

Study of Genetic Variation and Drought Tolerance in Commercial Rapeseed (*Brassica napus* L.) Cultivars

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Received: 03 August 2015

Accepted: 17 September 2015

Abstract

In order to identify drought stress tolerance in rapeseed, nine commercial rapeseed cultivars (Opera, Karaj1, Karaj2, Karaj3, Zarfam, Okapi, Talayeh, Licord and Modena) were evaluated under two conditions (normal and drought stress) in Kangavar region in the west Iran in 2014-2015 growing season. This research was carried out in a split plot experiment based on completely randomized design in four replications. Under drought stress conditions, irrigation was cut from end of flowering stage. Analysis of variance results showed that drought stress had significant effects on plant height, days to maturity, pods per main branch, pods per sub-branch, pods per plant, seeds per pod, 1000 seed weight, oil yield and seed yield. Seed yield reduced from 4036.9 to 2919.4 kg/ha (27.68%) and Oil yield reduced from 1840.8 to 1362.2 kg/ha (25.99%) caused by drought stress. Oil yield reduced from 1840.8 to 1362.2 kg/ha (25.99%) caused by drought stress. Meanwhile pods per sub-branch reduced more than other traits (27.93%). There were significant differences among cultivars for all studied traits. The Karaj3 and Talayeh cultivars showed the highest seed yield in normal (4632.5 kg/ha) and stress (3747.5 kg/ha) conditions respectively. The interaction effects of cultivar and irrigation conditions was significant just for plant height ($P<0.05$) and oil yield ($P<0.01$). The Karaj3 cultivar showed the highest MP (4172.5), GMP (4147.06), STI (1.055) drought indices. With consideration of correlation between indices and yield under stress and non-stress, these indices (except the SSI and TOL) were identified as the best drought indices for isolation and selection of tolerant cultivars.

Key words: *Brassica napus*; water deficit; seed yield; drought stress index

Introduction

Rapeseed (*Brassica napus* L.) is one of the main farming crops especially for its edible oil. The meal remains after oil extraction is valuable as a source of protein for the live-stock feed. In Iran, the production of the rapeseed is mostly limited by drought and soil salinity. Response to selection is resulted from significant genetic variation and high heritability (Shukla *et al.*, 2006).

Rapeseed is a main oilseed crop in many arid and semiarid areas where its yield is often

limited by water deficit and high temperatures during the reproductive development. Seed yield can be mostly limited even by the relatively short period of soil moisture shortage during the reproductive growth phase (Chaghakaboodi *et al.*, 2012a; Zakizadeh *et al.*, 2010; Zarei *et al.* 2012).

Irrigated rapeseed (*B. napus* L.) cultivation is currently expanding in rotation with winter cereals in Iran where its reproductive growth is frequently exposed to drought (Kahrizi and Allahvarand, 2012).

In rapeseed, the effect of water stress on crop depends on genotype, intensity and duration of stress, weather conditions and plant developmental stages. Severe stress reduces the duration of reproductive growth and stress during flowering or maturation stages leads to large yield losses (Chaghakaboodi *et al.*, 2012b; Chaghakaboodi *et al.*, 2012c).

Drought stress at any time during reproductive growth can decrease seed yield. The most important period to experience water deficit on many grain crops is throughout stem elongation and flowering (Garavandi *et al.*, 2011; Kakaie *et al.*, 2010; Ahmadi *et al.*, 2012; Zebarjadi *et al.*, 2012). The highest rapeseed yield reduce was resulted when water deficit occurred at flowering and pod developmental stages (Kahrizi and Allahvarand, 2012).

Henry and MacDonald (1978) showed that severe drought decreased oil and increased protein contents of rapeseed. Rahnema and Bakhshande (2006) reported that the highest seed yield reduction occurred when irrigation was only once applied in spring. Muhammad *et al.*, (2007) found that the highest seed yield was resulted with three times irrigation at early vegetative, flowering and seed formation.

Zirgoli and Kahrizi (2015) identified that the MP, STI and GMP indices as the best indices for isolation and selection of drought tolerant rapeseed cultivars.

The aim of this experiment was to determine the influences of drought stress on yield and yield components of commercial rapeseed (*Brassica napus* L.) cultivars in Kangavar in the west of Iran and is located in the easternmost part of Kermanshah Province.

The aim of current research is for study of genetic variation and drought tolerance in commercial rapeseed (*Brassica napus* L.) cultivars in Kangavar region.

Materials and Methods

In order to identify drought stress tolerance in rapeseed, nine commercial cultivars (Opera, Karaj1, Karaj2, Karaj3, Zarfam, Okapi, Talayeh, Licord and Modena) were evaluated

under two conditions (normal and drought stress) in Research Station Kangavar Region, Kermanshah Province, Iran in 2014-2015 growing season. The Kangavar climate is mild, and generally warm and temperate. The rain in Kangavar falls mostly in the winter. The climate here is classified as Csa by the Köppen-Geiger system. The average annual temperature in Kangavar is 13.2 °C. The rainfall here averages 423 mm.

Under drought stress conditions, irrigation was cut from flowering stage.

There is a high potential for expansion of rapeseed cultivation in this region as a promising alternative crop for diversification and economical use of land and water resources.

The quantity and type of applied fertilizers were determined. According to soil test, potash, phosphorus, and nitrogenous fertilizer were used, respectably, from potash sulfate source and urea source, in the form of basal and top-dressing fertilizers. Other stages of crop management were performed routinely.

Rapeseed seeds were planted by hand in four five-meter lines and 2 m width plots at 3 cm depth. Line spacing was 30 cm. Finally the plant density was 40-60 plants per m². Weeds were controlled from plots close to physiological maturity plants. Plots were harvested (eliminating edges) and sent to the laboratory to determining seed yield, seed oil and yield components. 10 plants were selected randomly to measure the plant height and number of branches per plant.

The amount of water applied was to restore the water to field capacity. Field capacity and permanent wilting point were previously measured by pressure plate.

This research was carried out in a split plot experiment based on randomized complete block design with four replications. The irrigation or stress conditions were layout in main plots and cultivars located in subplots. Under drought stress conditions, irrigation was cut from end of flowering stage.

Plant height, seeds per pod, pods per main branch, branches number per plant, pods per plant, 1000-seed weight seed, physiological

maturity date, seed yield, and oil yields were recorded. All plots were harvested during the 2nd week of June and number of branches and pods per plant, seeds per pod, 1000-seed weight and seed and oil yields were determined. Seed oil contents were determined by Soxhlet methods.

In order to consider susceptibility or resistance ratio of genotypes to water stress and evaluated main criteria for segregate genotypes, some indices were used as below:

1. Stress Tolerance Index (STI)

$$STI = \left[\frac{Y_p}{\bar{Y}_p} \right] \times \left[\frac{Y_s}{\bar{Y}_s} \right] \times \left[\frac{\bar{Y}_s}{\bar{Y}_p} \right] = \frac{Y_p \times Y_s}{(\bar{Y}_p)^2}$$

(Fernandez, 1992)

2. Stress susceptibility index (SSI)

$$SSI = \frac{1 - \left(\frac{Y_s}{Y_p} \right)}{SI}$$

(Fischer & Maurer, 1978)

Where SI is stress intensity and calculated as:

$$SI = 1 - \left(\frac{\bar{Y}_s}{\bar{Y}_p} \right)$$

3. Tolerance (TOL)

$$TOL = Y_p - Y_s$$

(Clarke *et al.*, 1992)

4. Mean productivity (MP)

$$MP = \frac{Y_p + Y_s}{2}$$

(Hossain *et al.*, 1990)

5. Geometric Mean Productivity (GMP)

$$GMP = \sqrt{Y_s \cdot Y_p}$$

(Fernandez, 1992)

6. Harmonic Mean (HAM)

$$HAM = \frac{2(Y_p \times Y_s)}{Y_p + Y_s}$$

(Fernandez, 1992)

7. Yield Index (YI)

$$YI = \frac{Y_s}{Y_p}$$

(Gavuzzi *et al.*, 1997).

The data were statistically analyzed for each season and then combined for both years by SAS and MSTATC softwares. Mean comparison was conducted using the Duncan's Multiple Range Test (DMRT).

Results

In order to study on effects of genotype and drought stress on rapeseed traits, analysis of variance was done.

Analysis of variance results showed that drought stress had significant effect on all studied traits (Table 1). Seed yield reduced from 4036.9 to 2919.4 kg/ha (27.68%) caused by drought stress. Oil yield reduced from 1840.8 to 1362.2 kg/ha (25.99%) caused by drought stress. Meanwhile pods per sub-branch reduced more than other traits (27.93%). Other reductions due to drought stress were as 1000 seed weight (9.52%), seed per pod (20.91%), pods per main branch (23.80%), pods per plant (26.34%), plant height (6.94%), pod length (3.01%) and days to maturity (2.86%) (Table 2).

There were significant differences among cultivars for all studied traits except pods per main branch (Table 1).

Means comparison using the Duncan's Multiple Range Test showed that the Karaj3 and Talaye cultivars showed the highest seed yield in normal (4632.5 kg/ha) and stress (3747.5 kg/ha) conditions respectively (Table 2). In overall Karaj3 showed the highest seed yield (4172 kg/ha) and oil (2000 kg/ha).

Means comparison confirmed that Karaj1 genotype had the highest plant height (172.0 cm) and plant height (172 cm). However there were not significant differences between this genotype with Karaj2 (Table 3).

Means comparison showed that Opera had the highest 1000 seeds weight (4.56 g) and pod length (6.8 cm). Okapi had the lowest (3.50)

for this trait. The Talaye cultivar showed the highest seed per pod (23.29), pods per sub-branch (62.50) and pods per plant (92.89) and oil yield (2032). The Modena and Okapi identified as the most late (265.6 days) and early (151 days to maturity) ripe cultivars.

The interaction effects of cultivar and irrigation conditions was significant just for plant height ($P < 0.05$) and oil yield ($P < 0.01$) (Table 1).

Drought resistance indices

Means comparison showed that the Karaj3 cultivar showed the highest MP (4172.5), GMP (4147.06) and STI (1.055) drought indices. The highest and lowest SSI belongs to Modena (1.6092) and Talaye (0.6533) cultivars respectively. Also the highest and lowest TOL belongs to Modena (1562.5) and Talaye (827.5).

Discussion

Analysis of variance showed that there was a significant difference among the genotypes for many traits. Since diversity is the basis for selection of superior and desirable varieties, population diversity can be considered for selection to supply superior genotypes.

In current research, the reduction of seed yield due to drought stress was estimated about 28%. Seed yield is a complex trait and is greatly influenced by various environmental conditions. Breeding programs depend on the knowledge of key traits, genetic systems controlling their inheritance, and genetic and environmental factors that influence their expression (Kahrizi *et al.*, 2010).

Various morphological and physiological characters contribute to seed yield. Each of these component characters has its own genetic systems. Further these yield components are influenced by environmental fluctuations. Therefore, it is necessary to separate the total variation into heritable and non-heritable components with the help of genetic parameters i.e. genotypic and phenotypic co-efficient

of variation, heritability and genetic gain (Kahrizi *et al.*, 2010).

Oil yield showed about 26% loss in drought than normal conditions. Then the oil content is influenced by soil moisture. Zirgoli and Kahrizi (2015) reported the oil yield content in drought conditions (Zirgoli and Kahrizi, 2015).

The Talaye cultivar had the highest oil content (2032 kg/ha). Results showed that the average oil content of rapeseed rape genotypes under stress conditions has been decreased compared to normal conditions (about 26%).

The results showed that in all varieties, 1000 seed weight were reduced under stress conditions (about 9.5%). Grain weight depends on cultivar, environmental conditions, place the seed on the plant and place the seeds in the fruit are different and will vary between 3 to 7 g. A part of the yield loss in stress conditions is related to the reduction of seeds weight.

In current research, the reduction of pods per plant due to drought stress was estimated about 26%. Mouhouche *et al.*, (1998) reported that number of pods per plant trait is a more sensitive index to drought stress. Drought stress especially at pod formatting stage is important for high yield and desired quality and it can gravely decrease the yield. Wright *et al.*, (1995) also showed that *B. napus*, sever reduction of dry matter of pod and it's numbers, resulted from more falling flowers and pods and this problem is more obvious at more severe stresses.

Average height of canola genotypes under stress conditions reduced compared to normal conditions (about 7%). It seems the fact that as canola plant is an indeterminate crop, the occurrence of drought during flowering stage, stop the vegetative growth and accelerate reproductive growth (Zirgoli and Kahrizi, 2015).

Table 1. Analysis of variance for studied traits of *Brassica napus* genotypes as split plot experiment based on randomized complete block design. Where Source of variation (SOV), degree of freedom (df), coefficient of variations (CV), plant height (PH), days to maturity (DTM), pods per main branch (PPMB), pods per sub-branch (PPSB), pods per plant (PPP), seed per pod (SPP), 1000 seeds weight (DSW) and pod length (PL). ^{ns}, * and ** indicate non-significant, significant in P<0.05 and P<0.001 respectively

SOV	df	Mean squares									
		Yield	DSW	PPMB	SPP	PPSB	PPP	PL	PH	DTM	oil yield
Block	3	0.165 ^{ns}	0.229 ^{ns}	6.710 ^{ns}	9.330 ^{ns}	119.000 ^{ns}	170.000 ^{ns}	1.320 ^{ns}	26.200 ^{ns}	1.190 ^{ns}	0.140 ^{ns}
Conditions (C)	1	22.478*	2.340**	1051.200**	447.000**	4765.000*	10266.000**	0.680	2483.000**	1065.6**	4.080**
Error (a)	3	1.289 ^{ns}	0.048	9.460	458.000	217.940	234.500	0.270	11.570	0.458	0.043
Genotype (G)	8	2.178**	0.880**	55.200 ^{ns}	16.330**	433.8*	635.400*	0.815*	452.800**	2.310*	0.782**
C × G	8	0.139 ^{ns}	0.160 ^{ns}	31.110 ^{ns}	5.480 ^{ns}	98.200 ^{ns}	248.000 ^{ns}	0.320 ^{ns}	108.000*	0.618 ^{ns}	0.175*
Error (b)	48	0.267	0.180	33.550	4.059	158.940	228.9	0.320	38.480	0.641	0.010

Table 2. Average of studied traits in normal and drought stress conditions and the loss percent of traits in rapeseed (*B. napus*)

	Normal conditions	Stress conditions	The loss percent
Seed yield (kg/ha)	4036.90	2919.4	27.68
Oil yield (kg/ha)	1840.80	1362.2	25.99
1000 seed weight	4.20	3.83	9.52
seed per pod	23.01	18.02	20.91
pods per main branch	32.09	24.45	23.80
pods per sub-branch	58.70	42.30	27.93
pods per plant	90.64	66.76	26.34
Plant height (cm)	169.11	157.36	6.94
Pod length (cm)	6.30	6.11	3.01
days to maturity	268.80	261.11	2.86

Table 3. Mean comparison of studied rapeseed cultivars for different traits in Duncan's new multiple range test (DMRT) method. Where plant height (PH), days to maturity (DTM), pods per main branch (PPMB), pods per sub-branch (PPSB), pods per plant (PPP), seed per pod (SPP), 1000 seeds weight (DSW) and pod length (PL)

Cultivar	Yield (kg/ha)	DSW (g)	PPMB	SPP	PPSB	PPP	PL (cm)	PH (cm)	DTM	Oil yield (kg/ha)
Opera	3765 ^{ab}	4.56 ^a	31.20	20.05 ^{bcd}	55.23 ^{ab}	86.40 ^{ab}	6.8 ^a	153 ^c	264.0 ^{bc}	1806 ^a
Karaj1	3522 ^b	3.82 ^{cd}	27.23	19.76 ^{bcd}	36.45 ^c	63.70 ^d	5.7 ^b	172 ^a	265.3 ^{ab}	1634 ^c
Karaj2	3333 ^b	4.05 ^{bc}	25.01	20.06 ^{bcd}	52.86 ^{ab}	77.90 ^{abcd}	6.1 ^b	170 ^a	265.1 ^{ab}	1539 ^{cd}
Karaj3	4172 ^a	4.10 ^{abc}	32.15	21.52 ^{ab}	52.35 ^{ab}	85.50 ^{abc}	6.4 ^{ab}	168 ^{ab}	265.4 ^{ab}	2000 ^a
Zarfam	3270 ^b	4.11 ^{ab}	26.91	21.02 ^{bc}	52.28 ^{ab}	81.20 ^{abc}	6.1 ^b	168 ^{ab}	263.0 ^c	1563 ^{cd}
Okapi	2767 ^c	3.50 ^d	28.30	21.24 ^{ab}	46.90 ^{bc}	75.00 ^{bcd}	6.2 ^b	151 ^e	265.3 ^{ab}	1162 ^d
Talaye	4161 ^a	4.35 ^{ab}	30.30	23.29 ^a	62.50 ^a	92.89 ^a	6.3 ^{ab}	161 ^{cd}	265.0 ^{abc}	2032 ^a
Licord	3585 ^b	3.62 ^{cd}	24.30	18.41 ^d	44.61 ^{bc}	68.93 ^{cd}	5.9 ^b	164 ^{bc}	264.6 ^{bc}	1574 ^{cd}
Modena	2726 ^c	4.05 ^{bc}	28.97	19.23 ^{cd}	48.67 ^{abc}	77.60 ^{abcd}	5.9 ^b	158 ^d	265.6 ^a	1089 ^e

Similar letters in each column show that there is not significant differences between cultivar at P=0.05

Table 4. The amounts of yields in normal and stress conditions and drought resistance indices in studied rapeseed cultivars

Cultivar	Yp	Ys	MP	GMP	STI	SSI	TOL
Opera	4220.0	3310.0	3765.0	3737.4	0.8571	0.7789	910.0
Karaj1	4260.0	2785.0	3522.5	3444.4	0.7280	1.2508	1475.0
Karaj2	3942.5	2725.0	3333.7	3277.6	0.6592	1.1155	1217.5
Karaj3	4632.5	3712.5	4172.5	4147.1	1.0550	0.7174	920.0
Zarfam	3775.0	2765.0	3270.0	3230.7	0.6404	0.9665	1010.0
Okapi	3232.5	2302.5	2767.5	2728.1	0.4567	1.0393	930.0
Talaye	4575.0	3747.5	4161.2	4140.6	1.0520	0.6533	827.5
Licord	4187.5	2982.5	3585.0	3534.0	0.7663	1.0395	1205.0
Modena	3507.5	1945.0	2726.2	2611.9	0.4186	1.6092	1562.5

Table 5. Correlation coefficients between studied drought resistance indices in rapeseed (*B. napus*)

Traits	Yp	Ys	MP	GMP	STI	SSI	TOL
Yp	1						
Ys	0.91**	1					
MP	0.97**	0.98**	1				
GMP	0.96**	0.98**	0.99**	1			
STI	0.95**	0.99**	0.99**	0.99**	1		
SSI	-0.62	-0.89**	-0.79*	-0.81**	-0.82**	1	
TOL	-0.28	0.66	0.51	0.54	-0.56	0.92**	1

* and ** indicate significant in P<0.05 and P<0.001 respectively

Also early reproductive growth and reduction in vegetative growth period in stress conditions leads to competition between vegetative and reproductive organs. Reduction of leaf area leads to decreasing in the photosynthetic materials. This situation accelerates more competition and decreased plant height. The plant height in different genotypes varies from 50 to 200 cm in height, but it usually is from 80 to 150 cm. Reduction in drought stress induc-

es to increasing in elements absorption and then more photosynthesis. Thus assimilates will be produced properly and allocated to the vegetative growth and then plant height is increased; because stress decreases photosynthesis through stomatal closure and other properties and consequently reduces growth (Zirgoli and Kahrizi, 2015).

Analysis of variance showed that there is significant variation among genotypes for pods per plant. The average pods per plant

of rapeseed rape genotypes under stress conditions have been decreased (about 26%). Number of pods per plant is sum of pods in main branch and branches. A rapeseed plant produced around 4000 flower buds in perfect conditions and the low density of flower buds. According to genotype, environment and density, 5 to 20 percent of them may be produce flower. Others will be abscised. Subsequently, 5 to 20 percent of flowers will be remained and 50 percent of them may be produce seed. In rapeseed, number of pods per plant is an important index for seed yield. It seems that main part of the yield loss in drought conditions is due to loss of pods per plant and number of seeds per pod. The number of pods per plants is sensitive to drought stress. Darjani *et al* (2013) indicated that the interaction of cultivar \times irrigation regime is effective on the number of pods per plant. Khalili *et al* (2012) demonstrated that drought stress reduced the number of pods per plant in rapeseed cultivars.

Opera cultivar had the highest pod length. The results showed that in all varieties, pod length was reduced under stress conditions. Khalili *et al* (2012) reported that rapeseed cultivars pod length decreased under drought stress.

Seeds per pod in all varieties were reduced under stress conditions (about 21%). Kimber *et al* (1995) demonstrated initial water stress during pod growth, affected the number of those. According to Gregorie (2007) abiotic stress by restricting the supply of assimilates for grain filling influence number of seeds per pod. Din *et al* (2011) reported that the number of seeds per pod cultivars significantly affected by stress during early stages of flower and pod filling. Darjani *et al* (2013) showed that the interaction of cultivar \times irrigation regime is effective on the number of seeds per pod.

There was a significant variation among the genotypes for days to seed maturity trait. Average days to seed maturity of rapeseed genotypes under stress conditions have been reduced; that indicate the effects

of environmental changes on days to seed maturity trait. The results indicate that stress limits the plant vegetative and reproductive growth. Also earlier reproductive growth and reduction of vegetative growth period in stress conditions leads to competition between the vegetative and reproductive organs and the seed yield is reduced.

The days to seed maturity is related to date flowering. The flowering initiation date could be changed by environmental changes. Early flowering as a desirable trait for canola cultivars can protect the plant against warm weather. Then pod and seed filling was occurred before the beginning of critical stresses. It also the early flowering prevents flowering period and seed filling synchrony with aphid activity (Zirgoli and Kahrizi, 2015).

Average days to end of flowering of rapeseed genotypes under stress conditions have been decreased. Early flowering in rapeseed is a mechanism of escape from stress. Water stress during the final stages of reproductive accelerates the senescence in the plant, since it is associated with a decrease in grain filling period. One of the plants strategies for dealing with drought is capacity for vegetative and extending of flowering period (Zirgoli and Kahrizi, 2015).

With consideration of correlation between drought resistance indices and yield under stress and non-stress, these indices (except the SSI and TOL) were identified as the best indices for isolation and selection of tolerant cultivars (Table 5).

Acknowledgements

The authors thank the Zagros Bioidea Co., Razi University Incubator for all supports.

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