genes (Zimmerli et al., 2008). It has been suggested that BABA primes plants by enhancing ABA and ethylene mRNA accumulation of early signaling intermediates (Zimmerli et al., 2008). Du et al. (2012) concluded that ABA accumulation after BABA application promotes desiccation tolerance by inducing stomatal closure, reducing water use, and enhancing the plant's antioxidant defense enzymes.

In this study we investigated activating the defense responses of young greenhouse grown plants with moderate salt stress and foliar BABA application. Pistachio (*Pistacia vera*), a species that is highly sensitive to transplant shock was used as the model plant. Earlier studies have demonstrated *P. vera* can tolerate relatively high soil salinity, up to 800 mg NaCl per kg of soil, and an EC of 8.7 dS m^{-1} in the irrigation water. This suggests *Pistacia* spp. have a well developed defense system against salt stress (Ferguson et al., 2002; Karimi et al., 2009; Tavallali et al., 2009; Karimi and Rahemi, 2012). We hypothesized activating this defense system with by priming with salt stress would decrease transplant shock from root injury and drought conditions. We also compared the efficacy of salt stress priming with that of BABA applications, hypothesizing that transplants could be primed by application of a safe stress signal molecule instead of subjecting them to salinity stress.

2. Material and methods

Germinated seeds of the *P. vera* genotype Ghazvini, a common Persian rootstock, were sown in 10.5 × 10.5 × 28.0 cm plastic pots filled with cocopeat + perlite (30–70, v/v). The pots were kept in a greenhouse with daily average, 25/20 °C and 40/55% day and night temperatures and humidity, and 16 h of light. Light intensity was reduced by a double layer of 25% light reduction shade cloth. The pots were irrigated to field capacity every other day. After two leaves developed, all the plants were irrigated with half strength Hoagland's solution. To avoid salt accumulation the pots were leached with 500 mL distilled water every 10 days. Sixty days after sowing, the plants were treated by adding 100 mM $CaCl_2$ and KNO_3, and 50 mM $CaCl_2$ + 50 mM KNO_3 to the nutrient solution. To prevent osmotic shock, the first two irrigation were at 50% treatment concentration. The 2.0 mM BABA treatment solution was sprayed on non-salinized plants simultaneously with the salt treatments. After the last salt and BABA treatments the pots were leached with 500 mL distilled water followed with half strength Hoagland's solution

Leaf relative water content (RWC) was determined in 20 uniform disks of fully developed young leaves based on fresh mass (FM), turgid mass (TM – after re-hydration for 24 h at 4 °C in the darkness), and dry mass (DM – after drying in 75 °C for 72 h) of the discs. The leaf RWC was calculated using the following equation (Eq. (1)).

$$RWC\% = 100 \times \frac{FM - DM}{TM - DM} \quad (1)$$

Leaf succulence was calculated using the water content of the leaf discs ($\frac{FM - DM}{18.01258}$) per area unit. The membrane thermostability index (MTI) was determined by measuring electrolyte leakage from 20 leaf discs from fully expanded leaves using the method of Arora et al. (1998), with slight modifications. The leaf discs were thoroughly washed in deionized water and kept in 40 °C water for 30 min. After measuring electrical conductivity (C1), the samples were boiled in water for 10 min and the electrical conductivity recorded as above (C2). The MTI was calculated using the following equation (Eq. (2)):

$$MTI = \left[1 - \left(C1/C2\right)\right] \times 100 \quad (2)$$

The leaf proline concentration was determined using the technique previously described by Bates et al. (1973). A 500 mg of sample of oven-dried leaves was extracted with 3% (w:v) aqueous sulfosalicylic acid for 48 h. After infiltration, the extract was reacted with ninhydrin reagent in boiling water for 30 min. The reaction mixture was extracted with toluene and the absorbance of chromospheres containing toluene was measured at 520 nm by spectrophotometry (Shimadzu, model 160A). The proline concentration was determined using an external standard curve.

The leaf hydrogen peroxide (H_2O_2) concentration was analyzed using the method of Velikova et al. (2000). ThelLeaf tissue was homogenized in trichloroacetic acid (TCA), centrifuged and the supernatant was combined with a potassium phosphate buffer and KI. The absorbance was measured at 390 nm using spectrophotometry (Shimadzu, model 160A). The lipid peroxidation was determined by calculating the quantity of malondialdehyde (MDA) made using the thiobarbituric acid reactive substances using the method of Heath and Packer (1968). The leaf samples were homogenized in TCA, and after centrifuging, the supernatants mixed with thiobarbituric acid (TBA) in 20% TCA. The mixtures were then heated to 95 °C for 30 min. After cooling and centrifugation at 10000*g* for 10 min, the supernatant absorbance was measured at 532 and 600 nm. The MDA content (nmol g^{-1} FW) was calculated using an extinction coefficient of 155 mM cm^{-1} after withdrawing the non-specific absorbance at 600 nm.

To monitor the rate of water loss (RWL) from detached leaves, two fully expanded leaves were excised from each plant, their petioles removed, and the leaves suspended in air at ambient laboratory conditions of low light intensity and 22.5 °C. The change in the leaf fresh mass was monitored by weighing after 390 min. The leaf fresh mass loss was expressed as the percentage of initial fresh mass. The rate of water loss (RWL) was calculated using Eq. (3) below.

$$RWL = \frac{FM_{Tx} - FM_{Tx+1}}{18.01258 \times DM \times (T_{x+1} - T_x)} \quad (3)$$

Where FM_{Tx} is leaf fresh mass (mg) at time Tx, FM_{Tx+1} is leaf fresh mass (mg) at time T_{x+1}, DM is leaf blade dry mass (mg), Tx is time (min.) when FM_{Tx} was determined, and T_{x+1} is time (min) when FM_{Tx+1} was determined. The number and size of the stomatal apertures on the adaxial and abaxial surfaces of healthy leaves were measured microscopically using midday epidermal cell imprints obtained with nail polish (Hilu and Randall, 1984). The size of the individual apertures was reported as width to length ratio by using image processing program Image J (http://imagej.net/Ops).

To evaluate the effect of the priming treatments the plants were carefully removed from the pots to avoid injury and washed with tap water. Then 30% of the root length was removed and the plants repotted in the same growing medium. The plants were incubated in a