Int. J. Sudan Research, Vol. 7, No. 1, 2017

EVALUATION OF THE IRON CONTRIBUTION TO SALT STRESS RESPONSES OF TWO *MALUS* SPECIES AND ITS POSSIBLE MECHANISMS

Siham Ibrahim Babiker, Meiling Zhang, Wei Liu, Yi Wang, Ting Wu, Xinzhong Zhang and Zhenhai Han*

Institute for Horticultural Plants,
China Agricultural University,
Yuanmingyuan West Road,
Haidian District, Beijing 100193, China
Tel.: +86-010-62732467
Fax: +86-010-62732467

E-mail: Sihba2003@yahoo.com

E-mail: zml.1989.jayou@163.com

E-mail: 1012226282@qq.com

E-mail: Wangyi@cau.edu.cn

E-mail: Cain77310@163.com

E-mail: zhangxinzhong999@126.com

E-mail: rschan@cau.edu.cn

*Corresponding author

ABSTRACT

Purpose: The study aimed to evaluate the effects of iron (Fe) availability on physiological and biochemical responses of salt stress in two apple species (*Malus xiaojinensis* and *Malus baccata*).

Design: The experiment was conducted under growth chamber conditions in a completely randomised design with three replications. Apple seedlings were exposed to 0 and 75 mM NaCl with either 100 μM Ferrozine (−Fe) or 40 μM FeNaEDTA (+Fe) in Hoagland nutrient solution. Roots and young leaves were collected after 0, 1, 3 and 6 days of treatments.

Findings: Under iron deficiency, salt stress increased the expression of *MdSOS1* and *MdAPX1* genes in roots and leaves of *M. xiaojinensis* and decreased the expression of *MxFIT*, *MxIRT* and *MxHA2* in roots, while the expression was slightly increased in *M. baccata*. Rhizosphere pH of both genotypes was increased by salt stress under examined iron levels. Under iron deficiency salt stress accumulated less Na^+, H_2O_2 and leakage in electrolyte, and more Fe^{2+}, K^+ in *M. xiaojinensis* tissues compared to *M. baccata* ones.

Value: The fi ndings indicated that under salt and iron stresses *M. xiaojinensis* tends to absorb more Fe^{2+} compared to *M. baccata*. Iron deficiency has a positive influence on *M. xiaojinensis* and could be used to minimise the negative impact of salt stress and thus contribute into enhancing salt stress tolerance.

***Keywords*:** apple; salt stress; *Malus xiaojinensis*; *Malus baccata*; salt stress tolerance; iron deficient; iron sufficient.

Reference to this paper should be made as follows: Babiker, S.I., Zhang, M., Liu, W., Wang, Y., Wu, T., Zhang, X. and Han, Z. (2017) 'Evaluation of the Iron Contribution to Salt Stress Responses of Two *Malus* Species and its Possible Mechanisms', *Int. J. Sudan Research*, Vol. 7, No. 1, pp.15–29.

INTRODUCTION

Salt stress is one of the most important stress factors that limit crop production. About 20% of the world's cultivated areas and 50% of crop land are salt affected (Flowers and Yeo, 1995). Fruit tree are one of the most affected crops by salt stress. Hence, developing and using of salt-tolerant rootstock is essential for sustaining production.

Malus xiaojinensis and *Malus baccata* are important apple rootstocks and are mainly using in Northern China (Han et al., 1994; Zha et al., 2014). *M. xiaojinensis* is salt-tolerant genotype (Han et al., 2006; Yin et al., 2010) in contrast to *M. baccata*, which is salt-sensitive one (Han et al., 2006). Also *M. xiaojinensis* was the most Fe-efficient genotype compared with *M. baccata* which is Fe-inefficient (Han et al., 1998).

Under salt stress, increasing Na^+ reduce the availability of K^+ thus lowering the K^+/Na^+ ratio in the cytosol and leading to inhibition of enzymatic activity (Zhu, 2003). Additionally, the concentration of Na^+ in the soil solution affects the transportation of water from soil to plant cells (Cabanero et al., 2006). Furthermore osmotic stress induces oxidative destruction of plant cells and enhanced generation of Reactive Oxygen Species (ROS) which including O_2, H_2O_2 and OH (Olías et al., 2009). These ROS are highly reactive and cause damage to membranes, protein and nucleic acids (Yasar et al., 2006); they also cause lipid peroxidation, protein denaturation and DNA mutation (Imlay and Linn, 1988).

Influx and efflux of Na^+ and K^+ through membranes is one of the mechanisms that plants use to tolerate salt stress (Gálvez et al., 2012). The Salt Overly Sensitive (*SOS1*) gene is located in the plasma membrane and is essential for plant salt tolerance (Shi et al., 2000). Mutant plants lacking a *SOS1* protein are particularly sensitive to NaCl stress (Zhu et al., 1998). However, overexpression of *SOS1* is restricted the accumulation of Na^+ in plant cells and improved salt tolerance (Yue et al., 2012). Moreover, *SOS1* is essential for maintaining ion homeostasis and partitioning of Na^+ in plant organs under salt stress (Olías et al., 2009).

To prevent cellular components damage by ROS, plants develop antioxidant mechanisms which can be enzymatic and non-enzymatic (Azevedo-Neto et al., 2006). Hydrogen peroxide (H_2O_2) is one of ROS that induces damages to plasma membrane (Mittler, 2002). Ascorbate Peroxidase Enzyme (APX) detoxifies H_2O_2 to H_2O and O_2 in the chloroplasts, cytosol and mitochondria (Shigeoka et al., 2002). APX activity has been shown to increase in response to iron stress (Vansuyt et al., 1997) and salt stress (Tsai et al., 2004). Over-expression of *APX* gene increased the protection to salt stress (Badawi et al., 2004). However, the expression was higher in salt tolerant cultivars than in sensitive ones (Mhadhbi et al., 2011).

Iron (Fe) is essential in plant growth and development as an important component and cofactor of many enzymes (Sharma, 2006). Despite the abundance of iron in the soils, it is not available for plants due to its existence in form of Fe^{3+} which has low solubility especially in soils with high pH, and its potentiality to react with reduced forms of oxygen (Guerinot and Yi, 1994). Iron deficiency significantly decreases fruit yield and quality (Tagliavini and Rombolà, 2001). Plant species evolved specific mechanisms to uptake iron effectively from soil 'strategy I and strategy II' (Kobayashi et al., 2006). *Malus* species use strategy I mechanism (Han et al., 1998), in this mechanism the solubility of iron is enhanced by H^+ efflux mediated by plasma membrane H^+-ATPase. In previous studies in cucumber (Santi et al., 2005) and Arabidopsis (Colangelo and Guerinot, 2004; Fox and Guerinot, 1998), they found that *CsHA1, AHA2* and *AHA7* were expressed strongly in roots under iron deficiency, additionally, *MxHA2* and *MxHA7* in *M. xiaojinensis* roots (Huai et al., 2012; Zha et al., 2014). In iron stress tolerant plants, iron reduction and transportation are induce by *FRO* and *IRT* genes, respectively. This uptake system is regulated by the transcription factor *FIT* (Colangelo and Guerinot, 2004; Yin et al., 2014).

Salt stress has a negative effect in the activities of root PM H^+-ATPase. Therefore root acidification was significantly reduced in plants subjected concurrently to salt and iron stresses (Rabhi et al., 2007). Witzel et al. (2009) stated that the expression of some proteins involved in iron uptake by barley plant was declined significantly under salt stress. Similarly, it was found that the relative uptake of iron by barley and rye decreased with increasing salinity and it was suggested that, at high salinity, the synthesis pathway of iron chelators and uptake systems for iron as well as the transporters for acquisition of iron in *B. subtilis* are partially inhibited (Hoffmann et al., 2002; Jumberi et al., 2001).

Application of some iron compounds like Fe-EDDHA was reported to enhance salt stress tolerance in some crops such as tomato (Chougui et al., 2004) and pea (Nenova, 2008). Also interaction effect between iron and salt stresses was found. These results are probably due to effect of iron on ionic stress or oxidative stress in plant cells. However, the effect through molecular mechanism of resistance to salt stress is a little in higher plant especially fruit trees. Therefore, the objective of this study is to evaluate the effect of iron availability through physiological, biochemical and molecular mechanisms of plant resistance in two apple genotypes with contrasting responses regarding salt tolerance and iron uptake efficiency.

MATERIALS AND METHODS

Plant materials and growth conditions

Seedlings of *M. xiaojinensis* and *M. baccata* were propagated on Murashige and Skoog (MS) medium containing 0.5 mg/L, 6-Benzylaminopurine (6-BA), and 0.5 mg/L Indole-3-Butytric Acid (IBA) and transplanted in a growth chamber at 25 ± 2°C and 14 hr photoperiod (2000 lx) for

one month, then returned to MS + 1.0 mg/L IBA for two weeks for rooting. Rooted seedlings were moved to Hoagland nutrient solution in plant growth chamber under a 16 hr light and 8 hr dark photoperiod, with 250 μmol quanta m^{-2} s^{-1} light intensity. Relative humidity was 85% and temperatures were 23–25°C and 19–21°C during the day and night, respectively with daily ventilation. At 8–9 mature leaves (fully expanded) stage, the plants were exposed to 0 mM (Z) or 75 mM NaCl (S) with either 100 μM Ferrozine (−Fe) or 40 μM FeNaEDTA (+Fe) in Hoagland nutrient solution. Experiment was conducted with three replications in a completely randomised design. Roots and young leaves were collected after 0, 1, 3 and 6 days of treatments.

RNA extraction and quantification

Roots and young leaves were ground and total RNA was extracted using a modified Cetyltrimethylammonium Bromide (CTAB) method (Zhang et al., 2005). The DNA was digested using DNaseI (Takara). RNA concentration and purity was measured with spectrophotometer (ThermoScientifi c, Nanodrop, 2000) and gel electrophoresis. Total RNA was reverse transcribed to obtain the fi rst-strand cDNA using an oligo-dT primer and M-MLV reverse transcriptase-based cDNA fi rst-strand synthesis kit (Takara). Primers for analysed genes were designed using Primer Premier 5 software (Biosoft International, Palo Alto, CA, USA) according to their sequences in apple genome, Based on the BLASTP results for those proteins encoded by examined genes in NCBI (http://www.ncbi.nlm.nih.gov/). Specifi city of the primers (Table 1) was evaluated with Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Real-time PCR was performed using an AB7500 Real-time PCR System (Applied Biosystems, Foster City, CA) in total volume 20 μL, containing 2 μL of cDNA template, 0.3 μL of each primer, 0.4 μL of 50 × Rox, 10 μL of SYBR Green Master Mix and 7 μL of ddH_2O. Real-time PCR cycling was conducted with initially preheating at 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec and 60°C for 34 sec (Kürkcüoglu et al., 2007). *β-Actin* was used as internal control, and relative expression of target genes was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Each sample was detected in three technical replicates.

Table 1 Primer sequences for the quantification of transcripts by Real-Time PCR

Gene		*Primer Sequences*
MxFIT	Forward	5′ – GGGAAACCATCAAGGAGGTCATA – 3′
	Reverse	5′ – AGCCATTCATCATAAGGTCAGA – 3′
MxIRT	Forward	5′ – TTGACAAGGGAGAAAACGGAGAC – 3′
	Reverse	5′ – ACAACTGAATGGACAATGATACCC – 3′
MxHA2	Forward	5′ – GCCAAGGGTGTGGAGAAAGA – 3′
	Reverse	5′ – CTAGCGCGATGCCAATTTCC – 3′
MdSOS1	Forward	5′ – ATGGCAGTTACCGTGCAGAA – 3′
	Reverse	5′ – CAAGGATGAGCAGAGCGACA – 3′
MdAPX1	Forward	5′ – AGCGAAGAGTACAAGACGGC – 3′
	Reverse	5′ – CGATGTCGAGGCCATTGTTG – 3′
β-Actin	Forward	5′ – TGGTGAGGCTCTATTCCAAC – 3′
	Reverse	5′ – TGGCATATACTCTGGAGGCT – 3′

Measurement of the rhizosphere pH

The pH of the hydroponic culture solution were measured near the surface of the root (Five plants/treatment) using a pH meter (Series 400 and 500, Beckman Coulter, USA). The solution was adjusted to 6.30.

Measurement of hydrogen peroxide (H_2O_2)

Hydrogen peroxide was measured according to method described by Mukherjee and Choudhari (1983). Root samples were ground and 0.2 g of it was mixed with a cold acetone and the homogenised was centrifuged with 3000 rpm at 4°C. The supernatant was precipitated using $Ti(SO4)_2$ and ammonium solution (NH_3) and washed 3 to 5 times using acetone. The pellet was dissolved in 4 ml of 1 mol/L sulfuric acid (H_2SO_4) and the absorbance was measured at wavelength of 415 nm against blank with a UV spectrophotometer (UV-2550, Shimadzu, Japan). Hydrogen peroxide (H_2O_2) was calculated from the standard curve plotted with known concentration of hydrogen peroxide.

Measurement of Na^+, K^+ and Fe^{2+} concentrations

Collected root and leave samples were washed with distilled water and dried in an oven at 105°C for 30 min, followed by 80°C for seven days. Samples were separately ground to powder and 0.1 g of it was digested with 8 ml of 70% HNO_3 in a microwave oven at 180°C for 1 hr. After cooled to room temperature, they were adjusted to 25 ml using deionized water. Fe^{2+}, Na^+ and K^+ concentrations were measured by inductively coupled plasma atomic emission spectrometry (ICP, type P-4010, Hitachi, Japan) as described by Gao et al. (2011).

Assay of Electrolyte Leakage (EL)

EL was measured according to method described by Lee et al. (2002). Fully expanded leaves were removed from plants and washed with deionized water. Leaves were cut into pieces (1 cm $\times$ 1 cm) and incubated in test tubes with 20 ml of deionizer water and shaken for 24 hr at room temperature (25°C). Electrical Conductivity (EC1) of bathing solutions was measured with an EC meter (DDS-12A, KNGYI equipment, Shanghai, China). The test tubes were capped and autoclaved at 120°C for 20 min. EC of incubation solution (EC2) was determined after solution was cooled to the room temperature. EL was calculated as the ratio of conductivity before autoclaved (EC1) divided by that after autoclaved (EC2) and expressed as percentage.

Statistical analysis

All data were evaluated by Analysis of Variance (ANOVA), used the statistical software package STATISTIX 8. Differences between treatments were separated by Least Significant Differences (LSD) tests at a probability level ≤ 0.05.

RESULTS

The rhizosphere pH and H_2O_2 content in roots

In the two genotypes, the rhizosphere pH was increased when plants grown under salt stress with either iron-deficient or iron-sufficient compared with those not exposed to salt stress (Figure 1a). In *M. xiaojinensis*, the pH was still within acidity range under iron deficiency and shifted to alkali range under iron-sufficient, while the pH was within alkali range under both iron conditions in *M. baccata*. This result indicated that the capacity of root acidification was more in *M. xiaojinensis* than in *M. baccata* even under salt stress.

Under salt stress, H_2O_2 content was significantly increased either under iron-deficient or iron-sufficient conditions related to control (Z, +Fe) with a greater extent in *M. baccata*. However, the lower accumulation was appeared under iron deficiency compared to that under iron-sufficient (Figure 1b).

Accumulation of Na^+, K^+ and Fe^{2+} in roots and leaves, and EL in leaves

Under iron treatments, salt stress signifi cantly increased Na^+ concentration in roots and leaves of the two genotypes. But Na^+ concentration of leaves exhibited higher than roots. However, the concentration in leaves of *M. baccata* was more compared to *M. xiaojinensis.* Under iron

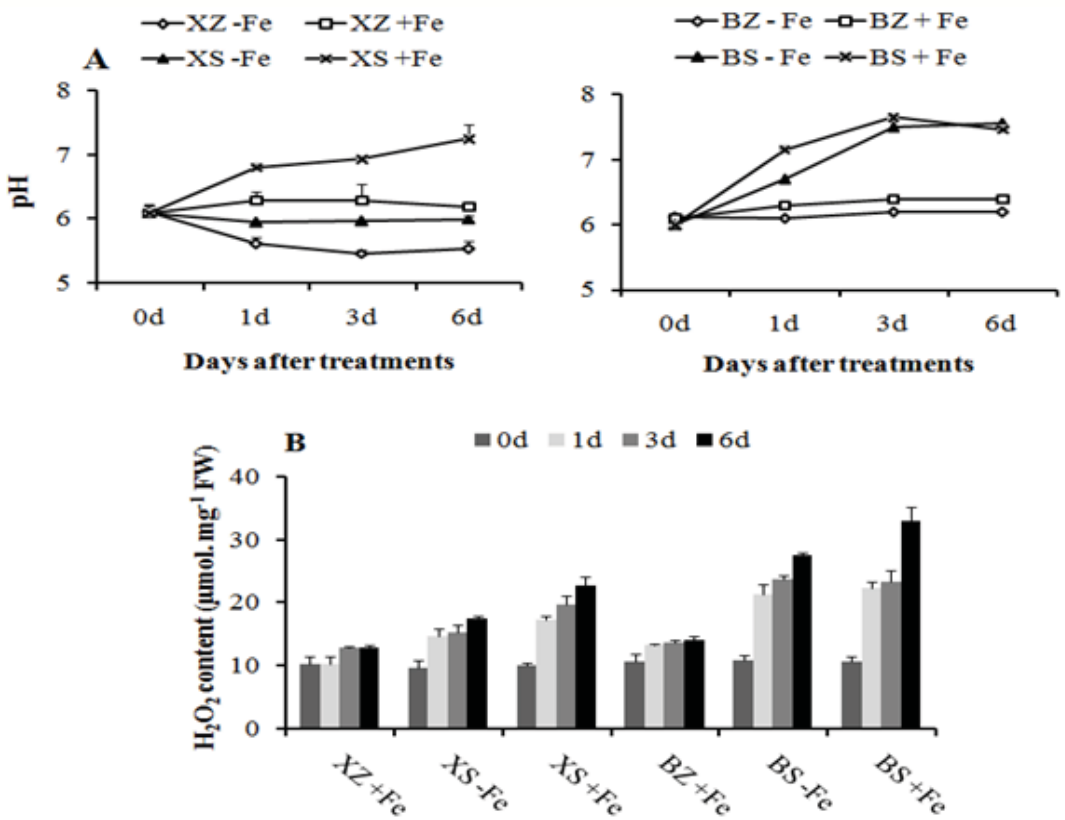

Figure 1 Rhizospheric pH and H_2O_2 content in roots of *M. xiaojinensis* (X) and *M. baccata* plants (B) hydroponically grown under salt stress (S) or without salt stress (Z) at iron-deficient (−Fe) or iron-sufficient (+Fe). (A) Rhizospheric pH. (B) H_2O_2 content. Mean values and standard deviations (bars) were obtained from three replicates

deficiency, salt stress decreased Na^+ accumulation by 52% in leaves of *M. xiaojinensis* compared to those under sufficient iron, but no different was observed in roots. In contrast in *M. baccata* tissues, Na^+ concentration was increased more under salt stress at deficient iron compared to sufficient one (Figure 2a, b).

Contrasting results were observed for K^+, where any increase in Na^+ was associated with decrease in K^+ and vice versa as shown in Figure 2c, d. Significant reduction was observed in the two genotypes of apple. In *M. xiaojinensis*, salt stress reduced K^+ concentration by 50% and 40% under iron-deficient in roots and leaves, respectively and by 68% under iron-sufficient in roots and leaves. In *M. baccata*, K^+ concentration reduced by more than 70% in both tissues at two iron conditions (Figure 2c, d).

Salt stress reduced Fe^{2+} concentration in the two genotypes either under deficient or sufficient iron. The reduction was clearly in *M. baccata* than in *M. xiaojinensis* tissues (Figure 2e, f). In *M. xiaojinensis* under deficient iron and salt stress condition, Fe^{2+} concentration was

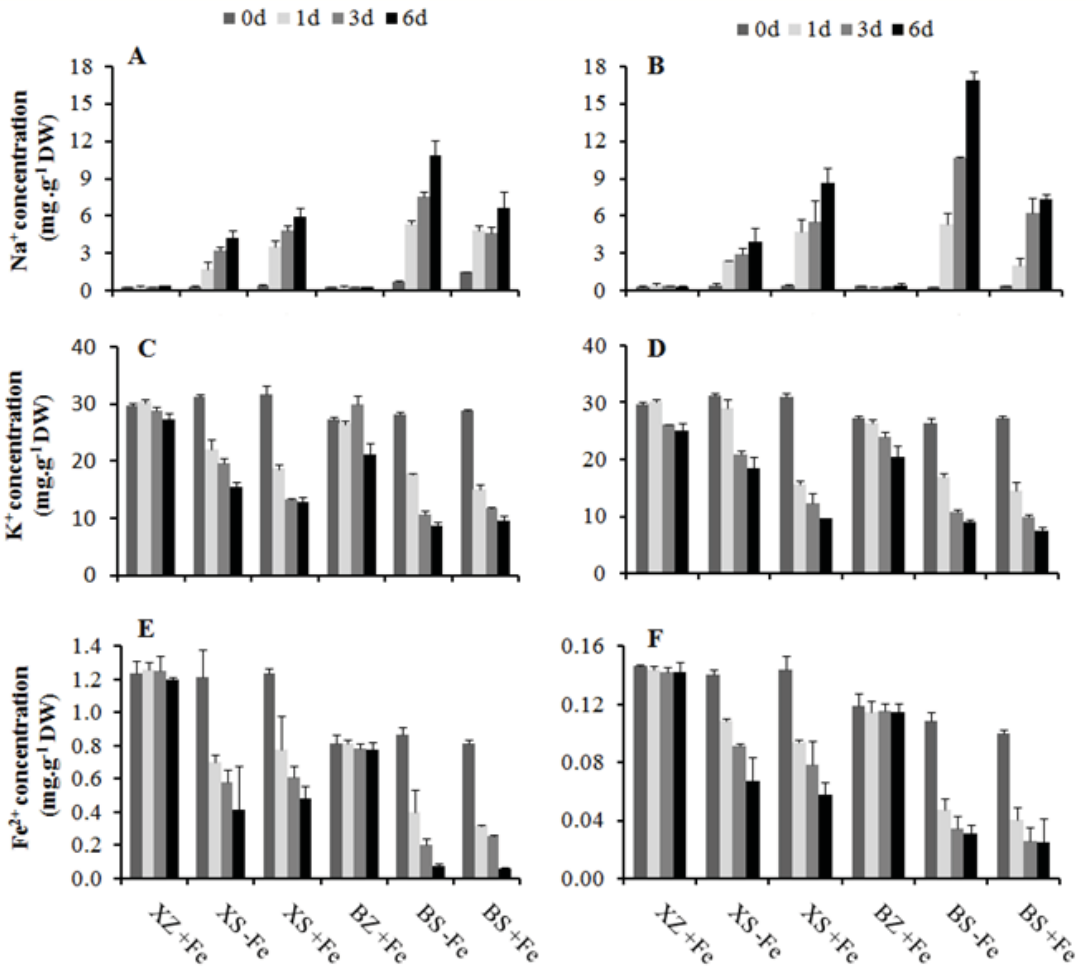

Figure 2 The concentrations of Na^+, K^+ and Fe^{2+} in roots and leaves of *M. xiaojinensis* (X) and *M. baccata* plants (B) hydroponically grown under salt stress (S) or without salt stress (Z) with deficient iron (−Fe) or sufficient iron (+Fe). (A, C and E) Na^+, K^+ and Fe^{2+} concentrations in roots, respectively. (B, D and F) Na^1, K^1 and Fe^{21} concentrations in leaves respectively. Mean values and standard deviations (bars) were obtained from three replicates

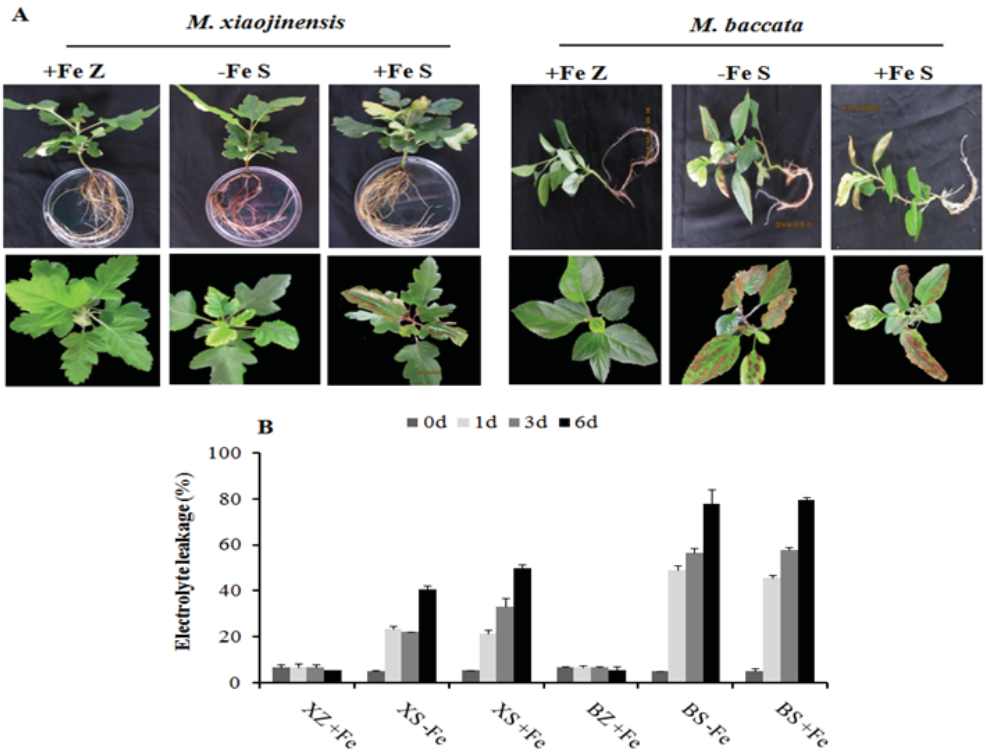

Figure 3 Growth phenotypes and leaves EL of *M. xiaojinensis* (X) and *M. baccata* plants (B) hydroponically grown under salt stress (S) or without salt stress (Z) with deficient iron (−Fe) or sufficient iron (+Fe). Mean values and standard deviations (bars) were obtained from three replicates

decreased by 52% in leaves and 56% in roots. While under sufficient iron the reduction was 60% in leaves and roots compared to control. However, in *M. baccata* the reduction was 78% and 92% in leaves and roots, respectively (Figure 2e, f).

Results showed that the two iron levels significantly affected EL in leaves of both genotypes when grown under salt stress compared to control, with a greater extent in *M. baccata* (Figure 3b). Under iron deficiency, EL was decreased in leaves of *M. xiaojinensis* than that under iron-sufficient. Compare with *M. xiaojinensi*, EL in *M. baccata* was greater by 52% under deficient iron and by 44% under sufficient one.

Expression analysis of MdSOS1, MdAPX1, MxFIT, MxIRT and MxHA2 genes

In order to investigate the response of these genes in salt stress tolerance genotype (*M. xiaojinensis*) and sensitive one (*M. baccata*), extraction RNA from roots and leaves were used and real-time qPCR was performed. The results indicated that the expression of *MdSOS1* gene was higher in roots (Figure 4a) compared to leaves (Figure 4b) and was more abundant in *M. xiaojinensis* than in *M. baccata.* Under salt stress, the expression of *MdSOS1* gene in *M. xiaojinensis* was higher at iron-deficient compared with that at iron-sufficient (Figure 4a, b) and seems to start directly after exposed to treatments and keeps increasing until 24 hr. However, a little expression was observed in the sensitive genotype. Without salt stress, no expression was detected even in tolerant genotype.

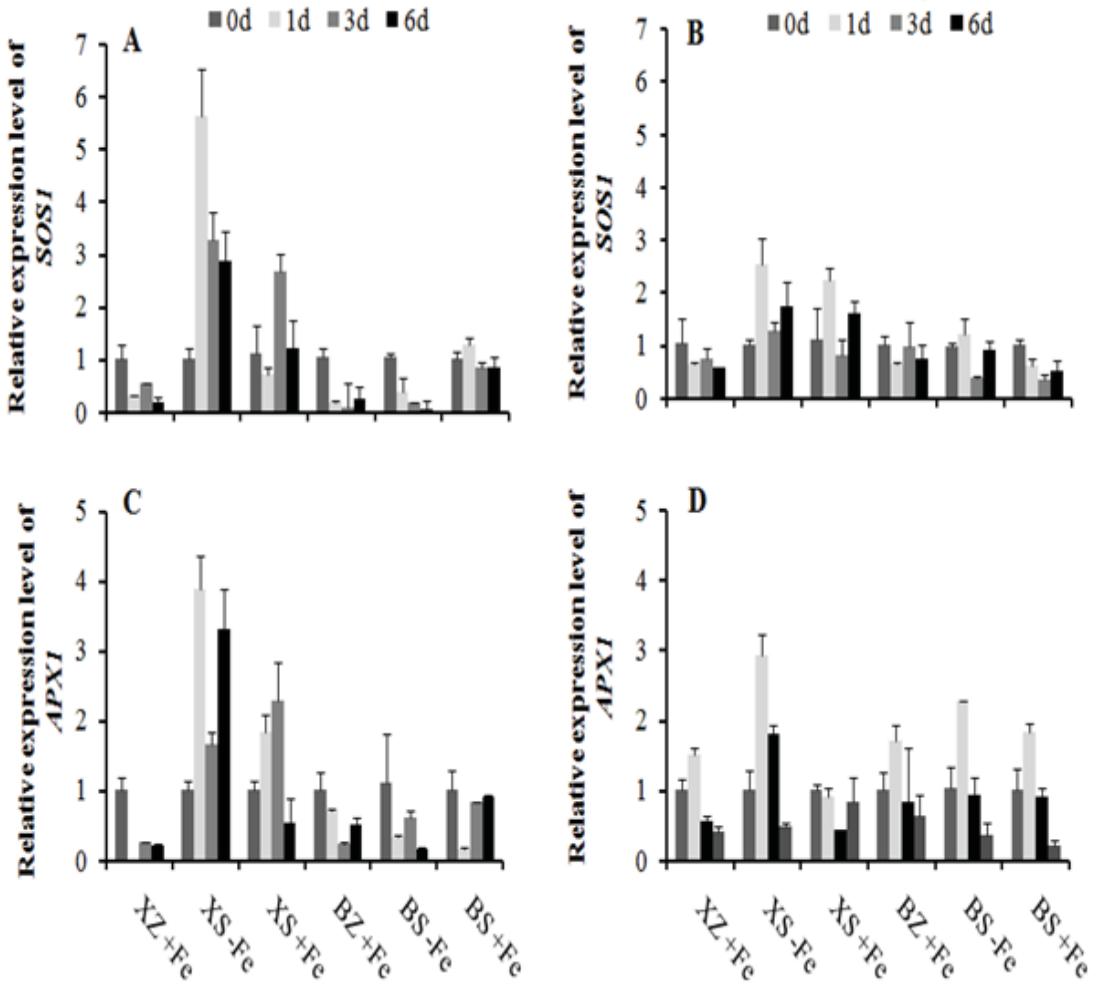

Figure 4 The relative expression levels of *MdSOS1* and *MdAPX1* in roots and leaves of *M. xiaojinensis* (X) and *M. baccata* plants (B) hydroponically grown under salt stress (S) or without salt stress (Z) with deficient iron (−Fe) or sufficient iron (+Fe). (A) Expression of MdSOS1 in roots (B) Expression of *MdSOS1* in leaves (C) Expression of *MdAPX1* in roots. (D) Expression of *MdAPX1* in leaves. Mean values and standard deviations (bars) were obtained from three replicates

Obtained results showed that the expression of *MdAPX1* gene in roots and leaves of *M. xiaojinensis* was observed under most of the treatments, with clear increase under salt stress with iron-deficient and the peaked was on the first day (Figure 4c, d). Furthermore some expression was observed in *M. baccata* under deficient and sufficient iron with abundant expression in leaves than in roots.

The results of evaluation of expression of *MxFIT*, *MxIRT* and *MxHA2* in roots of the two genotypes indicated that the expression of these genes was reduced in roots of *M. xiaojinensis* under salt stress at iron deficiency compared with those not exposed to salt stress, whereas, no significant difference was observed in roots of *M. baccata*. A little increase in the expression of *MxHA2* gene was observed in roots of *M. xiaojinensis* under iron-sufficient (Figure 5).

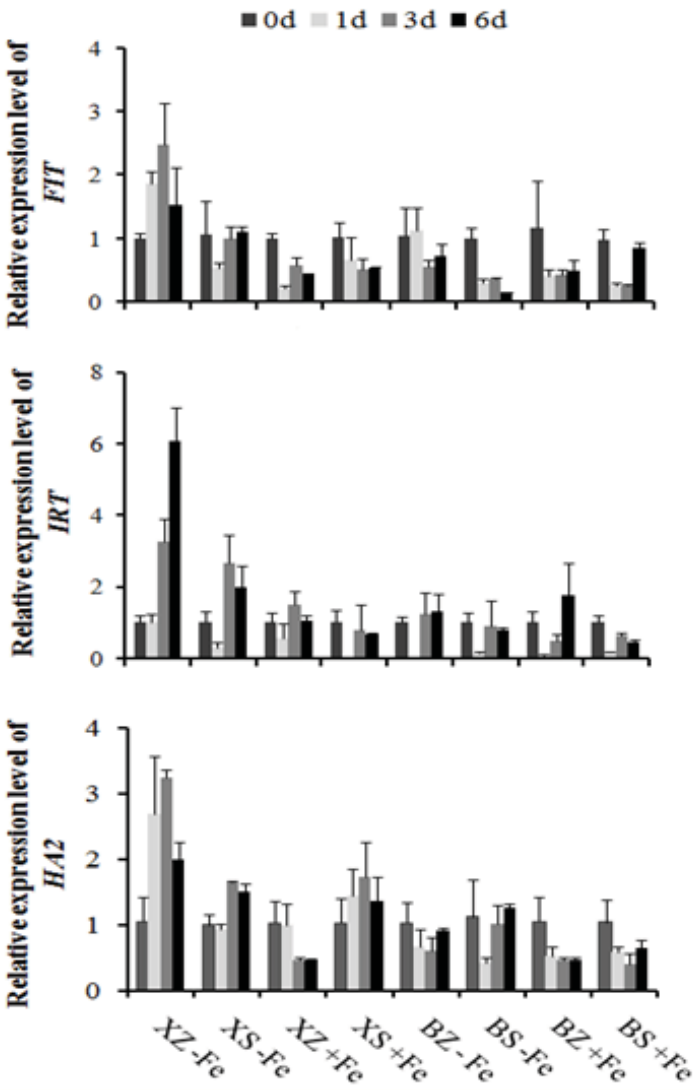

Figure 5 The relative expression levels of *MxFIT*, *MxIRT* and *MxHA2* genes in roots of *M. xiaojinensis* (X) and *M. baccata* plants (B) hydroponically grown under salt stress (S) or without salt stress (Z) with deficient iron (−Fe) or sufficient iron (+Fe). Mean values and standard deviations (bars) were obtained from three replicates

DISCUSSION

Salt stress induces ionic and oxidative stresses which damage plant tissues (Hasegawa et al., 2000). In agreement with this finding, obtained results showed damage in leaves and roots of both apple genotypes (Figure 3a) as indicated by accumulation of Na^+, K^+, H_2O_2 and EL in plant tissues. However, the responses to salt stress varied between two genotypes differing in salt tolerance.

Tsai et al. (2004) reported that salt stress enhance H_2O_2 accumulation in plant cell, which was in agree with this results. In contrast, other studies observed no effect on H_2O_2 content (Lee et al., 2001) or a significant reduction of H_2O_2 under salt stress (Kim et al., 2005). The

increment of H_2O_2 levels under salt stress and iron treatments indicated that, the accumulation of H_2O_2 is mainly due to osmotic stress induced by the external NaCl (Hernandez et al., 2010) and by free iron that enhancing the generation of ROS (Halliwell and Gutteridge, 1984). The reduction of H_2O_2 levels under salt stress at iron-deficient in root of *M. xiaojinensis* might be explain by less accumulated Na^+ in roots. Similarly, Lin and Kao (2001) found that NaCl stress lead to increase in H_2O_2 level in roots of rice seedlings, due to increase Na^+ concentration. Also could be due to high expression of *MdAPX1* gene, as reported by Badawi et al. (2004). However, the expression was higher in salt tolerant genotype than in sensitive one, similarly result was reported by Mhadhbi et al. (2011).

Premchandra et al. (1992) reported that environmental stresses have an effect on the permeability of plasma membrane due to its influence on lipid peroxidation. Loss of membrane stability induces increase in EL (Sairam et al., 2005). Liu et al. (2012) found that EL in tolerant genotype, *M. hupehensis*, was less increased than those in sensitive genotype, *M. prunifolia*. Similarly, this result showed less increase in EL in *M. xiaojinensis* than in *M. baccata*. The reduction of EL in *M. xiaojinensis* under salt stress at the deficient iron was more than at sufficient one, suggesting that under this condition *M. xiaojinensis* have better protective mechanism as can be seen from increment of *MdAPX* expression which detoxifies H_2O_2 and thus decreases lipid peroxidation and membrane damage.

The expression of *MdAPX* gene has been induced in plants by salt stress (Tsai et al., 2004) as well as iron stress (Vansuyt et al., 1997). The expression of *APX* was increased after 1 day of treatments. Similarly, reported that the expression of *APX* was increased significantly in roots of barley at 24 hr after exposed to NaCl (Kim et al., 2005).

Increased Na^+ concentration under salt stress and iron treatments, was identical with previous results by Yousfi et al. (2012) who stated that iron deficiency had a positive effect in Na^+ accumulation under salt stress in plant tissues compared with iron-sufficient, and the accumulation was less in shoots of wild barley (tolerant) than cultivated one (sensitive). Similar finding were reported by Liu et al. (2012). Also Yang (2013) reported that *M. xiaojinensis* have greater Na^+ restriction capability and higher K^+ selective absorption and transport capability than *M. baccata*. Moreover, the contrasting result for K^+ and Na^+ concentrations was earlier reported by Yasar et al. (2006), who stated that K^+ accumulation was high in organs in which Na^+ concentration was low and this can be explained by the competition between Na^+ and K^+ for the binding sites on the plasma membrane (Al-Harbi, 1995). Increased the expression of *MdSOS1* by salt stress under iron-deficient can contribute in the reduction of Na^+ and increase K^+ concentrations as reported by Yue et al. (2012).

The expression of *MdSOS1* was obviously correlated with low and high accumulated Na^+ in *M. xiaojinensis* and *M. baccata* tissues, respectively, as *M. xiaojinensis* is tolerant to salt stress (Yin et al., 2010) while *M. baccata* is sensitive (Han et al., 2006). These findings are in consistent with previous study by Liu et al. (2012). In addition, Yue et al. (2012) reported that over expression of *SOS1* in transgenic tobacco improved salt tolerance by reducing the accumulation of Na^+.

Increases the pH under salt stress can be attributed to the formation of biocarbonate compounds in the medium and thus decreased root acidity. The reduction of root acidity of *M. xiaojinensis* also could explain by the reduction in *MxHA2* expression due to the negative effect of salt stress on the H^+ pumping. Similarly Rabhi et al. (2007) and Wissal et al. (2011) attributed that to the negative effect of salinity in the activities of root PM H^+-ATPase that mediated H^+ efflux to increase the solubility of Fe^{3+} Moreover, the reduction in the expression

of *MxFIT* and *MxIRT* was probably correlated with the reduction in roots acidification; Witzel et al. (2009) stated that the expression of some proteins involved in iron uptake by barley plant was declined significantly under salt stress. Similarly, it was found that the relative uptake of iron by barley and rye decreased with increasing salinity and it was suggested that, at high salinity, the synthesis pathway of iron chelators and uptake systems for iron as well as the transporters for acquisition of iron in *B. subtilis* are partially inhibited (Hoffmann et al., 2002; Jumberi et al., 2001).

CONCLUSION

Under salt and iron stresses *M. xiaojinensis* tends to absorb more Fe^{2+} compared to *M. baccata*.

Iron deficiency has a positive influence on *M. xiaojinensis* and could be used to minimise the negative impact of salt stress and thus contribute into enhancing salt stress tolerance.

REFERENCES

Al-Harbi, A.R. (1995) 'Growth and nutrient composition of tomato and cucumber as affected by sodium chloride salinity and supplemental calcium', *Plant Nutrition*, Vol. 18, pp.1403–1416.

Azevedo-Neto, A.D., Prisco, J.T., Eneas-Filho, J., Abreu, C.E. and Filho, E.G. (2006) 'Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes', *Environmental and Experimental Botany*, Vol. 56, pp.87–94.

Badawi, G.H., Kawano, N., Yamauchi, Y., Shimada, E., Sasaki, R., Kubo, A. and Tanaka, K. (2004) 'Over-expression of ascorbate peroxidase in tobacco chloroplasts enhances the tolerance to salt stress and water deficit', *Physiologia Plantarum*, Vol. 121, pp.231–238.

Cabanero, F.J., Teruel, J.A. and Carvajal, M. (2006) 'New evidence about the relationship between water channel activity and calcium in salinity-stressed pepper plants', *Plant and Cell Physiology*, Vol. 47, pp.224–233.

Chougui, S., Yaklef, N. and Abdel Hamid, D. (2004) 'Interaction Iron-Salinity. 1- Relation of photosynthesis/ K+ (roots) with other morphological, physiological and biochemical aspects of tomato (*Lycopersicum esculentium* Mill). Arab Univ', *Journal of Agricultural Science*, Ain Shams University Cairo, Vol. 12, No. 2, pp.481–497.

Colangelo, E.P. and Guerinot, M.L. (2004) 'The essential basic helix-loop-helix protein FIT1 is required for the iron deficiency response', *Plant Cell*, Vol. 16, pp.3400–3412.

Flowers, T.J. and Yeo, A.R. (1995) 'Breeding for salinity resistance in crop plants. Where next?' *Australian Journal of Plant Physiology*, Vol. 22, pp.875–884.

Fox, T.M. and Guerinot, M.L. (1998) 'Molecular biology of cation transport in plants', *Annual Review. Plant Physiology. Plant Molecular Biology*, Vol. 49, pp.669–696.

Gálvez, F.J., Baghour, M., Hao, G., Cagnac, O., Rodríguez-Rosales, M.P. and Venema, K. (2012) 'Expression of *LeNHX* isoforms in response to salt stress in salt sensitive and salt tolerant tomato species', *Plant Physiology and Biochemistry*, Vol. 51, pp.109–115.

Gao, C.Q., Wang, Y.C., Jiang, B., Liu, G.F., Yu, L.L., Wei, Z.G. and Yang, C.P. (2011) 'A novel vacuolar membrane H+-ATPase c subunit gene (*ThVHAc1*) from *Tamarix hispida* confers tolerance to several abiotic stresses in *Saccharomyces cerevisiae*', *Journal of Molecular Biology*, Vol. 38, pp.957–963.

Guerinot, M.L. and Yi, Y. (1994) 'Iron: nutritious, noxious, and not readily available', *Plant Physiology*, Vol. 104, pp.815–820.

Halliwell, B. and Gutteridge, J.M. (1984) 'Oxygen toxicity, oxygen radicals, transition metals and disease', *Biochemestry*, Vol. 219, pp.1–14.

Han, Z.H., Shen, T., Korcak, R.F. and Baligar, V.C. (1994) 'Screening for iron-efficient species in the genus *Malus*', *Journal of Plant Nutrition*, Vol. 17, pp.579–592.

Han, Z.H., Shen, T., Korcak, R.F. and Baligar, V.C. (1998) 'Iron absorption by iron-efficient and -inefficient species of apples', *Journal of Plant Nutrition*, Vol. 21, pp.181–190.

Han, Z.H., Zhou, E.F., Xue, X.F., Bei, X., Wu, Z. and Bao, X. (2006) 'Na$^+$ compartmentalization in the leaves of *Malus zumi* and *Malus baccata* under salt stress', *Acta Botanica Boreali-Occidentalia Sinica*, pp.1378–1383.

Hasegawa, P.M., Bressan, R.A., Zhu, J.K. and Bohnert, H.J. (2000) 'Plant cellular and molecular responses to high salinity', *Annual Review of Plant Physiology and Plant Molecular Biology*, Vol. 51, pp.463–499.

Hernandez, M., Fernandez-Garcia, N., Diaz-Vivancos, P. and Olmos, E. (2010) 'A different role for hydrogen peroxide and the antioxidative system under short and long salt stress in *Brassica oleracea* roots', *Journal of Experimental Botany*, Vol. 61, pp.521–535.

Hoffmann, T., Schutz, A., Brosius, M., Volker, A., Volker, U. and Bremer, E. (2002) 'High-salinity-induced iron limitation in *Bacillus subtilis*', *Journal of Bacteriology*, Vol. 184, No. 3, pp.718–727.

Huai, X.T., Wang, Y., Zhang, Q., Zhang, X.Z. and Han, Z.H. (2012) 'Cloning and functional analysis of plasma membrane H+- ATPase gene (*MxHA2*) from *Malus xiaojinensis*', *Journal of Agricultural Biotechnology*, Vol. 20, No. 10, pp.1135–1142, DOI:10.3969/j.issn.1674-7968.2012.10.005.

Imlay, J.A. and Linn, S. (1988) 'DNA damage and oxygen radical toxicity', *Science*, Vol. 3, No. 240, pp.1302–1309.

Jumberi, A., Yamada, M., Yamada, S. and Fujiyama, H. (2001) 'Salt tolerance of grain crops in relation to ionic balance and ability to absorb microelements', *Soil Science and Plant Nutrition*, Vol. 47, No. 4, pp.657–664.

Kim, S.Y., Lim, J.H., Park, M.R., Kim, Y.J., Park, T.I. and Seo, Y.W. (2005) 'Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under saline stress peroxide in barley roots under saline stress', *Biochemistry and Molecular Biology*, Vol. 38, pp.218–224.

Kobayashi, T.I., Nishizawa, N.K. and Mori, S. (2006) 'Molecular analysis of iron-deficient graminaceous plants in Iron Nutrition in Plants and Rhizospheric Microorganisms', *Springer Dordrecht, The Netherlands*, pp.395–436.

Kürkcüoglu, S.K., Degenhardt, J., Lensing, J., Al-Masri, A.N. and Gau, A.E. (2007) 'Identification of differentially expressed genes in *Malus domestica* after application of the non-pathogenic bacterium *Pseudomonas fluorescens Bk3* to the phyllosphere', *Journal of Experimental Botany*, Vol. 58, pp.733–741.

Lee, B.H., Lee, H., Xiong, L. and Zhu, J.K. (2002) 'A mitochondrial complex 1 defect impairs cold- regulated nuclear gene expression', *Plant Cell*, Vol. 14, pp.1235–1251.

Lee, D., Kim, Y.S. and Lee, C.B. (2001) 'The inductive responses of the antioxidant enzymes by salt stress in the rice', *Plant Physiology*, Vol. 158, pp.737–745.

Lin, C.C. and Kao, C.H. (2001) 'Cell wall peroxidase activity, hydrogen peroxide level and NaCl- inhibited root growth of rice seedlings', *Plant Soil*, Vol. 230, pp.135–143.

Liu, C., Li, C., Liang, D., Wei, Z., Zhou, S., Wang, R. and Ma, F. (2012) 'Differential expression of ion transporters and aquaporins in leaves may contribute to different salt tolerance in *Malus* species', *Plant Physiology and Biochemistry*, Vol. 58, pp.159–165.

Livak, K.J. and Schmittgen, T.D. (2001) 'Analysis of relative gene expression data using real-time quantitative PCR and the $2^{\Delta\Delta CT}$ method', *Methods*, Vol. 25, pp.402–408.

Mhadhbi, H., Fotopoulos, V., Mylona, P.V., Jebara, M., Aouani, M.E. and Polidoros, A.N. (2011) 'Antioxidant gene–enzyme responses in *Medicago truncatula* genotypes with different degree of sensitivity to salinity', *Physiologia Plantarum*, Vol. 141, pp.201–214.

Mittler, R. (2002) 'Oxidative stress, antioxidants and stress tolerance', *Trends in Plant Science*, Vol. 7, pp.405–410.

Mukherjee, S.P. and Choudhari, M.A. (1983) 'Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings', *Physiologia Plantarum*, Vol. 58, pp.116–170.

Nenova, V. (2008) 'Growth and mineral concentrations of pea plants under different salinity levels and iron supply', *General and Applied Plant Physiology*, Vol. 34, Nos. 3–4, pp.189–202.

Olías, R., Eljakaoui, Z., Li, J., De Morales, P.A., Marin, M., Manzano, C., Pardo, J.M. and Belver, A. (2009) 'The plasma membrane Na$^+$/H$^+$ antiporter *SOS1* is essential for salt tolerance in tomato and affects the partitioning of Na$^+$ between plant organs', *Plant Cell and Environment*, Vol. 32, pp.904–916.

Premchandra, G.S., Saneoka, H., Fujita, K. and Ogata, S. (1992) 'Leaf water relations, osmotic adjustment, cell membrane stability, epi-cuticular wax load and growth as affected by increasing water deficits in Sorghum', *Journal of Experimental Botany*, Vol. 43, pp.1569–1576.

Rabhi, M., Barhoumi, Z., Ksouri, R., Abdelly, C. and Gharsalli, M. (2007) 'Interactive effects of salinity and iron deficiency in *Medicago ciliaris*', *Comptes Rendus-Biologies*, Vol. 33, No. 11, pp.779–788.

Sairam, R.K., Srivastava, G.C., Agarwal, S. and Meena, R.C. (2005) 'Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes', *Biologia Plantarum*, Vol. 49, pp.85–91.

Santi, S., Cesco, S., Varanini, Z. and Pinton, R. (2005) 'Two plasma membrane H$^+$- ATPase genes are differentially expressed in iron-deficient cucumber plants', *Plant Physiology and Biochemistry*, Vol. 43, pp.287–292.

Sharma, C.P. (2006) *Plant Micronutrients*, USA: Enfield, New Hampshire, Science Publishers.

Shi, H., Ishitani, M., Kim, C. and Zhu, J.K. (2000) '*The Arabidopsis thaliana* salt tolerance gene *SOS1* encodes a putative Na^+/H^+ antiporter', *Proceedings of the National Academy of Sciences, USA*, Vol. 97, pp.689–901.

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T. and Yabuta, Y. (2002) 'Regulation and function of ascorbate peroxidase isoenzymes', *Journal of Experimental Botany*, Vol. 53, pp.130–519.

Tagliavini, M. and Rombolà, A.D. (2001) 'Iron deficiency and chlorosis in orchard and vineyard ecosystems', *European Journal of Agronomy*, Vol. 15, pp.71–92.

Tsai, Y., Hong, C., Liu, L. and Kao, C. (2004) 'Relative importance of Na^+ and Cl^- in NaCl induced antioxidant systems in roots of rice seedlings', *Physiologia Plantarum*, Vol. 122, pp.86–94.

Vansuyt, G., Lopez, F., Inze, D., Briat, J.F. and Fourcroy, P. (1997) 'Iron triggers a rapid induction of *ascorbate peroxidase* gene expression in *Brassica napus*', *FEBS Letters*, Vol. 410, pp.195–200.

Wissal, M., Nahida, J., Marta, D., Chedly, A., Graziano, Z. and Mohamed, G. (2011) 'Responses of two lines of *Medicago ciliaris* to Fe deficiency under saline conditions', *Plant Growth Regulators*, Vol. 64, pp.221–230.

Witzel, K., Weidner, A., Surabhi, G.K., Borner, A. and Mock, H.P. (2009) 'Salt stress-induced alterations in the root proteome of barley genotypes with contrasting response towards salinity', *Journal of Experimental Botany*, Vol. 60, No. 12, pp.3545–3557.

Yang, H.B. (2013) 'Effect of NaCl stress on selective absorption and transportation of K^+ and Na^+ of roots in *Malus* species', *Research on Crops*, Vol. 14, No. 2, pp.535–539.

Yasar, F., Uzal, O., Tufenkc, S. and Yildiz, K. (2006) 'Ion accumulation in different organs of green bean genotypes grown under salt stress', *Plant Soil and Environment*, Vol. 52, No. 10, pp.476–480.

Yin, L.L., Wang, Y., Yan, M., Zhang, X., Xu, X.F. and Han, Z.H. (2014) 'Characterization of *MxFIT*, an iron deficiency induced transcriptional factor in *Malus xiaojinensis*', *Plant Physiology and Biochemistry*, Vol. 75, pp.89–95.

Yin, R., Bai, T., Ma, F., Wang, X., Li, Y. and Yue, Z. (2010) 'Physiological responses and relative tolerance by Chinese apple rootstocks to NaCl stress', *Scientia Horticulturae*, (Amsterdam), Vol. 126, pp.247–252.

Yousfi, S., Houmani, H., Zribi, F., Abdelly, C. and Gharsalli, M. (2012) 'Physiological responses of wild and cultivated barley to the interactive effect of salinity and iron deficiency', *ISRN Agronomy*, Vol. 10, pp.1–8.

Yue, Y., Zhang, M.C., Zhang, J.C., Duan, L.S. and Li, Z.H. (2012) '*SOS1* gene over expression increased salt tolerance in transgenic tobacco by maintaining a higher K^+/Na^+ ratio', *Plant Physiology*, Vol. 169, pp.255–261.

Zha, Q., Wang, Y., Zhang, X.Z. and Han, Z.H. (2014) 'Both immanently high active iron contents and increased root ferrous uptake in response to low iron stress contribute to the iron deficiency tolerance in *Malus xiaojinensis*', *Plant Science*, Vol. 214, pp.47–56.

Zhang, Y.G., Cheng, J.H., Xu, X.F. and Li, T.Z. (2005) 'Comparison of methods for total RNA isolation from *Malus xiaojinensis* LD-PCR amplification', *Biotechnology Information*, Vol. 4, pp.50–53.

Zhu, J.K. (2003) 'Regulation of ion homeostasis under salt stress', *Current Academy of Sciences*, USA, Vol. 97, pp.11632–11637.

Zhu, J.K., Liu, J. and Xiong, L. (1998) 'Genetic analysis of salt tolerance in Arabidopsis: evidence for a critical role of potassium nutrition', *Plant Cell*, Vol. 10, pp.1181–1191.

BIOGRAPHICAL NOTES

Siham Ibrahim Babiker is a Researcher of Horticultural Science, Fruit trees in Agricultural Research Corporation-Sudan. She obtained her PhD from China Agricultural University, China.

Meiling Zhang is currently working as Post Doctor at China Agricultural University. She obtained her PhD from China Agricultural University, China.

Wei Liu is a PhD candidate major in horticulture at the China Agriculture University. She has published several articles in regional journals in area related to garden and invention patents.

Yi Wang is currently working as Assistant Professor at the China Agriculture University. She obtained her PhD from the China Agriculture University. She has published several articles in regional and international journals in areas related to iron deficiency response.

Ting Wu is currently working as a teacher at China Agricultural University. She obtained her PhD from China Agricultural University, China.

Xinzhong Zhang is currently working as a professor at College of Agronomy and Biotechnology in China Agricultural University. He obtained his PhD from Shenyang Agricultural University, China. He taught lessons about breeding research of horticultural plants, Germplasm resources of fruit trees. He has published several articles in regional and international journals in areas related to inheritance and molecular marker of resistance to bot canker and ring rot in *Malus domestica*.

Zhenhai Han is currently working as a professor at College of Agronomy and Biotechnology in China Agricultural University. He obtained his PhD from China Agricultural University, China. He taught lessons for PhD students in the university. His research team is focused on the iron absorption mechanism in apples especially for *Malus xiaojinasis* and *Malus baccata*. He has published several articles and books in regional and international journals in areas related to apple rootstocks, biotic or abiotic stress, physiological and biochemical mechanisms as well as discovery and application of new apple rootstock.

ACKNOWLEDGEMENT

This paper is supported by the earmarked fund for China Agriculture Research System CARS-28), Key Laboratory of Beijing Municipality of Stress Physiology and Molecular Biology for Fruit Tree, Collaborative innovation center for eco-environmental improvement with forestry and fruit trees(CEFF-PXM2016-014207-000038)"