Effect of Drought Stress on proline content and Canola (Brassica napus L.) Antioxidants enzymes activity

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Abstract:
To study drought stress on seedling dry weight, prolin contents and evaluate of antioxidant enzymes activity in canola genotypes (Brassica napus l.), an e riment were evaluated at laboratory physiology, Shahed University. This experiment was carried out in factorial with CRD design with 4 replications in 2008. The first factor was including l vels of dough stress, one without stress as check (0) and the other with stress (-0.5 ,-1 and -1.5 Mpa) nd the second factor rapeseed genotypes of RGS and Modena were considered. To evaluate drought tolerance genotypes on the basis of Morphological aspects on root and stem dry weight, ascorbat peroxidase and Gayacol peroxidase activity in root and stem, change of osmolites include proline in seedling stage in growth environment with stress and comparison with control plants were computed. Analysis of variance showed that there were significant differences among drought stress, varieties and interaction drought*varieties in terms of traits under stress. Results showed significant differences among drought stress, genotypes and interaction between drought stress*genotypes with resp ct to all indices. The highest and the least amounts of root dry weight was related to Modena genot e in -1 Mpa stressed and RGS genotype non-stressed conditions, respectively 2.32 and 1.52g. Cor lation analysis between dry weights and all enzyme indices and proline content were significant, i which the most correlation was among proline content and antioxidants enzymes activity. There were ative significant between leaf and root antioxidants enzymes activity. Correlation coefficients between dry weight and indices in drought stress and proline was significant overall, result sho that in drought stress, plants attempt to increase proline content and Antioxidants enzymes activity in relating decrease organs dry weight. Besides, the sensitivity of stem than root in response to decrease of dry weight and antioxidant enzymes activity was more.

Key words**:** Rapeseed genotypes, Drought, seedling, Antioxidants enzymes activity, and praline content

Introduction

Rapeseed (*Brassica napus*), also known as **rape**, **oilseed rape**, **rapa**, **rappi**, **rapaseed** (and in the case of one particular group of cultivars, **canola**) is a bright yellow flowering member of the family Brassicaceae (mustard or cabbage family). The name derives from the Latin for turnip, *nāpus*, and is first recorded in English at the end of the 14th century.
Canola was developed through conventional plant breedi from rapeseed, an oilseed plant already used in ancient civilization. Canola oil is made at a cessing facility by crushing the rapeseed. Approximately 42% of a seed is oil. Canola oil is low in saturated fat, is high in monounsaturated fat, and has a beneficial omega-3 fatty acid profile; it has well established heart health benefits and is recognized by many health professional organizations**.** Canola is one of the most important oil seeds growing in many parts of Iran. It is very important to grow canola with high oil levels for agronomical benefits (Omidi *et al*., 2008). A large part of vegetable oil for human consumption Iran is imported to the country and hence cultivation and appropriate management of oil seeds to increase yield is very important (Omidi *et al*., 2010). In recent years, due to the adaptation of canola to different climatic conditions, its production has been the center of atte tion and its cultivation area has increased from 19 000 ha in 2000 to 300 000 ha in 2008 (Omidi *et al*., 2010). The presence of high concentration of active oxygen species (AOS), including superoxide radical (O2-·), hydroxyl radical (·OH) and hydrogen peroxide (H2O2), causes oxidative damage (Xiao and Zhang, 2004). Correspondingly plants will be induced to develop a defense and scavenging enzymes of active oxygen. One of the protective mechanisms is the enzymatic antioxidant system, which volves the sequential and simultaneous action of a number of enzymes including superoxide dis tase (SOD), peroxidase (POD) and catalase (CAT). Endogenesis antioxidant-glutathione (GSH) and AsA play important roles in scavenging enzymes of active oxygen. It has been revea d that chloroplast depends on the circulation of AsA-GSH to resist the contamination fro AOS (Zhang and Lei, et al., 2000). Therefore,

it is significant to determine the change in AsA-induced enzymatic antioxidant system, such as SOD, POD and CAT activities, and the malondialdehyde (MDA) oncentration, a general indicator of lipid peroxidation. The recent study was conducted in order to assess two canola varieties for their drought tolerance and to give more information on the significance of osmotic adjustment and osmolytes accumulation under drought stress.

Materials and methods

Biomass determination

To study drought stress on seedling dry weight, prolin contents and evaluate of antioxidant enzymes activity in canola genotypes (Brassica napus l.), an e riment were evaluated at laboratory physiology, Shahed University. This experiment was carried out in factorial with CRD design with 4 replications in 2008. The first factor was including l vels of dough stress, one without stress as check (0) and the other with stress (-0.5 ,-1 and -1.5 Mpa) nd the second factor rapeseed genotypes of RGS and Modena were considered. To evaluate drought tolerance genotypes on the basis of Morphological aspects on root and stem dry weight, ascorbat peroxidase and Gayacol peroxidase activity in root and stem, change of osmolites include proline in seedling stage in growth environment with stress and comparison with control plants were co .

Enzyme preparations and assays

Proline determination

Proline content was estimated using ninhydrin reaction method (Bates *et al*., 1973), and The Guaiacol peroxidase (GPx) activity was measured using a modification of the method of Chance and Maehly (1955). The APx activity was measured using a modification o the procedure of Nakano and Asada (1981).

Statistics

The results of the morphological and biochemical parameters were statistically analyzed by one-way ANOVA and Least Significant Difference (LSD) test to d rmine significant differences among group means (SAS Institute, 1988). A p-value of 0.05 was considered statistically sign icant. The statistical package SAS, version 9.1 (SAS/STAT Software for PC. SA Institute Inc., Cary, NC, USA) was used for all the applied statistical analyses (Steel and Torrie, 1980).

Results and analyze

Growth characteristics of oil rape seedlings under treatments

Analysis of variance showed that there were significant differences among drought stress, varieties and interaction drought-varieties in terms of traits under stress. Results showed significant differences among drought stress, genotypes and interaction betwee drought stress-genotypes with respect to all indices. Effects of Drought stress (D) treatments on the growth of canola genotypes is shown in Table 1-3.

The application of stress significantly affected plant growth components such as root and shoot dry weight (DW), proline content and antioxidant enzymes activity of canola genotypes (Table 1-3). Drought decreased the roots and shoots dry weight of c nola plants and this effect was particularly significant at high level of stress.

The highest and the least amounts of root dry weight was related to Modena genotype in -1 Mpa stressed and RGS genotype non-stressed conditions, respectively 2.32 and 1.52g. There were highly significant differences for root and shoot proline con nt for two factors (drought and genotype) studied. In this study, proline content in root was substantially higher than in shoot genotypes.

Correlation analysis between dry weights and all enzyme indices and proline content were significant, in which the most correlation was among proline content and antioxidants enzymes activity. There were negative significant between leaf and root antiox ants enzymes activity. Correlation coefficients between dry weight and indices in drought stress and proline was significant overall, result showed that in drought stress, plants attempt teo increase proline content and Antioxidants enzymes activity in relating decrease organs dry weight (data not shown).

Table 1. Analysis of variance for drought stress on plant characteristics of canola seedlings

S.O.V	root dry weight (gr)	shoot dry weight (gr)	Prolin Con.	Root Gua. peroxidase	Leaf Gua. peroxidase
water stress	**	**	**	**	**
Genotype	**	ns	**	**	**
water stress*Genotype	**	ns	**	**	**

* and **, significant at $P<0.05$ and $P<0.01$; ns, non-significant

Table 2. Main mean comparison of plant characteristics of canola seedlings under drought stress

water stress (MP)	Root dry weight (gr)	Shoot dry weight (gr)	Prolin Con.[2]	Root Gua. peroxidase[1]	Leaf Gua. peroxidase[1]
0	1.528d	1.728a	45.216d	9.501c	2.665d
-0.5	2.540a	0.578b	46.065c	7.046d	2.478c
-1	2.205b	0.884b	55.228b	9.620b	3.160b
-1.5	2.059c	0.859b	60.994a	9.954a	3.415a
(LSD)	0.116	0.328	0.383	0.039	0.042
RGS Genotype	1.722b	1.006a	58.699a	12.564a	4.559a
Modena Genotype	2.444a	1.018a	45.053b	5.496b	1.434b
(LSD)	0.082	0.232	0.271	0.028	0.03

Means followed by the same letters are not significantly different at $P\leq 0.05$. [1]($\mu molmin^{-1}$ g^{-1} FW) and [2](mmol/g Fw).

Table 3. Mean interaction comparison of canola seedlings genotypes under drought stress

Genotype	water stress (MP)	root dry weight (gr)	shoot dry weight (gr)	Prolin Con.	Root Gua. peroxidase[1]	Leaf Gua. peroxidase[1]
Modena	0	1.28e	**1.748a**	47.993e	13.045c	4.115d
	-0.5	1.753d	0.548b	5.0703c	10.028d	4.240c
	-1	1.860d	0.815b	63.303b	13.295b	4.768b
	-1.5	1.748d	0.913b	**72.798a**	**13.890a**	**5.115a**
RGS	0	1.528e	**1.708a**	42.440g	5.958f	1.215g
	-0.5	**3.328a**	0.608b	41.428h	4.065g	1.255g
	-1	2.550b	0.953b	47.153f	5.945f	1.553f
	-1.5	2.370c	0.805b	49.190d	6.018e	1.715e
(LSD)		0.164	0.464	0.542	0.055	0.06

Means followed by the same letters are not significantly different at $P\leq 0.05$. [1]($\mu molmin^{-1}$ g^{-1} FW) and [2](mmol/g Fw).

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