NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 39, 2022
First Online: March, 2022. DII 2067-5720 RAR 2022-117

ANTIOXIDANT ENZYME ACTIVITIES AND GRAIN YIELD OF RAPESEED (*Brassica napus* L.) OF RESPONSE TO LATE-SEASON DROUGHT AND SOWING DATE

Peyman Davami[1], Mojtaba Alavi Fazel[2*], Shahram Lak[2], Davood Habibi[3], Afshin Mozaffari[4]

[1]Ph.D. Student, Department of Agronomy, Khuzestan Science and Research Branch, Islamic Azad University, Ahvaz, Iran
[2]Department of Agronomy, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran
[3]Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, Iran
[4]Department of Agronomy, Ilam Branch, Islamic Azad University, Ilam, Iran
[*]Corresponding author. E-mail: alavifazel.2021@gmail.com

ABSTRACT

Drought stress and oxidative damage are among the most important factors that limit the growth and yield of drought crop plants. The global climate change has led us to adapting planting dates and select tolerant cultivars to encounter the new climatic conditions. To evaluate the effect of late-season drought stress under different planting dates on rapeseed cultivars, a 2- year field experiment was conducted as a factorial split-plot based on randomized complete block design (RCBD) in Karaj - Iran, from 2015 to 2017. Irrigation treatments were considered in the main plots and planting dates and cultivars as factorial were placed in subplots. Two planting dates were regular date (September, 26) (PD1) and late planting date (October, 26) (PD2). Irrigation was also carried out at two levels of normal irrigation (NI) and irrigation cut-off (II) from the pod formation stage onwards. Rapeseed cultivars included Tassilo, Elvise, Neptune and Okapi. Late-season drought stress and delayed planting date reduced anthocyanin (AN), guaiacol peroxidase (GPX) activity, and grain yield (GY), but increased malondialdehyde (MAD), in all rapeseed cultivars. The highest GY (3346.6 kg ha^{-1}) was obtained in NI conditions and PD1. According to the results of stepwise regression, AN, GPX, and MAD with a coefficient of determination (R^2=0.774) explained about 78% of the changes in the GY, among which MAD with a value of (-0.72) showed the greatest negative and significant impact on the GY. Therefore, Elvise can be introduced as a superior cultivar to be selected as a genotype that presents acceptable resistance under drought stress and late sowing in arid and semi-arid regions.

Keywords: drought stress, oxidative stress, proline, regression.

INTRODUCTION

Canola (*Brassica napus* L.) is a drought-tolerant oilseed plant with high oil quality spring and autumn genotypes (Dehghani et al., 2009). The expansion of rapeseed cultivation is a viable strategy to diversity crop rotations for production of edible oil and meal in temperature regions beside wheat and barley cultivation. Therefore, discovering high yielding genotypes with acceptable qualitative features should be considered. The development of rapeseed genotypes adapted to moderate cold temperatures in the arid and semi-arid regions is a step toward the mentioned cropping rotation strategy. Drought is one of the environmental stresses considered the most crucial factor in limiting the growth and production of crops in most parts of the world and including arid regions such as Iran (Mariani and Ferrante, 2017). The plant growth and development phases can be affected by changing the sowing date and such changes may have implications for plant tolerance to drought condition (Moslemi et al., 2011). The determination of the most suitable planting date can reduce the damage and negative effects of environmental stress on all vegetative and reproductive growth stages of plants. Since the occurrence of drought stress in plants increases the production of reactive oxygen species (ROS), the adaptation of plants to the destructive effects of drought stress depends by the strength of their antioxidant defense system

---

Received 14 October 2021; accepted 18 March 2022.

in these conditions (Amoah et al., 2019). Plant resistance to drought stress is at least in provet due to the ability to inhibit reactive oxygen species and reduce their destructive effects. Heat stress-induced oxidative stress in rapeseed led to a reduction in photosynthetic efficiency, stomatal conductance, carboxylation efficiency, electron transfer rate in photosystems, and a 85.3% decrease in grain yield as alteration in grain oil composition (Elferjani and Soolanayakanahally, 2018).

Proline is one of the important osmotic regulators in determining the drought stress resistance of plants (Azarpanah et al., 2013). Proline is involved in sweeping oxygen free radicals that damage cell membranes (Anjum et al., 2014). Leaf proline content (LPC) accumulates in many plants as a multifunctional amino acid in response to abiotic stress. Increasing LPC under stress protects cell membranes, proteins, cytoplasmic enzymes, inhibits ROS, and eliminates free radicals (Ghorbanli et al., 2013). In many crops, free LPC remarkably accumulates in response to low water potential such as drought in canola (Sabagh et al., 2019). Leaf relative water content is one of the factors affecting the continuation of rapeseed crop growth under water deficit stress (Raza et al., 2017). The reduction of LRWC under drought stress condition is the result of water deficiency in plant leaves (Shirani Rad et al., 2014). These unfavorable conditions increase the leakage of electrons into molecular oxygen by increasing the formation of ROS such as hydrogen peroxide (H_2O_2), superoxide, and hydroxyl. However, overproduction of reactive oxygen can cause oxidative stress that damages photosynthetic pigments, membrane lipids, proteins, nucleic acids, and normal metabolism (Salehi Shanjani et al., 2015). Drought stress during flowering and pod formation, the most drought-sensitive stages of rapeseed, accounted for 30.3% and 20.7% of yield loss, respectively (Norouzi et al., 2008).

By producing and accumulating anthocyanins in epidermal layers, plants can mitigate the effects of oxidative stress and influence the antioxidant capacity (Zhiliang et al., 2009). Meanwhile, oxidative stress stimulates the activity of antioxidant enzymes such as glutathione S-transferase and superoxide dismutase. Accumulation of anthocyanins in the leaves of some plants reduces osmotic potential of leaves and thus the leaf water potential and stomatal conductance (Cartea et al., 2011). MAD is a product of peroxidation of unsaturated fatty acids in phospholipids and is used as an index to assess oxygen free radicals plus membrane damage under drought stress (Katsuhara et al., 2005). Malondialdehyde increases under drought stress conditions, but the rate of increase in tolerant cultivars is far lower than in sensitive cultivars (Bhardwaj et al., 2012). Guiacol peroxidases uses oxidation of phenolic compounds such as guaiacol to detoxify and break down oxygenated water, and is also found in cytosols, cell walls, and vacuoles (Noctor et al., 2002). This enzyme plays a major role in making plants resistant to drought stress by scavenging ROS. Saving water, especially in the late-season (poding stage), which coincides with the growing water demand of spring cultivations, is significantly important, where farmers mostly do not have enough water to devote to both cultivations. Considering the importance of canola in diversifying cropping rotations, this experiment was conducted to study the biochemical parameters of a set of rapeseed cultivars of Iran under different sowing dates and irrigation regimes at the poding. The main goal of this study was to select an appropriate cultivar that can simultaneously tolerate early-season delayed planting and water deficit at the end of the growing season, with the lowest rate of yield loss.

MATERIAL AND METHODS

Experimental setup

This experiment was conducted at Pars Water and Soil Field, of Karaj, Iran, and 1320 above sea level. Based on weather statistics, the area falls into hot and dry with the semi-arid Mediterranean climate, having 150-180 days and occasionally up to 200 dry days. A factorial split-plot experiment in the form of a randomised complete block design

(RCBD) with three replications for two years (2015-2016 and 2016-2017) was conducted. Irrigation treatments were assigned the main plots and planting date and cultivars were placed in sub-plots. Two planting dates were conventional (PD1) and late planting (PD2). Irrigation was also carried out at two levels of normal irrigation (NI) and irrigation cut-off (II) from the pod formation stage onwards. Four winter cultivars of rapeseed were tested, including three rapeseed hybrids of Tassilo (originated from Germany), Elvise, Neptune, and Okapi (open pollinated cultivar) (originated in France). The reason for selecting these cultivars was to study and compare them for cultivation in cold temperate and semi-arid areas under late cultivation and terminal drought. Ten soil samples, at a depth of 0-30 cm, were randomly collected from different parts of the field using a rotary core drill and were poured into a bag and then mixed. Then, a sample was prepared from it and transferred to the soil laboratory. The results of field soil analysis are listed in Table 1.

Table 1. Chemical and physical characteristics of the experimental soil

Characteristic	Soil texture	OC (%)	pH	EC (dS m^{-1})	N (%)	P (ppm)	K (mg kg^{-1})
2015-16	Clay loam	0.53	7.7	2.2	0.09	9.7	168
2016-17	Clay loam	0.42	7.8	1.7	0.07	4.52	175

OC: organic carbon; EC: electrical conductivity; N: nitrogen; P: phosphorus; K: potassium.

Based on soil parameters, sufficient nitrogen (urea 100 kg ha^{-1}; one-third at sowing time, one-third at stem elongation stage and one-third at the beginning of flowering stage), phosphorus (75 kg ha^{-1} triple superphosphate at sowing time), and potassium (100 kg ha^{-1} potassium sulphate at sowing time) fertilizers were applied in two both years. Further, 2.5 liters per hectare of Butisan Star herbicide (41.6% suspension) was used after planting and before emergence to control a wide range of narrow- and broad-leaf weeds. Moreover the weeds were controlled by manual weeding following the emergence and establishment of rapseed seedlings. Experimental plots were 2 × 6 m, with rows 30 cm apart and plant spacing of 4 cm on the rows. Two lateral rows per plot were left aside as marginal rows and, thus, four middle rows were used for measuring (GY) attributes and biochemical traits. Furrow irrigation using siphon pipes was implemented on the basis of 80 mm evaporation from the surface of Class A Evaporation pan. The number of irrigations in NI and II treatments was 8 and 6 times, respectively, for which 5120 and 3840 m^3 ha^{-1} of water were used, respectively (after accounting for rainfall during the growth period for the two years). Oxydemeton methyl (Metasystox EC 25%, 1.5 L ha^{-1}) insecticide was applied at two stages, namely stemming and budding for the pest control, particularly aphid. All of the physiological traits were evaluated once per growing season, through the random selection of youngest mature leaves of the third internode from top of the plant, 10 to 15 days after stress implementation, between 11:00 to 13:00 per experimental plots in the pod formation stage.

Measurements

The proline content was measured by Bates et al. (1973) method. A quantity of 3.5 grams of plant fresh material (leaf) was weighed and after performing different stages and extraction, the content of LPC was measured at a wavelength of 520 nm in the spectrophotometer and the results are expressed in mg g^{-1} FW. Leaf relative water content was measured by the Ritchie et al. (1990) method. For this purpose, in the pod formation stage, three young and developed leaves were removed from the top three rows of each plot and transferred to the laboratory.

The fresh weight (FW), saturated weight (SW), and dry weight (DW) were weighed immediately and the relative content of leaf water was obtained in percentage using Eq. 1.

$$LRWC(\%) = (FW–DW/SW–DW)\times 100 \quad (1)$$

Anthocyanin extraction was performed according to Wagner (1979) method. Initially, 0.1 g of fresh leaf tissue was ground in a Porcelain mortar with 10 ml of acidic methanol (methanol and hydrochloric acid at a volume ratio of 1:99 cc). The resulting extract was kept in the dark at room temperature for 24 h and centrifuged (Model Spectra fuge 6C) at 12,000 rpm for 18 min. The absorption was read by a spectrophotometer (Model UV2100) at 550 nm. AN concentration was calculated using Eq. 2 and expressed as mg g^{-1} FW.

$$A = \varepsilon BC \quad (2)$$

A represents absorption rate at the wavelength of 550 nm, ε denotes extinction coefficient (equal to 300 $mMCm^{-1}$), B shows Cuvette diameter (1 cm), and C is the complex concentration in Mm. Malondialdehyde was measured according to Heath and Packer (1968) method, 0.2 g of fresh leaf tissue was ground with 2 ml of 0.1% trichloroacetic acid (TCA) solution. The resulting homogenates were centrifuged for 15 min at 12,000 rpm. Next, 2 ml of 20% trichloroacetic acid containing 0.5% thiobarbituric acid was added to 0.5 ml of the supernatant. The resulting mixture was incubated in a warm water bath at 96°C for 30 min and immediately cooled in crushed ice. The amount of this substance was calculated by changing the amount of adsorption at 532 and 600 nm and using the extinction coefficient of 155 cm^{-1} mM^{-1} and the results were expressed in terms of nanomoles per g fresh leaf weight (nmol g^{-1} FW).

For Measurement of antioxidant enzymes, the leaf enzymes were extracted by 0.1 M sodium phosphate buffer (pH=7) containing 2% polyvinyl pyrrolidine (PVP) with the supernatant used after 20-min centrifugation at 4°C at 20,000 rpm. Measurement of guiacol peroxidases activity was performed based on Chance and Maehly (1955) method and spectrophotometry at the wavelength of 470 nm for 2 min (Unit mg^{-1} protein). Upon physiological maturity, a 6 m^2 area of each plot was harvested from the soil surface and let to airdry for 4 days and used for determing the grain yield.

The harvest time for PD1 was around 10 June, and the PD2 was 22 June. The plants were aerated every other day to assure moisture loss. Eventually, the plant moisture decreased to nearly 12%. The grains were then separated from the collar-pruned plant siliques by a small experimental combine and they were poured into a cloth bag. They were then weighed after cleaning using a precise laboratory scale. Thus, the grain yield of each experimental plot was calculated, and finally, the GY was determined in kg ha^{-1}.

Statistical analysis

The effects of planting date, irrigation and cultivar were considered as fixed factors and statistical analysis was performed separately for the first and second year. After performing Bartlett test for homogeneity of variances, all the data were subjected to Analysis of Variance (ANOVA) using Statistical Analysis System (SAS) 9.2. Comparison of means was also done using LSD test at 5% probability level. Path analysis was implemented using PATH software.

RESULTS AND DISCUSSION

The proline content and relative water content of leaf

The results obtained from the analysis of variance showed that the LPC accumulation rate was significantly affected by interactions of irrigation × cultivar, planting date × cultivar and irrigation × cultivar and irrigation × planting date × cultivar at a probability level of 1%. The effect of planting date and interaction of irrigation × planting date was significant only at 5% level (Table 2). The results of comparing the means of interaction of irrigation × planting date × cultivar on proline accumulation showed that under irrigation cut-off conditions, the highest LPC accumulation with 18.88 mg g^{-1} FW belonged to Elvise cultivar at earlier planting date, while Tassilo cultivar showed a lower accumulation of LPC after applying the II conditions on both planting dates (Table 3). The results of analysis of variance showed that the effect of irrigation, cultivar, and irrigation interaction × cultivar, planting date × cultivar, and irrigation interaction × planting date × cultivar on LRWC was significant at the probability level of 1% (Table 2). Comparison of the means of irrigation interaction × planting date × cultivar showed that the highest and lowest LRWC was 90.99% and 66.06%, respectively, under NI and II conditions in Tassilo cultivar on the planting date of PD2. However, under NI conditions, no significant difference was observed among cultivars at different planting dates in terms of this trait (Table 3).

Table 2. ANOVA (mean of squares) for studied traits (2015-2017)

S.O.V.	d.f.	LPC	LRWC	AN	MAD	GPX	GY
Year (y)	1	0.02^{ns}	3.84^{ns}	0.004^{ns}	0.29^{ns}	8.6^{*}	446901.04^{ns}
Replication (r)	4	0.3	0.07	0.25	0.13	0.02	729.17
Irrigation (a)	1	1076.69^{**}	9448.6^{**}	144.1^{**}	9628.42^{**}	7084.52^{**}	3561251.04^{**}
y × a	1	0.02^{ns}	0.001^{ns}	0.04^{ns}	0.003^{ns}	4.73^{**}	8437^{ns}
Error	4	1.42	2.31	1.03	3.94	0.90	4315.23
Planting date (b)	1	2.21^{*}	3.48^{ns}	154.53^{**}	49.35^{**}	34.86^{**}	2978626.04^{**}
y × b	1	0.09^{ns}	0.05^{ns}	0.05^{ns}	0.07^{ns}	0.0002^{ns}	759.37^{ns}
a × b	1	2.26^{*}	1.26^{ns}	0.35^{ns}	43.15^{**}	126.25^{**}	3384.37^{ns}
y × a × b	1	0.07^{ns}	0.0004^{ns}	0.04^{ns}	0.42^{ns}	0.16^{ns}	551.04^{ns}
Cultivar (c)	3	19.789^{**}	11.71^{**}	101.26^{**}	67.97^{**}	852.59^{**}	489789.93^{**}
y × c	3	0.07^{ns}	0.05^{ns}	0.03^{ns}	0.06^{ns}	0.23^{ns}	201.04^{ns}
a × c	3	15.49^{**}	23.15^{**}	0.83^{ns}	38.19^{**}	152.79^{**}	5645.49^{ns}
b × c	3	2.58^{**}	22.68^{**}	2.10^{ns}	14.88^{**}	134.99^{**}	2453.82^{ns}
y × a × c	3	0.08^{ns}	0.04^{ns}	0.01^{ns}	0.017^{ns}	0.05^{ns}	87.15^{ns}
y × b × c	3	0.02^{ns}	0.05^{ns}	0.04^{ns}	0.05^{ns}	0.07^{ns}	89.93^{ns}
a × b × c	3	2.12^{**}	23.73^{**}	0.97^{ns}	4.63^{*}	156.01^{**}	18039.93^{*}
y × a × b × c	3	0.05^{ns}	0.04^{ns}	0.02^{ns}	0.08^{ns}	0.06^{ns}	14641.15^{ns}
Error	56	0.31	0.95	0.86	0.98	0.49	1716.66
C.v (%)		4.77	1.23	3.07	7.00	2.85	2.28

S.O.V.: sources of variations; d.f.: degree of freedom; LPC: leaf proline content; LRWC: leaf relative water content; AN: anthocyanin; MAD: malondialdehyde; GPX: guaiacol peroxidase; GY: grain yield; C.v: coefficient of variation; ns: not significant; $^{**}P<0.01$; $^{*}P\leq0.05$.

Table 3. Interaction of planting date, irrigation, and cultivar on studied traits in 2015-2017

Irrigation	Plantig date	Cultivar	LPC (mg g^{-1} Fw)	LRWC (%)	MAD (nmol g^{-1} FW)	GPX (U mg^{-1} protein)	GY (kg ha^{-1})
NI	PD1	Tassilo	8.68	90.99	3.03	15.96	3036.6
		Elvise	8.81	90.28	3.07	18.12	3346.6
		Neptune	8.07	90.04	3.09	16.29	3220.0
		Okapi	8.68	90.34	2.97	17.05	3103.3
	PD2	Tassilo	8.82	90.85	6.18	14.34	2683.3
		Elvise	8.74	90.30	4.41	17.27	3030.0
		Neptune	8.09	89.86	6.47	15.39	2870.0
		Okapi	8.59	90.06	6.18	16.06	3736.6
II	PD1	Tassilo	13.80	66.06	29.05	15.83	2913.3
		Elvise	18.88	74.55	19.24	40.19	3211.6
		Neptune	15.08	72.26	23.52	35.76	3081.6
		Okapi	14.48	70.36	25.83	35.20	2976.6
	PD2	Tassilo	14.40	70.84	24.46	33.89	2540.0
		Elvise	16.62	70.30	21.16	36.67	2880.0
		Neptune	14.28	69.64	26.28	34.91	2728.3
		Okapi	14.50	70.01	26.09	35.49	2603.3
LSD 5%			1.14	1.12	0.64	0.81	47.92

NI: normal irrigation; II: irrigation interruption from pod stage onwards; PD1: regular planting date (Sep., 26); PD2: late planting date (Oct., 26); LPC: leaf proline content; LRWC: leaf relative water content; MAD: malondialdehyde; GPX: guaiacol peroxidase; GY: grain yield. The means that their difference is larger than LSD are significantly.

Anthocyanin and Malondialdehyde

The results of analysis of variance revealed that the amount of anthocyanin in the studied canola cultivars was significantly affected by irrigation, planting date, and cultivar at the probability level of 1%, but the interactions between these treatments were not significant (Table 2). The results of mean comparison showed that the amount of AN in canola cultivars under irrigation cut-off with an average of 31.96 (mg g^{-1} FW) was significantly higher than the amount of AN accumulated in canola cultivars planted under NI conditions with an average of 29.51 mg g^{-1} FW. Planting canola cultivars at a later date (PD2) led to a significant reduction in the amount of AN accumulation compared to early planting (PD1) with the highest amount of AN in PD1 being 32.01(mg g^{-1} FW), while the lowest amount was obtained on PD2, 29.47 (mg g^{-1} FW). Elvise cultivar with an average of 33.49 (mg g^{-1} FW) had the highest AN accumulation, while Tassilo cultivar showed the lowest AN accumulation (28.2 mg g^{-1} FW).

The results of analysis of variance revealed that the effect of irrigation, planting date and cultivar and irrigation × planting date, irrigation × cultivar and planting date × cultivar interactions were significant at the probability of 1% for MAD and only irrigation × planting date cultivar interaction was significant at 5% level (Table 2). The amount of MAD was also affected by irrigation × planting date × cultivar interaction, where the highest amount with an average of 29.05 (nmol g^{-1} FW) in Tassilo cultivar was observed with II and on an earlier planting date (PD1) (Table 3).

Guaiacol peroxidase

The results of analysis of variance showed that GPX activity in canola at the probability level of 1% was significantly affected by irrigation, planting date and cultivar and, irrigation × planting date, planting date × cultivar, irrigation × cultivar and irrigation × planting date × cultivar interactions (Table 2). Irrigation × planting date × cultivar interaction showed that the highest activity of

GPX (40.19 Unit mg^{-1} protein) was in Elvise cultivar with II and planting date of PD1 (Table 3).

Grain yield

The results of analysis of variance revealed that grain yield was significantly affected by irrigation, planting date, and cultivar at the probability level of 1% and irrigation × planting date × cultivar interaction at the probability level of 5% (Table 2); Elvise cultivar had the highest grain yield in the earlier sowing date (PD1) and under normal irrigation with an average of 3346.67 kg ha^{-1} (Table 3).

Stepwise regression and Path analysis

In order to identify the most important enzymes affecting the grain yield, stepwise multiple regression analysis was used. GY was considered as a dependent variable and other traits were considered as independent variables. Among eight independent variables, three variables of AN, GPX, and MAD remained in regression model while the other variables were excluded. The adjusted coefficient of determination in the final model was 0.774, showing that 77.4% of the changes in GY were explained by the three variables introduced into the regression model, while 22.6% of the changes were related to other factors and relationships. The contribution and role of each of the variables introduced into the model in explaining the dependent variable can be obtained from beta values (β) or standard path coefficients (Table 4).

According to the standard path coefficients obtained from the third step, MAD with β of -0.72 had the highest negative and significant relationship with the GY. Thus, increasing MAD in canola can reduce the yield. MAD is a chemical marker which will increase under drought conditions in the plant and may cause cell wall degradation plus plant death. In this study, II from the silique formation stage onwards resulted in elevated MAD concentration. AN with beta coefficient of 0.61 had the strongest positive and significant relationship with the GY. AN play a significant role in stress adaptation by inhibiting free radicals (Gould, 2004). GPX also showed a significant inverse relationship with the GY with β of -0.25. Hence, GPX ranked the second after MAD in terms of had the most negative and significant effect on the GY. The regression equation was obtained for the GY as a dependent variable and the traits introduced into the model using the regression coefficients in Table 4.

In order to study as many interrelationships as possible between the traits introduced into stepwise regression model as well as to determine direct and indirect effects of each of them on the grain yield, Path analysis method was used based on cause and effect along with the order of occurrence and evolution of each studied canola trait. According to the results of analysis of direct and indirect effects of antioxidant enzymes on the GY (Table 5), a direct and positive effect was belonged AN (0.61) while a negative and significant direct impact was related to MAD (-0.72). The effects of AN and MAD were only direct and significant, while in addition to the direct effect (-0.25), the indirect effect of GPX on the GY was also significant (Figure 1).

Table 4. Stepwise regression for grain yield (dependent variable) and the other traits (independent variable)

Regression coefficients	Not standardized coefficients		Standard regression coefficient β	T	P
	B	Standard error			
Constant a	1359.69	175.27		7.76	0.000
Anthocyanin (X1)	67.36	6.24	0.61	10.79	0.000
Guaiacol peroxidase (X2)	-7.67	2.49	-0.25	-3.09	0.003
Malondialdehyde (X3)	-21.04	2.21	-0.72	-9.50	0.000
Regression equation	Y (grain yield) = 67.36 X1-7.68X2-21.04X3-1359.69				

RMSE=144.88 R=0.884 R^2=0.781 R^2 adj=0.774
RMSE: root-mean-square error; R: correlation coefficient; R^2: R-squared; R^2 adj: adjusted R-squared.

Table 5. Estimation of direct and indirect effects of antioxidant enzymes on the grain yield

Trait	Independent variable			Dependent variable
	Anthocyanin	Guaiacol peroxidase	Malondialdehyde	Grain yield
Anthocyanin	0.61^{**}	-0.12	-0.24	0.24
Guaiacol peroxidase	0.30^{*}	-0.25^{*}	-0.55^{**}	-0.50
Malondialdehyde	0.20	-0.19	-0.72^{**}	-0.71
Residual	-----	-----	-----	0.47
R²	-----	-----	-----	

R^2: R-squared. **P<0.01; *P≤0.05.

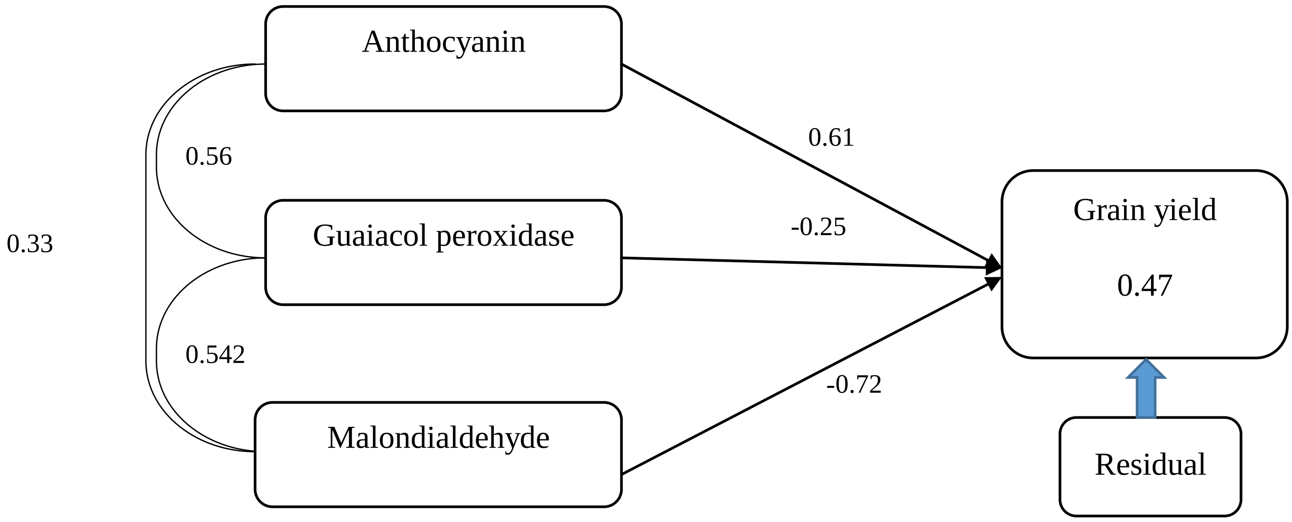

Figure 1. Path analysis of studied traits and GY

Cluster analysis

Using cluster analysis showed that the rapeseed cultivars were grouped into three groups based on mean yield, physiological and biochemical indices measured in well-watered and withholding irrigation treatments under different planting dates. Elvise cultivar, due to the highest yield and resistance to drought stress, was placed in the first group, Okapi and Neptune cultivars, respectively, were placed in the second and third groups in terms of similarity in physiological traits; also Tassilo cultivar was ranked last due to the lowest GY in well-watered and withholding irrigation treatments and both planting dates (Figure 2).

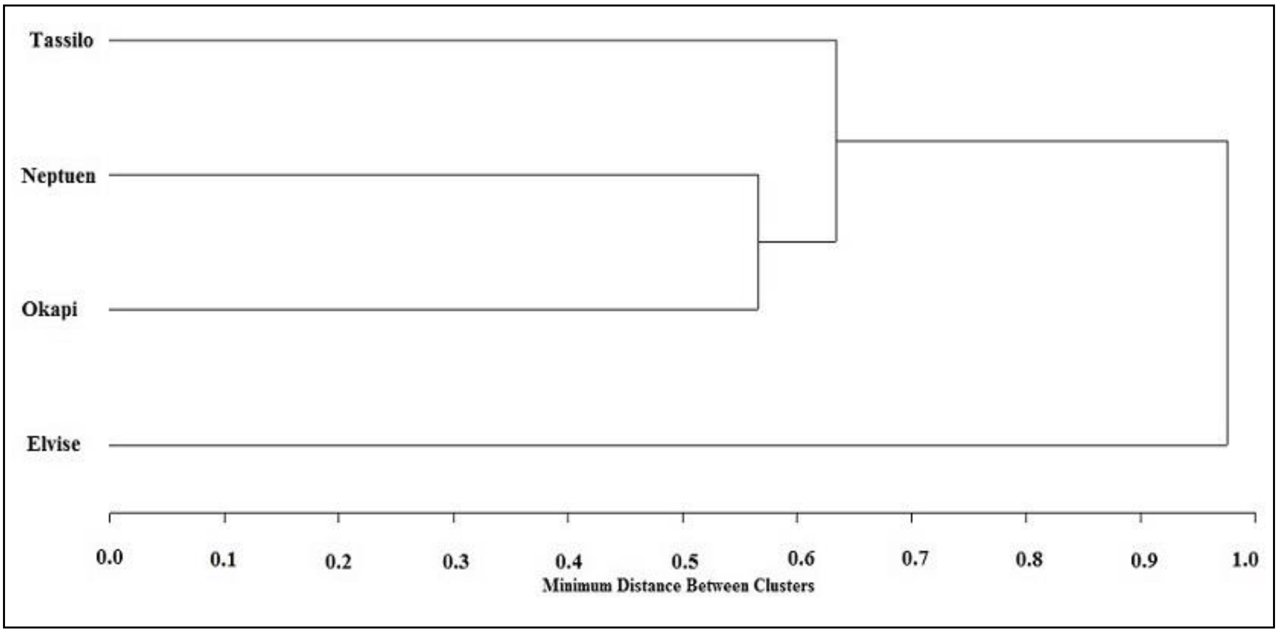

Figure 2. Dendrogram obtained from cluster analysis of physiological and biochemical traits measured in four rapeseed cultivars (SAS software)

Different plant species benefit from two sets of antioxidative defences, including antioxidative compounds and an enzymatic antioxidative system (Akbari-Kharaji et al., 2020). AN are water-soluble pigments belonging to Flanodi family made from Shikimic acid pathway and have antioxidant properties and act as free radical receptors, protecting plants against oxidative stress (Lin-Wang et al., 2010). The increase in AN in leaves and roots of drought stressed plants suggests that non-enzymatic strategies also play an important role in overcoming oxidative stress. AN as an antioxidant pigment reduced oxidative stress (Salehi-Lisar and Bakhshayeshan Agdam, 2016). Accumulation of secondary metabolites such as phenolic compounds and AN is also strongly affected by water stress, with evidence indicating that production of some of these matabolist incrases several times under stress. According to the result of present study, the amount of AN increased in the studied cultivars of canola with II. The results of previous studies have shown that accumulation of AN in plant leaves reduces osmotic potential of leaves and thus the water potential of leaf as well as stomatal conductance (Choinski and Johnson, 1993).

Environmental stresses stimulate accumulation of ROS, resulting in oxidative stress damage. Nevertheless, plants use an antioxidant system to cope with oxidative stress. MAD is a peroxidation product of unsaturated fatty acids in phospholipids. The level of lipid peroxidation is used as a marker of free radicals that are harmful to cell membranes under stress conditions. According to Moussa and Abdel-Aziz (2008), an increase in MAD under water shortage conditions indicates that drought causes membrane peroxidation through production of ROS**.** Degradation of cell membranes is one of the direct consequences of water shortage. Mirzaee et al. (2013) reported that MAD levels increased under drought stress, which is consistent with the results of the present study. The results of this study showed that drought stress was associated with increased levels of GPX activity. Increasing the severity and duration of drought stress may impair protein synthesis leading to a reduced the activity of most antioxidant enzymes, including GPX. Decreased GPX activity in some genotypes may also be due to increased levels of ascorbate peroxidase. On the other hand, high levels of ascorbate may have an inhibitory effect on GPX.

In a similar finding to this study, with a delay of canola planting time of one month, it was stated that grain yield may drop by 10-50% depending on the cultivar, and differences in canola cultivars in terms of GY may be attributed to different yield compoents such as number of branches, which reflects the number of siliques per plant and 1000-grain weight (Sharghi et al., 2011). According to Mostafavirad et al. (2012), delayed planting, in addition to shortening the grain filling period, reduces flowering and pollination due to exposure to hight temperaturer, which will finally lower the grain yield. Although the effect of delayed planting on yield reduction cannot be ignored, the impact of cultivar on the yield is also very significant (Moradi Aghdam et al., 2018). Thus genotypic differences and sowing date modifications in GY of rapeseed was not surprising. The results showed that drought stress was generally associated with higher levels of guaiacol peroxidase activity. However, prolonged drought has severely reduced the activity of most antioxidant enzymes, including guaiacol peroxidase due to impaired protein synthesis. This indicates that plants use their entire antioxidant system to cope with drought. GPX had a positive and significant indirect effect through AN along with a negative and significant indirect impact on canola GY through MAD. The indirect effect of GPX through MAD was greater than the indirect effect through AN. It can be concluded that the highest negative and significant impact on the GY was related to MAD. Increased cellular lipid peroxidation due to drought stress indicates the effective role of MAD in cell damage and finally cell death. GPX had also a negative and significant impact on the GY through correlation with MAD.

Drought stress conditions increase the amount of anthocyanin in leaf. Chajker-Scott (1999) stated that production and accumulation of anthocyanin in roots, stems, and especially leaf tissues may allow the plant to resist some environmental stresses such as drought and cold. Indeed, anthocyanin have the ability to prevent Photooxidation and damage caused by UVB radiation. AN is an important part of plant secondary metabolites and belongs to Flavonoid family, whose accumulation is known as a defense mechanism in plants; plants can respond to and adapt to stress by changing their cellular metabolism through various defense mechanisms (El-Tayeb, 2005). A direct relationship was found between MAD content and drought stress intensity (Sofo et al., 2004). Drought stress does not affect all organes of crops equally, where leaves, stems, and roots of crops are most affected by water stress respectively. Drought stress due to the production of ROS reduces the concentration of chlorophyll in plants (Jaleel et al., 2009). Reducing leaf area, premature leaf aging, and shortening the reproductive growth period for the plant would, cause abortion in flowers, lowers the number of seeds as well as the number and size of pods, and finally suppresses the final yield by reducing yield components (Din et al., 2011).

CONCLUSIONS

Delayed sowing and late-season drought stress are two common limiting factors in the growth and production of rapeseed in cold temperate regions with arid and semi-arid climates such as Iran. Given the ever-increasing water scarcity, it is important to select a cultivar capable of tolerating late-season drought stress and delayed sowing in these climates. Cessation of II after podding stage increased the content of anthocyanin pigment, and antioxidant enzymes activity. Elvise cultivar showed the highest anthocyanin, guiacol peroxidase and yield, respectively, the lowest malondialdehyde, levels between the studied cultivars. Besides, the results showed that malondialdehyde had the highest degree of correlation and the most direct negative effect on yield. Drought stress conditions among the studied traits. guiacol peroxidase had a positive and significant indirect effect through AN and a negative and significant indirect effect on canola yield through malondialdehyde. Therefore, Elvise can be introduced as a superior cultivar to be cultivated to withstand late-season drought stress prevalent arid and semi-arid climates.

REFERENCES

Akbari-Kharaji, M., Ehsanzadeh, P., Gholami Zali, A., Askari, E., Ahmad Rajabi-Dehnavi, A., 2020. *Ratooned fennel relies on osmoregulation and antioxidants to damp seed yield decline with water limitation*. Agronomy for Sustainable Development, 40: 9.

Amoah, J.N., Ko, C.S., Yoon, J.S., Weon, S.Y., 2019. *Effect of drought acclimation on oxidative stress and transcript expression in wheat (Triticum aestivum L.)*. Journal of Plant Interactions, 14(1): 492-505.

Anjum, N.A., Arena, C., Singhgill, S., 2014. *Reactive oxygen species (ROS) and response of antioxidants as ROS scavengers during environmental stress in plant*. Frontiers in Environmental Science, Lausanne, Switzerland.

Azarpanah, A., Alizadeh, O., Dehghanzadeh, H., 2013. *Investigation on proline and carbohydrates accumulation in Zea mays L. under water stress condition*. Extreme life, Biospeology & Asterobiology, International Journal of the Bioflux Society, 5(1): 47-54.

Bates, L.S., Walderen, R.D., Taere, I.D., 1973. *Rapid determination of free pyrroline for water stress studies*. Plant Soil, 39: 205-207.

Bhardwaj, J., and Yada, S.K., 2012. *Comparative study on biochemical parameters and antioxidant enzymes in drought tolerant and a sensitive variety of Horsegram (Macrotyloma uniflorum) under drought stress*. American Journal of Plant Physiology, 7: 7-22.

Cartea, M.E., Francisco, M., Soengas, P., Velasco, P., 2011. *Phenolic compounds in Brassica vegetables*. Molecules, 16: 251-280.

Chajker-Scott, L., 1999. *Enviromental significance of anthocyaninsin plant strees responses*. Photochemistry and Photobiology, 70(1): 1-9.

Chance, B., and Maehly, A.C., 1955. *Assay of catalase and peroxidase*. Methods in Enzymology, 2: 764-775.

Choinski, J.S., and Johnson, M., 1993. *Changes in photosynthesis and water ststus of developing leaves of Brachystegia spiciformis Benth*. Tree Physiology, 13: 17-27.

Dehghani, G., Malek Shhi, F., Alizadeh, B.A., 2009. *Study of drought tolerance indices in canola (Brassica napus L.) genotypes*. Isfahan University of Technology, 13: 77-90.

Din, J., Khan, S.U., Ali, I., Gurmani, A.R., 2011. *Physiological and agronomic response of canola varieties to drought stress*. Journal of Animal and Plant Sciences, 21: 78-28.

Elferjani, R., and Soolanayakanahally, R., 2018. *Canola responses to drought, heat, and combined stress: shared and specific effects on carbon assimilation, seed yield, andoilcomposition*. Plant Science, 9: 1224.

El-Tayeb, M.A., 2005. *Response of barley Gains to the interactive effect of salinity and salicylic acid*. Plant Growth Regulation, 45: 215-225.

Ghorbanli, M., Gafarabad, M., Amirkian, T., Allahverdi Mamaghani, B., 2013. *Investigation of proline, total protein, chlorophyll, ascorbate and dehydro ascorbate changes under drought stress in Akria and Mobil tomato cultivars*. Iranian Journal of Plant Physiology, 3(2): 651-658.

Gould, K., 2004. *Nature's Swiss Army Knife: The diverse protective roles of anthocyanins in leaves*. Journal of Biomedicine and Biotechnology, 5: 314-332.

Heath, R.L., and Packer, L., 1968. *Hotoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation*. Archives of Biochemistry and Biophysics, 125(1): 189-198.

Jaleel, C.A., Paramasivam, M., Wahid, A., Farooq, M., Al-Juburi, H.J., Somasundaram, F., Panneerselvam, R., 2009. *Drought stress in plants: A review on morphological characteristics and pigment compositions*. International Journal of Agriculture and Biology, 11: 100-105.

Katsuhara, M., Otsuka, T., Ezaki, B., 2005. *Salt stress induced lipid peroxidation is reduced by glutation S transferase but this reduction of lipid peroxides is not enough for a recovery of root growth in Arabidopsis*. Plant Science, 169: 369-373.

Lin-Wang, K., Bolitho, K., Grafton, K., Kortstee, A., Karunairetnam, S., McGhie, T.K., Espley, R.V., Hellens, R.P., Allan, A.C., 2010. *An R2R3 MYB transcription factor associated with regulation of the anthocyanin biosynthetic pathway in Rosaceae*. Plant Biology, 10: 50.

Mariani, L., and Ferrante, A., 2017. *Agronomic management for enhancing plant tolerance to abiotic stresses-drought, salinity, hypoxia, and lodging*. Horticulture, 3(4): 52.

Mirzaee, M., Moieni, A., Ghanati, F., 2013. *Effects of drought stress on the lipid peroxidation and antioxidant enzyme activities in two canola (Brassica napus L.) cultivars*. Journal of Agricultural Science and Technology, 15: 593-602.

Moradi Aghdam, A., Seyfzadeh, S., Sehrani Rad, A.H., Valadabadi, S.A., Zakerin, H., 2018. *The effect of irrigation cut on physiological characteristics and grain yield of rapeseed cultivars under different planting dates*. Journal of Plant Physiology, 10(38): 59-76.

Moslemi, Z., Habibi, D., Asgharzadeh, A., Ardakani, M.R., Mohammadi, A., Sakari, A., 2011. *Effects of super absorbent polymer and plant growth promoting rhizobacteria on yield and yield components of maize under drought stress and normal conditions*. Afr. J. Agric. Res., 6: 4471-4476.

Mostafavirad, M., Shariati, F., Mostafavi Rad, S., 2012. *Evaluation of sowing date influence on quantitative and qualitative yield in four rapeseed cultivars adapted to cold regions in Arak, Iran. Electron*. Journal of Plant Production, 5(2): 159-167.

Moussa, H., and Abdel-Aziz, S.M., 2008. *Comparative response of drought tolerant and drought sensitive maize genotypes to water stress*. Australian Journal of Crop Science, 1: 31-36.

Noctor, G., Gomez, L., Vanacker, H., Foyer, C.H., 2002. *Interactions between biosynthesis, compartmentation, and transport in the control of glutathione homeostasis and signaling*. Journal of Experimental Botany, 53: 1283-1304.

Norouzi, M., Toorchi, M., Salekdeh, G.H., Mohammadi, S.A., Nishabouri, M.R., 2008. *Effect of water deficit on growth, grain yield and osmotic adjustment in rapeseed*. J. Food Agric. Environ., 6: 132-138. https://doi.org/10.3168/jds.2007-0441

Raza, M.A.S., Shahid, A.M., Saleem, M.F., Khan, I.H., Ahmad, S., Ali, M., Iqbal, R., 2017. *Effects and management strategies to mitigate drought stress in oilseed rape (Brassica napus L.): a review*. Zemdirbyste, 104: 85-94.

Ritchie, S.W., Nyvgen, H.I., Halady, A.S., 1990. *Leaf water content and gas exchange parameters of two wheat genotypes differing in drought resistance*. Crop Sci., 30: 105-111.

Sabagh, A.E., Hossain, A., Barutçular, C., Islam, M.S., Ratnasekera, D., Kumar, N., Meena, R.S., Gharib, H.S., Saneoka, H., Teixeira da Silva, J.A., 2019. *Drought and salinity stress management for higher and sustainable canola (Brassica napus L.) production: A critical review*. Aust. J. Crop Sci., 13: 88-96.

Salehi Shanjani, P., Izadpanah, M., Ramezani Yeganeh, M., Rasoulzadeh, L., Kavandi, A., Sardabi, F., Pahlavani, M.R., Amirkhani, M., Seyedian, S.E., 2015. *Comparison of the effect of drought stress on osmotic regulation, peroxidase, polyphenol peroxidazopigments in different seed samples of false chamomile and yellow chamomile Anthemis tinctoria Tripleurospermum servanes*. Journal of Plant Research (Iranian Journal of Biology), 28(1): 126-139.

Salehi-Lisar, S.Y., and Bakhshayeshanagdam, H., 2016. *Drought stress tolerance in plants: causes, consequences and tolerance*. In: Hossain, M.A., Wani, Sh.H., Bhattacharjee, S., Burritt, D.J., Phan Tran, L.S. (eds.), Drought stress tolerance in plants, Vol. 1. Springer Press, London: 35-50.

Sharghi, Y., Shirani Rad, A.H., Ayeneh Band, A., Noormohammadi, G., Zahedi, H., 2011. *Yield and yield components of six canola (Brassica napus L.) cultivars affected by planting date and water deficit stress*. Plant Physiology and Biochemistry, 10(46): 9309-9313.

Shirani Rad, A.H., Bitarafan, Z., Rahmani, F., Taherkhani, T., Moradi Aghdam, A., Nasresfahani, S., 2014. *Evaluation of spring rapeseed (Brassica napus L.) cultivars for different planting date and irrigation regimes*. Plant Sci., 24: 1166-1172.

Sofo, A., Dichio, B., Xiloyannis, C., Masia, A., 2004. *Lipoxygenase activity and praline accumulation in leaves and roots of olive tree in response to drought stress*. Plant Physiology, 121: 56-58.

Wagner, G.J., 1979. *Content and vacuole/extravacuole distribution of neutral sugars, free amino acids, and anthocyanins in protoplast*. Plant Physiology, 64: 88-93.

Zhiliang, H., Baowu, W., Paul, W., Ralphenia, D.P., 2009. *Identification of anthocyanins in muscadine grapes with HPLC-ESI-MS. LWT*. Food Science and Technology, 42: 819-824.