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CONTROLLED AGEING FOR STORABILITY ASSESSMENT AND PREDICTING SEEDLING EARLY GROWTH OF CANOLA CULTIVARS (*Brassica napus* L.)

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ABSTRACT

Seed aging decreases the quality of seed and grain and results in agricultural and economic losses. Accelerated aging is one of the most useful tests used for the evaluation of seed vigour and storability. Present experiment was carried out for investigation the effect of different periods of seed accelerated ageing treatment (0, 24, 48, 72 and 96 h) on germination and seedling early growth of four canola (*Brassica napus* L.) cultivars. Applied cultivars were including of Okapi, Orient, Fornax and SLM046. Accelerated ageing was conducted at 40 °C and 100% relative humidity. At the end of ageing periods 1 g of seed was used for Electrical Conductivity test. Rest of seeds were located in Petri dish on whiteman paper then transformed to germination parameters decreased; however the sensitivity of cultivars to ageing periods was different. Maximum germination percentage, vigour index and also the longest shoot was obtained under 96 h from cv. Fornax, whereas the minimum vigour was observed from cv. Okapi. Result of EC test revealed that the highest solute leakage of the seeds was related to cv. Okapai which followed by cv. Orient that could be result of severe membrane damages.

Keywords: canola, cultivars, accelerated ageing, early growth, germination.

INTRODUCTION

High-quality seeds are of great socio-economic significance because seeds provide the majority of our food supply and are important sources of animal and industrial feedstock. High quality seeds are characterized by maintaining a high germination rate and stable content after storage. Seed germination, seedling emergence and crop establishment are important aspects of canola production, and are the main components of seed/seedling vigour (Devaiah *et al.*, 2007). A major concern of growers is that deterioration of some seed cultivars, leading to loss of vigour, may be undetected before planting.

However seeds gradually deteriorate during prolonged storage and lose viability (McDonald, 1999). In addition, incorrect sowing date or harvesting period under warm and humid conditions can induce seed ageing. This ageing is manifested as reduction in germination percentage and those seeds that do germinate produce weak seedling (Veselova and Veselovsky, 2003). The survival of various seed lots or cultivars may differ when seeds are stored under identical condition. However with consider to this point, grower before planting need a procedure for assessment of storability and viability of seeds for each area and selecting the best cultivars.

The accelerated aging (AA) test is acknowledged as one of the most employed tests to evaluate the physiological potential of various species of seeds, providing information of high degree of consistency (TeKrony, 1995). The principle of this method is based on the artificial acceleration of the deterioration rate of the seeds, by exposing them to high temperature and relative humidity levels, which are considered as the most prominent environmental factors with respect to the intensity and velocity of deterioration (McDonald, 1999). In this situation, low-quality seeds deteriorate more rapidly than vigorous ones, presenting a differentiated decrease in viability. Many factors affect the behavior of seeds that are submitted to the test; the interaction between temperatures/exposure periods is one of the aspects most frequently studied, but another aspect that must be considered in the accelerated aging test, is the interaction between cultivars/exposure.

Through much of the literature there is an assumption that oxidative reactions are responsible for the deteriorative changes observed in aged seeds. Four types of oxidations are known which might reasonably contribute to the progress of seed ageing. These include free radical oxidations, enzymic dehydrogenation, aldehyde oxidation of proteins, and Maillard reaction (Bernal-lugo and Leopold 1998). Free radicals attack membrane lipids, and cause major disruption of their viscosity and their permeability (Van Zutphen and Cornwall, 1973). Increase of solute leakage at this situation would be due to damaged membrane.

Recently Electrical Conductivity (EC) test has been optimized for vigour evaluation of soybean, field bean and French bean and this can be suitable method for assessment of ageing intensity and estimation of cultivars storability.

The objective of this work was to determine the effect of different accelerated ageing periods on membrane integrity (EC test) and germination behavior of four canola cultivars.

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MATERIAL AND METHODS

Commercial canola (*Brassica napus* L.) seeds cvs. Okapai, Orient, Fornex and SLM046, produced by Seed and Plant Improvement Institute of Karaj, were used in this study. The standard germination test (AOSA, 1998) was conducted on 100-seed samples of each cultivar at 20 ± 1 °C for 7 d on moistened Whiteman papers in dark growth chamber. Only normal seedlings were counted.

The AA test was performed according to the method known as "gerbox" (11 x 11 x 3 cm), in a chamber; samples containing three grams of seeds were utilized, distributed so as to form a simple layer over the surface of the metallic screen suspended inside each plastic box (internal compartment), containing 40 Ml water. The boxes, were covered with lids, remained inside the chamber during four aging periods (24, 48, 72 or 96 h), at 40±1°C and 100% relative humidity. During the aging period, the seeds absorbed moisture from the humid environment within the inner chamber. Seeds were then placed for germination according to methodology described for the germination test. The evaluation was performed seven days after sowing and the results were expressed as mean percentage, germination index, shoot and root length and vigour index of normal seedlings for each lot.

Seedling vigour index (SVI) was calculated following modified formula of Abdul-Baki and Anderson (1973):

SVI = [seedling length (cm) × germination percentage]

The germination index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) by following formulae:

GI =	No. of germinated seed	++	No. of germinated seed	
	Days of first count		Days of final count	

Electrical conductivity test: Solute leakage of the seeds was estimated by soaking 1 g seeds in 50 mL of deionised water at 25°C in an incubator. The experiment was replicated for 3 times. The electrical conductivity of seed leachates was measured by conductivity meter (Jenway-4010) after 24 h soaking.

The experiment was laid out in Completely Randomized Design with tow factor factorial (4×5) arrangement, with three replications. First factor was canola cultivars and second was periods of ageing. The data were analyzed using a statistical package, MSTATC average compared by Dunkan method, at 5% level of probability.

RESULTS AND DISCUSSIONS

Analysis variance showed that effect of ageing period on all germination traits was significant. Behavior of germination and early growth of the canola cultivars significantly was different (P>0.05) and the interactions between ageing period and cultivar were significant for all assessed traits except the seedling dry weight (Table-1).

Mean comparison for germination percentage showed that cv. Okapai had minimum germination percentage at 24 h ageing, whereas maximum germination for this period was recorded from cv. Fornax and SLM046. Similar trend was observed for 48, 72 and 96 h ageing (Table-2). Evaluation the shoot length of canola cultivars under ageing periods revealed the same results (Table-3). In general results indicate that cultivars which had high quality at the initiation of test (control) could maintain this superiority during the different ageing periods.

Analyzing the effect of ageing period on root length showed that during the all ageing periods cv. Orient had the longest root, while the shortest root was observed from cv. SLM046 (Table-4). Although cv. SLM046 had the longest shoot during the prolonged ageing periods, but had the shortest root. This could be attributed to different sensitivity of the early growth traits against the ageing treatments.

Results of ageing periods on seedling vigour index and germination index confirmed the pervious obtained results. Both cv. Orient and Fornex were better than other cultivars (Figures 1 and 2).

This test classified the cultivars with the best and worst performance and and evaluated the seeds vigor and their storability. Therefore, it was observed that cv. Orient had high quality and was resistant against the deterioration, whereas cv. Okapi was identified as having a lower physiological potential.

 Table-1. Analysis variances of the effects of accelerated ageing (AA) treatments on germination parameter and seedling early growth of four canola cultivars.

Variance sources	df	Germination (%)	EC (%)	GI (%)	Root length (%)	Shoot length (%)	SVI (%)	Seedling dry mass (%)
Cultivars (C)	3	*	*	*	*	*	*	NS
AA period (P)	4	*	*	**	**	**	**	*
С×Р	12	**	*	*	*	*	**	NS
LSD 0.05 (lnt) (12 df)	-	10.3	29.87	34.21	17.64	31.53	16.48	26/74

* Significant at the 0.05 level, ** Significant at the 0.01 level and NS = not significant.

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Accelerated	Cultivars				
ageing period	Okapi	Orient	Fornax	SLM046	
Control	70 ^{cd}	82.5 ^{ab}	88.3 ^a	90 ^a	
24 h	56.7 ^{ef}	64.2 ^{de}	81.7 ^{ab}	83.7 ^{ab}	
48 h	26.7 ^{it}	50.8 ^f	76.7 ^{bc}	70.8 ^{cd}	
72 h	11.7 ^j	37.5 ^g	74.3 ^{bc}	51.7 ^f	
96 h	6.3 ^k	16.7 ^{ig}	58.3 ^{ef}	20.8 ^{hi}	

Table-2. The effects of different accelerated ageing treatment on germination percentage of canola cultivars.

* Figures not sharing the same letters in the same column differ significantly at p< 00.05

Table-3. The effects of different accelerated ageing treatment on shoot length (mm) of canola cultivars.

Accelerated	Cultivars				
ageing period	Okapi Orient		Fornax	SLM046	
Control	33.6 ^{bc}	37.6 ^{bc}	42.7 ^b	61.6 ^a	
24 h	21.5 ^{cde}	24.9 ^{cde}	34.2 ^{bc}	48.7 ^{ab}	
48 h	13.9 ^{efg}	16.2 def	27.4 ^{cd}	23.2 ^{cde}	
72 h	9.31 ^{gh}	13.5 ^{efg}	19.6 def	14.7 ^{efg}	
96 h	7.4 ^{gh}	8.1 ^{gh}	15.4 ^{efg}	9.7 ^{gh}	

* Figures not sharing the same letters in the same column differ significantly at p< 00.05

Table-4. The effects of different accelerated ageing treatment on root length (mm)	
of canola cultivars.	

Accelerated	Cultivars				
ageing period	Okapi	Orient	Fornax	SLM046	
Control	73 ^{ab}	79.9 ^a	75 ^a	59.6 ^{bc}	
24 h	59.2 ^{bc}	56.2 ^{bc}	50.7 ^{cd}	62.3 ^b	
48 h	43.7 ^{de}	54.4 ^{bc}	39.2 ^{ef}	38.7 ^{ef}	
72 h	19.6 ^{gh}	34.7 ^{ef}	24.7 ^{fg}	26.9 ^{eg}	
96 h	12.1 ^{ghi}	24.3 ^{fg}	16.8 ^{gh}	9.3 ^{hi}	

* Figures not sharing the same letters in the same column differ significantly at p < 00.05



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Figure-1. Influence of different duration of accelerated ageing treatments on germination index of canola cultivars. The bars with different alphabets are statistically different.

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Figure-2. Influence of different duration of accelerated ageing treatments on seedling vigour index of canola cultivars. The bars with different alphabets are statistically different.

As angiospermous seeds approach maturity, they characteristically accumulate soluble sugars (Amuti and Pollard, 1977). These solutes are known to contribute to the development of tolerance to desiccation and to longevity itself (Bernal-Lugo and Leopold, 1995). The protective effect of these polyols is thought to occur through maintaining the structural integrity of membranes, and providing stability for macromolecules such as proteins (Crowe and Crowe, 1986). In addition, as water is withdrawn from a solution of sugars, the sugars form a glassy state, and this can serve as a physical stabilizer (Franks *et al.*, 1991). Because of the extremely high viscosity that can be obtained in the glassy state, deteriorative reactions should be suppressed. Different responses of canola cultivars to accelerated ageing treatments my explained by their capacity for sugar accumulation that occur during seed filling period. Results of EC test showed that maximum

Solute leakage of the seeds was recoded for cv. Okapai and also revealed that ageing periods lower than 72 h were less efficient to separate cultivars into different vigour levels (Table-5.)

 Table-5. The effects of different accelerated ageing treatment on electrical conductivity of canola cultivars seed leachates (dS/m).

Accelerated	Cultivars				
ageing period	Okapi	Orient	Fornax	SLM046	
Control	28.15 ^{cde}	25.9 ^{de}	20.15 ^e	21.85 ^e	
24 h	38 bcd	28.25 ^{cde}	19.85 ^e	24.75 ^{de}	
48 h	41.65 ^{bc}	32.45 ^{cd}	22.8 ^e	27.05 ^{de}	
72 h	46.15 ^{abc}	42.95 bcd	25 ^{de}	31.45 ^{cde}	
96 h	50.40 ^{ab}	61.75 ^a	25.09 ^{de}	37.3 ^{bcde}	

* Figures not sharing the same letters in the same column differ significantly at p< 00.05

In this study, the 72 and 96 hour periods had a higher sensitivity in identifying cultivars that possessed different levels of physiological potential. The information that obtained here should help in the decision for the selection of cultivars for sowing at different regions, evaluation of storage potential, use in quality control programs, and also as an aid in developing selection methods to be used in plant breeding.

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