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Full Length Research Paper

Growth and physiological responses of five *Malus* species to the pH of hydroponic solutions

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We characterized the growth and physiological responses of five wild *Malus* species indigenous to China to acid and alkaline abiotic stresses. At the six-leaf stage, seedlings of *Malus sieversii*, *Malus prunifolia*, *Malus robusta*, *Malus mandshurica* and *Malus hupehensis* were transferred to hydroponic systems in which the solution pH was 5.5, 7.0 or 8.5. Fresh and dry weights, plant heights and root lengths were measured over time. After 20 days of treatment, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), ascorbic acid (AsA), and glutathione (GSH) contents were determined, as well as the rate of superoxide radical ($O_2^{\cdot-}$) generation and antioxidant enzyme activities in the roots. When the pH was raised from 7.0 to 8.5, seedlings of *M. sieversii* displayed significant increases in their growth parameters whereas values for all other traits were significantly decreased. The opposite trend was observed with *M. prunifolia* and *M. hupehensis*. When the pH was decreased from 7.0 to 5.5, seedlings of *M. sieversii* showed a significant decline in their dry weights but a significant improvement in root MDA, H_2O_2, AsA, and GSH contents, $O_2^{\cdot-}$ generation and enzyme activities. In conclusions, these results suggest that growth is not severely inhibited for *M. prunifolia*, *M. mandshurica*, and *M. hupehensis* under acidic conditions (pH 5.5) or alkaline conditions for *M. sieversii*, *M. robusta* and *M. mandshurica* (pH 8.5). In fact, *M. sieversii* grew better at pH 8.5, whereas growth was significantly inhibited for *M. sieversii* and *M. robusta* at pH 5.5 and for *M. prunifolia* and *M. hupehensis* at pH 8.5.

Key words: Antioxidant enzymes, antioxidants, growth traits, hydroponics pH, *Malus* species, oxidative stress.

INTRODUCTION

All plants are subjected over time to various biotic and abiotic stresses within their natural environment. Apple production is susceptible to fungi, viruses, replant disease, drought, water-logging, sunburn, wind, low temperatures, and acidification or alkalinization of the soil (Schrader et al., 2001, 2003; Manici et al., 2003).

Therefore, the selection and utilization of multi-resistance wild plant resources is an effective means for modifying current cultural varieties for adaptation to sub-optimal conditions, and for improving their yield and quality (Mano and Takeda, 1998; Iriki et al., 2001). About 80% of all wild *Malus* species originates from China (Zhou, 1999). These scarce and valuable plants are now widely collected, studied and utilized for apple rootstocks. For example, *Malus sieversii* has a rich biodiversity suitable for complex and varied terrains and landscapes, as well as unique types of ecosystems (Geibel et al., 2000; Dzhangaliev, 2003; Cao et al., 2004; Liu et al., 2004; Yan et al., 2008). This species, recognized as the major progenitor of the domestic apple is characterized by various fruit sizes and shows good resistance to *Erwinia amylovora* (fire blight) and drought (Forsline and Aldwinckle, 2004; Ma et al., 2008). Likewise, *Malus hupehensis*, *Malus robusta* and *Malus sieboldii* have

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Abbreviations: APX, Ascorbate peroxidase; **AsA,** ascorbic acid; **CAT,** catalase; **DW,** dry weight; **FW,** fresh weight; **GR,** glutathione reductase; **GSH,** glutathione; **GSSG,** oxidized glutathione; **MDA,** malondialdehyde; **POD,** peroxidase; **SOD,** superoxide dismutase.

Table 1. Origins of five *Malus* species for testing effects of hydroponics pH.

Code	Specie	Locality	Mean annual precipitation (mm)	Temperature (℃)	Normal range of soil pH
A	*M. sieversii*	Gongliu, Xinjiang (43° 28' N, 82° 8' E)	256	7.4	8.0-8.5
B	*M. prunifolia*	Fuping, Shaanxi (34° 46' N, 109° 10' E)	588	10.6	6.5-7.6
C	*M. robusta*	Huailai, Hebei (40° 24' N, 115° 32' E)	413	10.5	7.5-8.5
D	*M. mandshurica*	Xingcheng, Liaoning (40° 38' N, 120° 41' E)	600	8.7	7.0-8.1
E	*M. hupehensis*	Pingyi, Shandong (35° 7' N, 117° 25' E)	785	13.2	6.5-7.5

higher tolerance to root-zone hypoxia while *M. sieversii* and *Malus toringoides* are more sensitive to such stress (Bai et al., 2008). Soil degradation, that is, the decline and loss of soil functions, is becoming more serious worldwide, threatening agricultural yields and terrestrial ecosystems (Chen et al., 2002). Like other crops, apple production in China is affected by soil salinization from drought in the northwest Loess Plateau area and by acidification due to rapid industrialization on the southwest plateau.

To overcome this problem, growers must select and utilize acid- or alkaline-tolerant wild *Malus* species, an approach that has been adopted with other crops (Bal and Dutt, 1986; Ashraf and Waheed, 1993; Nevo et al., 1993; Gossett et al., 1994; Zhen et al., 2010, 2011). Because of their demonstrated resistance to multiple stresses, *M. sieversii*, *Malus prunifolia, M. robusta, Malus mandshurica* and *M. hupehensis* are desirable seedling rootstocks for various apple production areas in China. Therefore, they were selected as materials for our research of their tolerance to acidic or alkaline growing conditions. Here, we focused on their relative changes in biomass and accumulations of MDA and soluble proteins when seedling roots were exposed to different pH values in hydroponic solutions.

MATERIALS AND METHODS

Plant materials

Five species of *Malus* -- *sieversii*, *prunifolia*, *robusta*, *mandshurica* and *hupehensis* – that originates from different climatic regions in China were used in these experiments (Table 1). Seeds were surface-sterilized in 0.3% (v/v) H_2O_2 for 20 min, then rinsed several times with sterile H_2O (Zhang et al., 2007). They were then stratified at 4℃ for 85 days. The germinants were sown in plastic pots (9 cm diameter, 12 cm high; 3 seeds per pot) filled with sterilized sand. All pots were placed in a greenhouse at the College of Horticulture, Northwest A&F University, Yangling (34° 20′ N, 108° 24′ E). Plants were grown under natural light, at 25/20℃ (day/night) and 65 to 80% relative humidity. They were watered weekly with Hoagland (1920) nutrient solution (pH 6.0 ± 0.2). When they reached the six-leaf stage, uniform seedlings were selected and transferred to a hydroponic system that consisted of plastic containers (70 × 50 × 20 cm) filled with 20 L of half-strength Hoagland nutrient solution (pH 7.0, electrical conductivity 1.2 ms cm^{-1}).

Nine containers were used for each species (50 seedlings per container were evenly separately fixed on the polyethylene bubble board through the hole), and all were placed in a controlled growth room (12 h photoperiod, 25/20℃ day/night and a photon flux density of 140 to 160 µmol m^{-2} s^{-1}). The seedlings were allowed to acclimate to these hydroponic conditions for 5 days.

pH treatments

To evaluate the effects of solution pH (5.5, 7.0 or 8.5) on seedling performance, each of the five species were treated in three replicated hydroponics containers. Values of pH for each solution were adjusted to acid (pH 5.5) or alkaline (pH 8.5) conditions with 76 ml of 1 M HCl or 46 ml of 1 M NaOH, respectively. The third level (pH 7.0), for the control was achieved by combining the solutions into one container filled with 20 L of half-strength Hoagland nutrient solution. These solutions were renewed every 5 days.

Measurements of seedling growth

Starting values were recorded on day 0 of the treatment period for fresh (FW) and dry weights (DW) of the whole plant, plant heights and root lengths. For FW, 10 seedlings per species were randomly sampled and weighed. These samples were then oven-dried at 70℃ for at least 72 h and re-weighed to establish their average initial DW. Another 10 seedlings randomly selected from each species, treatment, and replicate were marked and measured for initial values of plant height and root length. After 20 days of treatment, those previously marked seedlings were again assessed for FW, DW, height and root length. Another 10 seedlings randomly chosen from each species, treatment, and replicate were partitioned into root and stem portions, then quickly frozen in liquid nitrogen and stored at –70℃.

Determination of MDA, H_2O_2, and $O_2^{\cdot-}$ contents

We monitored lipid peroxidation by measuring the accumulation of malondialdehyde (MDA) according to the method of Baziramakenga et al. (1995), with some modifications. Root samples (0.5 g) were homogenized in 5 ml of 0.1% trichloroacetic acid (TCA) in phosphate buffer solution (PBS, pH 7.8) and centrifuged (12,000×g, 15 min). Supernatant (1 ml) was added to 0.5% thiobarbituric acid in 4 ml of 20% TCA. The mixture was placed in a water bath at 100℃ for 10 min, then quickly cooled in an ice bath for 15 min. Samples were centrifuged (12,000×g, 5 min), and absorbance of the supernatant was measured at 450, 532 and 600 nm. Hydrogen peroxide (H_2O_2) was measured according to a method described by Patterson et al. (1984). Frozen roots were homogenized in acetone, at a ratio of 1 g of sample: 2 ml of ice-cold acetone. Titanium reagent (2% $TiSO_4$) was added to a known volume of extract supernatant to give a Ti concentration of

2%. The Ti–H_2O_2 complex, together with untreated Ti was then precipitated by adding 0.2 ml of 17 M ammonia solution per ml of extract. The precipitate was washed five times with ice-cold acetone by re-suspension, then drained and dissolved in 3 ml of 2 M H_2SO_4. Absorbance of the solution was measured at 410 nm against blanks that had been prepared similarly but without plant tissue.

The generation rate for the superoxide radical ($O_2^{\cdot-}$) was determined by the method of Elstner and Heupel (1976), with some modification. Briefly, 1 g of root tissue was ground with 4 ml of 65 mM PBS (pH 7.8) and centrifuged (5000×g, 10 min). The supernatant (1 ml) was mixed with 0.9 ml of 65 mM PBS (pH 7.8) and 0.1 ml of 10 mM hydroxylamine hydrochloride, and was incubated at 25°C for 20 min. Afterward, 17 mM sulfanilamide and 7 mM α-anaphthylamine were added to that mixture, followed by incubation at 25°C for 20 min. Light absorbance was measured at 530 nm.

A standard curve with the nitrogen dioxide radical (NO_2^-) was used to calculate the rate of $O_2^{\cdot-}$ generation.

Extraction and assay of antioxidant enzyme activities

Antioxidant enzymes were extracted from apple roots according to the method of Yu et al. (2003), with some modifications. Samples (0.5 g) were homogenized in 8 ml of 0.1 M phosphate buffer (pH 7.5) containing 2% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged (12,000×g, 20 min) and the supernatant was used for enzyme analysis. All assays were carried out at 2 to 4°C. Superoxide dismutase (SOD) activity was measured as described by Beauchamp and Fridovich (1971), with minor modification. The assay medium (3 ml) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid disodium salt (EDTA-Na), 12 mM L-methionine, 2 µM riboflavin, and 75 µM nitrotetrazolium blue chloride; the riboflavin was added last. Tubes were shaken and exposed to a photosynthetic photon flux of 50 µmol m^{-2} s^{-1} for 15 min. The reaction was initiated and terminated by turning the light on and off, respectively. Absorbance was measured spectrophotometrically at 560 nm; tubes containing the assay mixture but lacking root extract (control) also were illuminated to determine the net A_{560nm}. Peroxidase (POD) activity was measured according to the method of Sofo et al. (2004), with some modifications.

The reaction solution (3 ml) contained 2.9 ml of 50 mM phosphate buffer (pH 7.0), 50 µl of 10 mM guaiacol, 10 µl of 40 mM H_2O_2 and 40 µl of crude enzyme extract. The increase in absorbance at 470 nm, due to the oxidation of guaiacol was measured at 20°C. Catalase (CAT) activity was assayed by monitoring the decrease in A_{240nm} (Aebi, 1984). The mixture contained 50 mM phosphate buffer (pH 7.0) and 30% H_2O_2 (w/v), and the mixture was started by adding the solution to 10 µl of crude extract. Activities of ascorbate peroxidase (APX) and glutathione reductase (GR) were assayed using the method described by Ma and Cheng (2003). Root tissues (0.5 g) were extracted with 8 ml of 50 mM PBS (pH 7.5) containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol, 0.1% triton X-100 and 2% PVP-3000. The homogenates were centrifuged at 16,000×g for 20 min at 4°C and the supernatants were collected. APX activity was monitored at 290 nm in 3 ml of a reaction mixture containing 50 mM Hepes-KOH (pH 7.6), 0.1 mM EDTA, 1 mM H_2O_2, 0.5 mM reduced ascorbate (AsA) and 0.05 ml enzyme extract. The reaction was initiated by adding the peroxide.

One unit of APX activity was the amount of APX catalyzing the oxidation of 1 mmol ascorbate per min. GR activity was monitored at 340 nm in 3 ml of a reaction mixture containing 100 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1 mM oxidized glutathione (GSSG), 0.2 mM NADPH and 0.1 ml enzyme extract. The reaction was initiated by adding NADPH. One unit of GR activity was defined as the amount of reduction of 1 mmol GSSG per min.

Determination of AsA and GSH contents

Samples (0.5 g) were homogenized in 8 ml of 6% (w/v) TCA that had been pre-cooled on ice. The extract was then centrifuged at 12,000×g for 20 min. The AsA content was determined according to the method of Kampfenkel et al. (1995) while GSH was assayed as described by Griffith (1980). Non-protein thiols were extracted by homogenizing the samples in 5% (w/v) sulfosalicylic acid on ice before centrifugation (10,000×g, 20 min). The supernatants were collected for analysis. GSH content was determined in neutralized samples after GSSG was reduced with yeast-GR, 5,5-dithio-bis-nitrobenzoic acid and NADPH. Readings were taken with a spectrophotometer at 412 nm.

Statistical analysis

All data were subjected to ANOVA using a SPSS Statistical Package (version 13.0). Results were presented as means ± standard deviation (SD).

RESULTS

Effects of pH on growth

Growth of *M. sieversii* seedlings was significantly stimulated at pH 8.5 compared with that at pH 7.0 (control). Fresh weights, dry weights, and root lengths were significantly increased at 111.39, 114.30 and 86.47%, respectively (Figure 1a, b, d). Although, heights also changed, their increase did not differ significantly from the control (Figure 1c). By contrast, growth of *M. prunifolia* seedlings was significantly inhibited at pH 8.5 compared with their performance at pH 7.0. Values for FW, DW, height, and root length at the higher pH were 56.99, 49.73, 37.07 and 53.73% lower, respectively, compared with measurements at pH 7.0 (Figure 1a to d). Seedlings of *M. hupehensis* grown at pH 8.5 were significantly smaller than those at pH 7.0, with FW, DW, height, and root length being 56.99, 49.73, 37.54 and 41.36% less, respectively, than the control. Likewise, dry weights for *M. sieversii* and *M. robusta* were 22.16 and 44.97% less, respectively, at pH 5.5 than at pH 7.0.

Meanwhile, no significant difference from the control was found for growth of *M. robusta* and *M. mandshurica* at pH 8.5 or for *M. prunifolia*, *M. mandshurica*, and *M. hupehensis* seedlings at pH 5.5.

Effects of pH on MDA and H_2O_2 contents, and $O_2^{\cdot-}$ generation rate

Compared with the control (pH 7.0), MDA and H_2O_2 content and the rate of $O_2^{\cdot-}$ generation by roots from *M. sieversii* grown at pH 8.5 were reduced significantly by 65.54, 32.12 and 39.84%, respectively (Figure 2a to c). However, those same parameters were increased significantly in *M. prunifolia* roots at pH 8.5, rising by 172.53, 129.53 and 119.58%, respectively, versus the control. For *M. hupehensis* at pH 8.5, MDA, H_2O_2, and $O_2^{\cdot-}$ generation

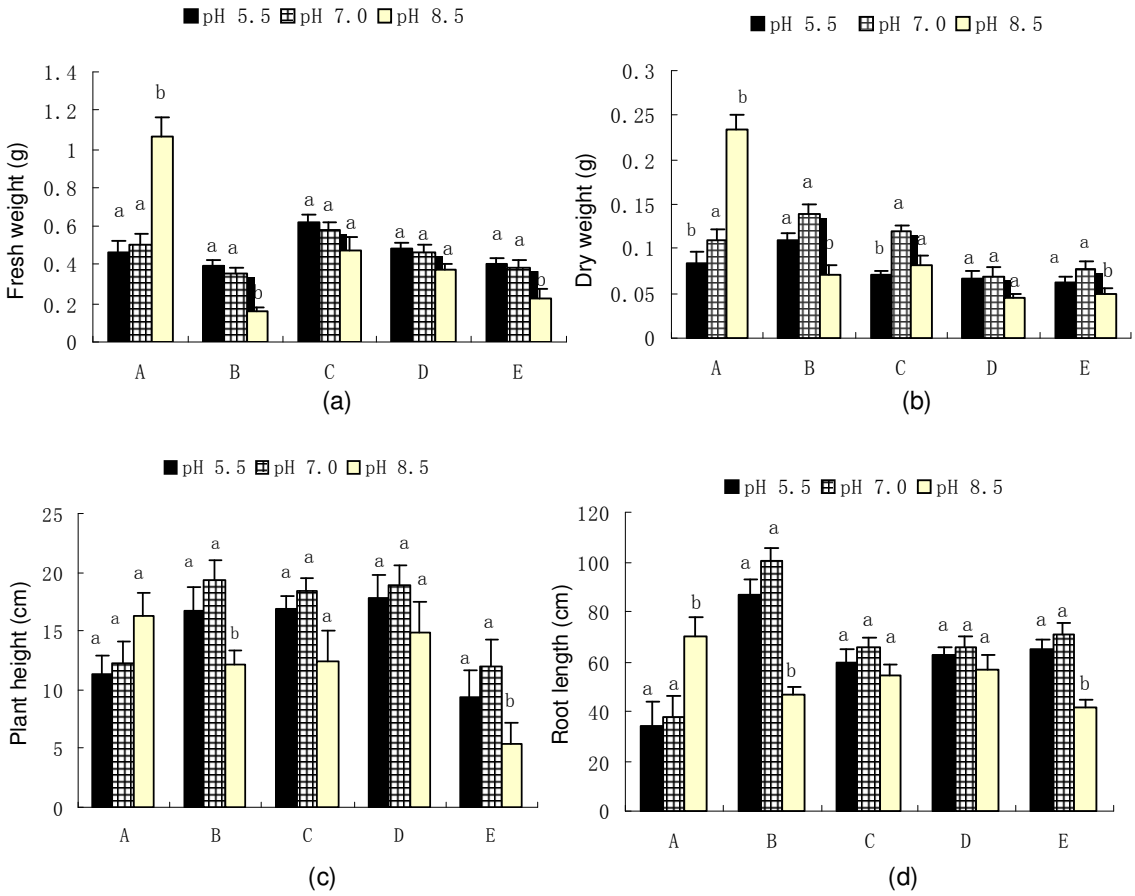

Figure 1. Fresh weights (a), dry weights (b), plant heights (c), and root lengths (d) from *Malus sieversii* (A), *M. prunifolia* (B), *M. robusta* (C), *M. mandshurica* (D), and *M. hupehensis* (E) grown hydroponically at pH 5.5, 7.0 (control) or 8.5. Seedlings were sampled on Days 0 and 20 of treatment. Values are means of 3 replicates ± standard error (SE). Significant difference ($P<0.05$ level) was tested between control and pH 5.5 or pH 8.5 for each dependent variable and indicated by different letters above bars.

also were significantly enhanced by 110.36, 171.06 and 154.44%, respectively, as were those for *M. sieversii* at pH 5.5 (43.71, 65.33 and 15.78%, respectively). Roots from *M. robusta* grown at pH 5.5 also showed significantly higher values for MDA and H_2O_2 contents, and $O_2^{\cdot-}$ generation, rising by 74.40, 82.41 and 61.11% respectively, over the control. By comparison, those parameters did not differ from the control for seedlings of *M. robusta* and *M. mandshurica* at pH 8.5 and 5.5 or for *M. prunifolia*, *M. mandshurica* and *M. hupehensis* at pH 5.5.

Effects of pH on SOD, POD, CAT, APX and GR activities

Activities of the SOD, POD, CAT, APX and GR enzymes from roots of *M. sieversii* at pH 8.5 were 33.86, 24.46, 20.55, 26.76 and 32.91%, respectively lower than those grown at pH 7.0 (Figure 3a to f). By contrast, these activities were increased statistically within *M. prunifolia* roots treated at pH 8.5, being enhanced by 65.28, 52.02, 45.89, 70.69 and 67.36%, respectively, over the control. At pH 8.5, those respective activities in *M. hupehensis* were significantly higher, by 80.50, 55.90, 72.49, 107.70 and 86.90%, than for control seedlings. This response was also true for *M. sieversii* (significant increases of 57.62%, SOD; 24.69%, APX; and 26.23%, GR) and for *M. robusta* (49.75%, SOD; 26.68%, POD; 27.22%, CAT; 56.10%, APX and 33.33%, GR). However, when compared with the control, no significant change in activity was found for *M. robusta* and *M. mandshurica* at pH 8.5 or for *M. prunifolia*, *M. mandshurica* and *M. hupehensis* at pH 5.5.

Effects of pH on AsA and GSH contents

Ascorbate and glutathione contents in *M. sieversii* roots were 46.83 and 43.40% lower, at pH 8.5 than at pH 7.0 respectively (Figure 3f, g), while those of *M. prunifolia* at pH 8.5 were significantly higher than the control, by 44.10%

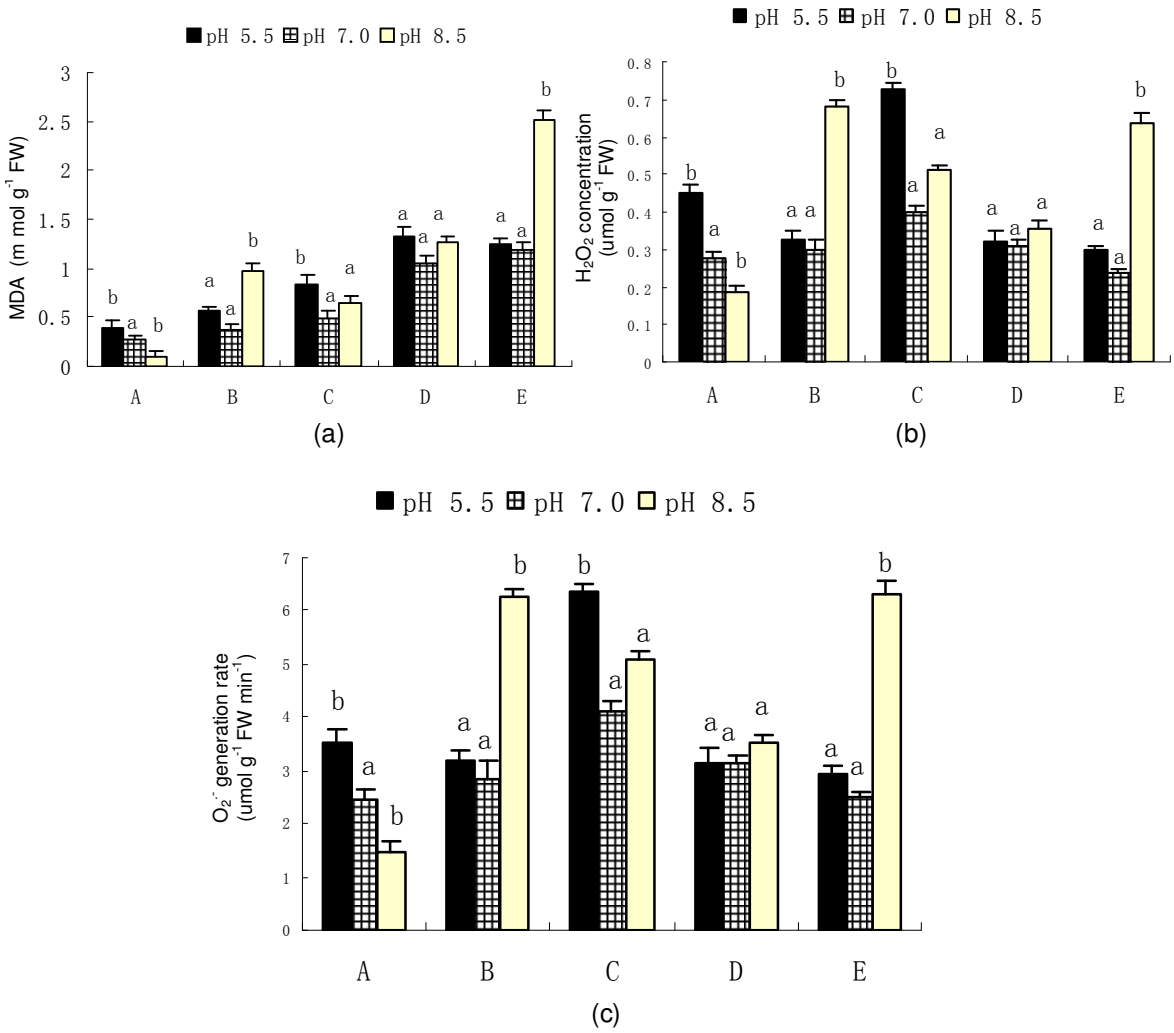

Figure 2. MDA (a) and H_2O_2 (b) content, and rate of $O_2^{\cdot-}$ generation (c) in roots from *Malus sieversii* (A), *M. prunifolia* (B), *M. robusta* (C), *M. mandshurica* (D), and *M. hupehensis* (E) grown hydroponically at pH 5.5, 7.0 (control) or 8.5. Seedlings were sampled on Day 20 of treatment. Values are means of 3 replicates ± standard error (SE). Significant difference ($P<0.05$ level) was tested between control and pH 5.5 or 8.5 treatment for each dependent variable and indicated by different letters above bars.

(AsA) and 58.72% (GSH). This was also true for *M. hupehensis*, with significant increases of 75.42% (AsA) and 92.23% (GSH). At a pH of 5.5, those contents in *M. sieversii* were also significantly higher than those from the control; that is, by 35.10% (AsA) and 49.20% (GSH). The same trend was found with *M. robusta* (40.63%, AsA and 52.35%, GSH). As it was found with enzyme activities, changing the pH value of the solution from 7.0 did not significantly influence AsA or GSH contents in *M. robusta* and *M. mandshurica* at pH 8.5 or *M. prunifolia*, *M. mandshurica* and *M. hupehensis* at pH 5.5.

DISCUSSION

We monitored the tolerance of five wild species of *Malus* when exposed to acid or alkaline conditions in a hydroponics system. All have long been used in China as seedling apple rootstocks or base rootstocks for dwarf inter-stem nursery trees (Zhou, 1999). Our findings are useful both for understanding the underlying resistance physiology of these important but scarce resources and for the more applied goal of developing elite dwarf multi-resistance apple rootstock that is better suited to modern production approaches. Acid and alkali resistance can be evaluated by determining relative changes in plant biomass, the accumulation of MDA and H_2O_2, and the rate of $O_2^{\cdot-}$ generation (Hannon and Bradshaw, 1968; Dhindsa et al., 1981; Francois et al., 1986; Zhu, 2001; Apel and Hirt, 2004; Gong et al., 2011). The generation of oxidative stress in plant cell is because an imbalance between reactive oxygen species (ROS) and their removal

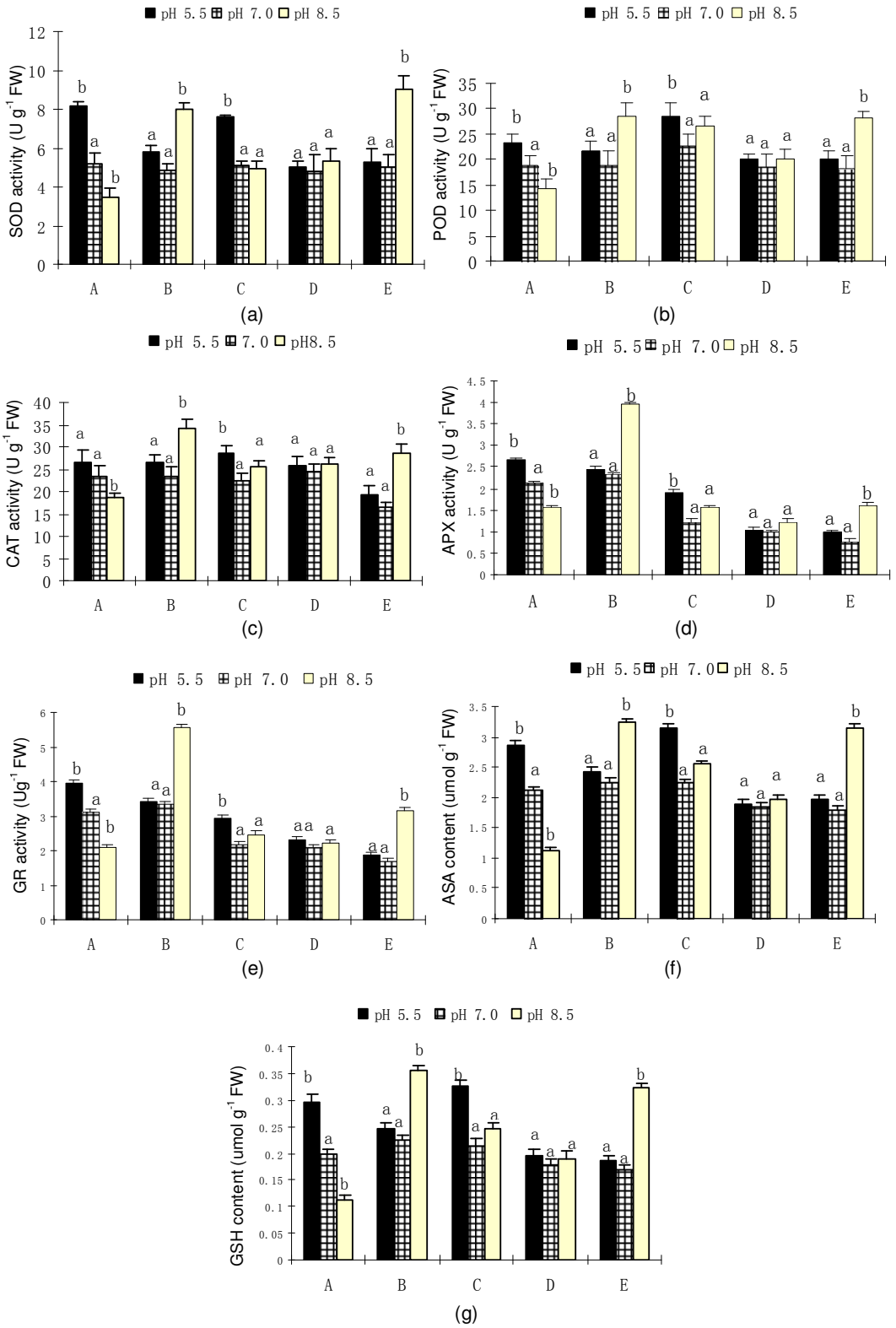

Figure 3. Activities of SOD (a), POD (b), CAT (c), APX (d), and GR(e), and AsA (f) and GSH (g) content in roots from *Malus sieversii* (A), *M. prunifolia* (B), *M. robusta* (C), *M. mandshurica* (D), and *M. hupehensis* (E) grown hydroponically at pH 5.5, 7.0 (control) or 8.5. Seedlings were sampled on day 20 of treatment. Values are means of 3 replicates ± standard error (SE). Significant difference ($P<0.05$ level) was tested between control and pH 5.5 or 8.5 treatment for each dependent variable and indicated by different letters above bars.

removal makes macromolecules and membranes damaged, thus leads to the reduction of plant growth. The control plant possesses its own active antioxidant defense systems (antioxidative enzymes such as SOD, CAT, and APX, as well as nonenzymatic antioxidants such as AsA and GSH) through which production and removal of ROS is in balance. SOD can convert $O_2^{\cdot-}$ into H_2O_2 and O_2; moreover, CAT, and APX can reduce H_2O_2 into H_2O and O_2 (Asada, 1999). The defense capability of antioxidant systems was related to plant species (Bai et al., 2010; Zhen et al., 2010, 2011). Here, we identified *M. sieversii* as being alkali-tolerant because it had significant increases in fresh and dry weights, plant heights, and root lengths, as well as less accumulation of MDA and H_2O_2 and a lower rate of $O_2^{\cdot-}$ generation at a higher pH (8.5).

In contrast, *M. robusta* and *M. hupehensis* were found to be alkali-sensitive and *M. sieversii* and *M. mandshurica* were acid-sensitive based on their significant reductions in fresh and dry weights, heights, and root lengths when the solution pH was altered. They also showed greater accumulations of MDA and H_2O_2 and a faster rate of $O_2^{\cdot-}$ generation when the pH was adjusted from a neutral 7.0. The significant increases in antioxidant enzyme activities, and contents of ascorbate and glutathione led to those significant accumulations and enhanced $O_2^{\cdot-}$ generation. Under acidic conditions, fresh weights, plant heights, and root lengths were not significantly reduced in *M. sieversii* and *M. mandshurica*, although they showed declines in dry weights, MDA and H_2O_2 accumulations and $O_2^{\cdot-}$ generation because those plants more easily absorbed water.

Conclusion

In summary, *M. sieversii*, *M. mandshurica* and *M. robusta* showed normal growth at pH 7.0 to 8.5; *M. prunifolia*, *M. robusta*, and *M. hupehensis*, at pH 5.5 to 7.0. Therefore, we recommend that *M. sieversii*, *M. mandshurica*, and *M. robusta* are the best selections for general rootstocks but that *M. robusta* and *M. hupehensis* should be avoided in regions with high pH. Furthermore, seedling rootstocks would be best chosen from *M. prunifolia*, *M. robusta*, and *M. hupehensis* whereas *M. sieversii* and *M. mandshurica* are not suitable for soils with low pH values. We also believe that *M. sieversii* would be appropriate as a parent for breeding alkali-tolerant apple dwarf rootstock.

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REFERENCES

Aebi H (1984). Catalase *in vitro*. Meth Enzymol., 105: 121-126.

Apel K, Hirt H (2004). Reactive oxygen species: Metabolism, oxidative stress and signal transduction. Annu Rev Plant Biol., 55: 373-399.

Asada K (1999). The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol., 50: 601–639.

Ashraf M, Waheed A (1993). Responses of some local/exotic accessions of lentil (*Lens culinaris* Medic.) to salt stress. Crop Sci, 170: 103-112.

Bai TH, Ma FW, Li CY, Shu HR, Han MY, Wang K (2008). Physiological responses and analysis of tolerance of apple rootstocks to root-zone hypoxia stress (in Chinese). Sci Agric Sin, 41(12): 4140-4148.

Bai TH, Li CY, Ma FW, Feng FJ,Shu HR (2010). Responses of growth and antioxidant system to root-zone hypoxia stress in two *Malus* species. Plant and Soil, 327: 95-105.

Bal AR, Dutt SK (1986). Mechanism of salt tolerance in wild rice (*Oryza coarctata* Roxb.) Plant Soil, 92: 399-404.

Baziramakenga R, Leroux GD, Simard RR (1995). Effects of benzoic and cinnamic acids on membrane permeability of soybean roots. J Chem Ecol., 21: 1271-1285.

Beauchamp C, Fridovich I (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analyt Biochem, 44: 276-287.

Cao H, Xu XF, Han ZH, Wang XW, Guo TQ, (2004). Changes of physiological characteristic on photosynthesis in *Malus* seedling leaves during water stress(in Chinese). Acta Hortic Sin 31: 285-290.

Chen J, Tan M (2002). Soil degradation: A global problem endangering sustainable development. J Geog Sci., 12(2): 243-252.

Dhindsa RS, Plumb-Dhindsa P, Throne TA (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. J Exp Bot., 32: 93–101.

Dzhangaliev AD (2003). The wild apple tree of Kazakhstan. Hortic Rev 29: 63-303.

Elstner EF, Heupel A (1976). Inhibition of nitrite formation from hydroxylammonium-chloride: A simple assay for superoxide dismutase. Analyt Biochem, 70: 616-620.

Forsline PL, Aldwinckle HS (2004). Evaluation of *Malus sieversii* seedling populations for disease resistance and horticultural traits. Acta Hortic, 663: 529-534.

Francois LE, Maas EW, Donovan TJ, Youngs VL (1986). Effect of salinity on grain yield and quality, vegetative growth, and germination of semi-dwarf and durum wheat. Agron, J., 78: 1053.

Geibel M, Dehmer KJ, Forsline PL (2000). Biological diversity in *Malus sieversii* populations from Central Asia. Acta Hortic, 538: 43-49.

Gong XL, Liu C, Zhou M, Hong MM, Luo LY, Wang L, Wang Y, Cai JW, Gong SJ, Hong FS (2011). Oxidative damages of maize seedlings caused by exposure to a combination of potassium deficiency and salt stress. Plant Soil, 340: 443–452.

Gossett DR, Millhollon EP, Lucas MC (1994). Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci, 34: 706-714.

Griffith OW (1980). Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Analyt Biochem, 106: 207-212.

Hannon N, Bradshaw AD (1968). Evolution of salt tolerance in two contrasting species of grass. Nature, 220: 1342-1343.

Hoagland DR (1920). Optimum nutrient solutions for plants. Science, 52: 562-564.

Kampfenkel K, van Montagu M, Inzé D (1995). Extraction and determination of ascorbate and dehydroascorbate from plant tissue. Analyt Biochem, 225: 165-167.

Liu J, Zhou QH, Sun HW, Qiu ZL, Wang YW, Sun ZX (2004). Study on the phenotype biodiversity of Xinjiang wild apples (*Malus sieversii*) (in Chinese). J Fruit Sci., 21: 285-288.

Ma FW, Cheng LL (2003). The sun-exposed peel of apple fruit has higher xanthophyll cycle-dependent thermal dissipation and antioxidants of the ascorbate glutathione pathway than the shaded peel. Plant Sci 165: 819-827.

Ma W, Ma FW, Mi F, Ma H, Shu R (2008). Morphological and physiological responses of two contrasting *Malus* species to exogenous abscisic acid application. Plant Growth Regul, 56: 77-87.

Manici LM, Ciavatta C, Kelderer M, Erschbaumer G (2003). Replant

problems in South Tyrol: Role of fungal pathogens and microbial population in conventional and organic apple orchards. Plant Soil, 256: 315-324.
Mano Y, Takeda K (1998). Genetic resources of salt tolerance in wild *Hordeum* species. Euphytica, 103: 137-141.
Nevo E, Krugman T, Beiles A (1993). Genetic resources for salt tolerance in the wild progenitors of wheat (*Triticum dicoccoides*) and barley (*Hordeum spontaneum*) in Israel. Plant Breed, 110: 338-341.
Iriki N, Kawakami A, Takata K, Kuwabara T, Ban T (2001). Screening relatives of wheat for snow mold resistance and freeing tolerance. Euphytica, 122: 335-341.
Patterson BD, Macrae EA, Ferguson IB (1984). Estimation of hydrogen peroxide in plant extracts using titanium (IV). Anal. Biochem., 139: 487-492.
Richards CM, Volk GM, Reilley AA, Henk AD, Lockwood DR, Reeves PA, Forsline PL (2009). Genetic diversity and population structure in *Malus sieversii*, a wild progenitor species of domesticated apple. Tree Genet Genom 5: 339-347.
Schrader LE, Zhang J, Duplaga WK (2001). Two types of sunburn in apple caused by high fruit surface (peel) temperature. Plant Health Prog. doi:10.1094/PHP-2001-1004-01-RS.
Schrader L, Sun J, Felicetti D, Seo JH,Jedlow L, Zhang J (2003). Stress-induced disorders: Effects on apple fruit quality. Washington Tree Fruit Postharvest Conference, Wenatchee, WA, USA. http://postharvest. tfrec.wsu.edu/PC2003A.pdf.
Sofo, A, Dichio, B, Xiloynnis, C, and Masia, A (2004). Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. Plant Sci., 166: 293-302.
Volk GM, Richards CM, Reilley AA, Henk AD, Forsline PL, Aldwinckle HS (2005). Ex situ conservation of vegetatively propagated species: Development of a seed-based core collection for *Malus sieversii*. J Amer Soc Hortic. Sci., 130: 203-210.
Yan R, Long H, Song Q, Chen Y (2008). Genetic polymorphism of *Malus sieversii* populations in Xinjiang, China. Genet Resour Crop Evol., 55: 171-181.
Yu JQ, Ye SF, Zhang MF, Hu WH (2003). Effects of root exudates and aqueous root extracts of cucumber (*Cucumis sativus*) and allelochemicals, on photosynthesis and antioxidant enzymes in cucumber. Biochem. Syst. Ecol., 31: 129-139.
Zhang JH, Mao ZQ, Wang LQ, Shu HR (2007). Bioassay and identification of root exudates of three fruit tree species. J. Integr. Plant Biol., 49: 257-261.
Zhen A, Bie ZL, Huang Y, Liu ZX, Lei B (2011). Effects of salt-tolerant rootstock grafting on ultrastructure, photosynthetic capacity, and H_2O_2-scavenging system in chloroplasts of cucumber seedlings under NaCl stress. Acta Physiol. Plant, 33: 2311–2319.
Zhen A, Bie ZL, Huang Y, Liu ZX, Li Q (2010). Effects of scion and rootstock genotypes on the antioxidant defense systems of grafted cucumber seedlings under NaCl stress. Soil Sci. Plant Nutr., 56: 263–271.
Zhou Z (1999). The apple genetic resources in China: The wild species and their distribution, informative characteristics and utilization. Genet. Resour. Crop Evol., 46(6): 599-609.
Zhu JK (2001). Plant salt tolerance. Trends Plant Sci., 6: 66-71.