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Research Report

Antioxidant Mechanism and Lipid Peroxidation Patterns in Leaves and Petals of Marigold in Response to Drought Stress

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Abstract. In this study, malondialdehyde (MDA), relative conductivity (RC), superoxide anion (O_2·-), hydrogen peroxide (H_2O_2), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) as well as ascorbic acid (AsA), glutation (GSH) and carotenoid (Car) were analyzed in plants under drought condition to investigate the enzymatic and non-enzymatic antioxidant defense mechanisms of leaves and petals, respectively. Two different drought resistance marigold cultivars (*Tagetes erecta* L. cv. Chokdee and *Tagetes erecta* L. cv. Discovery) treated with 6-day drought stress were used at early flowering stage. Results indicated that drought treatment increased MDA, RC, O_2·- and H_2O_2 contents in the two cultivars, especially in drought-sensitive cultivar 'Discovery'. In contrast, 'Chokdee' had higher level antioxidative enzyme activities and more non-enzymatic antioxidants than those in 'Discovery'. SOD, POD, CAT, APX activities and non-enzymatic antioxidants (GSH and AsA) in the leaves and petals were increased at the beginning treatment, and decreased later. The activity of CAT in leaves and petals, APX in petals and AsA in petals on day 6 after treatment were lower than those in control, while Car in the two cultivars decreased consistently during drought stress treatment. In addition, all the antioxidant enzyme activities in the leaves were higher than those in petals, but AsA and GSH were accumulated at lower levels in leaves than those in petals of the both cultivars. Furthermore, significant linear relationships were found between antioxidative enzymes and reactive oxygen species (ROS), as well as in non-enzymatic antioxidants and ROS. In conclusion, drought tolerance of 'Chokdee' was correlated with eliminating the O_2·- and H_2O_2 and maintaining lower lipid peroxidation as well as higher membrane stability by increasing activities of antioxidative enzymes and the amount of non-enzymatic antioxidants. Furthermore, different drought response mechanisms were involved in leaves and petals of marigold under drought stress.

***Additional key words*:** antioxidant enzymes, membrane permeability, non-enzymatic antioxidants, reactive oxygen species, *Tagetes erecta* L.

Introduction

Drought stress is one of the most important factors that affect the growth and development of plants. In normal conditions, a variety of reactive oxygen species (ROS) such as superoxide anion (O_2·-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH·) produced continuously as byproducts of various metabolic pathways that are localized in different cellular compartments like chloroplast and mitochondria (Navrot et al., 2007; Sun 2004). However, drought, heat and other environmental stresses induced sudden increase and accumulation of ROS could cause lipid peroxidation and lead to cell death (Imlay, 2003). It is known that antioxidant mechanisms, both enzymic and nonenzymic constituents, are involved in the protection of the plants against ROS. The antioxidant enzyme systems include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutation reductase (GR) and dehydroascorbate reductase (DHAR) (Fover et al., 1994; Reddy et al., 2004). SOD is believed to play a crucial role in antioxidant defense because it catalyzes the dismutation of O_2·- into H_2O_2 whereas CAT and POD destroy H_2O_2 (Scandalios, 1993). Furthermore, previous studies demonstrated that antioxidant enzyme activities are positively correlated with plant stress

tolerances, which has been reported in several field-grown crops, such as pea (Moran et al., 1994), maize (Wang et al., 2002), wheat (Keles et al., 2004) and rice (Wang et al., 2007). The nonenzymatic system is composed of low molecular metabolites, such as ascorbic acid (AsA), glutation (GSH), carotenoid (Car) and flavonoids and in which AsA is the most abundant, powerful and water soluble antioxidant acts to prevent or in minimizing the damage caused by ROS (Athar et al., 2008). Noctor et al. (1998) also confirmed that AsA and GSH could directly detoxify superoxide and hydroxyl radicals and thus contribute to nonenzymatic ROS scavenging. The Car is involved in protecting the photosynthetic apparatus by quenching $^1O_2$ and other harmful free radicals which are synthesized during photosynthesis (Collins, 2001).

Although many studies have shown that the induction of antioxidant machinery is important against environmental stresses, most of them only focused on the leaves to drought tolerance and few studies investigated leaf and petal systems at the same time, especially in ornamental plants.

Marigold (*Tagetes erecta* L.) belongs to Asteraceae family and is normally used for a bedding plant, cut flower, or coloring agent in poultry feed to obtain yellow egg yolks (Bao, 2003). In addition, this species planted as intercrops or in rotation with crops to reduce the effects of infested soil (Alexander and Waldenmaier, 2002). Due to the importance of this species, the detailed research is needed. However, there are no data available regarding to the relationship between antioxidant defense mechanism and drought stress in marigold cultivars. Therefore, this study aims to investigate antioxidant defense mechanism responsible for differential drought tolerance in two marigold cultivars, *Tagetes erecta* L. cv. Chokdee and *Tagetes erecta* L. cv. Discovery and to characterize the drought response pattern of leaves and petals at early flowering stage in marigold. The results would improve our understanding in marigold breeding and the mechanisms of marigold drought tolerance in regions arid and semi-arid areas. For these purposes we studied malondialdehyde (MDA), relative conductivity (RC), hydrogen peroxide (H_2O_2) and superoxide anion (O_2·-). superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), ascorbic acid (AsA) and carotenoid (Car), as well as glutation (GSH) in leaves and petals.

Materials and Methods

Plant Material and Cultivation

The experiments were conducted in a greenhouse at horticulture college of Northwest A&F University, Yangling (34°12′-34°20′ N; 108°-108°7′ E, elevation 560 m), Shaanxi, P. R. China in 2010. Drought tolerant cultivar (*Tagetes erecta* L. cv. Chokdee) and drought sensitive cultivar (*Tagetes erecta* L. cv. Discovery) were sown on 14 June and transferred to culture pots (14 × 16 cm) with a mixture of peat mass and soil (1:2, v/v) at four-leaf seedlings. The average air temperature and relative humidity (RH) during treatment was 25℃ and 60%, respectively. After 8 weeks, 72 pots of similar size plants (34-40 × 18-22 cm, height × crown diameter) were selected and placed in a greenhouse with mean temperature 23-30/16-19℃ (day/night), and RH between 70/50% (morning/late afternoon) during the experiment. Subsequently, all plants were exposed to two treatments, the regular irrigation (one time everyday) and 6 days of continuous drought in the dry season. Soil water content decreased from 37% (control) to 5% under drought stress treatment. Three replicates per treatment and each replicate include 3 pots. The experiments were performed using third-fourth leaves from each individual plant, which were excised at 0 d, 2 d, 4 d, 5 d, and 6 d.

Measurements of Physiological Parameters

MDA was extracted using trichloroacetic acid (ACA) and calculated according to Gao (2000). RC was determined using a DSS conductivity meter according to the method of Gao (2000). H_2O_2 content was measured by the method of Alexieva et al. (2001). The formation rate of O_2·- was determined as described by Ke et al. (2002).

The extraction medium for enzymes (SOD, POD, CAT, APX) was made of 8 mL of ice-cold phosphate buffer (0.05 M pH 7.8) and 1% (W/V) PVP. Leave and petal tissues (0.5 g, respectively) were ground, and centrifuged at 12,000 g for 20 min at 4℃. Supernatant was used for determination of enzymes. SOD, POD and CAT activities were measured according to Gao (2000). The activity of APX was determined as in Nakano (1981).

The extraction buffer was 8 mL of 10% TCA with 1% PVP (W/V). AsA was assayed according to Tanaka et al. (1985). GSH was determined according to Lu et al. (1999). Car was measured by following the method of Gao (2000). Leaf samples (0.02 g) were kept in 5 mL of 80% acetone for 24 h and then absorbance were recorded using spectrophotometer.

Statistical Analysis

Statistical analysis was tested using the SPSS 18.0 software. Mean of results were compared using Duncan test at 0.05 levels.

Results

MDA and RC

Drought stress increased MDA of leaves compared with

control (Fig. 1A). It was increased markedly in 'Discovery' cultivar (1.32-fold) on day 4 when compared to day 0, which is higher than that in 'Chokdee'. The MDA of leaves was increased by 2.19-fold in 'Discovery' on day 6, whereas 'Chokdee' was increased by 1.34-fold. Similarly, 'Chokdee' and 'Discovery' of petals in MDA showed significant differences (Fig. 1B). The MDA of petals were increased by 2.01 and 1.24-fold in 'Discovery' and 'Chokdee' on day 4 respectively. As the stress developed, MDA of 'Discovery' was 3.29-fold on day 6 compared to the reference ones, while 'Chokdee' increased by 1.75-fold.

RC of leaves was significantly different between the two cultivars under drought stress (Fig. 2A). The RC of leaves showed transient change in 'Discovery', with an increase of 1.50-fold compared to control after 2 days of drought stress. The RC of leaves was increased rapidly in 'Discovery' (2.68-fold) on day 6, while 'Chokdee' showed lower increase rate (1.76-fold). A significant higher amount of RC was also observed in petals of 'Discovery' than that in 'Chokdee' (Fig. 2B). The RC of petals in 'Discovery' sharply increased up to 33.77%, while RC in 'Chokdee' only rose to 20.56% on day 4. After 6 days' drought stress, 'Discovery' and 'Chokdee' increased by 2.10 and 1.49-fold, respectively.

A significant positive correlation was found between MDA and RC in leaves and petals of the two marigold cultivars under drought stress ($r_{0.01}$ = 0.948, $r_{0.01}$ = 0.955) (Table 1).

H_2O_2 and Formation Rate of O_2·-

Drought stress increased H_2O_2 of leaves (Fig. 3A). After 2 days drought stress, a significant increase (54.89%) in H_2O_2 of leaves was observed in 'Discovery' compared with control but not in 'Chokdee' cultivar. 'Discovery' accumulated 280.45% on day 6 which is much higher than control, whereas 'Chokdee' was 139.67%. A similar tendency was observed in petals (Fig. 3B). The H_2O_2 of petals significantly increased by 94.27% in 'Discovery' on day 4, however, slightly increased by 14.47% in 'Chokdee'. The effect on H_2O_2 of petals in 'Chokdee' was smaller than in 'Discovery' on day 6 (77.45 and 274.01%, respectively).

Alteration in formation rate of O_2·- of leaves in response to drought treatment were shown in Fig. 4A. Both two cultivars showed a significant increase with stress developed, but 'Discovery' has clearly higher than 'Chokdee'. 'Discovery' and 'Chokdee' showed the rate of increase (41.07 and 5.11%, respectively) on day 6 with respect to control.

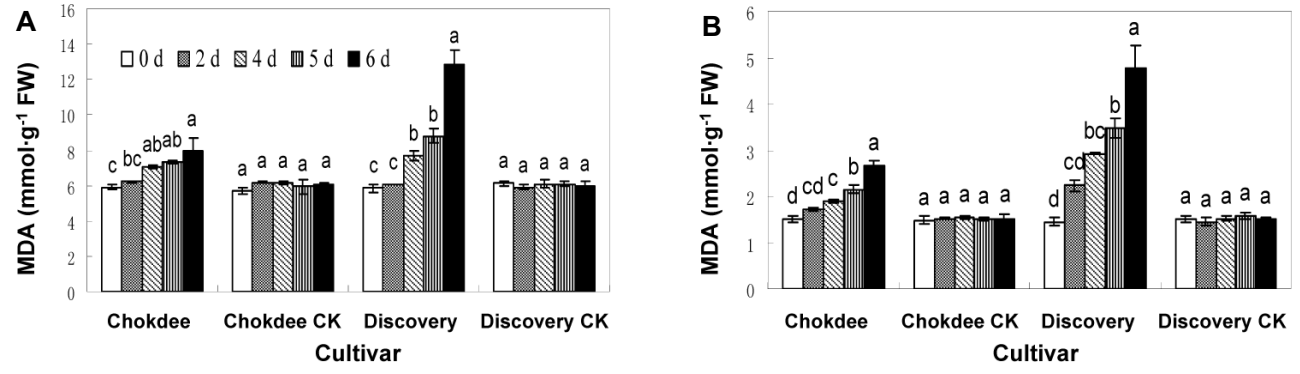

Fig. 1. Effect of drought stress on MDA of leaves (A) and petals (B). Bars represent standard errors of three replications. Different letters on top of bars represent significantly differences from the non-stressed one, at $P < 0.05$.

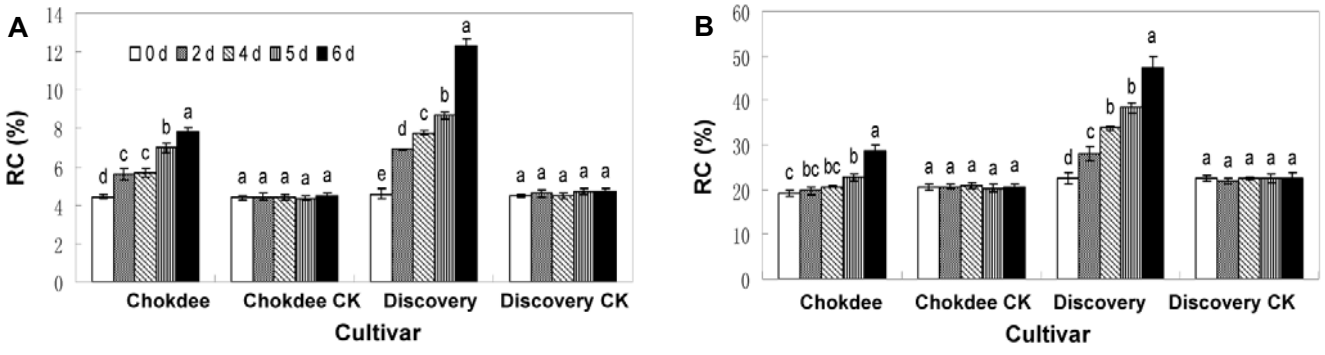

Fig. 2. Effect of drought stress on RC of leaves (A) and petals (B). Bars represent standard errors of three replications. Different letters on top of bars represent significantly differences from the non-stressed one, at $P < 0.05$.

Similarly, significantly higher $O_2 \cdot -$ of petals was observed in 'Discovery' than that in 'Chokdee' (Fig. 4B). 'Discovery' showed a significant increase in petals on day 2, while the significant increase was on day 5 in 'Chokdee'. 'Discovery'

Table 1. Correlations of physiological index in leaves.

A	SOD	POD	CAT	APX	AsA	GSH	Car	H_2O_2	$O_2 \cdot -$	MDA	RC
SOD	1										
POD	0.875**	1									
CAT	0.667*	0.447	1								
APX	0.536	0.627	-0.046	1							
AsA	0.847**	0.950**	0.471	0.575	1						
GSH	0.596	0.460	0.230	0.498	0.322	1					
Car	-0.077	-0.203	0.663*	-0.574	-0.138	-0.246	1				
H_2O_2	-0.342	-0.148	-0.844**	0.221	-0.187	-0.060	-0.849**	1			
$O_2 \cdot -$	-0.791**	-0.552	-0.705*	-0.153	-0.524	-0.328	-0.603	0.880**	1		
MDA	-0.348	-0.229	-0.843**	0.147	-0.251	-0.068	-0.790**	0.922**	0.888**	1	
RC	-0.288	-0.189	-0.797**	0.189	-0.174	-0.013	-0.833**	0.962**	0.893**	0.948**	1
B	SOD	POD	CAT	APX	AsA	GSH	H_2O_2	$O_2 \cdot -$	MDA	RC	
SOD	1										
POD	0.918**	1									
CAT	0.739*	0.817**	1								
APX	0.730*	0.738*	0.896**	1							
AsA	0.705*	0.728*	0.885**	0.786**	1						
GSH	0.569	0.728*	0.641*	0.490	0.499	1					
H_2O_2	-0.490	-0.365	-0.734*	-0.601	-0.645*	-0.063	1				
$O_2 \cdot -$	-0.620*	-0.381	-0.568	-0.595	-0.747*	0.021	0.969**	1			
MDA	-0.408	-0.330	-0.694*	-0.595	-0.768**	-0.027	0.967**	0.975**	1		
RC	-0.573	-0.439	-0.598	-0.614	-0.746*	-0.034	0.968**	0.987**	0.955**	1	

*Significant at $P < 0.05$, **Significantat $P < 0.01$.

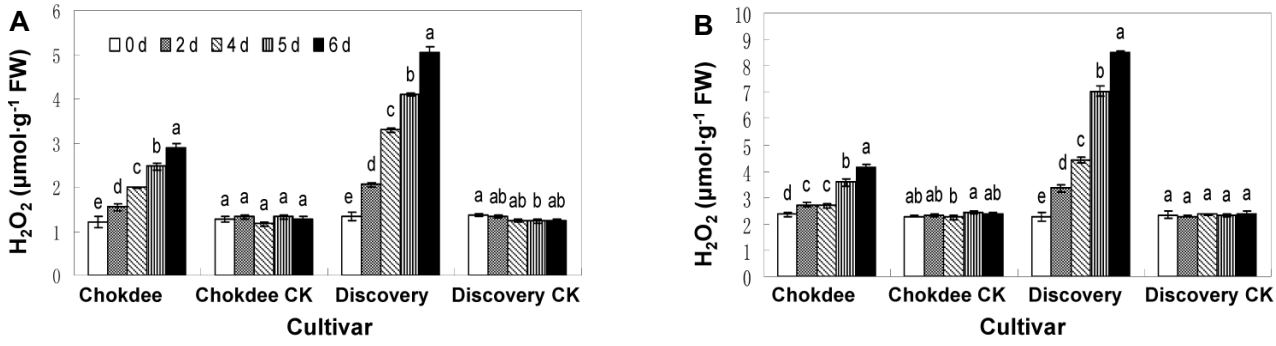

Fig. 3. Effect of drought stress on H_2O_2 of leaves (A) and petals (B). Bars represent standard errors of three replications. Different letters on top of bars represent significantly differences from the non-stressed one, at $P < 0.05$.

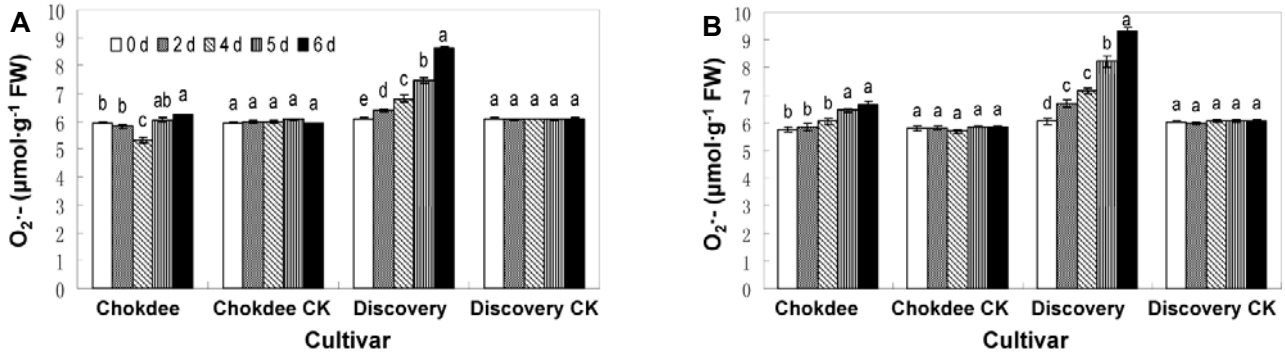

Fig. 4. Effect of drought stress on $O_2 \cdot -$ of leaves (A) and petals (B). Bars represent standard errors of three replications. Different letters on top of bars represent significantly differences from the non-stressed one, at $P < 0.05$.

and 'Chokdee' increased by 53.38 and 15.66% under drought stress 6 days, respectively.

The path coefficient analysis showed a significant positive correlation between H_2O_2 and O_2·- in leaves and petals ($r_{0.01}$ = 0.880, $r_{0.01}$ = 0.969) (Table 1).

Antioxidant Activities (SOD, POD, CAT, and APX)

Drought stress resulted in increase of SOD activity, both in leaves and petals (Fig. 5). 'Chokdee' showed a significantly higher SOD activity of leaves than that in 'Discovery'. The SOD activity of leaves in the two cultivars was increased initially, and then decreased from 4 d to 6 d. However, the SOD activity of 'Chokdee' still remained higher than that in 'Discovery' on day 6 (1.17 and 1.00-fold, respectively) compared to control. The response of SOD activity to drought stress in petals was similar to that in leaves, but with a lower level of SOD activity in petals than in leaves (Fig. 5B). The SOD activity in petals was 1.26-fold higher in 'Chokdee' on day 4 than that in the control, while it was 1.02-fold higher in 'Discovery' than that in the control. The SOD activities of leaves and petals were negatively correlated with the change of O_2·- formation rate ($r_{0.01}$ = 0.791, $r_{0.05}$ = 0.620, respectively) (Table 1).

Effect of drought stress on POD activity of leaves differed greatly in leaves of the two cultivars (Fig. 6A). The POD activity of leaves in the two cultivars went up to the peak on day 4, then showed decline. However, the POD activity in 'Chokdee' was higher than in 'Discovery'. In 'Chokdee', the activity increased markedly by 44.17% after 6 days drought stress treatment, while in 'Discovery', the increase was slight (0.54%). Meanwhile, the POD activity of petals in 'Chokdee' increased by 110.00% in comparison with 0 d, whereas it was only 11.11% higher in 'Discovery' than on day 0. Furthermore, the POD activity of petals was lower than that of leaves in the two cultivars.

We also observed that CAT activity of the leaves rose firstly, and then declined under water deficit conditions (Fig. 7A). The decrease of CAT activity in leaves was significant after 5 d in the case of 'Discovery', but remaining significantly higher in the case of 'Chokdee'. At the end of drought stress, the CAT activity of leaves decreased by 45.21% in 'Discovery', whereas the decrease in 'Chokdee' was 10.14%, as compared to their controls. It was also observed in petals of the two cultivars (Fig. 7B). The CAT activity in petals of 'Discovery' and 'Chokdee' peaked at 8.53 and 11.91 U·min^{-1}·g^{-1} FW on day 4, respectively. But

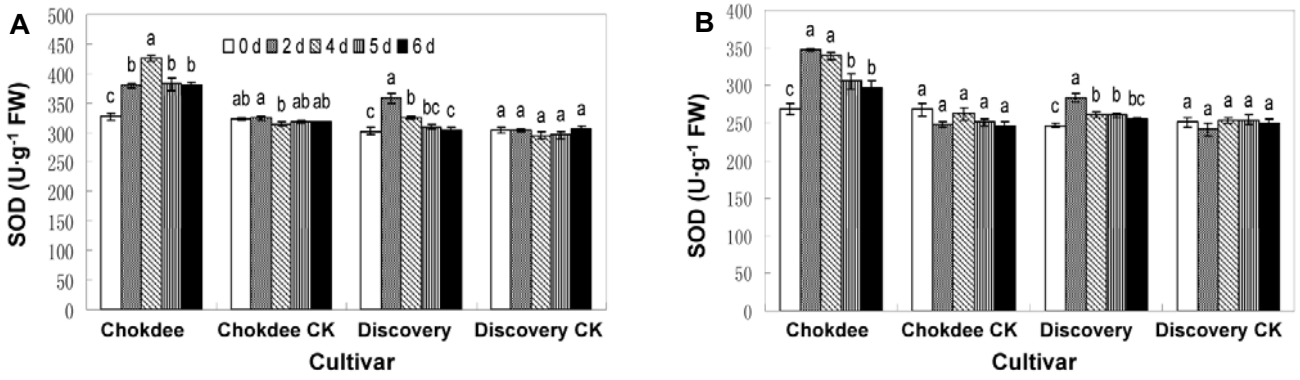

Fig. 5. Effect of drought stress on SOD of leaves (A) and petals (B). Bars represent standard errors of three replications. Different letters on top of bars represent significantly differences from the non-stressed one, at $P < 0.05$.

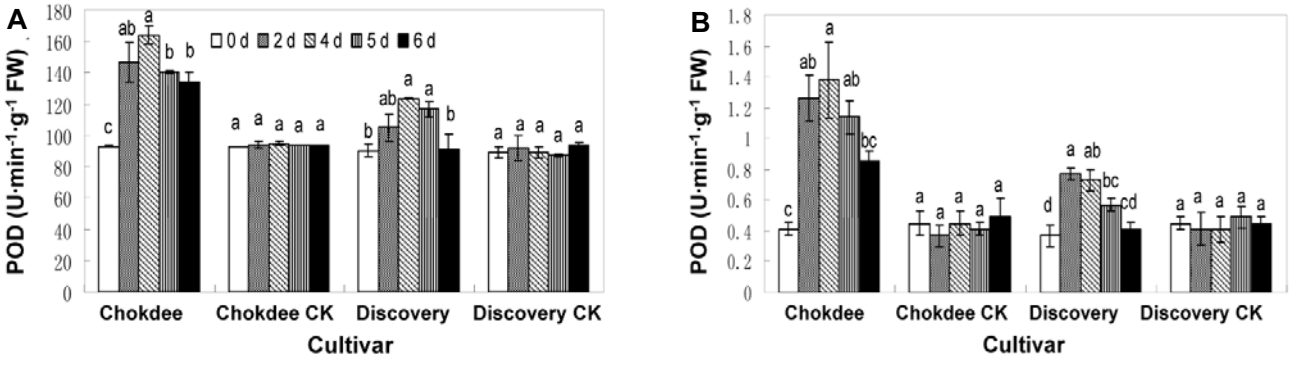

Fig. 6. Effect of drought stress on POD of leaves (A) and petals (B). Bars represent standard errors of three replications. Different letters on top of bars represent significantly differences from the non-stressed one, at $P < 0.05$.

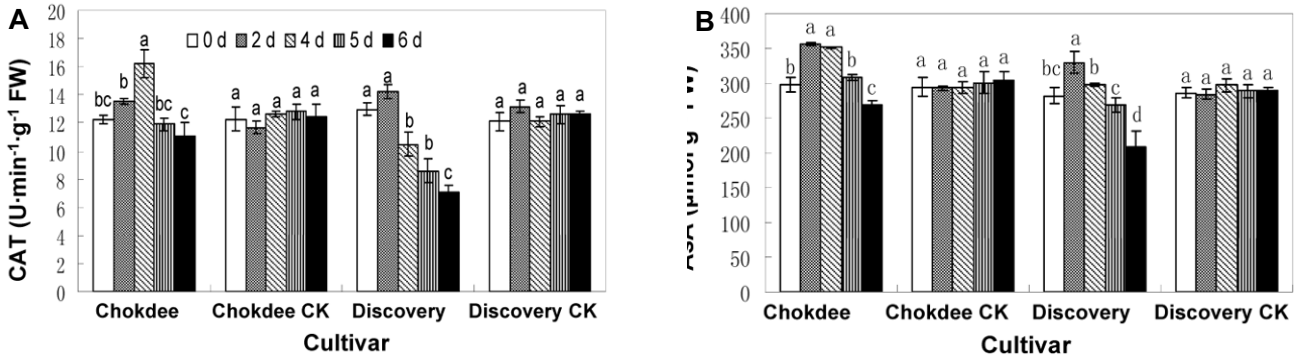

Fig. 7. Effect of drought stress on CAT of leaves (A) and petals (B). Bars represent standard errors of three replications. Different letters on top of bars represent significantly differences from the non-stressed one, at $P < 0.05$.

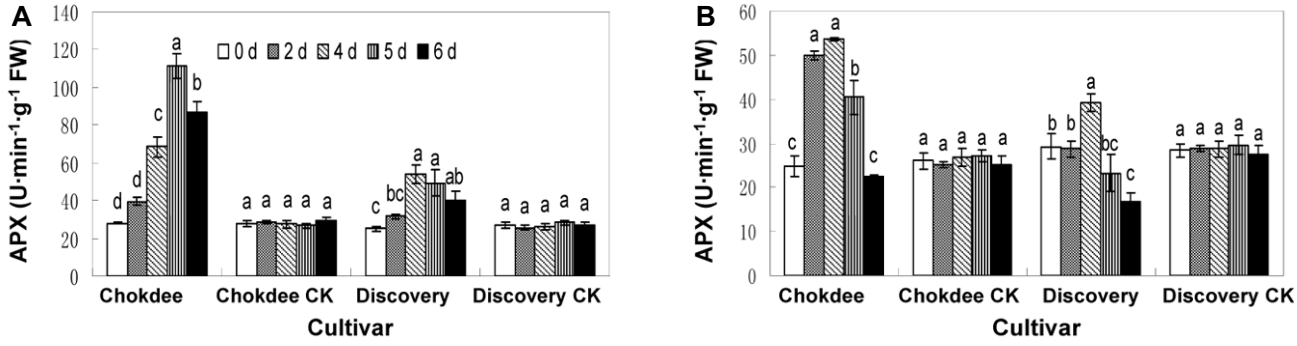

Fig. 8. Effect of drought stress on APX of leaves (A) and petals (B). Bars represent standard errors of three replications. Different letters on top of bars represent significantly differences from the non-stressed one, at $P < 0.05$.

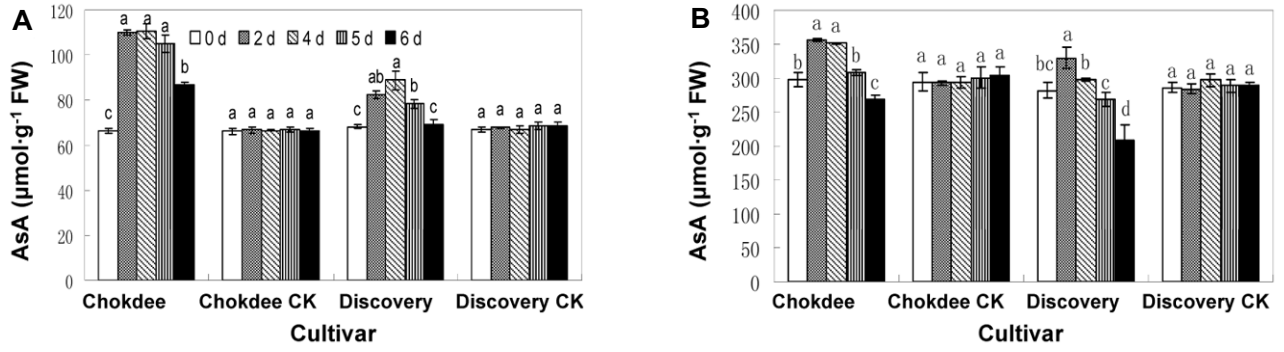

Fig. 9. Effect of drought stress on AsA of leaves (A) and petals (B). Bars represent standard errors of three replications. Different letters on top of bars represent significantly differences from the non-stressed one, at $P < 0.05$.

a considerable drop was detected with drought stress in petals of 'Discovery' on day 5. After drought treatment 6 days, 'Discovery' and 'Chokdee' showed significant decrease 74.08 and 4.17%, respectively. The level of CAT in leaves and petals were negatively correlated with H_2O_2 ($r_{0.01}$ = 0.844, $r_{0.05}$ = 0.734, respectively) (Table 1).

Under drought conditions, APX activity of leaves in the two cultivars rose firstly, and then declined on 5-6 d (Fig. 8A). There was significantly higher APX activity in 'Chokdee' than in 'Discovery'. 'Discovery' presented the maximum rate of increase (114.29%) on day 4, whereas APX activity of leaves in 'Chokdee' went up to peak and increased by 298.57% on day 5. After 6 days, the APX activity of leaves in 'Discovery' changed to 40.40 $U \cdot min^{-1} \cdot g^{-1}$ FW, while 'Chokdee' maintained high lever (86.80 $U \cdot min^{-1} \cdot g^{-1}$ FW). As severe drought stress developed, the APX activity of petals increased first but decreased afterwards. 'Discovery' and 'Chokdee' exhibited reduction (42.46 and 9.68%, respectively) in APX of petals on day 6 compared to 0 d.

Antioxidants (AsA, GSH, and Car)

Drought stress induced an increase in AsA of leaves, but

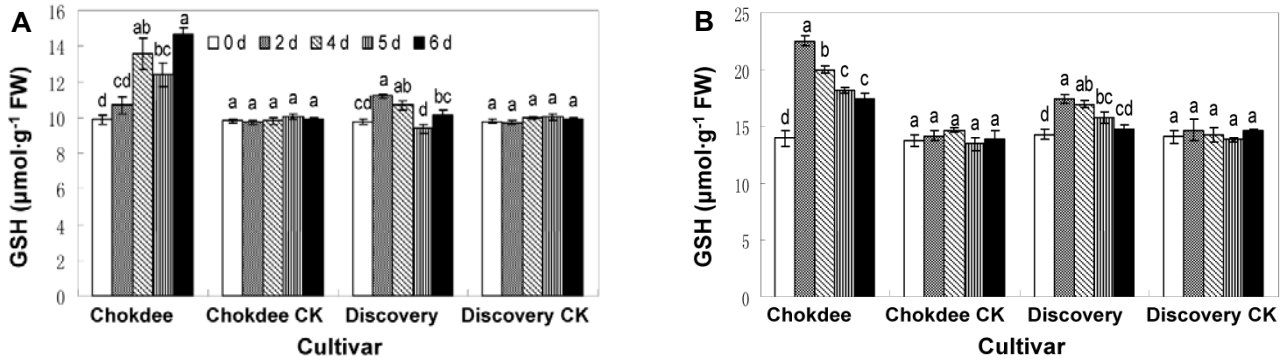

Fig. 10. Effect of drought stress on GSH of leaves (A) and petals (B). Bars represent standard errors of three replications. Different letters on top of bars represent significantly differences from the non-stressed one, at $P < 0.05$.

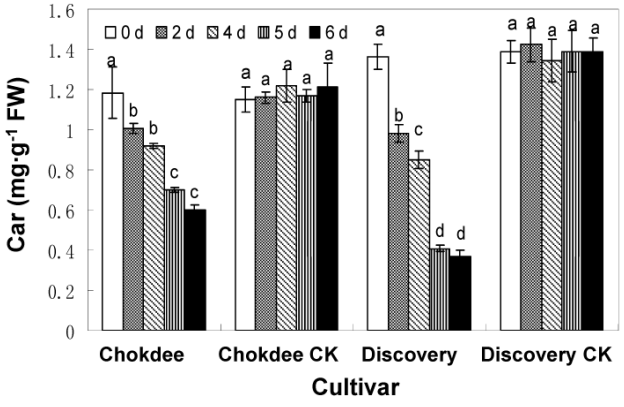

Fig. 11. Effect of drought stress on Car of leaves. Bars represent standard errors of three replications. Different letters on top of bars represent significantly differences from the non-stressed one, at $P < 0.05$.

the difference was significant under drought treatment between the two cultivars (Fig. 9A). The AsA of leaves was 1.31-fold higher in 'Chokdee' on day 6 than control, whereas it was 1.01-fold higher in 'Discovery' than control. The reduction of AsA in the two cultivars was observed in petals (Fig. 9B). A transient decline of AsA in 'Discovery' was observed. After 6 days, the AsA of petals in 'Discovery' decreased by 26.27%, while it declined 9.92% in 'Chokdee'.

The two cultivars exhibited a similar GSH of leaves in control conditions. However, drought stress induced increase of GSH of leaves, and the increase was more markedly in 'Chokdee' than in 'Discovery' (Fig. 10A). Moreover, the content of GSH increased at the early stage and then decreased gradually. The GSH in 'Chokdee' and 'Discovery' increased by (48.28 and 3.89%, respectively) on day 4 in comparison with 0 d. A similar tendency was also observed in petals (Fig. 10B). The GSH of petals in 'Chokdee' and 'Discovery' increased by 61.39 and 21.88% on day 2, respectively. Nevertheless, the GSH decreased gradually from 2 d to 6 d, in particular, 'Discovery' decreased gradually to the level of the control.

The Car of the two cultivars was significantly reduced during the experimental period (Fig. 11). During water stress, 'Discovery' showed a rapidly decline in Car content from 0 d to 4 d. The reduction in Car of 'Discovery' and 'Chokdee' was 77.92 and 48.77% on day 6, respectively, as compared to control. Additionally, The Car of leaves was negatively correlated with H_2O_2 and MDA ($r_{0.01} = 0.849$, $r_{0.01} = 0.790$, respectively) (Table 1).

Discussion

Drought stress conditions trigger a wide variety of plant responses, ranging from physiological and metabolic processes to change. Environmental stresses result in accelerated production of ROS, which may cause lipid peroxidation and consequent membrane injuries since peroxidation of membrane lipids would enhance membrane fluidity and electrolyte leakage (Thompson et al., 1987). MDA is an indicator of lipid peroxidation and MDA together with RC to evaluate the drought stress in plants (Sun et al., 2010; Zlatev et al., 2006). This study observed that drought sensitive cultivar 'Discovery' showed significantly higher MDA and RC in leaves and petals than those in drought tolerant cultivar 'Chokdee'. The results indicated that drought tolerant marigold cultivar maintains higher membrane stability and lower lipid peroxidation under drought treatment, which was in agreement with previous studies (Li et al., 2006; Wang et al., 2007).

Plant tolerance to environmental stresses is associated with decomposing of $O_2\cdot-$ and H_2O_2 of plants subjected to stress (Demiral and Turkan 2005; Turkan et al., 2005). Mittler (2002) reported that the damage of cell membranes may attribute to high H_2O_2 levels, which could accelerate the Haber-Weiss reaction, increasing the formation and therefore prompting lipid peroxidation. Qian et al. (2010) suggested that that $O_2\cdot-$ and H_2O_2 of leaves in Buchloe dactyloides were quantitatively regressive to the concentration of PEG-6000 over time course. In our experiment,

drought stress resulted in the increase of O_2·- and H_2O_2 in leaves and petals. Moreover, sensitive cultivar 'Discovery' accumulated higher O_2·- and H_2O_2 than in drought tolerant cultivar 'Chokdee'. Additionally, H_2O_2 concentration in the petals was higher than that in leaves. The differential responses of H_2O_2 accumulation between the leaves and petals may be connected with different protective mechanisms in marigold. The enhanced eliminating ability for O_2·- and H_2O_2 in tolerant cultivar inhibited the accumulation of ROS and thus protected the plant from lipid peroxidation oxidative damage under drought stress. Therefore, in comparison to the tolerant cultivar, more MDA was accumulated in drought sensitive cultivar under drought stress. These results are in accordance with reports on alfalfa (Wang et al., 2009), pepper (Hu et al., 2010), and Thomson navel orange (Tajvar et al., 2011).

Plants have an efficient system for decomposing reactive oxygen species, using antioxidative enzymes such as SOD and APX in chloroplasts (Asada, 1999). The increasing of SOD activity induces the higher tolerance to oxidative stress (Bowler et al., 1991). In this study, antioxidative enzyme activities increased under drought treatment, especially for SOD and POD, which indicates that drought stress induces the expression of the enzymes in marigold. Antioxidative enzymes activity declined significantly with increasing drought stress. Bai et al. (2006) reported a similar result. Additionally, our results showed that the two cultivars responded to drought stress differently in terms of activities of antioxidative enzyme. The enzyme activities in drought tolerant cultivar 'Chokdee' increased more markedly than that in drought sensitive cultivar 'Discovery'. The similar results were observed in wheat (Dhanda et al., 2004; Shao et al., 2005) and rice (Guo et al., 2006). Moreover, enzyme activities in leaves of the two cultivars were higher than that in petals. This implied that antioxidant enzymes would be predominantly responsible for controlling oxygen in leaves.

CAT is a main enzyme to eliminate H_2O_2 in the mitochondrion and microbody (Shigeoka et al., 2002). APX is a component of the ascorbate-glutathione pathway, which plays a key role in scavenging H_2O_2 (Foyer et al., 2005). In our experiment, the CAT and APX activities in leaves and petals increased in the early periods of drought treatment, and then declined significantly with drought stress. The results suggested that the increase of enzyme activities in marigold is a defensive response to water stress, while this self-regulation level had less with progress of water deficit. The reduction of CAT would result in accumulation of H_2O_2 and increase in lipid peroxidation, thereby enhancing MDA and causing damage in plants. In addition, the enzyme activities in leaves and petals of the two cultivars have different changing time course.

The balance between SOD and CAT activities in cells is crucial for determining the steady-state level of O_2·- and H_2O_2 (Mathur et al., 2009). In this study, there were more significant linear trends to antioxidative enzymes and reactive oxygen species. A negative correlation was found between SOD and O_2·-, moreover, CAT was negatively correlated with H_2O_2 and MDA, indicating that the antioxidative enzyme activities were significantly correlated to drought resistance of marigold cultivars under drought stress. Besides, there was a significant positive correlation among SOD, POD, CAT and APX. It was noted that the synergistic reactions of SOD-POD-CAT-APX system effectively against oxidative damage in marigold cultivars subjected to drought stresses. This result was in good agreement with the result of Cavalcanti et al. (2004) and Han et al. (2010).

AsA and GSH not only act as substrates in the ascorbate-glutathione cycle, but also perform non-enzymatically in the eliminating O_2·- and H_2O_2. Their higher contents have been owed to alleviate of the injury by ROS (Schonhof et al., 2007). Liu et al. (2009) reported that Paraquat (PQ) and polyethylene glycol (PEG) significantly increased the contents of GSH and AsA in cucumber leaves. There is evidence that AsA and GSH increased and Car declined in different plants under drought stress, such as Calycanthus chinensis (Ke et al., 2007). In our study, it was noted that drought tolerant marigold cultivar had higher AsA, GSH, and Car levels than that in drought sensitive cultivar 'Discovery'. Furthermore, there was a negative correlation between Car and H_2O_2, and also between AsA and H_2O_2.

In conclusion, drought tolerance in marigold 'Chokdee' is associated with diminishing oxidative injury. The antioxidant system of marigold functioned at higher antioxidative enzymes and high non-enzymatic antioxidant contents to eliminate the O_2·- and H_2O_2 efficiently under drought. In addition, we concluded that the antioxidant enzyme activities in leaves were higher than that in petals, whereas nonenzymic constituents (AsA and GSH) in petals were accumulated more markedly than that in leaves. Antioxidant metabolisms of leaves and petals in marigold respond differently to drought stress.

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