Evaluation of drought stress effects on germination parameters of rapeseed (*Brassica napus*) through cold test

Hamideh Khalaj[1], A. H. Shirani Rad[2], S. A. Sadat Noori[1], E. Allah Dadi[1], GH. Abass. Akbari[1], M. R. Labbafi[1]

[1]*Department of agronomy; Abouraihan Campus, University of Tehran, Iran*
[2]*Assistant professor of Seed and Plant Improvement Institute, Karaj, Iran*
Email: hamideh_6285@yahoo.com

Abstract

In order to evaluate the effects of drought stress applied at different growth stage on germination and seedling growth of 6 rapeseed cultivars a factorial experiment was carried out in a completely randomized design with 4 replications. The studied factors were less irrigation stress at 4 levels (normal irrigation, irrigation until flowering, irrigation until padding and seed filling stages) and 6 winter rapeseed cultivars (SLM046, Licord, Opera, Zarfam, Orient, Okapi). The cold test was performed on four 100-seed replications of each treatment by exposing the samples to 5°C for 5 days on moistened blotter papers and then transferred to 22°C for 5 days germination. After 5 days the root and shoot length of seedling, root/shoot ratio, fresh and dry seedling weight, normal and abnormal seedling, MTG[1], MDG[2] and FGP[3] were measured. Analysis of variance showed significant difference between less irrigation stress levels for shoot and root length, root/shoot ratio, fresh and dry seedling weight ($p \leqslant 0.01$) and abnormal seedlings ($p \leqslant 0.05$). Cultivars effect were significant for abnormal seedlings ($p \leqslant 0.01$) and FGP, MTG and seedling dry weight ($p \leqslant 0.05$). Root/shoot ratio, abnormal seedling and seedling fresh weight among traits were significant by cultivar × less irrigation stress interaction. Comparing of data means showed that normal irrigation had the maximum shoot height. Less irrigation seed filling stages increased seedling dry weight, root length and MDG. Among cultivars Licord and Orient had the most MDG and Zarfam had the most seedling dry weight. SLMO46 had the height shoot length, Licord and Zarfam had most FGP. Opera had the highest root/shoot ratio in less irrigation after seed filling stage. Maximum fresh weight produced by Zarfam in normal irrigation. SLMO46 had the lowest abnormal seedling in normal irrigation.

Key words: Rapeseed, Cold test, Less irrigation stress, seed vigour

Introduction

The two major environmental factors that currently reduce plant productivity are drought and salinity (Serrano *et al*., 1999). Some seed lots treated by stresses for an extended period may have reduced germination and vigor. Seed quality factors are very important, because other than purchasing decisions, the grower has no control over a poor quality seed lot. Although management can account for minor discrepancies in cultural and pest related factor
(Wang, 2005). Drought conditions occur ubiquitously during the growing season of many plants, and in the case of crops, it can have a profound negative effect on agricultural quality and productivity (Wang *et al*., 2005). Seed quality factors include seed maturity, seed size, chlorophyll content and genetics. Genetic plays an important role in germination, seed and seedling vigor (Anonymous, 2007).According to the Odiemah results (1988) cold test is suitable for recognizing effect of stress. Seed germination is an important stage in the life history of plant, affecting seedling development, survival, and population dynamics (Bewley and Black, 1994; Bewley, 1997; Wang, 2005). Seed vigor is hard to define, but is the sum of those properties which determine the activity and performance of the seed during germination and emergence. A germination test is a seed viability test only. It will not provide you with a measurement of all the properties required to give vigorous growth and consequently better establishment (Phillips and Edwards, 2006). A study was conducted in 2005-2006 in which cold test were evaluated to determine vigor and germination of canola (*Brassica napus* L.) seeds. The second aim was related to less irrigation after flowering, padding and seed filling stages and this amount of water used to start spring culture. In this study had been selected six common canola cultivars in Iran, included of Orient, Okapi, Licord, SLMO46, Opera and Zarfam used which origin of this canola cultivar is Iran.

Materials and Methods

Seed Quality Determination

The canola seeds cultivars (SLM046, Licord, Opera, Zarfam, Orient and Okapi) were obtained from Seed and Plant Improvement Institute, Karaj, Iran. This experiment was performed on four 100 seed replications of each cultivar. The seeds samples exposed to 5ºc for 5 days on moistened blotter papers and then transferred to growth chamber with 22±2ºc for 5 days on moistened blotter papers for germination (Elias and Copeland, 1997). After 24 hour normal seedlings were counted and

[1] Mean Time Germination
[2] Mean Daily Germination
[3] Finally Germination Percentage

finally percent of normal seedlings was recorded. At the end of experiment thirty plants from each box were randomly chosen and tagged for subsequent sampling. Root, shoot and whole plant of seedling measured and they weighted for fresh weight and then samples dried in oven 70ºc for 24 h for measured dry weight.

Daily record used to estimate as follows:

FGP is the Finally Germination Percentage. FGP is the last recorded before sampling.

MTG is the Mean Germination Time, and estimate as follow

$$MTG = \frac{\sum (nidi)}{\sum ni}$$

Where di=days after sowing, ni=number of germ in di, $\sum ni$ = total germ during 7 days.

$$MDG = \frac{\text{Final germination percentage}}{\text{Germination term}}$$

MDG is the mean germination days and it is speed germination days index. MDG estimate as follow

Experimental Design and Statistical Analyses

All data were subjected to analysis of variance (ANOVA) appropriate to a factorial form on Complete Randomized Design with four replications for laboratory experience in 2005 with four replications factors. The studied factors were less irrigation at 4 levels (normal irrigation, irrigation until flowering, irrigation until padding and seed filling stages) and 6 winter rapeseed cultivars (SLM046, Licord, Opera, Zarfam, Orient, Okapi). All experience data were using SAS 9.1 and the treatment means were tested by Duncan Multiple Range (DMR) and drawing picture with Excel 2003.

Result and discussion

The analysis of variance showed, less irrigation had significant effect on abnormal seedling ($p \leq 0.05$); root and shoot length of seedling, root/shoot ratio; seedling fresh and dry weight ($p \leq 0.01$). Effect of Cultivars were significant in MTG, FGP, seedling dry weight ($p \leq 0.05$) and abnormal seedling ($p \leq 0.01$). Interaction of less irrigation × cultivar showed significant differences ($p \leq 0.05$) for abnormal seedling, seedling fresh weight and root/shoot ratio (Table 1). Generally less irrigation caused to increase root length, seedling dry weight and MDG; but shoot length decreased (Table 2). Perez *et al*. (1994) as the same results declared that the good seed in cold test produced more seedling weight than other seeds. Root length under applying less irrigation in cold test increased, Burris (1975) and Burris and Navratil (1979) confirmed this result. MDG in less irrigation after seed filling increased that it may related to decreasing retransferred of photosynthetic material. Among cultivars, Zarfam had the maximum seedling dry weight and MTG (Table 3), it may be related to adaptation this cultivar for Iran climate. Zarfam in normal irrigation (2.767 gr), SLMO46 in Less irrigation after flowering (2.437 gr), Orient in Less irrigation after padding (2.138 gr) and Zarfam in Less irrigation after seed filling (1.955 gr) had the highest seedling fresh weight (Table 4). Orient in normal irrigation (1.364), Okapi in Less irrigation after flowering (1.557), Zarfam in Less irrigation after padding (1.727) and Opera in Less irrigation after seed filling (1.789) were the maximum in root/shoot ratio (Table 4). Licord and Zarfam in normal irrigation (3.25), SLMO46, Orient and Opera in Less irrigation after flowering (3.5), Licord in Less irrigation after padding (5.75) and Zarfam in less irrigation after seed filling (4.75) had the most abnormal seedling (Table 4).

Conclusion

Less irrigation caused to increase root length, seedling dry weight and MDG; but shoot length decreased. Genetic plays an important role in germination, seed and seedling vigor and were differences among cultivars. Among cultivars Zarfam were the best cultivar under less irrigation stress situation in Iran or the same region. Root/shoot ratio is the best character for determination of the best cultivar(s) for different situation. Result of Cold test showed that, less irrigation can be occurred and this amount of water used to start spring culture.

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Table 1. Source of variance of traits affected by Less irrigation stages and canola cultivar under cold test condition

S.O.V	DF	Root Length (cm)	Shoot Length (cm)	Root/shoot ratio	Seedling dry Weight (gr)	Seedling fresh Weight (gr)	MDG	MTG	FGP	Abnormal seedling
		MS								
Less irrigation stages (A)	3	2.22 **	4.06 **	0.72 **	18.6 **	0.016 **	0.02 ns	1.7 ns	2.22 ns	0.04 *
Cultivar (B)	5	0.36 ns	0.30 ns	0.03 ns	7.07 *	0.002 ns	0.02 ns	1.6 *	3.95 *	0.04 **
A × B	15	0.223 ns	0.28 ns	0.04 *	3.04 ns	0.002 *	0.04 ns	3.69 ns	1.37 ns	0.02 *
Error	72	0.40	0.15	0.02	3	0.001	0.03	5.	1.66	0.01
CV		8.03	7.49	9.9	2.78	6.4	11.67	0.29	1.31	17.74

×× significant at the 0.01 level of probably, × significant at the 0.05 level of probably, ns no significant

Table 2. mean traits for four Less irrigation stages under cold test condition

Less irrigation stages	Root Length (cm)	Shoot Length (cm)	Root/shoot ratio	Seedling Fresh weight (gr)	Seedling dry weight (gr)	MDG	MTG	FGP	Abnormal seedling
Normal irrigation	7.57 b	5.84 a	1.29 b	2.36 a	0.063 b	2.5091 ab	40.163 a	98 a	2.65 b
Less irrigation after flowering	7.73 b	5.49 b	1.37 b	2.22 a	0.065b	2.5085 b	51.094 a	98.08 a	3.04 ab
Less irrigation after padding	7.94 ab	4.91 c	1.62 a	1.92 b	0.084a	2.5129 ab	41.621 a	98.72 a	3.62 a
Less irrigation after seed filling	8.28 a	5.04 c	1.65 a	1.88 b	0.082a	2.5135 a	43.92 a	98.12 a	3.72 a

Mean followed by the same letters in each column are not significantly different (Duncan multiple rang 5 %)

Table 3. mean traits for six canola cultivars under cold test condition

Cultivar	Root length (cm)	Shoot length (cm)	Root/shoot ratio	Seedling fresh weight (gr)	Seedling dry weight (gr)	MDG	MTG	FGP	Abnormal seedling
Licord	8.04 a	5.24 ab	1.54 a	1.91 b	0.066 b	2.51 a	36.885 a	98.43 a	3.81 a
SLMO46	7.72 a	5.56 a	1.41 b	2.15 a	0.075 ab	2.51 ab	42.698 a	97.37 b	2.62 b
Okapi	7.83 a	5.36 ab	1.47 ab	2.13 ab	0.08 ab	2.50 ab	45.239 a	98.33ab	2.53 b
Orient	7.8 a	5.15 b	1.49 ab	2.05 ab	0.068 b	2.51 a	45.294 a	98.06 ab	3.18 ab
Zarfam	7.79 a	5.30 ab	1.49 ab	2.21 a	0.086 a	2.50 b	51.021 a	98.87 a	3.62 a
Opera	8.13 a	5.29 ab	1.53 a	2.07 ab	0.07 b	2.51 ab	44.806 a	98.26 ab	3.66 a

Mean followed by the same letters in each column are not significantly different (Duncan multiple rang 5 %)

Table 4. Interaction of traits measurements for six canola cultivars and Less irrigation stages

Less irrigation	Canola cultivar	Seedling fresh weight	Root/shoot ratio	Abnormal seedlings
Normal irrigation	Licord	1.778 k	1.339 efgh	3.25 g
	SLMO46	2.445 c	1.292 fgh	1.75 l
	Okapi	2.555 b	1.235 gh	2.333 ij
	Orient	2.368 d	1.364 efgh	2.25 j
	Zarfam	2.767 a	1.248 gh	3.25 g
	Opera	2.353 d	1.296 fgh	3 h
Less irrigation after flowering	Licord	2.353 d	1.436 defgh	2 k
	SLMO46	2.437 c	1.197 h	3.5 f
	Okapi	2.370 d	1.557 abcde	3.333 fg
	Orient	1.880 hi	1.287 fgh	2.5 i
	Zarfam	2.368 d	1.357 efgh	3.5 f
	Opera	2.122 e	1.449 defg	3.5 f
Less irrigation after padding	Licord	1.840 ij	1.718 abc	5.75 a
	SLMO46	1.972 fg	1.538 bcde	2.25 j
	Okapi	1.883 hi	1.689 abc	2 k
	Orient	2.138 e	1.495 cdef	4.25 d
	Zarfam	1.775 k	1.727 abc	3 h
	Opera	1.923 gh	1.604 abcd	5 b
Less irrigation after seed filling	Licord	1.793 jk	1.687 abc	4.25 d
	SLMO46	1.842 ij	1.618 abcd	3 h
	Okapi	1.982 f	1.444 defg	2.5 i
	Orient	1.820 jk	1.764 ab	3.75 e
	Zarfam	1.955 fg	1.631 abcd	4.75 c
	Opera	1.945 fg	1.789 a	3.5 f

Mean followed by the same letters in each column are not significantly different (Duncan multiple rang 5 %)