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Physiological characteristics, antioxidant enzyme activities, and gene expression in 2 spring canola (*Brassica napus* L.) cultivars under drought stress conditions

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Abstract: This study examined the influence of drought stress during the flowering stage of 2 canola cultivars on some physiological characteristics, antioxidant activities, and gene expression of antioxidant enzymes. Significant differences were observed among interactions of cultivars and levels of drought stress in total shoot dry material, relative water content percentage, proline, carbohydrates, gene expression, and activities of antioxidant enzymes. Moreover, a significant positive correlation was indicated between shoot dry materials with relative water content percentage, sucrose, catalase activity, ascorbate peroxidase activity, and cytosolic ascorbate peroxidase relative gene expression ratio. Antioxidant enzymatic activity and the association between gene transcript levels and activity seem to play roles in protecting plants from oxidative damage in drought stress. The lowest ascorbate peroxidase activity was measured under normal conditions in the cultivar Option (409 nmol ascorbate oxidation per min/g protein), and the highest ascorbate peroxidase was observed in RGS003 (600 nmol ascorbate oxidation per min/g protein) in high stress. Additionally, in the case of relative gene expression ratio the lowest cytosolic ascorbate peroxidase was observed under normal conditions in Option (1.05), and the highest cytosolic ascorbate peroxidase was seen in RGS003 (1.48) in high stress. According to the results of the current study, RGS003 seems to be more tolerant than Option.

Key words: Antioxidant enzyme, *Brassica napus*, drought stress, gene expression, physiological traits

1. Introduction

Drought stress is the main limiting factor to plant growth and productivity in many regions of the world (Borsani et al., 2001). Drought induces oxidative stress resulting from increased production of reactive oxygen species (ROS), including superoxide (O_2^-), singlet oxygen ($^1O_2$), hydroxyl radical (OH*), and hydrogen peroxide (H_2O_2), which can attack lipids, proteins, carbohydrates, and nucleic acids (Smirnoff, 1993). Drought stress inhibits the photosynthesis of plants and causes changes in chlorophyll contents and components and damage to the photosynthetic apparatus (Nayyar and Gupta, 2006). Moreover, it inhibits photochemical activities and decreases activities of enzymes in the Calvin cycle in photosynthesis (Monakhova and Chernyadev, 2002). Several enzymes detoxify ROS resulting from stress. Superoxide dismutase (SOD) (EC. 15.1.1) is the first defense enzyme that converts superoxide to H_2O_2, which can be scavenged by catalase (CAT) (EC 1.111.1.6) and different classes of peroxidases (POX) (EC. 1.111.1.7) and ascorbate peroxidase (Bowler et al., 1992). Catalase, peroxidases, and the ascorbate–glutathione cycle (AsA-GSH cycle), which involves 4 enzymes including ascorbate peroxidase, glutathione reductase (GR), monodehydroascorbate reductase (MR), and dehydroascorbate reductase (DR), are the major components scavenging H_2O_2, the product of dismutation of O_2 by SOD at different cellular compartments (Asada, 1999). Previous studies have indicated that higher activity levels of antioxidant enzymes may contribute to better drought tolerance by increasing protection capacity against oxidative damage (Sharma and Dubey, 2005). However, alterations in antioxidant enzyme activities under drought stress are dependent on plant species, cultivar, and stress intensity and duration (DaCosta and Huang, 2007). Abedi and Pakniat (2010) reported that under drought stress conditions peroxidase activity increased in all cultivars of rapeseed, but catalase activity increased in some cultivars and decreased in others. Hojati et al. (2011) demonstrated that under drought stress CAT activity increased in leaves and roots of safflower compared with well-watered plants. POX activity also increased in all drought treatments when compared with the control (Hojati et al., 2011). CAT activity increased in both cultivars under drought stress when compared with the

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control in wheat (Shao et al., 2005). Limited research has focused on gene expression patterns in conjunction with the underlying enzymes promoting drought tolerance. The over- expression of SOD, if accompanied by enhanced H_2O_2 scavenging mechanisms like CAT and POX enzyme activities, is considered an important antidrought mechanism in crops with oxidative stress during drought stress conditions (McKersie et al., 1999). Understanding the association of antioxidant enzyme activity and gene expression with genetic variation in drought tolerance is important for further understanding molecular factors controlling antioxidant defense for drought tolerance (Xu et al., 2011). The current study examined antioxidant enzyme responses to drought stress and transcript levels associated with drought in 2 spring canola cultivars. Our aim was to compare physiological responses and changes in antioxidant enzyme activities and gene expression patterns in response to 3 levels of drought stress.

2. Materials and methods

This experiment was carried out in a greenhouse of the Agricultural Biotechnology Research Institute of Iran (ABRII), located in Karaj (Iran) at 35°48′N latitude, 50°57′E longitude, 1320 m above sea level, in the 2011–2012 growing season. The sowing date was 11 November 2011. This factorial experiment was based on a completely randomized design and was conducted in 4 replications (2 plants in each pot and 4 pots per replication). Factors included different canola cultivars, namely RGS003 (tolerant cultivar) and Option (sensitive cultivar), and drought stress including normal irrigation (75AW) (available water), moderate stress conditions (50AW), and high stress conditions (25AW). For supplying the crop nutrient requirements, fertilizers of urea, superphosphate, and potassium nitrate were used. Plants were kept in optimum conditions of soil moisture before drought stress was implemented at 65 days (the beginning of the flowering stage). To apply water, the weight method was used. Once every day all pots were weighed using a precision portable balance device with an accuracy of ±5 g. Then the moisture of the pots based on the soil moisture curve was set. This method is easily adapted for potted plants. Irrigation was based on 25%, 50%, and 75% of AW for nonstress, moderate stress, and high stress conditions, respectively. AW was calculated using the following formula:

$$AW = FC - PWP,$$

where FC is soil field capacity and PWP is soil permanent wilting point. Upon completion of the maturity stage, expanded young leaves of all plants were simultaneously harvested. Shoot dry material and relative water content (RWC) percentage (Ritchie et al., 1990) were determined. Proline and total soluble sugars amount were measured by spectrophotometer (Schlegel, 1956; Bates et al., 1973). Stomata conductance was determined by porometer. A chromatographic system consisting of a Knauer HPLC (USA) equipped with a 50-µL sample loop, degasser, quaternary pump, column oven, and RI detector was used for carbohydrate analyses. Chromatographic separations were carried out by Eurokat column (250 × 4.6 mm, i.d. 5 µm) at 40 °C. A gradient elution was used in this study. The mobile phase was pure water with pH 2.5 for 15 min. Enzyme activity was determined by spectrophotometer using the method reported by Zhang and Kirkham (1996) and Bian and Jiang (2009). Gene expression was performed using a reverse transcriptase polymerase chain reaction (RTPCR). Total RNA from the canola leaves was isolated using Plant RNeasy Mini kit (Qiagen, Chatsworth, CA, USA) and treated with DNase (Turbo DNA- Free Kit; Ambion, Austin, TX, USA) to remove contaminating genomic DNA. RNA was reverse transcribed with the Ready-to-go RT-PCR beads. The synthesized cDNA was subjected to PCR for 35 cycles using primers for *CAT2*, g *POD*, cytosolic *APX*, and 18S (as internal control) for PCR obtained from Bian and Jiang (2009). Aliquots of individual PCR products were resolved through agarose gel electrophoresis, images were captured by Quantity One (Bio-Rad Laboratories, Hercules, CA, USA), and bands were determined using the Discovery Series Quantity One (Bio-Rad Laboratories) (Xu et al., 2011). Effects of watering treatment, cultivars, and their corresponding interactions were determined by analysis of variance according to the general linear model procedure of SAS (Version 9.0). Differences between means were separated by Duncan's multiple range tests at the 0.01 and 0.05 P levels.

3. Results and discussion

3.1. Shoot dry material

Analysis of variance (Table 1) showed significant differences between cultivars and different levels of stress ($P \leq 0.01$) and their interactions ($P \leq 0.05$). The lowest and highest shoot dry material were observed in Option (24.10 g per plant) under high stress conditions (25AW) and in RGS003 (51.60 g per plant) under normal irrigation (75AW), respectively (Figure 1). In both cultivars, stress conditions significantly reduced shoot dry material. The results showed that RGS003 could be more tolerant to drought stress. In drought stress conditions, the reduction in shoot dry matter could be due to decreases in cell turgor pressure, plant leaf area, and photosynthetic rate because of biochemical limitations caused by water deficiency (reduced photosynthetic pigments, especially chlorophylls) (Lawler and Cornic, 2002). Stress conditions caused a reduction in shoot dry weight that depended on the severity of the stress.

Table 1. Analysis variance of shoot dry weigh, RWC%, stomata conductance, proline, total soluble sugar, sucrose, glucose, and fructose in 2 spring canola cultivars under drought stress.

S.O.V.	d_f	Mean of square							
		Shoot dry material	RWC%	Stomata conductance	Proline	Total soluble sugar	Sucrose	Glucose	Fructose
Cultivars	1	789.23**	101.16**	0.7026$^{n.s.}$	0.09486$^{n.s.}$	142.25*	0.48**	0.12**	0.21**
Drought	2	412.01**	175.16**	40.05**	0.1237**	86.54*	0.82**	0.23**	0.51**
Cultivars × drought	2	85.23*	39.166*	3.332$^{n.s.}$	0.0270**	18.44$^{n.s.}$	0.18*	0.2*	0.15**
Error	15	14.20	9.34	3.724	0.0019	18.06	0.2	0.069	0.2
C.V.		1208	9.3	13.2	14.0	5.0	10.2	8.3	7.2

**: Significant at 1% probability level; *: Significant at 5% probability level, $^{n.s.}$: no significant differences

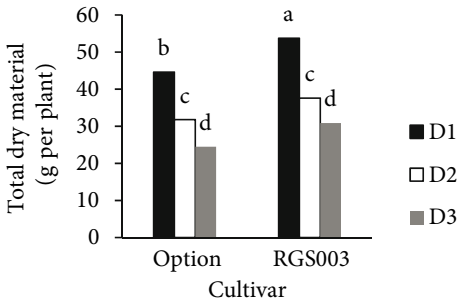

Figure 1. Interaction of drought stress (D1: normal conditions, D2: moderate stress, D3: high stress) and cultivars on total dry material in 2 spring canola cultivars by Duncan's multiple range test.

3.2. RWC percentage and stomatal conductance

Based on the results of analysis of variance of RWC shown in Table 1, there were significant differences ($P \leq 0.01$) among the RWC levels of tested cultivars and different levels of stress and among interaction ($P \leq 0.05$) under stress conditions. As shown in Figure 2, RGS003 with normal irrigation (88%) had significantly higher RWC than Option under high stress conditions (71.5%). The results showed that RGS003 can be more tolerant and Option is a susceptible cultivar under drought stress conditions. Yadav and Bhushan (2001) reported that under drought stress the amount of RWC decreased; thus, there is a close relation between RWC and crop yield.

Drought stress had significant effects on stomatal conductance among cultivars (Table 1) ($P \leq 0.01$), but there were no significant differences among different levels of stress and interaction. It seems that under normal conditions, because of regular irrigation, the cells' suitable water status increased stomatal conductance.

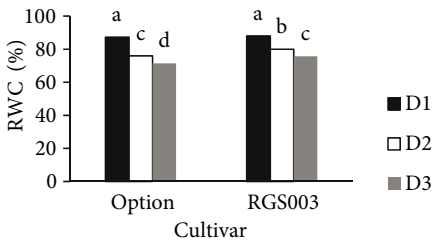

Figure 2. Interaction of drought stress (D1: normal conditions, D2: moderate stress, D3: high stress) and cultivars on RWC% in 2 spring canola cultivars by Duncan's multiple range test.

3.3. Proline

The results shown in Table 1 indicated significant differences ($P \leq 0.05$) between cultivars and different levels of stress and their interaction ($P \leq 0.01$) in leaves. The more tolerant cultivar (RGS003) in the processing of osmotic adjustment probably increased the proline in the leaves under drought stress (Figure 3). RGS003 and Option in all treatments of drought stress (except normal conditions) had high proline amounts. In normal conditions, due to sufficient water in leaf cells, the proline

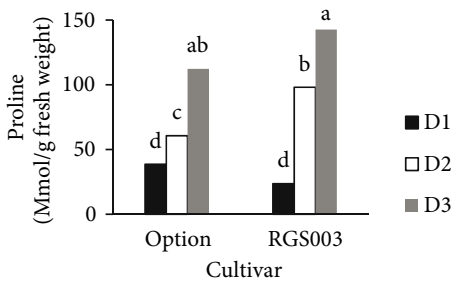

Figure 3. Interaction of drought stress (D1: normal conditions, D2: moderate stress, D3: high stress) and cultivars on proline in 2 spring canola cultivars by Duncan's multiple range test.

in leaves was low. The highest proline was observed under high stress conditions (25AW) in RGS003 (142.63 nmol/g fresh weight), and the lowest proline was observed in the RGS003 cultivar under normal conditions (23.68 nmol/g fresh weight) (Figure 3). Cechin et al. (2006) investigated the response of photosynthesis and proline under stress in leaves of the safflower oil plant and showed that drought stress increased proline accumulation in young leaves. Din et al. (2011) found that metabolic factors such as free proline content in leaves increased significantly under severe drought stress at both the reproductive and the productive stage.

3.4. Total soluble sugar and carbohydrates

The results showed that there were significant differences ($P \leq 0.05$) among the cultivars and different levels of stress and interaction of cultivars. It seems that increasing total soluble sugar to reduce osmotic potential is a resistance mechanism of plants against moderate stress. Reduced osmotic potential upon the occurrence of stress can be one way to induce tolerance against drought stress and prevent cell dehydration.

Su et al. (2004) expressed that increased soluble sugars have a direct relationship with tolerance and stability of performance under stress conditions. In determining the physiological response of cabbage to salinity and drought, they declared that the amount of the total soluble sugar increase and the osmotic potential are related directly to osmotic adjustment. In this case, cultivars take up ions such as Na^+ into the cell (Maggio et al., 2005). Therefore, decreasing the amount of osmotic potential will probably increase turgor pressure. Under high stress, a reduction in the amount of soluble sugar was seen, probably because anaerobic respiration was increased in high stress, and the plants preferred to activate anaerobic respiration to convert other products for their survival. Furthermore, since there were no differences between cultivar and interaction of different levels of stress in stomatal conductance, under drought stress, the stomata were not completely closed, and there was uptake of CO_2 for aerobic respiration. In the tolerant cultivar (RGS003) under moderate stress conditions, total soluble sugar was higher than that of the sensitive cultivar (Option), probably to achieve better osmotic adjustment. Under high stress conditions in both cultivars, a decrease in the amount of total soluble sugar was seen.

Different levels of drought stress and cultivars had a significant effect on the amount of sucrose ($P \leq 0.01$), and there was a significant difference among the interactions of cultivars and different levels of drought stress ($P \leq 0.05$) (Table 1). As shown in Figure 4, sucrose content at normal irrigation (334 µmol/g dw) was higher than in Option (328.5 µmol/g dw). The lowest sucrose content was observed in Option under moderate stress conditions (300.75 µmol/g dw), lower than that of RGS003.

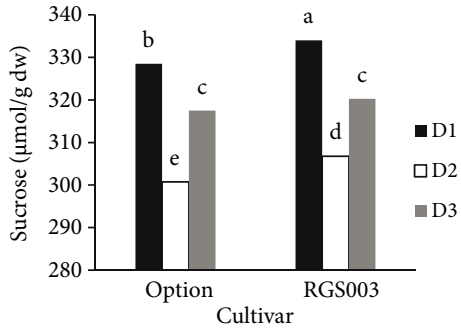

Figure 4. Interaction of drought stress (D1: normal conditions, D2: moderate stress, D3: high stress) and cultivars on sucrose in 2 spring canola cultivars by Duncan's multiple range test.

Figure 5 shows that the lowest glucose was observed in RGS003 (77.25 µmol/g dw) and Option (77.5 µmol/g dw) under normal conditions, and the highest glucose was measured in RGS003 (139.5 µmol/g dw) under moderate stress conditions (50 AW).

Analysis of variance of fructose showed that there were significant differences ($P \leq 0.01$) among cultivars and different levels of stress and their interaction. As can be seen in Figure 6 the lowest fructose level was observed in Option (105.25 µmol/g dw) under normal irrigation. Figure 6 shows that fructose level was increased under moderate and high stress conditions in RGS003 and Option.

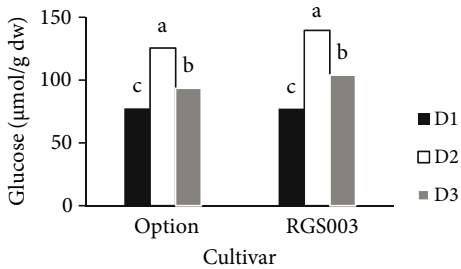

Figure 5. Interaction of drought stress (D1: normal conditions, D2: moderate stress, D3: high stress) and cultivars on glucose in 2 spring canola cultivars by Duncan's multiple range test.

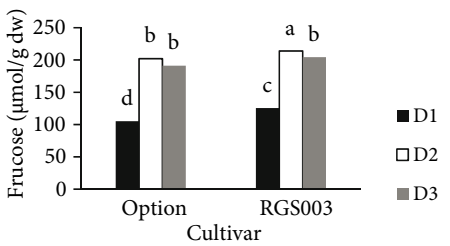

Figure 6. Interaction of drought stress (D1: normal conditions, D2: moderate stress, D3: high stress) and cultivars on fructose in 2 spring canola cultivars by Duncan's multiple range test.

Under moderate stress conditions, the amount of fructose and glucose increased, because sucrose converts to glucose and fructose. These compounds are osmoprotectant for cell protection from the harmful effects of stress. Muller et al. (2012) showed the level of sucrose decreased under moderate water deficit, but concentrations of glucose, fructose, and trehalose were significantly enhanced and that of raffinose decreased in all variants of drought.

3.5. Catalase and guaiacol peroxidase and ascorbate peroxidase activities

As can be seen in Table 2, significant differences ($P \leq 0.01$) were observed between cultivars and different levels of stress and their interaction ($P \leq 0.05$) in leaves. Analysis of corresponding interactions by Duncan's multiple range test showed that the lowest catalase activity under normal conditions was measured in Option (1037 nmol per min/g protein), and the highest catalase in RGS003 (2141 nmol per min/g protein) was measured under high stress conditions (25 AW) (Figure 7). There were no significant differences in the amount of catalase activity between the tolerant cultivar (RGS003) and the sensitive cultivar (Option) in moderate stress (50AW), but under high stress conditions (25AW) there were significant differences. Analysis of variance of guaiacol peroxidase activity showed that there were significant differences ($P \leq 0.01$) among different levels of stress, cultivars, and their interaction ($P \leq 0.05$). The guaiacol peroxidase in RGS003 (4302.5 nmol per min/g protein) was significantly higher than the guaiacol peroxidase under moderate stress conditions in Option (2466 nmol per min/g protein) (Figure 8). Additionally, there were significant differences among different levels of stress, cultivars, and their interaction ($P \leq 0.05$). Our results showed that the lowest ascorbate peroxidase was seen under normal conditions in Option (409 nmol ascorbate oxidation per min/g protein), and the highest ascorbate peroxidase was seen in RGS003 (600 nmol ascorbate oxidation per min/g protein) under high stress conditions (25 AW) (Figure 9). Higher levels of enzyme activities in the tolerant cultivar could be due to its higher resistance. Under drought stress, CO_2 fixation and $NADP^+$ recovering at the Calvin cycle decrease and cause the production of harmful free radicals and damage to the cell membrane. Guaiacol peroxidase and catalase increase in several cycles of physiological responses to environmental stresses. In these conditions, hydrogen peroxide is produced in the process of stress and is detoxified in living organisms by catalase and glutathione peroxidase (Dat et al., 2000). Increasing guaiacol peroxidase and catalase activities increase resistance against harmful free radicals under stress conditions (Jin et al., 2006).

3.6. Gene expression of CAT2, gPOX, and cytosolic APX

The number of enzyme activities in canola may be related to the gene transcript level. In fact, changes in enzyme activities probably correlate directly with the expression of genes. For example, in both resistant and susceptible cowpea (*Vigna unguiculata*), the leaf cytosolic glutathione reductase gene was upregulated by drought stress and directly related to stress intensity (Contour-Ansel et al., 2006). In this study, we saw that the relative expression ratio in *CAT2*, *gPOD*, and cytosolic *APX* increased under stress conditions. These results can show the relationship of enzyme activity and transcript levels. Analysis of variance of relative expression ratio in *CAT2* showed that there were significant differences ($P \leq 0.05$) among different levels of stress, cultivars, and their interaction. From the data in Figure 10, it is apparent that the lowest *CAT2* relative expression under normal conditions was in RGS003 (2.07), and the highest *CAT2* relative expression was in RGS003 (4.90) under high stress conditions (25 AW) (Figure 10). In Option, the relative expression ratio was higher under moderate stress conditions than under

Table 2. Analysis variance of CAT activity, gPOX activity, APX activity, and *CAT2*, g*POX*, and cyt *APX* relative gene expression ratio in 3 spring canola cultivars under high drought stress.

S.O.V.	d_f	Mean of square					
		Cyt *APX* R.G.E.R.	g *POX* R.G.E.R.	*CAT2* R.G.E.R.	APX activity	gPOX activity	CAT activity
Cultivars	1	2.29*	21.12**	9.52*	1324.21*	15,980.09**	9023.12**
Drought	2	2.91**	18.19**	10.97*	1198.01*	17,912.98**	1121.45**
Cultivars × drought	2	2.70*	9.21*	8.34*	1219.90*	9121.15**	2098.61*
Error	15	0.51	0.78	0.89	310.96	1312.45	651.13
C.V.		3.9	6.5	8.1	13.9	12.3	10.41

**: Significant at 1% probability level; *: Significant at 5% probability level, n.s.: no significant differences

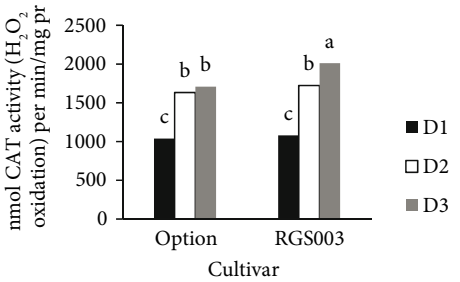

Figure 7. Interaction of drought stress (D1: normal conditions, D2: moderate stress, D3: high stress) and cultivars on CAT activity in 2 spring canola cultivars by Duncan's multiple range test.

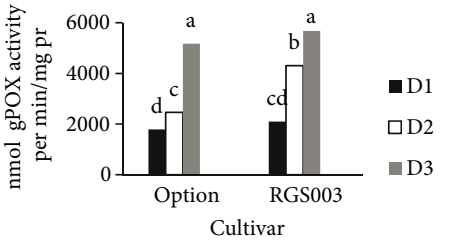

Figure 8. Interaction of drought stress (D1: normal conditions, D2: moderate stress, D3: high stress) and cultivars on gPOX activity in 2 spring canola cultivars by Duncan's multiple range test.

high stress conditions. In RGS003, however, the relative expression ratio was higher in high stress conditions than under moderate stress conditions. There were significant differences ($P \leq 0.01$) among different levels of stress, cultivars, and their interaction ($P \leq 0.05$) in the relative expression ratio of guaiacol peroxidase. In analyzing the corresponding interactions by Duncan's multiple range test, the lowest guaiacol peroxidase was in Option (1.01) under normal conditions, and the highest guaiacol peroxidase was in RGS003 (5.62) under high stress conditions (25AW) (Figure 11). Our analysis showed that the lowest cytosolic ascorbate peroxidase was under normal conditions in Option (1.05), and the highest cytosolic ascorbate peroxidase was in RGS003 (1.48) under high stress conditions (25AW) (Figure 12). It seems likely that the induction of cyt *APX* expression plays an important role in removing H_2O_2 and minimizing photo-oxidative damage. An *Arabidopsis thaliana* gain-of-function mutant with constitutively higher *APX2* expression was more drought tolerant than wild- type plants and exhibited improved water use efficiency (Rossel et al., 2006). Transgenic rice plants (indica rice 'Pusa Basmati-1') with stable expression of the Cyt *Cu/ZnSOD* gene from the mangrove plant (*Avicennia marina*) also revealed better abiotic stress tolerance in comparison with

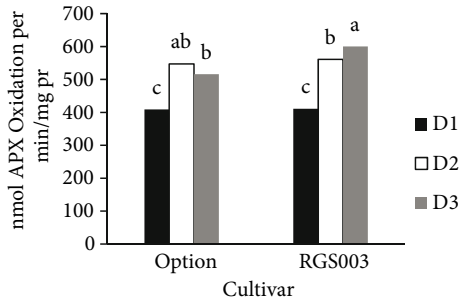

Figure 9. Interaction of drought stress (D1: normal conditions, D2: moderate stress, D3: high stress) and cultivars on APX activity in 2 spring canola cultivars by Duncan's multiple range test.

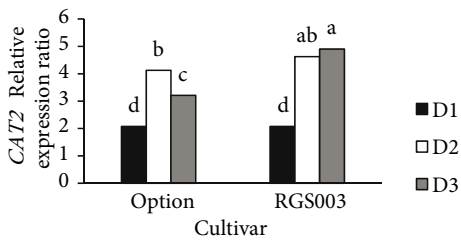

Figure 10. Interaction of drought stress (D1: normal conditions, D2: moderate stress, D3: high stress) and cultivars on *CAT2* relative gene expression ratio in 2 spring canola cultivars by Duncan's multiple range test.

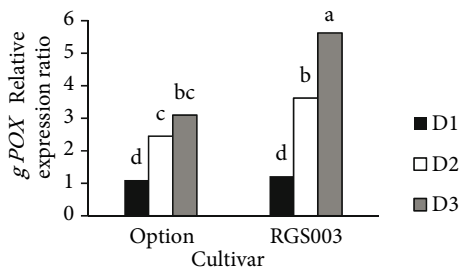

Figure 11. Interaction of drought stress (D1: normal conditions, D2: moderate stress, D3: high stress) and cultivars on *gPOX* relative gene expression ratio in 2 spring canola cultivars by Duncan's multiple range test.

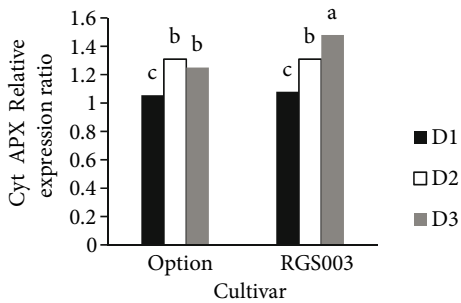

Figure 12. Interaction of drought stress (D1: normal conditions, D2: moderate stress, D3: high stress) and cultivars on Cyt *APX* relative gene expression ratio in 2 spring canola cultivars by Duncan's multiple range test.

Table 3. Correlation between shoot dry material, RWC percentage, stomatal conductance, proline, total soluble sugar, carbohydrates, catalase, guaiacol peroxidase, ascorbate peroxidase activities, and gene expression in 2 spring canola cultivars under water deficit stress.

	Shoot dry material	RWC%	Stomatal conductance	Proline	Total soluble sugar	Glucose	Fructose	Sucrose	Cat activity	Pox activity	Apx activity	*Cat2* gene Exp	*Pox* gene Exp	Cyt *Apx* gene Exp
Shoot dry material	1													
RWC%	0.621*	1												
Stomatal conductance	0.289	0.42	1											
Proline	0.658*	0.721**	0.341	1										
Total soluble sugar	0.521	0.698*	0.342	0.317	1									
Glucose	0.214	0.031	0.270	0.123	0.621*	1								
Fructose	0.431	0.412	0.050	0.123	0.630*	0.234	1							
Sucrose	0.821**	0.512	0.289	0.549	0.701*	−0.043	−0.136	1						
Cat activity	0.613*	0.340	0.239	0.128	0.348	0.312	0.035	0.628*	1					
Pox activity	0.547	0.256	0.132	0.541	0.025	0.219	0.321	0.560	0.121	1				
Apx activity	0.609*	0.659*	0.198	0.723**	0.563	0.341	0.098	0.112	0.654*	0.423	1			
Cat2 gene Exp	0.512	0.221	0.086	0.624*	0.217	0.132	−0.035	0.187	0.516	0.121	0.567	1		
Pox gene Exp	0.419	0.239	0.189	0.452	0.123	−0.143	0.012	0.560	0.023	0.571	0.419	0.139	1	
Cyt *Apx* gene Exp	0.641*	0.231	0.432	0.238	0.453	0.223	0.115	0.212	0.618*	0.190	0.634*	0.128	0.137	1

**: Significant at 1% probability level; *: Significant at 5% probability level, n.s.: no significant differences

untransformed control plants (Prashanth et al., 2008). The most striking results to emerge from the data are that RGS003 is probably more tolerant than Option, because the relative expression ratio was higher in this cultivar than in Option. Moreover, the amount of enzyme activates was higher in RGS003 than in Option. A comparison of the results reveals that antioxidant enzymes like CAT, gPOX, and APX might play important protective roles in cellular survival in severe water deficit. At both enzymatic activity and gene transcript levels, it is interesting to note that these enzymes could play roles in protecting plants from oxidative damage in drought stress.

3.7. Correlation between traits under high drought stress

The most interesting finding was that, under high stress conditions, significant positive correlations were observed between shoot dry materials with RWC%, sucrose, catalase activity, APX activity, and cyt *APX* relative gene expression ratio (Table 3).

3.8. Conclusion

Oxidative damage is an important injury that could decrease plant yield. Tolerant cultivars had mechanisms against these damages, such as antioxidant enzymes that could decrease the destructive effects. We observed that antioxidant enzymes in the tolerant cultivar at both activity and transcript levels were higher than those in the sensitive cultivar. The tolerant cultivar also had a higher yield than the sensitive one. The amount of enzyme activities in canola can be related to the gene transcript level. In fact, the number of changes in enzyme activities correlates directly with the expression of genes. Therefore, antioxidant enzymatic activity and the association between gene transcript levels and activity could play roles for protecting plants from oxidative damage in drought stress.

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