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Annals of Biological Research, 2013, 4 (6):56-60
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Investigate the changes of superoxide dismutase in the seedling stage of enduring and sensitive to drought cultivars of canola

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ABSTRACT

In order to study the relationship between activity of antioxidant enzymes with drought tolerance, an experiment were performed using 8 different cultivars of Canola with different degrees of drought tolerance in a randomized complete block design with three replications in three level of drought stress (Non stress or 100% field capacity), moderate stress (75% field capacity) and high stress (50% field capacity). Traits measurement was carried out in three stages, with an interval of about 5 days. Data analysis was conducted as composite in environment and time. The experimental was measured in pots of 35 cm diameter and a depth of 38 which amount of plant available water was by weighting method and based on the extent determining of FC (Field Capacity) and PWP (permanent wilting extent) using plaster blocks device. 10 seeds were planted in each pot. Plants were sparse after a three-leaf stage and remained 5 plants per pot. Tensions began when the plants had five leaves. This experiment was performed as greenhouse at Ardabil city in the summer of 2012. Enzymatic extraction was performed by method of Sairam et al [1]. Superoxide dismutase activity was measured by method Giannopolities and Ries [2], and was calculated the method of Asada et al [3]. Results of variance analysis showed the difference between genotypes was no significant at all stress and stages levels, which this experiment agree with results of some studies and were inconsistent with several other studies. Mean comparison showed that with the passage of time increased enzyme activity. Stress intensity was not affected on superoxide dismutase activity. Enzyme activity in susceptible cultivars in more severe stress was higher than resistant cultivars.

Key words: Antioxidant, canola, drought stress, the seedling stage, superoxide dismutase

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INTRODUCTION

Canola (Brassica napus) is a plant belonging to the genus of Brassica and the family Brassicaceae which is considered one of the most important oil-producing plants. Canola is an old plant and the information and documents of the cultivation of this plant indicate in India two thousand years before Christ. The canola oil was used as lights oil, edible oil and forage plant in feed for livestock. Oilseeds are the main crops that are placing in second row of agricultural products in terms of the importance. Oilseeds besides providing of the requirement calorie supply fatty acids and some essential vitamins needed by human and animals. Nowadays, one of the most important sources of edible oil is Canola that due to the types of spring and autumn, and intermediate, it has well adapted to environmental adverse conditions such as cold and drought. Today, much of the world's Canola production occur under dry land conditions and therefore plant responses to water stress is considered as major problem [4]. Basically, Canola during germination and at flowering and pod development stage is sensitive to drought [5]. Drought is one of the major factors limit production of crop in worldwide [6]. In addition arid and semiarid areas of low rainfall,

distribution of rainfall varies from year to year and therefore it is difficult to predict the extent and distribution. Under such conditions, the grain yield shows different fluctuations in successive years. Therefore modification of advanced cultivars for arid and semi-arid only by selection has not been much successful for grain yield [7, 8]. Drought is one of the main factors in the production of reactive oxygen in plants. Closing of stomata for water store minimizes CO2 of the leaf inside and is reduced carbon assimilation for oxygen optical breathing. The process of stomata closing and reinforcement of the electron transfer to oxygen produce the reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, and singlet oxygen [9, 10]. Photosynthetic electron transport system is the main source of reactive oxygen production in plant tissues [3], which have the potential to produce singlet oxygen and superoxide. These toxic oxygen species are highly reactive which in the absence of protective mechanisms, normal cellular metabolism disrupts through oxidative damage, including lipid peroxidation, membrane damage, degradation of proteins, inactivate enzymes, pale pigments and DNA damage [11, 12].

The bulk of the damage resulting from non-biological stress with oxidative damage is related at cellular level, which one of the primary purposes of many oxidative stresses are cytoplasm membranes [13, 14]. Supreme plants contain active oxygen scavenging systems are including antioxidant enzymes such as superoxide dismutase (SOD), Ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) and some antioxidants with low non-enzymatic molecular weight [15]. Super oxide dismutase was identified for the first time by Mann and Kleilin [16] and during that time was thought to be a storage protein. The enzyme was originally known erythrocuprein, indofenol oxidase and tetrazolium oxidize until its catalytic role was discovered by McCord and Fridovich [17]. It is known that superoxide dismutase, catalyses dismutation superoxide to hydrogen peroxide and oxygen, [18]. After understanding the basic concepts, it is interesting to know the common issue to all biotic and abiotic environmental stresses is production of reactive oxygen species [19, 20]. An issue that is sometimes overlooked in plants in the study of environmental stresses is that the activated forms of oxygen analyze proteins and nucleic acids and these are reactions can be very dangerous [21]. The purpose of this experiment was to investigate the changes in superoxide dismutase in the seedling stage of tolerant and sensitive to drought cultivars of canola.

MATERIALS AND METHODS

The experiment were performed using 8 different cultivars of Canola with different degrees of drought tolerance (Table 1), in a randomized complete block design with three replications in three level of drought stress (Non stress or 100% field capacity), moderate stress (75% field capacity) and high stress (50% field capacity). Traits measurement was carried out in three stages, with an interval of about 5 days. So the time factor also entered test and data analysis was conducted as composite in environment and time. The amount of plant available water was measured by weighting method and based on the extent determining of FC (Field Capacity) and PWP (permanent wilting extent) using plaster blocks device. 10 seeds were planted in each pot. Plants were sparse after a three-leaf stage and remained 5 plants per pot. Tensions began when the plants had five leaves and leaves were completely developed. Leaf sampling for measurements traits were performed day before irrigation and superoxide dismutase activity was measured and while determination of superior varieties were determined traits relationship with drought resistance of varieties.

Table 1- Canola cultivars used in the experiment

Row	Cultivar	Reaction against drought	Row	Cultivar	Reaction against drought
1	Slm046	Enduring	5	Modena	Sensitive
2	Orient	Enduring	6	Talaye	Sensitive
3	Es Hydromel	Enduring	7	Opera	Sensitive
4	Es Betty	Enduring	8	Zarfam	Sensitive

Extraction and measurement of antioxidant enzymes

Leaves were powdered in masonry mortar containing Nitrogen and then enzymes extraction was performed by Sairam et al method [1]. Measurements were performed in (Non stress or 100% field capacity), moderate stress (75% field capacity) and high stress (50% field capacity) and after the cultivars were five-leaf was conducted in three stages. For extraction of superoxide dismutase and catalase 0.5 g powder was stirred in 10 ml of cold phosphate buffer 0.1 M (PH=7.5) containing 0.5 mM EDTA. For extraction of ascorbate peroxidase 0.5 g powder was stirred in 10 ml of cold phosphate buffer 0.1 M (PH=7) containing 0.5 mM Ascorbic acid. This mixture was filtered using a soft cloth and then were transferred to micro tubes containers micro tubes for centrifuge (All the

above procedures were performed in the refrigerator). Intended solution was centrifuged at a power 4˚ C 20000 × g for 15 minutes. The clear liquid of supernatant was used to measure enzyme activity.

Superoxide dismutase

Superoxide dismutase activity was measured by Giannopolities and Ries method [2]. 3 ml reaction mixture containing 13 mM methionine, 75 micro mol nitroblue tetrazolium Chloride (NBT), 2 micro mol riboflavin, 50 mM phosphate buffer (PH=7.8) and 0 to 50 micro liters of the enzyme extract. The reaction was initiated by turning on the fluorescent lamps. The reaction solution was placed below two 15-watt fluorescent bulbs with a height of 20 cm for 10 minutes and the reaction was terminated by turning off the lights. Then the reaction solution was wrapped through the black cloth to measure absorption. Absorbance were measured at a wavelength of 560 nm with a Spectrophotometric SHIMADZU Model UV-120-02 made in Japan. Enzyme did not added to one of dishes and was created the maximum color. One of the dishes was not irradiated with light and not made any color and was considered as Blank. One unit of SOD activity were considered as the amount of enzyme required for 50% preventing of photochemical revival of Nitro Blue tetrazolium Chloride and was calculated by Asada et al [3].

$$UnitSOD = \frac{V}{v} - 1$$

In which:

V = is the absorption rate of a reaction without enzyme (maximum response).

v = is the absorption rate of the reactions containing enzyme.

RESULTS AND DISCUSSION

The results of combined variance analysis of the trait are presented at (Table 2) in three stages. The effect of environment (drought stress) for the traits studied was no significant at the 5% level. There were no significant differences among cultivars. The interaction of stress × genotype was no significant at the 5% level. No significant genotype × environment interaction for superoxide dismutase trait can be due to low intensity [7]. Superoxide dismutase ($p = 0.06$) had a tendency to change over time. The interaction of stress × time superoxide dismutase showed a tendency to significance ($p = 0.091$). This means that the characteristic response than time was different in three environments without stress, stress 75% and 50% field capacity. The interaction of time × genotype was no significant at superoxide dismutase trait. The interaction of time × genotype × stress was significant. Coefficient of variation (CV) was low in all parameters tested and was showing acceptable accuracy of experiments and low experiment error. So test failure could not be considered reason for their non-significant. ANOVA discovers only large differences between genotypes but if differences are subtle and accurate it will not be successful to detect them but will show in mean comparison themselves [22].

The results of mean comparison showed that with the passage of time increased enzyme activity (Table 3). Stress intensity was not affected on superoxide dismutase activity. The third stage had highest value of superoxide dismutase enzyme activity. Results showed that stress has no effect on the activity of superoxide. In the research of Bartoli et al [23], Superoxide dismutase and catalase activity was not affected by drought. Non stress environments, 75% and 50% field capacity with separation of each step showed that drought stress did not change in the superoxide dismutase activity at first stage with increasing stress. The initial increase in the activity of these enzymes can be due to excessive accumulation of reactive oxygen species in the starting stress [24]. The second stage of sampling did not change increasing stress of superoxide dismutase activity. In the third stage, measurements of superoxide dismutase activity increased with increasing salinity.

In the grouping investigation of this trait was observed that there was significant stress between resistant cultivars and susceptible varieties apart from the 75% farm capacity (stage 3) at all levels which indicated difference between resistant and susceptible cultivars (Table 4). At first stage, the amount of Superoxide dismutase activity in susceptible varieties is more than resistant varieties. At second stage in the serve stress 50% farm level, the amount of Superoxide dismutase activity in resistant varieties is more than susceptible varieties that this condition is governing in the third stage (Table 5). This is perhaps a strategy that resistance cultivars in non-stress condition spending grows without energy to produce Enzymes and produce enzyme in the necessary conditions but these conditions is opposite in sensitive cultivars. Selote and Khanna-Chopra, stated that the resistant species for adaptation and more deal with drought raise the level of superoxide dismutase activity [25]. Therefore we can

determine the drought resistant varieties in the different crops with determine the level of this enzyme activity. The results of these researches are similar to obtained results in two stages (stages 2 and 3).

Table 2- Results of variance analysis of superoxide dismutase in reproductive stages and genotypes

CV	DF	MS
		superoxide dismutase
Stress	2	13.77 ^{ns}
Error 1	6	0.0034
Genotype	7	3.879 ^{ns}
Genotype× Stress	14	9.568 ^{ns}
Stage	2	174.86 ^{ns}
Stage× Stress	4	16.49 ^{ns}
Stage × Genotype	14	2.98 ^{ns}
Stage × Repeat	4	0.0048 ^{ns}
Stage × Genotype× Stress	28	7.4**
Error 2	134	0.008
CV (%)	-	2.16

*ns, * and **, respectively, non-significant and significant in the 5% and 1% level*

Table 3- Mean comparison of traits between the two stress conditions and without stress at different measurements stages

Time	Environment	superoxide dismutase
First stage	--	2.65 C
Second stage	--	3.76 B
Third stage	--	5.72 A
	100% field capacity	3.63 A
	75% field capacity	4.008 A
	50% field capacity	4.502 A
First stage	100% field capacity	2.4 A
	75% field capacity	2.38 A
	50% field capacity	3.17 A
Second stage	100% field capacity	4.19 A
	75% field capacity	3.33 A
	50% field capacity	3.75 A
Third stage	100% field capacity	4.29 B
	75% field capacity	6.31 A
	50% field capacity	6.58 A

Means with a common letter do not show significant differences in the each column

Table 4- Analysis of Variance of the orthogonal comparisons between resistant and sensitive cultivars in the traits of superoxide dismutase

superoxide dismutase		First stage			Second stage			Third stage		
		Stress level			Stress level			Stress level		
SOV	DF	50% field capacity	75% field capacity	100% field capacity	50% field capacity	75% field capacity	100% field capacity	50% field capacity	75% field capacity	100% field capacity
Orthogonal comparison	1	1.47**	0.35**	3.16**	0.07**	0.38**	10.02**	23.71**	0.03ns	14.68**
Error	13	0.0008	0.0009	0.014	0.00077	0.0013	0.0009	0.001	0.0017	0.0008

Table 5- Mean Comparison of the resistant and sensitive cultivars in terms of the superoxide dismutase trait

superoxide dismutase	First stage			Second stage			Third stage		
	Stress level			Stress level			Stress level		
Group	50% field capacity	75% field capacity	100% field capacity	50% field capacity	75% field capacity	100% field capacity	50% field capacity	75% field capacity	100% field capacity
Enduring	2.92 B	2.26 B	2.037 B	3.81 A	3.21 B	3.55 B	7.57 A	6.27 A	3.51 B
Sensitive	3.42 A	2.50 A	2.76 A	3.69 B	3.46 A	4.84 A	5.58 B	6.34 A	5.07 A

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