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Research paper

Changes in root architecture and endogenous hormone levels in two *Malus* rootstocks under alkali stress

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ABSTRACT

High soil pH is a serious agricultural problem in northern China, where it affects apple metabolism, specifically root development. To explain the physiological responses of apple plants to alkali stress (non-stressed control, pH 6.0 ± 0.1, versus alkali stress treatment, pH 9.0 ± 0.1), we used hydroponics culturing to study the performance of two rootstocks with contrasting degrees of alkali tolerance. These trials included two alkaline salts (Na_2CO_3:$NaHCO_3$; 1:1, v:v) that were intended to simulate soil conditions in northern China. For both rootstocks, values for fresh and dry weights, plant heights, root lengths, and root activity were decreased in response to stress treatment while relative electrolyte leakage in the roots was increased when compared with their respective controls. However, the amplitude of this response was much smaller in the alkali-tolerant *Malus prunifolia* than in the alkali-sensitive *M. rockii*. The effects of alkali stress on endogenous hormones also varied significantly between stress-treated rootstocks and their controls. After 15 days of alkali stress treatment, for *M. prunifolia* (AT), compared with the control, the contents of dihydrozeatin riboside (DHZR) and jasmonic acid (JA) were decreased while the contents of indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellin (GA_3) and zeatin riboside (ZR) were increased in the roots, among which the contents of ABA, ZR and JA in the roots were significantly different from their controls. As for *M. rockii* (AS), the contents of ABA, IAA, JA and DHZR were decreased while the content of GA_3 and ZR were increased in the roots, among which ABA, IAA, JA and ZR in the roots were significantly different from the controls. However, there were significant differences in the contents of ABA and IAA in the roots between two apple rootstocks seedlings under alkali stress. Especially for the IAA levels in the roots, compared with their controls, was increased by 30.0% in *M. prunifolia* but decreased by 17.8% in *M. rockii*. After 15 days of alkali stress treatment, the root architecture of *M. rockii* was also more seriously influenced by alkali stress compared with *M. prunifolia*. Compared with their controls, the descent range of each index of the root architecture of *M. rockii* was almost twice as high as that of *M. prunifolia*. Especially for the number of root tips, the decrease amplitude of *M. rockii* was 17.6 times greater than that of *M. prunifolia*. These differences in root architecture might be explained by changes in the activity of hormones, especially for IAA.

1. Introduction

Soil salinization and alkalization are major sources of abiotic stress that limit crop productivity worldwide. In fact, soil alkalinity affects 434 million ha of agricultural land in more than 100 countries (Jin et al., 2006; Li et al., 2015). When such alkalization results from excess $NaHCO_3$ or Na_2CO_3, the consequences can be more severe than when the cause is neutral salts such as NaCl or Na_2SO_4 (Shi and Sheng 2005; Shi and Wang 2005; Chen et al., 2009). In northeastern China, alkalinized pasture covers more than 70% of the available land area and continues to expand (Kawanabe and Zhu 1991; Wang et al., 2015a). Alkaline soils are characterized by elevated pH, a high percentage of exchangeable sodium, poor fertility and very low water content (Lv et al., 2012). This challenge is one of the most important limiting factors to the development of agricultural production. Although more attention is now being paid by agricultural ecologists to the mechanisms and potential causes of salt stress, less focus has been given to the link with alkali stress.

Apple (*Malus* sp.) is a deciduous fruit tree that is primarily cultivated in arid and semi-arid areas. In China, the most productive region

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Abbreviations: ABA, abscisic acid; AS, alkali-sensitive; AT, alkali-tolerant; CTKs, cytokinins; DHZR, dihydrozeatin riboside; DW, dry weight; FW, fresh weight; GA, gibberellin; IAA, indole-3-acetic acid; JA, jasmonic acid; REL, relative electrolyte leakage; ZR, zeatin riboside

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is the northwestern Loess Plateau, which features abundant sunlight, deep soils, and a wide gradient in daytime and nighttime temperatures. However, reduced rainfall and higher evaporation in that area have led to soil salinization and alkalization that are not conducive to apple growth. Therefore, selecting the most appropriate rootstock is of great importance. The effects of alkali stress are not clear for apple but have been reported for several other agricultural crops, such as *Oryza sativa* (Wang et al., 2015a; Wei et al., 2015), *Avena sativa* (Bai et al., 2013; Gao et al., 2014), *Hippophae rhamnoides* (Chen et al., 2009), *Glycine soja* (Lv et al., 2012), *Solanum lycopersicum* (Wang et al., 2011), *Helianthus annuus* (Shi and Sheng 2005), *Gossypium* sp. (Chen et al., 2011), *Leymus chinensis* (Shi and Wang 2005; Wang et al., 2015b), and *Triticum aestivum* (Yang et al., 2008; Guo et al., 2015).

Endogenous phytohormones play crucial roles in regulating the developmental processes and signal networks involved in plant responses to a series of biotic and abiotic stresses (Robert-Seilaniantz et al., 2007; Bai et al., 2011). They include ethylene, abscisic acid (ABA), gibberellins (GAs), auxins such as indole-3-acetic acid (IAA), and cytokinins (CTKs). The most active CTKs are dihydrozeatin riboside (DHZR) and zeatin riboside (ZR) (Bai et al., 2011). Fluctuations in phytohormone levels not only have an impact on plant adaptability to environmental changes, but also influence growth, development, and physiological/biochemical metabolism (Albacete et al., 2008). Alkali stress causes the stress hormone ABA to accumulate in the leaves but be released from the roots into the soil solution unless prevented by Casparian bands. In addition, a deficiency of ABA makes plants extremelysensitive to several types of stress (Degenhardt et al., 2000). As a regulator of root growth, auxins modulate biosynthesis, distribution, and polar transport in plants (Dharmasiri et al., 2005; Li et al., 2015). Thimann (1936) found that this class of hormones can both stimulate root initiation and inhibit root elongation. Likewise, ethylene can block root elongation during periods of alkali stress and can contribute to plant responses to drought, water stress, phosphate starvation, salinity, and excess aluminum (Li et al., 2015). Finally, early metabolic disorders of CTK may be a major reason for inhibited shoot growth and leaf senescence when soil temperatures are high (Bai et al., 2011), while wheat plants show increased cold-hardiness when their GA contents are reduced (Sun et al., 2010).

The root is the first organ to sense alkaline conditions, a source of stress that inhibits the development of primary roots (Li et al., 2015). We previously described difference in alkali tolerance among 17 *Malus* rootstock species, with ‘Shandingzi’ apple (*M. baccata*) being the most sensitive and ‘Fupingqiuzi’ (*M. prunifolia*) being the most tolerant (Zhang et al., 2016). We also found that ‘Lijiangshandingzi’ apple (*M. rockii*) had poor tolerance. The extent of damage related to alkali stress was closely associated with the degree of tolerance by each rootstock. Our preliminary study indicated that the root system was strongly influenced by such stress, but we were unable to determine its effect on apple root architecture in that investigation. Transcription factors and plant hormones are key regulators of primary root branching and root responses to environmental changes (Petricka et al., 2012). Therefore, based on our earlier findings, we examined two apple rootstocks of *M. prunifolia* and *M. rockii* and compared their responses to alkali treatment with respect to root architecture and levels of endogenous phytohormones.

2. Materials and methods

2.1. Plant materials and experimental design

Two genotypes of apomictic apple rootstocks were used, ‘Fupingqiuzi’ (*Malus prunifolia*; alkali-tolerant, or “AT”) and ‘LijiangShandingzi’ (*M. rockii*; alkali-sensitive, or “AS”). Beginning in early January of 2015, both rootstocks were propagated from seeds that were first stratified in sand at 0–4 °C for 50–60 d. Once this germination process had been accelerated, budlets (approximately 0.3 cm long) were sown in square plastic pots (9 cm × 9 cm × 10 cm) containing nursery substrate. The seedlings were grown outdoors for approximately two months under natural light and temperature conditions. At the 7- to 8-true-leaf stage, uniformly sized plants were transferred to a hydroponics system and cultured according to the method described by Bai et al. (2008). Each plastic basin (52 cm × 37 cm × 15 cm) contained 13 L of a 1/2-strength Hoagland nutrient solution (Hoagland and Arnon 1950), which was aerated with an air pump for 1-h intervals that were then interrupted for 0.5 h each time. The dissolved oxygen concentration was maintained at 8.0–8.5 $mg\ L^{-1}$. Culturing conditions included a 14-h photoperiod and day/night temperatures of 22–24 °C/15 to 18 °C.

On 12 June 2016, uniformly sized seedlings were randomly assigned to two groups (control treatment and stress treatment) and transplanted into six hydroponics basins. Each basin (three per treatment) contained 56 seedlings, for a total of 168 seedlings per treatment. After one week of pre-treatment, to allow the plants to adapt to the new growing conditions, the experimental period began, with each basin considered a single replicate (i.e., three replicates per treatment group).

2.2. Simulated alkaline conditions

Alkaline stress conditions (Treatment) in the pot substrate were created by adding 1 $mol\ L^{-1}$ $NaHCO_3$ and 1 $mol\ L^{-1}$ Na_2CO_3 to 1/2-strength Hoagland solution, at a 1:1 vol ratio. The pH value was adjusted to 9.0 ± 0.1, as measured with a digital pH meter. For the control treatment (Control), seedlings were placed in standard 1/2-strength Hoagland solution and the pH was adjusted to 6.0 ± 0.1 by adding concentrated sulfuric acid. The solution was exchanged every 5 d and the pH of the control and stress treatment solutions was adjusted daily to 6.0 ± 0.1 and 9.0 ± 0.1, respectively.

2.3. Evaluation of physiological indices

After 15 d of treatment, plant heights and root lengths were recorded. The seedlings were then harvested and separated into root, shoot, and leaf portions to determine their individual fresh weights (FWs)($n = 20$). Finally, enzymes were deactivated by exposing the tissues to 105 °C for 15 min. Dry weights (DWs) were measured after the samples were oven-dried at 75 °C to a constant weight.

The roots sampled from 10 plants per treatment were used to analyze a series of physiological indexes. Electrical conductivity was evaluated based on relative electrolyte leakage (REL), which was calculated according to a method described previously (Dionisio-Sese and Tobita 1998). Root activity was monitored by the triphenyl tetrazolium chloride reduction method (Comas et al., 2000).

2.4. Analysis of root architecture

After 15 d of treatment, root samples were washed with distilled water, then separated and evaluated with a root scanner (EPSON PERFECTION V700 PHOTO, SEIKO EPSON CORP, JAPAN) ($n = 20$). The morphological features of interest included total length, surface area, average diameter, volume, number of root tips, number of root forks, and the proportion of root types, analyzed by WinRHIZO root imaging software (REGENT INSTRUMENTS, CANADA).

2.5. Determining concentration of endogenous phytohormones

On Days 0, 3, 6, 9, 12, and 15 d of treatment, new roots were sampled, then immediately frozen in liquid nitrogen and stored at −80 °C. Endogenous phytohormones ABA, IAA, GA_3, JA, ZR, and DHZR were extracted and purified by an indirect ELISA technique that was modified from a method described by Bai et al. (2008).