Central Asian Journal of Plant Science Innovation **1**(1): 1–9 (2021)

RESEARCH PAPER

Study of relationship between some agro-physiological traits with drought tolerance in rapeseed (*Brassica napus* L.) genotypes

Zeinab Chaghakaboodi [1]*, Mehdi Kakaei [2], Alireza Zebarjadi [1]

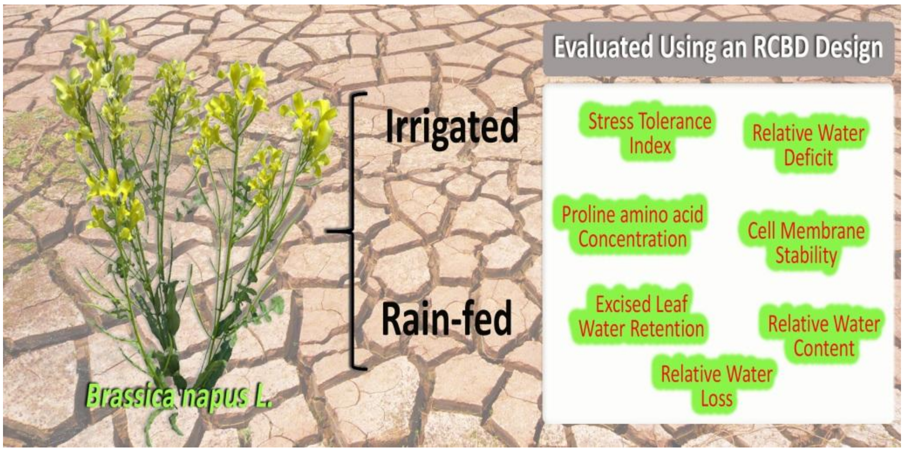
[1] Department of Production Engineering and Plant Genetics, Faculty of Science and Agricultural Engineering, Razi University, Kermanshah, Iran

[2] Departement of Plant Breeding & Genetic, Payam-e-Noor University, Hamedan, Iran

Highlights

- Data showed that genotypes under in rain-fed conditions had the highest value of PC in compared to the irrigated conditions.
- The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species.
- RWC of the leaves is a better indicator of water stress than other growth or biochemical parameters of the plants.

Graphical Abstract



Article Info

Receive Date: 03 November 2020
Revise Date: 31 January 2021
Accept Date: 13 February 2021
Available online: 27 February 2021

Keywords:
Brassica napus
Drought tolerance
Physiological traits
Rainfed condition

Abstract

Drought is one of the non-biological stresses that cause damage to crops and orchards. Due to limited water and low rainfall, half of the area under cultivation is rainfed. Rapeseed (Brassica napus) is one of the most important oilseeds that has a wide range of climate adaptation. In order to evaluate some agro-physiological traits for identification of drought tolerance in rapeseed, fourteen different rapeseeds genotypes differing in yield performances were evaluated using an RCBD design with three replications under two different environments (irrigated and rainfed) in two years. Stress Tolerance Index (STI), Proline amino acid Concentration (PC), Cell Membrane Stability (CMS), Relative Water Content (RWC), Excised Leaf Water Retention (ELWR), Relative Water Loss (RWL), Relative Water Deficit (RWD) and SPAD were determined in order to find out whether these physiological traits could be used as the indicators of drought tolerance. The result of combined analysis of variance showed a highly significant difference among years, genotypes, and between genotypes × years interaction for most of the traits. The results of correlation showed a highly significant between stress tolerance index, grain yield and oil yield in both conditions. There was a significant correlation between relative water content and relative water deficit. It can be concluded that most agro-physiological traits in Rapeseed are affected by genotype, year and interactions.

10.22034/CAJPSI.2021.01.01

E-ISSN: 2783-1310

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* Corresponding author: z_chaghakaboodi@yahoo.com (Z. Chaghakaboodi)

1. Introduction

Rapeseed (*Brassica napus* L.) is the second most important source of edible oil production in the world after soybeans (Farooq and E-Azam, 2001). In this plant, the stages of flowering and the formation of pods are among the most sensitive stages to drought stress, which are faced with drought stress in most agricultural areas of the country. Crop yield is influenced by plant genetic structure, environmental conditions and the effects of their interaction (Turner, 1986; Turner et al., 2003). However, all living and non-living stresses are important factors in reducing production. Drought stress is the most important factor limiting crop production in agricultural systems in arid and semi-arid regions (Robertson and Holland, 2004). The relative moisture content plays an important role in regulating the stomatal conductance and thus the photosynthetic rate of the plant. The results of some studies on canola showed that the relative water content of RWC leaves in irrigation treatments was higher than stress treatments but decreased under RWC drought stress conditions. STI as The most desirable drought resistance index in the study of resistance in spring, spring safflowers were introduced in different regions. SSI and TOL indices for selection of resistant cultivars Drought in canola was reported to be unsuitable. Increasing the amount of proline in stress conditions is one of the criteria for tolerance in plants and proline can play a protective role for proteins and enzymes in stress conditions. Increasing the amount of free proline is one of the defense mechanisms of plants against drought stress (Hoque et al., 2008). The effectiveness of a breeding program fundamentally depends on the direction and importance of the relationship between performance and performance components, and its performance and formation process depend on genetic, environmental, and agronomic factors as well as their interaction. In a study, the stability of cell membranes (CMS) under stress conditions was stated as the main factor of drought resistance. The leaf age, leaf position in the stem and the intensity of drought stress to be effective in damaging cell membranes. The cell membrane stability (CMS) for 4 types of peanuts by PEG polyethylene glycol test was determine and result show that drought tolerance was correlated with electrical conductivity. The effect of drought stress on the plant is a function of genotype, during the drought period, climatic conditions and plant growth stage. The time of onset of drought stress is more important than the severity of drought. Drought stress does not occur suddenly but slowly compared to many stresses, so time dimension plays an important role in terms of survival in drought stress conditions (Gunes et al., 2008). The different stages of canola growth have different sensitivities to drought and its contribution to yield is different. This can be easily determined by rainfall removal or irrigation tests at different stages of the life cycle. Also, the lack of irrigation significantly, canola yield low and reduced grain yield by 45 to 58% compared to irrigation. Decreased chlorophyll content has been reported under drought stress and maintaining chlorophyll concentration under drought stress helps stabilize photosynthesis under these conditions (Tripathy et al., 2000). Farshadfar et al., 2008, reported that photosynthetic durability and maintenance of chlorophyll concentration under stress conditions are among the physiological indicators of drought tolerance (Farshadfar et al., 2008).

This study aims to screen for drought tolerance of autumn rapeseed genotypes under water stress conditions using physiological traits (Ghasempour and Rangin, 2007).

2. Materials and Methods

In this study, 14 rapeseed (*Brassica napus* L.) were planted under deficit water and normal conditions. The experiment was conducted at the Research Farm, Agricultural Faculty of Razi University, Kermanshah, Iran. The traits were in a randomized complete block design (RCBD) with three replications. The yield (kg/ha) was obtained by converting the seed yield per plot to hectares. Normal plots were irrigated three times, at the bud formation, flowering and grain filling stages, while stressed plot received no water other than rainfall. Origin and characters of genotype are given in (Table1).

Table 1. Origin and Characters of genotypes.

No.	Genotypes	Origin	Appearance
1	Geronimo	Rosticafrance (European=Winter)-(Mexican-China-Canadian=Spring)	Winter
2	Celecious	Sralof	Winter
3	Milena	Germany	Winter
4	Sahra	Danisco	Winter
5	Sunday	Danisco	Winter
6	Zarfam	Iran	Winter
7	Dante	Germany	Winter
8	SLM-046	Germany	Winter
9	Talaye	Iran	Winter
10	Talent	Germany	Winter
11	ARC2	U.S.A	Winter
12	Opera	SW-sweden	Winter
13	ARC5	U.S.A	Winter
14	Licord	Germany	Winter-Spring

2.1. Cell membrane stability (CMS)

First, the developed leaves were separated. The middle part of the leaves was cut into one-centimeter pieces and washed three times with distilled water. The leaf pieces were placed in containers containing 25 ml of distilled water (control) or 24 ml of 40% solution of PEG6000. The samples were then incubated at 10 °C for 24 h. The dishes were taken out of the incubator and the liquid inside the container was emptied and the leaves were washed. Controlled and PEG-treated samples were again immersed in distilled water at 10 °C for 24 h. Electrical conductivity was measured. Containers containing the sample and distilled water were then autoclaved for 15 minutes and their final electrical conductivity was recorded. Then the percentage of cell membrane damage was calculated based on the following formula (Sullivan, 1972).

Injury (%) $=1- \{[1-T_1/T_2] / [1-C_1/C_2]\} \times 100$ CMS (%) $= 1 – I$ (also in %)

T_1 and T_2 = first and second conductivity measurement of desiccation treatment, respectively.

C_1 and C_2 = first and second conductivity measurement of control, respectively.

2.2. Stress tolerance index (STI)

Stress tolerance includes performance potential in non-stress environments, performance in stress environments and SI stress intensity. STI estimation is based on the geometric mean of productivity and is due to the fact that it has a single rank correlation (one) with the geometric mean of productivity (Fernandez, 1992).

$STI = (Yp)\ (Ys) / (\bar{Y}p)\ 2$

2.3. Free proline content (PC)

0.1 g of fresh flag leaf was extracted on the proline content based on Bates (1973) method and the proline concentration of the samples was determined using a spectrophotometer using specific concentrations of pure proline as a control at 520 nm using UV spectrophotometer (Bates et al., 1973).

2.4. Relative water content (RWC)

First, the fully developed leaves are separated and taken to the laboratory. Then their weight is recorded as wet weight. The samples are then immersed in distilled water for 24 hours and their weight is recorded as saturated weight after 24 hours. The leaves are placed in an oven at 70 °C for 24 hours and their dry weight is measured. The relative water content of the leaf is calculated by including the numbers obtained by weighing in the formula below (Bennett et al., 1987; Matin et al., 1989).

RWC= (FW – DW) / (TW – DW) ×100

2.5. Relative water deficit (RWD)

RWD (%) = 100 – RWC (Tourneux et al., 2003).

2.6. Excised leaf water retention (ELWR)

It was calculated based on the following equation, in which FW2 and WW2, respectively, in which the initial weight of the leaves and the weight of wilting are after 4 hours (Gavuzzi et al., 1997).

ELWR (%) = [1 – ((FW –WW4h)/FW))] × 100

2.7. Relative water loss (RWL)

To measure the amount of water lost in each plot, several plants were selected and several leaves were developed from each plant, they were separated and their wet weight (FW) was recorded. The samples were then placed in the laboratory for 6 hours to obtain their wilting weight (WW). The samples were then placed in an oven at 70 ° C for 48 hours to measure the dry weight (DW) of the samples and then the amount of water lost was calculated using the following formula (Gavuzzi et al., 1997).

RWL (%) = [(FW – WW) / (FW – DW)] × 100

2.8. Relative chlorophyll content (RCC)

Leaf chlorophyll was measured using a chlorophyll meter (SPAD_502, Japan). Statistical analyses were performed using SPSS 16, SAS 9.2 and MSTAT-C software.

3. Results and Discussion

The results of combined analyses of variances indicated the highly significant differences among genotypes for all traits exception Grain yield, RWC, RWD and ELWR, significant differences among years for all traits exception ELWR and significant differences between year's × genotypes interaction for all traits except Oil %, Grain yield, ELWR and RWL (Table 2). Mean comparison of interaction effects years and genotypes for Oil yield, Proline Concentration, RWC, RWD and SPAD in rapeseed were performed. Mean comparison of interaction effects years and genotypes for Oil yield (Fig. 1) showed that oil yield for all genotypes in first years higher than second years because of grain yield in first years higher than second years. In our study, there were significant ($P< 0.01$) differences among genotypes for proline concentration (PC). Data showed that genotypes under deficit water conditions had the highest value of PC compared to the normal conditions. PC of the genotypes increased under drought stress conditions on average by 73.13 comparing to normal conditions. Increases in PC have been also reported by (Khan et al., 2001; Kocheva et al., 2004). Mean comparison of interaction effects years and genotypes for PC showed that PC for all genotypes in first years higher than second years except genotype no 14 (Licord) that for this genotype PC for second years higher than first years (Fig. 2). Data showed that drought-tolerant genotypes had the highest value of RWC in compared to the other genotypes. The values were 77.77, 79.72 and 89.90, 94.73 for deficit water and normal conditions respectively. A decrease in the RWC in response to drought stress has been noted in a wide variety of plants (Nayyar and Gupta, 2006; Sinclair and Ludlow, 1985). Reported that RWC of the leaves is a better indicator of water stress than other growth or biochemical parameters of the plants. RWC of the leaves is very responsive to drought stress and has been shown to correlate with drought tolerance (Colom and Vazzana, 2003). Drought tolerant genotypes showed higher RWC rather than drought-sensitive genotypes (El-Tayeb, 2006; Ouk et al., 2006). Thus, RWC is not an indicator of drought tolerance. Mean comparison of interaction effects years and genotypes for RWC (Fig. 3) showed that relative water content for all genotypes in second years higher than first years. Mean comparison of interaction effects years and genotypes for RWD showed that relative water deficit for all genotypes in first years higher than second years (Fig. 4).

Table 2. Combined analysis of variance for traits of *brassica napus* genotypes.

S.O.V	df	Oil (%)	Oil yield	Grain yield	Proline	RWC	ELWR	RWL	RWD	SPAD
Year	1	741.85**	46040371.76**	35057920.00**	314670.03**	29092.78**	1.397^{ns}	6767.83**	28992.23**	3150.67**
Error I	4	13.06	4221.17	200795.83	1095.19	168.88	18.72	44.85	171.00	21.14
Genotype	13	43.92**	172928.04**	1052724.43^{ns}	26788.82**	200.98^{ns}	92.47^{ns}	315.48*	225.95^{ns}	144.91**
Gen×year	13	34.90^{ns}	179974.88**	1033754.51^{ns}	27774.99**	437.48**	102.82^{ns}	217.39^{ns}	506.66**	120.42**
Error II	136	28.80	53924	1969142	7598.38	142.09	112.76	173.94	137.62	38.09
C.V.	-	10.60	35.15	58.39	76.96	13.96	17.06	26.09	80.07	13.11

ns, * and **: Not significant, significant at the 5% and 1% levels of probability, respectively.

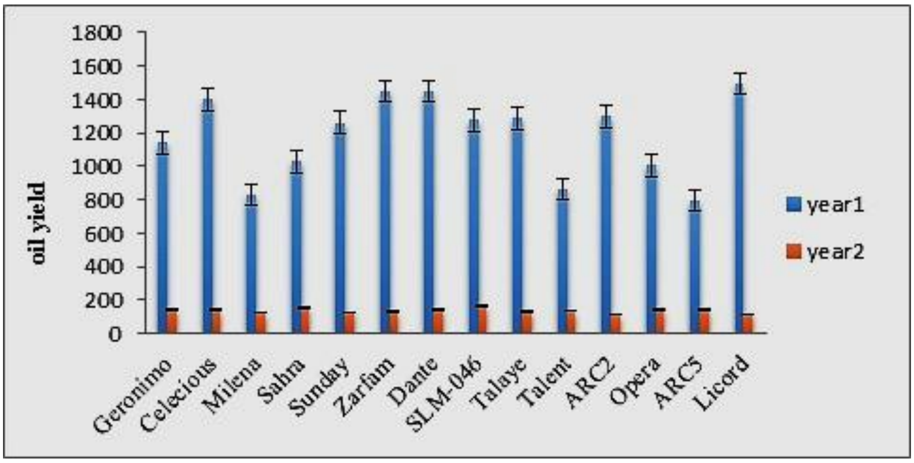

Figure 1. Mean comparison of interaction effects years and genotypes for oil yield in rapeseed (Duncan's =0.01).

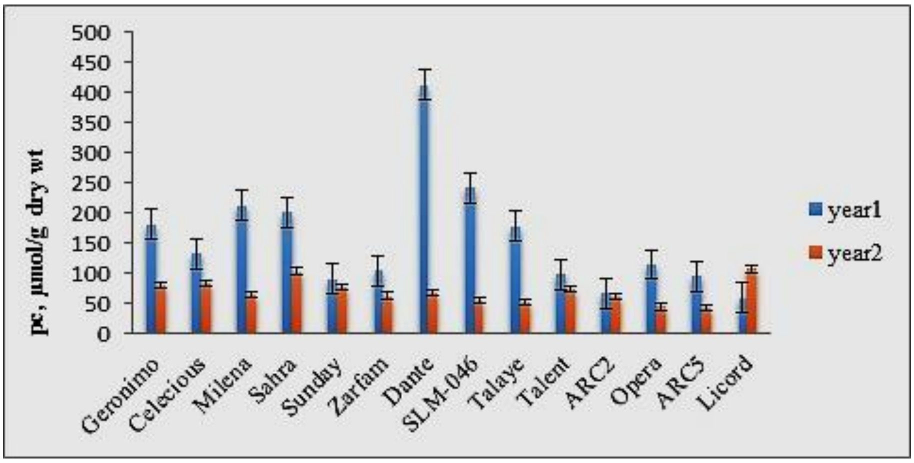

Figure 2. Mean comparison of interaction effects years and genotypes for PC in rapeseed (Duncan's =0.01).

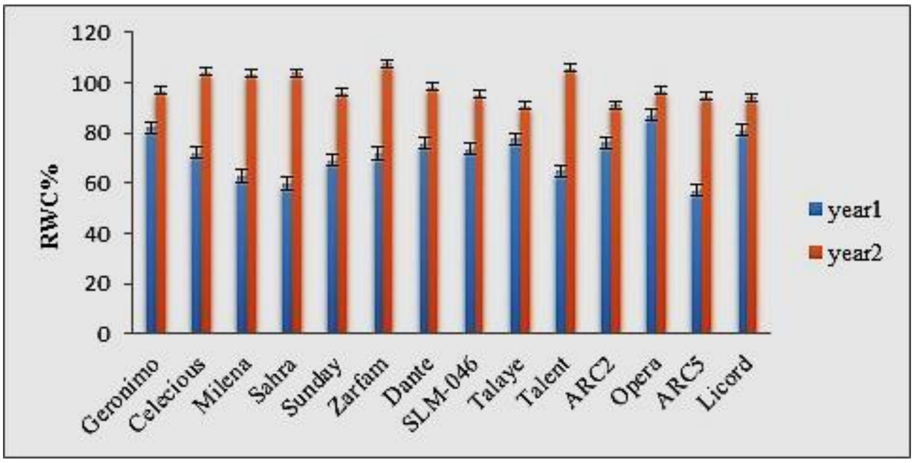

Figure 3. Mean comparison of interaction effects years and genotypes for RWC in rapeseed (Duncan's =0.01).

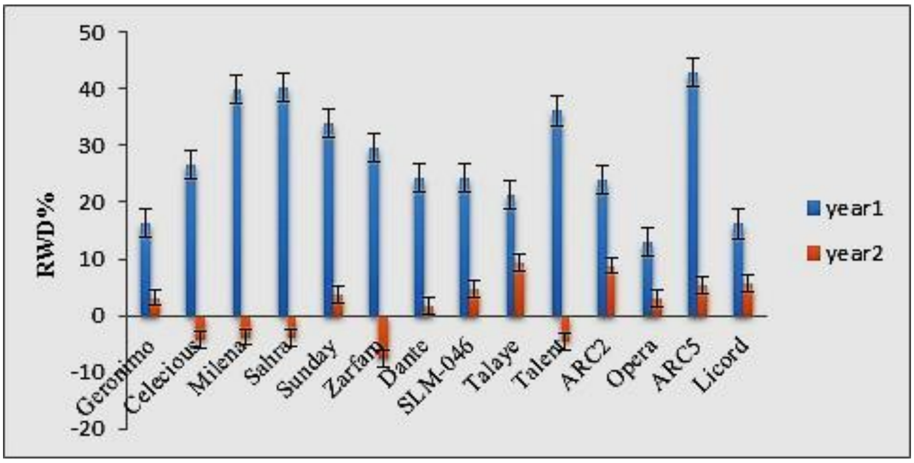

Figure 4. Mean comparison of interaction effects years and genotypes for RWD in rapeseed (Duncan's =0.01).

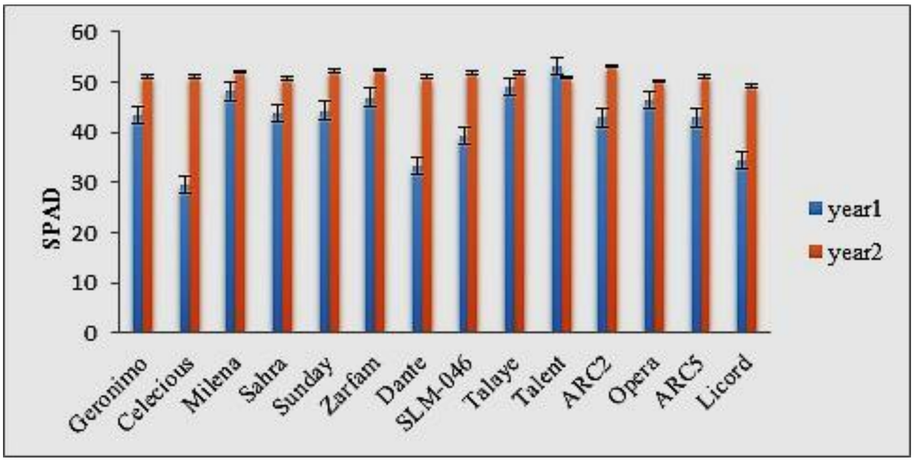

Figure 5. Mean comparison of interaction effects years and genotypes for SPAD in rapeseed (Duncan's =0.01).

SPAD under deficit water condition was decreased significantly than normal conditions. The drought stress caused a large decline in chlorophyll content, in all sunflower varieties (Manivannan et al., 2007). The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species. Mean comparison of interaction effects years and genotypes for SPAD (Fig. 5) showed that SPAD for all genotypes except genotype no 10 (Talent) in second years higher than first years. There was no significant correlation between SPAD and STI. Thus, based on the results of this study, SPAD was not an indicator of drought tolerance (Gunes et al., 2008). Correlation analysis (Table 3) showed that RWC and RWD negatively and significantly ($P< 0.01$) correlated under deficit water ($r = -0.989^{**}$) and under normal ($r = -0.994^{**}$) conditions. Although, RWC had positive and significant ($P< 0.05$) correlation with grain yield ($r = 0.610^{*}$), oil yield ($r = 0.654^{*}$) and STI ($r = 0.549^{*}$) under normal conditions, it had a negative and significant ($P< 0.05$) correlation ($r = -0.565^{*}$) under deficit water conditions. STI was positively and significantly ($P< 0.01$) correlated with grain yield ($r = 0.785^{**}$), oil yield ($r = 0.851^{**}$), RWC (0.549*) and negative and significant correlation ($P< 0.05$) with RWL (-0.643*) and RWD (-0.550*) under normal and was positively and significantly ($P< 0.01$) correlated with oil yield($r = 0.900^{**}$) and grain yield ($r =0.952^{**}$) under deficit water condition. The correlation among RWL, RWD and STI was negatively (Table 3). That means that drought-tolerant genotypes had higher RWC and lower RWL and RWD. Therefore these genotypes which maintained higher RWC under deficit water conditions is believed to be more droughts tolerant and gave higher yielding than others. The superior performance of drought tolerant soybean, maize and wheat under water stress environment are attributing to osmoregulation when stress set in (Bennet et al., 1987; Schonfeld et al., 1988). These cultivars difference in RWC could be used to select high yielding genotypes that maintain cell turgor under water stress environment to give a high relative yield. The observed relationship between Yp and Ys with STI was in consistent with those reported by Fernandez, 1992 STI varied in different genotypes between 0.256 to 0.505 and genotype no 7 (Dante) exhibits the high STI and grain yield was found drought tolerance (Fernandez, 1992). There was no significant correlation between STI and PC of the genotypes under deficit water conditions (Zarei et al., 2007). Stepwise regression analysis of STI (dependent) and measured physiological traits under stress conditions (independent) showed that grain yield best explained differences in drought tolerance. Because there is a positive and strong correlation between STI and Grain yield. Therefore, based on stepwise regression analysis, this trait can be considered the best indicator of drought tolerance in rapeseed genotypes (Table 4).

Table 3. Simple correlation coefficients of the traits in *brassica napus* genotypes.

	RWC	**ELWR**	**RWL**	**RWD**	**SPAD**	**Oil (%)**	**Oil yield**	**Grain yield**	**PC**	**STI**	**CMS**
RWC		0.255	-0.565*	-0.989**	0.002	0.273	0.192	0.107	-0.093	0.262	-
ELWR	-0.398		-0.506	-0.264	0.013	0.022	-0.263	-0.299	-0.293	-0.200	-
RWL	-0.386	-0.234		0.588*	0.372	-0.050	-0.445	-0.375	0.144	-0.475	-
RWD	-0.994**	0.354	0.426		0.044	-0.185	-0.235	-0.181	0.077	-0.336	-
SPAD	-0.186	0.178	0.172	0.194		-0.172	-0.287	-0.227	-0.010	-0.270	-
Oil (%)	0.099	-0.629*	0.425	-0.056	0.084		-0.004	-0.338	-0.107	-0.328	-
Oil yield	0.610*	-0.013	-0.530	-0.614*	-0.225	0.004		0.936**	0.354	0.900**	-
Grain yield	0.654*	0.120	-0.502	-0.657*	-0.284	-0.370	0.828**		0.336	0.952**	-
PC	-0.373	0.358	-0.396	0.352	-0.042	-0.388	-0.071	-0.138		0.329	-
STI	0.549*	0.166	-0.643*	-0.550*	-0.184	-0.414	0.785**	0.851**	0.163		-
CMS	-0.291	0.308	0.074	0.282	0.203	0.009	0.385	0.385	0.385	-0.197	

Notes: * and **—significant at 5 and 1% levels of probability, respectively. Lower diagonal represents the genotypes grown under irrigated and upper diagonal represents the genotypes grown under rain-fed conditions.

Table 4. Stepwise regression analysis for STI and traits in *brassica napus* genotypes.

Dependent variable	**Independent variable**	**Unstandardized coefficient**	**Standardized coefficient**	**t**	**Collinarity**	**statistics**
					tolerance	VIF
STI	Grain yield	0.000	0.952	10.725**	1.000	1.000

Notes: *R*2 = 0.952; adjusted *R*2 = 0.898. **: significant at 1% levels of probability.

4. Conclusion

In this research, there were significant (P< 0.01) differences among genotypes for proline concentration (PC). Data showed that genotypes under deficit water conditions had the highest value of PC compared to the normal conditions. PC of the genotypes increased under drought stress conditions on average by 73.13 comparing to irrigated conditions (Khan et al., 2001; Kocheva et al., 2009). Data showed that drought-tolerant genotypes had the highest value of RWC compared to the other genotypes. The values were 77.77, 79.72 and 89.90, 94.73 for deficit water and normal conditions respectively. A decrease in the RWC in response to drought stress has been noted in a wide variety of plants (Nayyar and Gupta, 2006; Sinclair and Ludlow, 1985). Reported that RWC of the leaves is a better indicator of water stress than other growth or biochemical parameters of the plants. RWC of the leaves is very responsive to drought stress and has been shown to correlate with drought tolerance (Colom and Vazzana, 2003). Drought tolerant genotypes showed higher RWC rather than drought-sensitive genotypes (El-Tayeb, 2006). Thus, RWC is not an indicator of drought tolerance. SPAD under deficit water condition was decreased significantly than normal conditions. The drought stress caused a large decline in chlorophyll content, in all sunflower varieties (Manivannan et al., 2007). The decrease in chlorophyll under deficit water is mainly the result of damage to chloroplasts caused by active oxygen species.

Acknowledgments

The authors thank Razi University for providing financial supports for this research project.

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How to cite this paper:

Chaghakaboodi, Z., Kakaei, M., Zebarjadi, A., 2020. Study of relationship between some agro-physiological traits with drought tolerance in rapeseed (*Brassica napus* L.) genotypes. *Cent. Asian J. Plant Sci. Innov.*, **1**(1), 1-9.