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Investigation of Seed Vigor and Germination of Canola Cultivars under less Irrigation in Padding Stage and after it

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Abstract: The laboratory experiment was a factorial based on a Completely Randomized Design with four replications during 2005. Factors included two irrigation levels (normal irrigation (irrigation after 60 mm evaporation from class a pan) and less irrigation in padding stage and after it) and six winter cultivars (Licord, Okapi, Orient, SLMO64, Zarfam and Opera). The seeds used in this experiment produced under less irrigation (moderate drought stress) and they were three months in storage. The seed vigor was tested in laboratory experiment by three vigor tests, including Standard Germination test, cold test, Accelerated Ageing Test. Result showed that the accelerated aging test had significant differences on all of character in this study. Mean Comparing, showed canola cultivars had significant effect on Root/ Shoot ratio, abnormal seedling, hard seed, seedling fresh weight, MTG and FGP. Less irrigation levels had significant effect on total and shoot length, ratio root/shoot length and MTG.

Key words: Seed germination, canola, vigor test, less irrigation, padding stage

INTRODUCTION

Drought conditions occur ubiquitously during the growing season of many plants and in the case of crops, it could have a profound negative effect on crop productivity (Phillips and Edwards, 2006). Limited water supplies may make it impossible to grow traditional annual crops, such as corn, winter canola and beans. Less irrigation had negative effects on winter rapeseed yield and quality (Gilliland and Hang, 2003).

Seed quality was affected by different parameters such as environmental factors, genetic, moisture and fertility of soil (Soleimani *et al*., 2000). Seed germination is an important stage in the life history of plants, affecting seedling development, survival and population dynamics. Germination, seed and seedling vigor are affected by many factors, including stresses, seeding practices, Genetic (Anonymous, 2007).

The standard germination test is a seed viability test in best situation for germination and showed speeds of germination only (AOSA, 1988). For these reasons used the other laboratory test such as the accelerated aging (seeds are under high temperature and relative humidity) and the cold test (seeds are under cool condition), subject seeds to stress as means of determining their suitability for planting. We conducted a study in 2005 in which 3 vigor testes were evaluated to determine their vigor and germination canola (*Brassica napus* L.) seeds.

The laboratory experiment was conducted in physiology laboratory of aburaihan campus for investigation of seed vigor and germination of canola cultivars. The major aims of this study were related to less irrigation in padding and after it; this amount of water used to start spring culture and determination the best cultivar(s) under less irrigation. In this study, six common winter canola cultivars in Iran were used included Licord, SLMO46, Okapi, Orient, Opera and Zarfam (which origin of Zarfam cultivar is Iran).

MATERIALS AND METHODS

Laboratory experiment and seed quality determination: In order to evaluation the effects of less irrigation applied in padding stage and after it (irrigation until padding) on canola seed germination and vigor, an experiment was conducted during 2005 at the physiology laboratory of Abouraihan Campus, University of Tehran at Pakdasht area. Six winter canola cultivars (Licord, Okapi, Orient, SLMO64, Zarfam and Opera) and three ordinary vigor tests (standard germination, accelerated aging and cold tests) were evaluated.

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The standard germination test (AOSA, 1988) was conducted on four groups of 100 seeds of each cultivar at 20±2°C for 7 days on moistened blotter papers. Only normal seedlings were counted and then thirty plants from each box were randomly chosen and tagged for subsequent sampling.

The cold test (Elias and Copeland, 1997) was performed on four groups of 100 seeds of each cultivar by exposing the samples to 5°C for 5 days on moistened blotter papers and then transferred to 20±2°C for 5 days. The mean time germination and daily germination speed of normal seedlings were recorded. Thirty plants from each box were randomly chosen and tagged for subsequent sampling.

The accelerated aging test was conducted by aging seeds at 42°C for 48 h (Elias and Copeland, 2001) using the wire-mesh tray method (Johnston *et al*., 2002). A single layer of seeds from each sampling date of each cultivar was placed on 10×10×3 cm copper wire mesh tray inside a 11×11×3.5 cm plastic box containing 2 cm water (about 100 mL) above the bottom of the box. Following incubation, the seeds were germinated at 22°C for 7 days as described above. The percent of normal seedlings was recorded and in 7th day, abnormal seedling recorded and then thirty plants from each box were randomly chosen and tagged for subsequent sampling. Lengths of whole plant and roots samples measured and then were dried in oven at 70°C for 24 h for measuring seedling dry weight. Daily record used to estimate as follows:

DGS (Day Germination Speed) is time to need for germination a seed when ever reduce it, increase germination speed. The speed of germination is based on the rate of radical protrusion per unit time. The theory behind the speed of germination is that a relatively short time for radical protrusion is a characteristic of vigorous seeds that permits them to germinate and emerge from the soil quicker than less vigorous seeds.

MDG (Mean Daily Germination) is contrast to Day Germination Speed (time to need for germination a seed), MDG is mean germination days and it is speed germination day index.

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

MTG (Mean Time Germination):

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

Where:

di = Days after sowing,
ni = No. of germ in di,
Σ ni = Total germ during 7 days.

Final Germination Percentage (FGP) is the maximum percentage which occurred for seed lots.

Experimental design and statistical analyses: All data were subjected to analysis of variance (ANOVA) appropriate to a factorial form on Completely Randomized Design with four replications during. The studied factors were less irrigation at two levels (normal irrigation, less irrigation in padding stage and after it) and 6 winter rapeseed cultivars (SLM046, Licord, Opera, Zarfam, Orient, Okapi). All data were analyzed with SAS (version 9.1) and the treatment means were tested by Duncan Multiple Range (DMR) and drawing figures with Excel 2003. Figures showed contrast among irrigation levels, tests and cultivars (treated compared showed with the same color).

RESULTS AND DISCUSSION

Irrigation levels effect: Analysis of variance (Table 1) showed that irrigation levels had significant effect ($p<0.01$) on root/shoot ratio, Whole seedling height, shoot height and Mean Time Germination (MTG). Data means of laboratory measurements showed that less irrigation in padding stage and after it had positive effect on hard seed, abnormal seedling, MTG, whole seedling height and shoot height of canola seedlings (Table 2).

Hard seed coat make kind of dormancy that Seeds can not absorb water and germinated. Based on Wang *et al*. (2005), reported that increase of abnormal seedling showed decline of vigor seeds, less irrigation in padding stage and after it caused to increase of abnormal seedling (Table 2).

Seeds were under less irrigation on padding and after it need to more MTG compare to seeds under control condition (Table 2), similar to these results Karim and Naylor (2001) reported that less irrigation increase mean time of germination.

Seeds under less irrigation on padding and after it had the most whole seedling height (Table 2), the result of Abba and Lovato (1998) was different with the result of this study. Abba and Lovato (1998) reported that whole seedling height was an important indicator for showing high seed quality. The result of this study showed less irrigation increased shoot height (Table 2), increasing of shoot height cause lessening tolerant of plant in drought stress condition (Wang *et al*., 2005).

The results showed that less irrigation in padding stage and after it caused to decline root/shoot ratio and Mean Daily Germination (MDG) (Table 2). Plants need to long roots in drought stress and decline of root/shoot ratio showed low tolerant of plants in this situation. In this study less irrigation in padding stage and after it

Table 1: Source of variance of laboratory measurements (MS) under different irrigation levels, vigor tests and winter canola cultivars

SV	df	Hard seed	Abnormal seedling	Root/shoot ratio	Seedling fresh weight	Seedling dry weight	Whole seedling height
Rep	3	0.16^{ns}	0.036^{ns}	0.041^{ns}	0.023^{ns}	0.0004^{ns}	1.02^{ns}
Irrigation	1	0.18^{ns}	0.213^{ns}	0.163**	0.006^{ns}	0.0008^{ns}	21.77**
Vigor test	2	0.35^{ns}	63.600**	0.053^{ns}	2.121**	0.0016*	23.73**
Cultivar	5	0.24^{ns}	0.295^{ns}	0.046*	0.402**	0.0002^{ns}	1.13^{ns}
Vigor test×Irrigation	2	0.88*	13.470**	1.409**	1.371**	0.0007^{ns}	20.27**
Vigor test×Cultivar	10	0.19^{ns}	0.248^{ns}	0.022^{ns}	0.086^{ns}	0.0002^{ns}	1.49^{ns}
Irrigation×Cultivar	5	0.12^{ns}	0.243^{ns}	0.070^{ns}	0.171^{ns}	0.0003^{ns}	1.33^{ns}
Vigor test×Cultivar×Irrigation	10	0.13^{ns}	0.094^{ns}	0.005^{ns}	0.105^{ns}	0.0002^{ns}	0.78^{ns}
Error	105	0.18	0.130	0.0201	0.083	0.0003	1.28
CV		38.31	11.530	9.510	13.180	2.4500	7.72

Table 1: Continued

SV	df	Shoot height	Root height	Mean time germination	Daily germination speed	Mean daily germination	Finally germination (%)
Rep	3	0.05^{ns}	0.98^{ns}	0.0008^{ns}	0.000013^{ns}	0.74^{ns}	0.83^{ns}
Irrigation	1	9.92**	2.30^{ns}	0.0069^{ns}	0.000013^{ns}	0.40^{ns}	0.36**
Vigor test	2	33.15**	93.31**	0.010**	0.00038**	11.59**	69.83**
Cultivar	5	0.15^{ns}	1.06^{ns}	0.0010^{ns}	0.000049^{ns}	0.93^{ns}	9.58^{ns}
Vigor test×Irrigation	2	18.08**	5.11**	0.0011^{ns}	0.000039^{ns}	0.44^{ns}	2.46^{ns}
Vigor test×Cultivar	10	0.23^{ns}	0.76^{ns}	0.0004^{ns}	0.000032^{ns}	0.72^{ns}	3.23^{ns}
Irrigation×Cultivar	5	0.56^{ns}	0.99^{ns}	0.0019^{ns}	0.000016^{ns}	0.80^{ns}	2.92^{ns}
Vigor test×Cultivar×Irrigation	10	0.22^{ns}	0.23^{ns}	0.0007^{ns}	0.000034^{ns}	0.96^{ns}	2.90^{ns}
Error	105	0.25	0.64	0.0008	0.00004	1.11	2.46
CV		8.57	9.13	0.97	0.87	18.21	1.6

**Significant at the 0.01 level of probably, *Significant at the 0.05 level of probably, ns: No significant

Table 2: Means comparison of traits in different irrigation levels

Irrigation	Seedling dry weight (g)	Seedling fresh weight (g)	Hard seed	Abnormal seedling	Ratio root /shoot length	Root height (cm)	Shoot height (cm)	Seedling height (cm)	Mean germination time	Daily germination speed	Mean daily germination	Finally germination (%)
Normal irrigation (Control)	0.082a	2.192a	0.85a	10.97a	1.53a	8.65a	5.66b	14.32b	3.022948b	0.032a	37.97a	97.5493a
Less irrigation after padding	0.089a	2.178a	1.07a	11.33a	1.45b	8.86a	6.20a	15.06a	3.037512a	0.033a	33.53a	97.6338a

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

decreased MDG, his result as the result of Hunter *et al.* (1984) confirmed high seed quality had more MDG than low seed quality.

Vigor tests effect: Analysis of variance (Table 1) showed that vigor tests had significant effect on abnormal seedling, weights, heights, MTG, Daily germination Speed (DGS), MDG and Finally germination percentage (FGP). Data means of laboratory measurements showed that standard germination test had positive effect on abnormal seedling, root/ shoot ratio, MTG, seedling fresh and dry weight, whole seedling height, shoot and root height of seedlings (Table 3).

Te result of this study showed that in standard germination test abnormal seedling increased (Table 3) but against our result, the result of Ghassemi *et al.* (1997) in maize and sorgum showed accelerate aging test condition caused to increase abnormal seeds.

Standard germination test in this study showed root and shoot height increased, root increased more than shoot height for this reason whole seedling height and root/shoot ratio increased too (Table 3).

Abba and Lovato (1998) and Ellis *et al.* (2000) had the same result and they showed increase of whole seedling height and root/shoot ratio in standard germination test.

The results showed that cold test condition caused to increase DGS, MDG and FGP (Table 3).

Cold test caused to decrease Mean Time Germination in compare to standard germination and accelerate aging tests that may due to cold stress in cold test (Table 3). Because of increasing DGS, MDG in Cold test, mean time for germination decreased and this result were agreement with Tys and Jankowski (2002), Stankova and Stankov (2001) and Hunter *et al.* (1984), they reported cold test condition is as cold stress and stimulated seeds to germination.

Cultivar effect: Analysis of variance (Table 1) showed that cultivar had significant effect on root/shoot ratio ($p<0.05$) and seedling fresh weight ($p<0.01$). Data means of canola cultivars showed that among cultivars, Zarfam had the most root/shoot ratio, Finally Germination Percentage (FGP) and seedling fresh and dry weight (Table 4). In this study Zarfam were the best cultivar

Table 3: Means comparison of traits in different germination tests

Vigor test	Seedling dry weight (g)	Seedling fresh weight (g)	Hard seed	Abnormal seedling	Root /shoot length	Root height (cm)	Shoot height (cm)	Mean germination time	Daily germination speed	Mean daily germination	Finally germination (%)
Standard test	0.089a	2.42398a	0.7b	17.64a	1.52333a	10.3283a	6.8827a	3.04049a	0.027b	28.85b	98.2708a
Aging test	0.093a	2.01407b	1.26a	12.15b	1.49478ab	8.1372b	5.5041b	3.036547a	0.034b	32.12b	96.1702b
Cold test	0.074b	2.10067b	0.93ab	3.22c	1.45826b	7.7461c	5.388b	3.013436b	0.035a	41.88a	98.3191a

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

Table 4: Means comparison of traits in 6 canola cultivars

Cultivars	Seedling dry weight (g)	Seedling fresh weight (g)	Hard seed	Abnormal seedling	Ratio Root /shoot length	Root height (cm)	Shoot height (cm)	Seedling height (cm)	Mean germination time	Daily germination speed	Mean daily germination	Finally germination percentage
Licord	0.079a	2.03421c	0.75ab	11.91a	1.55333a	8.9417a	5.8250a	14.7663a	3.033088ab	0.034a	32.34a	97.5417bc
SLMO46	0.087a	2.26179ab	1.00ab	10.47b	1.48375ab	8.7925a	6.0025a	14.7975a	3.026950ab	0.035a	31.87a	97.1667bc
Okapi	0.086a	2.07159c	0.86ab	11.28ab	1.48773ab	8.6677a	5.8700a	14.5368a	3.041517a	0.033a	34.32a	97.6957abc
Orient	0.080a	2.20208bc	1.37a	10.54ab	1.52542a	8.9663a	5.9425a	14.9104a	3.028508ab	0.031a	37.84a	96.7500c
Zarfam	0.090a	2.41259a	0.63b	11.90ab	1.42348b	8.5291a	6.0422a	14.95717a	3.021567b	0.029a	36.47a	98.6250a
Opera	0.092a	2.13218bc	1.13ab	10.83ab	1.47783ab	8.6400a	5.9487a	14.5883a	3.030222ab	0.033a	32.57a	97.7826ab

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

Table 5: Interaction of laboratory measurements for two levels irrigation and standard germinations, cold and accelerate aging tests condition

Vigor test	Irrigation	Hard seed	Abnormal seedling	Root/shoot ratio	Root height (cm)	Shoot height (cm)	Whole seedling height (cm)	Seedling fresh weight (g)
Standard germination test	Normal irrigation (control)	1.10b	3.66c	1.70a	10.55a	6.23b	16.78b	2.25b
	Less irrigation in padding stage and after it	0.94b	4.75a	1.34cd	10.11a	7.53a	17.64a	2.59
Accelerate aging test	Normal irrigation (control)	1.02b	2.93d	1.59b	7.71c	4.87d	12.59e	2.01c
	Less irrigation in padding stage and after it	1.39a	3.99b	1.41c	8.53b	6.08bc	14.61c	1.87d
Cold test	Normal irrigation (control)	1.15ab	1.75f	1.62b	7.92c	4.93e	12.85de	2.28b
	Less irrigation in padding stage and after it	1.12b	2.04e	1.30d	7.57c	5.84c	13.42d	1.92c

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

because Zarfam had produced the highest root/shoot ratio and the lowest hard seed (Table 4). Root length is a good evidence for viability growth and stability seeds in field (Ellis *et al*., 2000). The result of this study showed that Zarfam cultivar seeds germinated quickly because they had less MTG and Zarfam had the most Finally Germination Percentage (FGP) (Table 4) and seedling of Zarfam cultivar had the most seedling fresh and dry weight (Table 4). Seedling fresh and dry weights are important parameter for predicting of viability of seeds in field (Verma *et al*., 1999).

Interaction of vigor test and irrigation: Analysis of variance (Table 1) showed that interaction of irrigation and vigor test were significant on hard seed ($p<0.05$), abnormal seedling, root/shoot ratio and seedling fresh and dry weight, whole seedling weight, root and shoot height ($p<0.01$).

The result of this study showed that less irrigation in padding stage and after it under accelerate aging test made the most hard seeds (Table 5) and this result were not agree with Perez *et al.* (1994), their results showed drought stress under cold test had the most hard seeds.

The result of interaction of irrigation and vigor test in this study confirmed that less irrigation in padding stage and after it caused to increase abnormal seedling (Table 5), this result were agree to Machado *et al.* (2001) result in wheat (*Triticume aestivume*) and *Faseolus vulgais*.

Generally the result of three vigor test were the same, seeds that produced under less irrigation produced longer shoot than root and root/shoot ratio decreased (Table 5), the research of Mendonca *et al.* (2000) and Vieira *et al.* (1991) confirmed present result. Mendonca *et al.* (2000) reported that seeds of broccoli (*Brassica oleraceae* L.) produced under drought stress had short root.

Less irrigation in padding stage and after it produced seeds that their fresh weights declined in accelerate aging test and cold test but standard germination test had different results, may these results due to different vigor

test conditions, cold stress in cold test and temperature and moisture in accelerate aging test (Verma *et al*., 2001).

CONCLUSION

The results of present study showed that seeds produced under less irrigation in padding stage and after it had shorter root and longer shoot and they need more time for germination, these parameters cause to decrease seed quality and we do not recommend less irrigation in this stage of canola growth.

Among vigor tests standard germination test had the best condition for seed germination and we use it only for comparing seeds produced under deficit and normal irrigations in laboratory experiment. For comparing seed vigor and germination need to do other tests, accelerate aging test were better than cold test.

Among cultivars Zarfam had the most root/shoot ratio, finally germination percentage, it may due to compatibility of Zarfam cultivar to Iran situation.

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