DOI : 10.5958/0974-0112.2017.00069.X

Indian J. Hort. 74(3), September 2017: 346-350

Influence of six dwarfing interstocks on the 'Fuji' apple under drought stress

X.L. Li, J.K. Zhang, M.J. Li, B.B. Zhou, Q. Zhang and Q.P. Wei*

Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (North China), Ministry of Agriculture, Beijing Academy of Forestry & Pomology Sciences, Beijing 100093, P.R. China

ABSTRACT

Investigation of growth and physiology of scion on various interstocks to drought stress is a valuable process for apple drought-tolerance stock selection. In this attempt, the 'Fuji' apple cultivar grafted on six dwarfing interstocks, including of M series (M26, M9-T337), CG series (CG24) and SH series (SH1, SH6 and SH40) on *Malus hupehensis* var. Pingyiensis Jiang rootstock were maintained as normal (-10 to -20 Kpa) (control) , moderate (-30 to -40 Kpa) and severe drought (-50 to -60 Kpa) stress levels. After 50 days treatment, comprehensive evaluation by subordinate function value (SF) was employed based on trunk growth, net photosynthetic rate, leaf water potential *etc*., and drought tolerance of 'Fuji' apple was ranked as following interstocks: SH6 > SH40 > SH1> M9-T337 > M26 > CG24. Moreover, the determination of 'Fuji' apple in responding to drought stress revealed that three antioxidant enzymes (SOD, APX and DHAR) and four genes (*DREB2A*, *ZAT10*, *SOD1*, *APX1*) constitute the putative key components for drought regulation.

Key words: Apple, drought stress, dwarfing interstock, antioxidant, drought-related gene.

INTRODUCTION

Apples are one of the most widely cultivated and economically important fruits worldwide. With the development of dwarfing, high-density cultivation method in modern production systems, the importance of dwarfing interstocks has been widely recognized (Pereira-Lorenzo *et al.*, 5). Since the 1960s, an increasing number of dwarf apple rootstocks have been developed, examples of these include the M series (Malling, UK), MM series (Malling Merton, UK), O series (Ottawa, Canada), CG series (Cornell-Geneva, United States) and SH series (Shanxi, China) *etc*. However, the screening for high drought-tolerance interstocks remained unsatisfactory, especially in arid and semi-arid areas, where drought has been a major limiting factor in apple cultivation. For investigating the influence of different interstocks to the 'Fuji' apple under drought stress, and laying a foundation for tolerant interstock identification, the most widely used dwarfing interstocks in China from the M series (M26, M9-T337), CG series (CG24) and SH series (SH1, SH6, SH40) were used in this study. And the putative effectors for drought tolerance from physiology and molecular components were detected and analysed comprehensively.

MATERIALS AND METHODS

The investigation was carried out on 3-year-old 'Fuji' apple (*M. domestica* Borkh.) trees grafted on six types of dwarfing interstocks (M26, M9-T337, CG24, SH1, SH6 and SH40) on the base rootstock *Malus hupehensis* var. Pingyiensis Jiang separately, at the Beijing Academy of Forestry & Pomology Sciences (39°56´N, 116°56´E), Beijing, China. Soil water potential were designed for control (control, -10 to -20 Kpa), moderate drought stress (T1, -30 to -40 Kpa) and severe drought stress (T2, -50 to -60 Kpa), and monitored using data loggers (WatchDog-1000 series, Spectrum Tech. Inc., USA). During 50 days treatments, Trunk diameter and shoot length were measured and trunk growth rate (TGR) and shoot growth rate (SGR) were calculated according to the following formula: TGR = [(Final diameter) – (initial diameter)] / [initial diameter] × 100; SGR = [(Final shoot length) – (initial shoot length)] / [initial shoot length] × 100.

All leaf tissue evaluations were performed using uniform fully expanded mature leaves from the mid-stem area of each replicates per 'Fuji' apple. Leaf water potential (LWP) was measured with a Scholander-type pressure chamber (Soil-Moisture Equipment Corp, CA, USA) at 5:00 a.m. and leaf relative electrolytic leakage (REC) was assessment according to Bolat *et al*. (1). Net photosynthetic rate (*P*n) and relative chlorophyll content (RCC) was measured using a CI-340 handheld photosynthesis system (Camas, WA, USA) and SPAD-502 chlorophyll meters (Konica Minolta, Osaka, Japan) separately, at 10:00 a.m. under clear weather conditions. Meanwhile, leaves were cut off and ground to powder used for antioxidant enzymes activity detection, and total

*Corresponding author's E-mail: diccaas@caas.cn

**Beijing Key Laboratory of New Technique in Agricultural Application, Beijing 102206, P.R. China

RNA extraction used for gene expression analysis by Real-time qPCR, performed on a Bio-Rad C1000™ thermal cycler with a CFX384™ Real Time system (Bio-Rad, USA). Statistical analyses were carried out using SPSS 11.0. Differences among treatments were separated by least significant difference (LSD) tests at a probability levels p = 0.05.

RESULTS AND DISCUSSION

Soil moisture had a direct effect on scion growth, whilst interstock had an inhibitory effect on water transport from root to scion; the water conductivity of different interstocks determined the trunk and shoot growth, leaf water potential, as well as *P*n, which in turn were reflections of scion drought tolerance ability during rootstock-scion interaction (Martínez-Ballesta *et al.*, 3). Our experiments showed that after 50 days of severe drought stress (T2), significant deference of TGR of 'Fuji' apple on all six interstocks was observed, from those on CG24 and SH1 interstocks showed the smallest TGR, implying they are highly sensitivity to drought stress, whereas those on SH6 performed the highest TGR in all three treatments (Table 1). Besides, drought stress led to significantly decreasing in SGR of all scions except those on M9-T337. The 'Fuji' apple on M26 and CG24 showed more than 50% lower SGR compared with their controls, and those on SH series showed higher SGR than others in treatment T2. Generally, drought stress restrained trunk growth and shoot elongation, whereas for those 'Fuji' apple on the interstocks M26 and SH6, SGR inhibited significantly under moderate drought stress, but TGR was affected slightly and even were promoted, suggesting drought stress not only influenced the production but also the partitioning of dry matter to various plant parts.

As a productivity indice, *P*n indicated the carbohydrate accumulation capacity and then are directly related to abiotic-stress tolerance of plant. Under drought stress, we found that *P*n was not significantly affected in treatment T1 with the exception of those on CG24 (Fig. 1A), whereas more than 60% decrease was observed in treatment T2 to all plants, especially the scions grafted on CG24 decreased to just 12.6% of the control, suggesting diverse stability in drought tolerance. On the contrary, the REC of 'Fuji' apple was about 50% higher in treatment T2 than those of the control except those on M26 and SH6 (Fig. 1B). The REC level of scion on CG24 interstock increased to about 3-fold, suggesting more serious leaf cell membrane damage existed than those on other interstocks.

LWP is usually considered to be a result of a balance between soil moisture and transpiration. Here, we addressed the relationship between LWP and dwarfing interstock in the pre-requisite of the same soil water content, base rootstock and scion cultivar. As shown in Fig. 1C, 'Fuji' apple exhibited divergence in LWP among six dwarfing interstocks in each treatment. Comparatively, the relative steadiness of LWP both in T1 and T2 was observed on the scions of SHs interstocks, whereas, those on other interstocks with 2.9- to 4.5-fold increment, suggesting a weaker ability in leaf water conservation. Meanwhile, drought stress led to double impact on the RCC of 'Fuji' apple as indicated in Fig. 1D. Moderate drought stress had a slight improvement on RCC to all 'Fuji' apple detected, whereas, severe drought stress caused significantly decrease, especially for those on M26.

Evaluation of drought tolerance is usually conducted based on comprehensive analysis of limited indices, and tolerance levels are ranked relatively, but not showed by soil water potential or drought duration (Liu *et al.*, 2). Based on above six indices from treatment T2, comprehensive evaluation by subordinate function value (SF) was employed to the tolerance analysis of 'Fuji' apple on different interstocks under drought stress. Further, the larger of SF in each indice or average for each interstock, the more stability of 'Fuji' apple to drought stress. Thus, we ranked the drought tolerance of 'Fuji' apple as

Table 1. Trunk growth rate (TGR) and shoot growth rate (SGR) of 'Fuji' apple under drought stress treatments.

Interstock	TGR (%)			SGR (%)		
	Control	T1	T2	Control	T1	T2
M26	5.25a	4.95a	2.03b	3.27a	2.55b	1.58c
M9-T337	5.03a	2.76b	2.61b	1.81a	1.66a	1.22a
CG24	8.81a	4.23b	3.60c	3.42a	2.24b	0.90c
SH1	8.78a	6.86b	4.84c	3.47a	2.32ab	1.90b
SH6	11.54a	12.04a	8.95b	2.39a	1.77b	1.61b
SH40	9.63a	7.66b	5.39b	3.09a	1.89b	1.66b

Control, plants grown at a normal soil water potential (-10 to -20 kPa); T1, moderate drought stress (-30 to -40 kPa); T2, severe drought stress (-50 to -60 kPa). The means followed by the different letters in a row indicate significant differences at 0.05 level (LSD test).

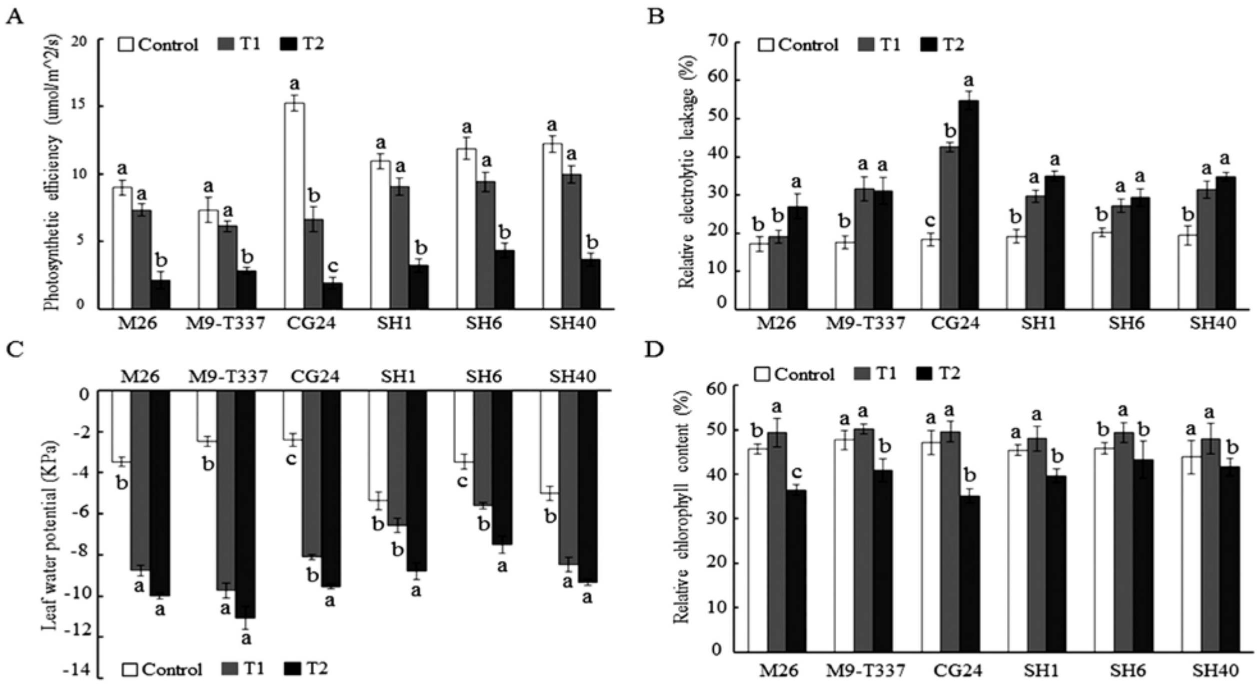

Fig. 1. Influence of six dwarfing interstocks on the leaf physiology parameters of 'Fuji' apple under drought stress. Control, plants grown at a normal soil water potential (-10 to -20 kPa); T1, moderate drought stress (-30 to -40 kPa); T2, severe drought stress (-50 to -60 kPa). Different letters in a group column indicate significant differences at 0.05 level (LSD test).

following interstocks: SH6 > SH40 > SH1> M9-T337 > M26 > CG24 (Table 2).

Antioxidant enzymatic systems play important roles in scavenging harmful oxygen species induced by multiple biotic and abiotic stresses. In this study, we assessed the activity changes of four types of antioxidant enzymes, including SOD, CAT, APX and DHAR in 'Fuji' apple grafted on SH6. With the process of drought stress treatment, the activities of antioxidant enzymes were usually induced and followed by decrease due to oxidation damage (Wang *et al.*, 6), which was also well reflected in the changes of SOD, CAT and DHAR activities in the present study (Fig. 2). However, the levels of APX activities fail to be induced by drought stress and declined throughout the stress treatments, performing a higher-sensitivity response to drought stress.

A number of genes have been identified and demonstrated to be key regulars for drought tolerance (Xiao *et al.*, 7). In this study, we cloned 12 drought-related genes from 'Fuji' apple and determined by real-time qPCR. Results showed that the mRNA levels of four antioxidant enzyme genes were all induced by drought stress, and the changes of *DHAR2* gene

Table 2. Comprehensive evaluation of the adaptability of 'Fuji' apple on interstocks to drought stress by subordinate function value (SF).

Interstock	TGR	SGR	*P*n	REC	LWP	RCC	Av. of SF	Order
M26	0.00	0.68	0.09	1.00	0.30	0.16	0.37	5
M9-T337	0.08	0.32	0.37	0.85	0.00	0.71	0.39	4
CG24	0.23	0.00	0.00	0.00	0.43	0.00	0.11	6
SH1	0.41	1.00	0.53	0.71	0.64	0.56	0.64	3
SH6	1.00	0.71	1.00	0.91	1.00	1.00	0.94	1
SH40	0.49	0.76	0.72	0.72	0.49	0.80	0.66	2

TGR = trunk growth rate; SGR, shoot growth rate; *P*n = net photosynthetic rate; REC = relative electrolytic leakage; LWP = leaf water potential; RCC = relative chlorophyll content.

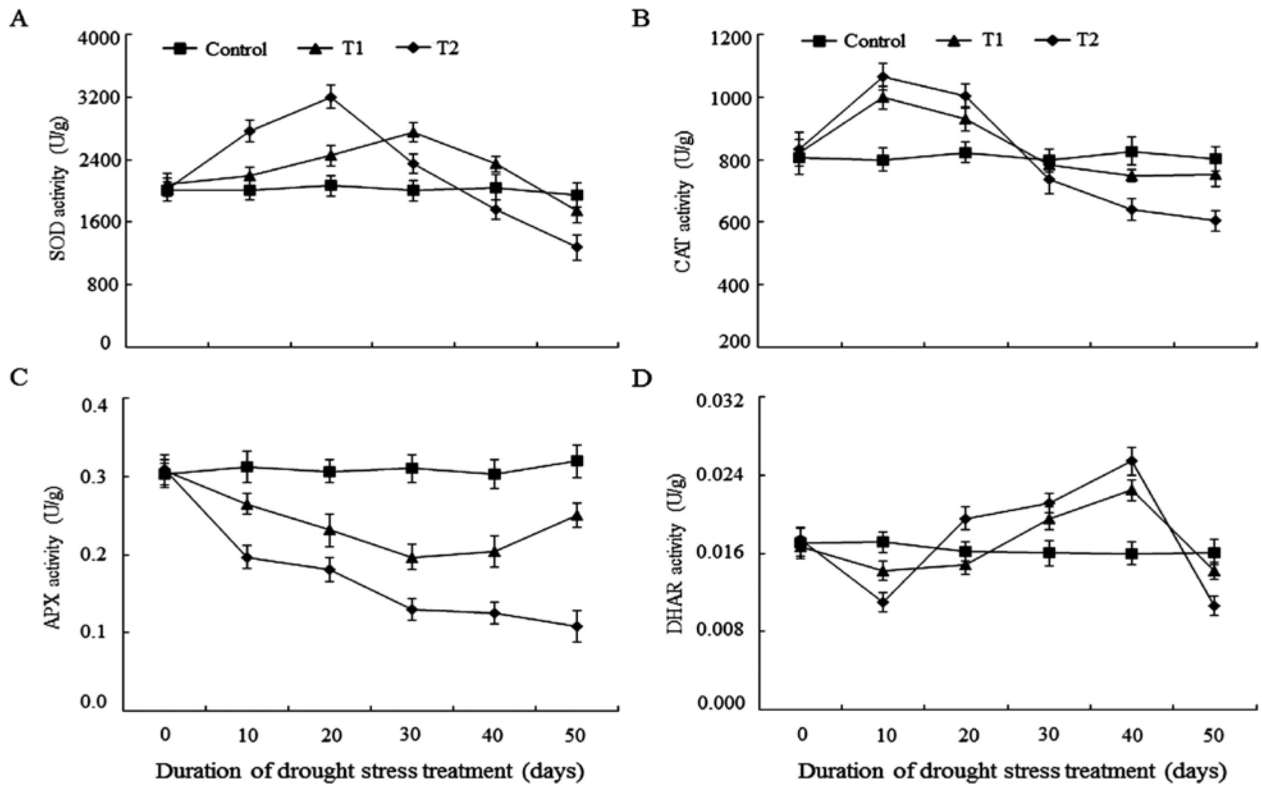

Fig. 2. Antioxidant enzyme activities of 'Fuji' apple under drought stress. (a) SOD, superoxide dismutase; (b) CAT, catalase; (c) APX, ascorbate peroxidase; and (d) DHAR, dehydroascorbate reductase.

expression showed a well conformity with enzyme activities (Fig. 3). Regarding DREB gene family, it was shown that *DREB1A*, *DREB2A* and *DREB2B* gene mRNA levels were upregulated significantly in treatment T2, whereas *DREB2C* were continuously down-regulated in both treatments. Besides, the expression of *NHX1*, *NPK1* and *LOS5* genes in treatment T2 peaked at 40 days and then decreased to initial levels, performing insensitive and none strong response to drought stress. Among the twelve genes, the expression of *SOD1*, *APX1*, *DREB2A* and *ZAT10* genes showed better positive reflection in response time and mRNA levels to drought stress than other genes detected. In treatment T2, the expression of *DREB2A* peaked at 20 days and then decreased sharply until the end of drought treatment. In contrast to the short-term action of *DREB2A*, *ZAT10* increased up to peak at 20 days and then decreased smoothly, which indicated a difference at functional stages during drought stress. The regulation pathway from *DREB2A* to *ZAT10* and then to antioxidant enzyme genes deserves further explorations (Mittler *et al.*, 4).

Overall, we investigated the influence of dwarfing interstocks on the 'Fuji' apple under drought stress, and ranked of six interstocks according to SF values calculated from six indices. Due to the restrictions of indice number, observation years and rootstocks *etc*., additional experiments are needed to further determine the precise interaction between interstocks and 'Fuji' apple under drought stress. Further, the studies on mechanisms of drought response regulation and exploration of key drought-tolerance components involved in drought tolerance are still inadequate.

ACKNOWLEDGEMENTS

This work was supported by the Beijing Natural Science Foundation (Project No. 6174037); and the Beijing Municipal Education Commission (Project No. CEFF-PXM2017014207000043).

REFERENCES

1. Bolat, I., Dikilitas, M., Ercisli, S., Ikinci, A. and Tonkaz, T. 2014. The effect of water stress on some morphological, physiological, and biochemical characteristics and bud success on apple and Quince rootstocks. *Sci. World J.* 2014: 1-8.

2. Liu, B.H., Cheng, L., Liang, D., Zou, Y.J. and Ma, F.W. 2012. Growth, gas exchange, water-use

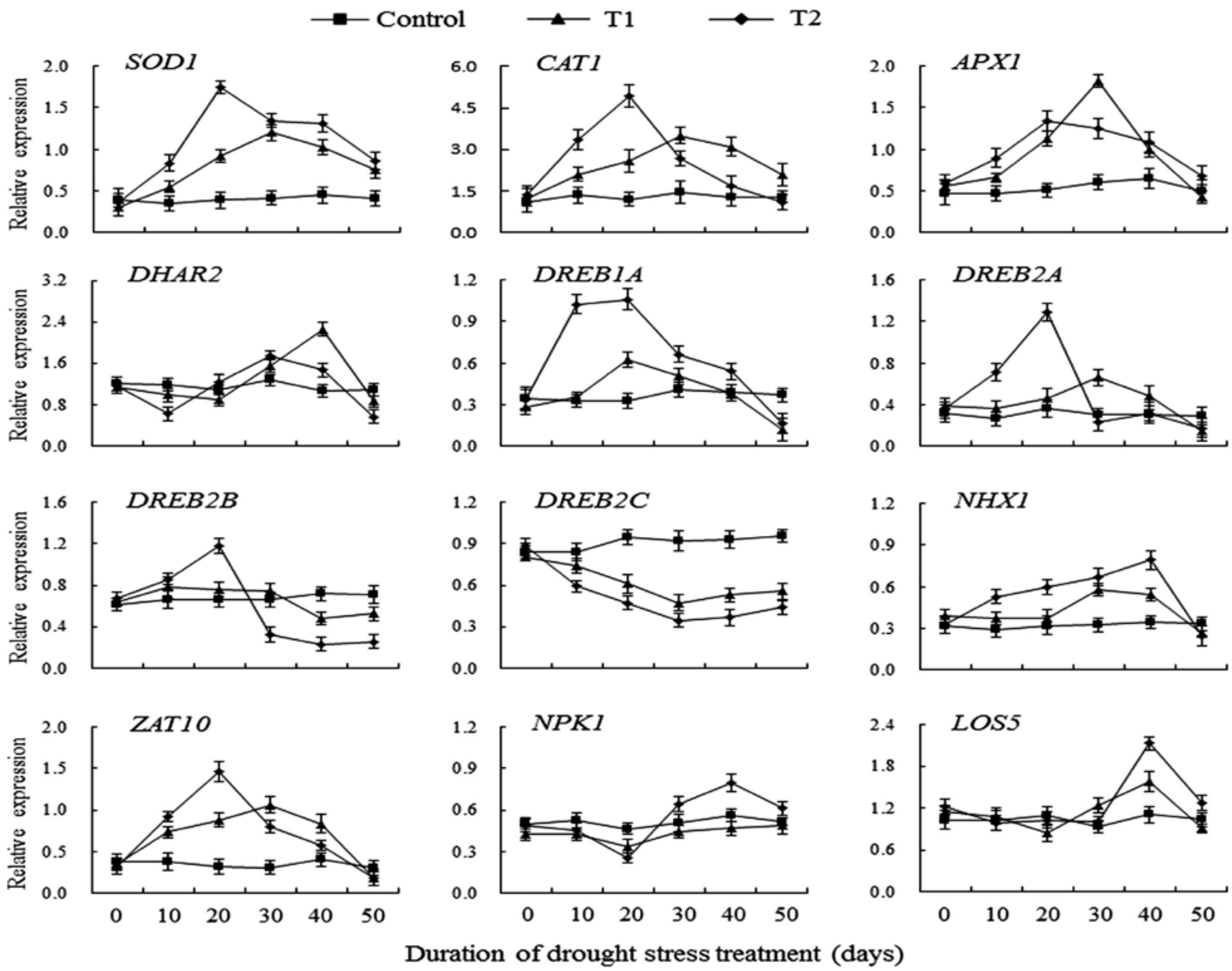

Fig. 3. Expression of 12 drought-related genes in 'Fuji' apple on SH6 interstock under drought stress.

efficiency, and carbon isotope composition of 'Gale Gala' apple trees grafted on 9 wild Chinese rootstocks in response to drought stress. *Photosynthetica*, **50**: 401-10.

3. Martínez-Ballesta, M.C., Alcaraz-López, C., Muries, B., Mota-Cadenas, C. and Carvajal, M. 2010. Physiological aspects of rootstock-scion interactions. *Scientia Hort.* **127**:112-18.

4. Mittler, R., Kim, Y., Song, L., Coutu, J., Coutu, A., Ciftci-Yilmaz, S., Lee, H., Stevenson, B. and Zhu, J.K. 2006. Gain- and loss-of-function mutations in Zat10 enhance the tolerance of plants to abiotic stress. *FEBS Lett.* **580**: 6537-42.

5. Pereira-Lorenzo, S., Ramos-Cabrer, A.M. and Fischer, M. 2009. Breeding apple (*Malus* x *domestica* Borkh). **In**: *Breeding Plantation Tree Crops: Temperate Species*, Jain, S.M. and Priyadarshan, P.M. (Eds.), Springer, New York, pp. 33-81.

6. Wang, S.C., Liang, D., Li, C., Hao, Y.L., Ma, F.W. and Shu, H.R. 2012. Influence of drought stress on the cellular ultrastructure and antioxidant system in leaves of drought-tolerant and drought-sensitive apple rootstocks. *Plant Physiol. Biochem*. **51**: 81-89.

7. Xiao, B.Z., Chen, X., Xiang, C.B., Tang, N., Zhang, Q.F. and Xiong, L.Z. 2009. Evaluation of seven function-known candidate genes for their effects on improving drought resistance of transgenic rice under field conditions. *Mol. Plant.* **2**: 73-83.

Received : July, 2016; Revised : June, 2017;
Accepted : July, 2017