Abscisic acid and kinetin seed treatments improved germination characteristics of aged seeds in canola cultivars (*Brassica napus)* under water stress

Reza Tavakkol-Afshari, Saeideh Rashidi

Department of Agronomy, College of Agriculture, University of Tehran, Karaj, Iran Email: tavakkol@ut.ac.ir

Abstract

The use of high-quality seed is essential for good stand establishment and yield in any crop. A major concern of seed business and rape seed growers alike is that deterioration of some seed lots leading to loss of vigour. Seed germination, seedling emergence and crop establishment are important aspects of rape seed production. In Iran, water is the most common abiotic stress to which rape seeds are exposed. The objectives of this study was to assess the effects of exogenous application of abscisic acid (ABA) and cytokinin (CYT) seed treatment on improvement of germination characteristics of aged seeds of rape cultivars under drought stress. The experiments were carried out at seed and biotechnology laboratories of College of Agriculture, University of Tehran during 2004-2005. A completely randomized design with three replications was conducted. The rape seed cultivars Licord (winter variety) and Option 500 (spring variety) were included. The seeds were aged using the accelerating-aging test. Characteristics such as germination percentage (%), germination index and germination speed were measured in all experiments. The results revealed that seed aging or deterioration and drought stress reduced the seed vigor, germination %, and speed of germination. Under the accelerate-aging test and drought stress, rape seed cultivars differed significantly. For accelerate-aging and drought stress treatments, germination percentage, germination index, and speed of germination of Licord cultivar was significantly higher than Option cultivar. The treatment of these various aged seeds with ABA and CYT hormones improved drought tolerance. Higher concentrations of these two hormones increased the level of tolerance. Aged seeds performed better under ABA treatments.

Key words: abscisic acid, aged seed*,* *Brassica napus*, drought stress, kinetin,

Introduction

The use of high-quality seed is essential for good stand establishment and yield in any crop. Seed germination, seedling emergence and crop establishment are important aspects of canola production and are the main components of seed/seedling vigour. A major concern of seed business and canola growers alike is that deterioration of some seed lots leading to loss of vigour (Ellias and Copeland, 2001). Biochemical deterioration during seed ageing has been studied mostly under accelerated ageing conditions using high temperature and high seed water content (McDonald, 1999). Under such storage conditions, seeds typically lose their viability within a few days or weeks. Water is the most common abiotic stress to which crops are exposed. Germination and seedling establishment secure plant survival and are very important phases of plant life. Germination rate decreases with decreasing external water potential and for each species there is a critical value of water potential below which germination will not occur. It has also been shown that water stress alters the level of plant hormones. Gibberellic acid improved the activity of phosphatase enzymes which play important roles in adaptation of germinating seeds (Sharma *et al*., 2004). The objectives of this study was to assess the effects of exogenous application of abscisic acid (ABA) and cytokinin (CYT) seed treatment on improvement of germination characteristics of aged seeds of canola cultivars under drought stress.

Materials and Methods

Two spring and winter canola cultivars, namely, Option 500 and Licord, were used in this study. This experiment was conducted in Seed and Plant Biotechnology Labs, Department of Agronomy, University of Tehran. The germination test was conducted on 50-seed samples of each cultivar at 25 °C for seven days on moistened blotter papers. Tests were replicated three times. Only normal seedlings were counted. The accelerated aging test was conducted by aging seeds at 40 °C and 90% RH for 48, 120, and 192 hours using the wire-mesh tray method (McDonald and Phaneendranath, 1978). Following incubation, the seeds were germinated at 25 °C for seven days. For plant hormone assessment, seeds were exposed to media containing 0, 10, 50, 100 µM of abscisic acid (ABA) and Kinetin for 24 hours. Hormone treatments conducted separately. Then the treated aged and control seeds were placed in Petri dishes containing polyethylene glycol (PEG) 6000 at osmotic potential of 0, -4, -8, -12, -16 bars at 25 °C for seven days under dark conditions. The percentage of germination, germination speed and germination index were recorded for all treatments. A randomized complete block design was used for all experiments. Data were analyzed using Minitab and SAS program. Multiple range tests was used for mean analysis.

Results

Effects of accelerated seed aging on germination characteristics of canola cultivars

The germination percentage and germination index of two canola cultivar were declined after accelerated aging. Eight days of aging reduced the germination percentage of Licord and Option by almost 24%. Option cultivar lost its viability faster than Licord after two days, however, after eight days of aging these two cultivars reached similar level of germination. The results of this part indicated that aging treatments can provide appropriate deterioration treatments for the next experiments.

Table 1. Effect of seed aging on germination percentage and germination index of two canola cultivars.

Accelerated aging treatment (days)	Germination (%)		Germination Index	
	Licord	Option 500	Licord	Option 500
Control	98a	94b	0.82a	0.79a
2	95ab	85c	0.80ab	0.72c
5	87c	81d	0.73c	0.69d
8	75e	71f	0.62e	0.59f
mean	89	83	0.74	0.70

Means with similar alphabet are not significant at 5%.

Effects of simulated drought conditions on germination characteristics of canola aged seeds

On increasing the concentration of PEG in the Petri dishes from -4 to -16 bars, the germination characteristics decreased, in comparison to control (Table 2) for all aging treatments. This reduction was much higher for most deteriorated seeds. At osmotic potential of -4 bars, aged seeds had reduction up to 20%. At eight days of aging, germination percentage reduced by 20%. At -8 bars, the germination percentages of 2, 5, and 8 days of aging treatment reduced by 32, 40, and 54%, respectively. However, for control seeds, the germination decreased by only 26%. The similar pattern was detected for germination speed and germination index.

Table 2. Effects of drought stress on germination %, germination index and germination speed of canola aged seeds.

Aging treatment (days)	Drought level (bar)														
	0			-4			-8			-12			-16		
	%	GI	GS	%	GI	GS	%	GI	GS	%	GI	GS	%	GI	GS
0	97a	0.81a	51.71a	82d	0.64d	38.99d	71g	0.54f	35.27e	33k	0.28j	24.15h	14n	0.1mn	15.5k
2	90b	0.77b	47.58b	77e	0.58e	36.83e	61h	0.44h	31.9f	30kl	0.20k	20.92i	80	0.09n	14kl
5	84c	0.71c	42.64c	71g	0.51g	35.37f	50i	0.39i	29.34g	21m	0.16l	19.16j	6pq	0.08n	13.27m
8	73f	0.60e	37.31d	58i	0.44h	31.51g	33k	0.30j	24.63h	19n	0.13m	17.56k	4q	0.06o	11.94n
Mean	86	0.72	44.81	72	0.54	35.68	54	0.42	30.29	26	0.19	20.45	8	0.08	13.66

%, GI, GS, represent, germination percentage, germination index, and germination speed, respectively.
Means with similar alphabet are not significant at 5%.

Effects of kinetin and abscisic acid on germination characteristics of canola seeds

Kinetin and abscisic acid were found to be effective in promoting germination characteristics of canola seeds as compared to control (Table 3). In comparison to control, seed treatment with kinetin and abscisic acid at 100 µM increased germination by 22 and 28%, respectively. Abscisic acid promoted germination characteristics most effectively. For germination speed, kinetin increased germination by 20%, while abscisic acid promoted by 29%. For germination index, this increase for kinetin and abscisic acid was 26% and 34%, respectively.

Table 3. Effects of kinetin and abscisic acid on germination characteristics of canola seeds.

Hormone concentrations (µM)	Kinetin			Abscisic acid		
	%	GI	GS	%	GI	GS
0	49d	0.39d	28.85d	49a	0.40a	28.88a
10	55c	0.44c	31.43c	59b	0.48b	33.67b
50	59b	0.49b	33.39b	65c	0.54c	37.56c
100	63a	0.53a	36.12a	69d	0.61d	41.09d
Mean	57	0.46	32.45	61	0.51	35.3

%, GI, GS, represent, germination percentage, germination index, and germination speed, respectively.
Means with similar alphabet are not significant at 5%.

Abscisic acid promoted germination of aged canola seeds most effectively

Percentage of germination of canola seeds was improved by kinetin and abscisic acid. This improvement was detected for all hormone concentrations. However, abscisic acid promoted germination better than kinetin at higher levels of deterioration. The highest concentration of abscisic acid and kinetin (100 µM) improved germination of aged seeds at eight days by 49% and 31%, respectively. For control treatment, this increase for abscisic acid and kinetin was about 22% and 18%, respectively.

Table 4. Effects of kinetin and abscisic acid concentrations on percentage of germination of canola aged seeds.

Hormone concentration (μM)	Accelerated aging test (days)							
	0		2		5		8	
	Kinetin	ABA	Kinetin	ABA	Kinetin	ABA	Kinetin	ABA
0	59ef	59c	53ghi	54c	46ik	47ab	37i	37a
10	64bcd	68g	58efg	63d	52hi	57c	44k	49bc
50	68ab	72j	62cde	68gh	57fg	63de	50ij	57c
100	72a	76k	65bc	72i	61def	67f	54g	73de
Mean	66	69	60	64	54	58	46	54

Means with similar alphabet are not significant at 5%.

Canola cultivars respond differently to abscisic acid seed treatment

Analysis of variance detected a significant interaction between ABA and canola cultivars. ABA seed treatment increased percentage of germination and germination speed of both cultivars. Licord cultivar performed better under all ABA concentrations as compared to Option cultivar. Germination percentage of Licord and Option at 100 μM of ABA increased up to 27 and 30%, respectively. Moreover, 100 μM of ABA improved germination speed by almost 26%.

Table5. Effects of abscisic acid concentrations on germination percentage and germination speed of canola cultivars.

Abscisic acid concentrations (μM)	Germination (%)		Germination speed	
	Licord	Option	Licord	Option
0	52e	47f	30.98a	28.84b
10	62cd	57d	34.62b	31.84c
50	67bc	63c	39.52c	34.9d
100	71a	68b	42de	38.51e
Mean	63	59	36.78	33.52

Means with similar alphabet are not significant at 5%.

Discussion

Seeds of good quality are undamaged seeds that produce viable and vigorous seedlings without defects under various environmental conditions also after storage (Dickson, 1980). Depending on storage conditions, storage time and genotype seeds gradually lose vigor and viability. A common interpretation of the physiology of this seed deterioration cannot be given because the causes are assumed to be due to a variety of processes. For instance, mechanical damage that occurs in the field is different than physiological damage occurring during storage, which in itself may have different components (McDonald, 1999). Seed deterioration in agronomic crops is a problem that has been unintentionally aggravated by domestication because wild species often retain a high seed quality for many years (Moore and Halloin, 2000). Considerable evidence indicates that repair of DNA, RNA, protein, membranes and enzymes occurs during imbibition (Kalpana and Madhava Rao, 1997; Dell'Aquila and Tritto; 1991, Petruzzeli, 1986). The plant hormone ABA affects a wide range of processes in plants. Examples are: altered gene expression, tolerance to cold stress, inhibition of growth, and tissue-specific stress responses like the closure of stomata. Furthermore, the plant hormone plays a role in seed maturation and dormancy. The various mutants used to elucidate the role of ABA in these processes have been reviewed by Leung and Giraudat (1998), Koornneef et al. (1998), and Finkelstein et al. (2002). The many pleiotropic effects of the abi3 mutants indicate that this protein affects many processes during seed maturation and germination, including the storability of seeds. Clerkx et al. (2003) reported a decrease in viability and vigor by storing the seeds in two relative humidities (RHs) for a prolonged period. At 60% RH, the mutant lost germinability, but storage at 32% RH showed no decrease of germination although seed vigor decreased. The decrease in viability and vigor could be related to an increase in conductivity, suggesting membrane deterioration. This was not affected by light conditions during imbibition, expected to influence the generation of active oxygen species. They suggested that during seed maturation, ABI3 regulates several processes: acquiring dormancy and long-term storability and loss of chlorophyll. The possibility of exogenous application of ABA on activation of this gene is very strong.

The observed reduction in endogenous cytokinins under water stressed conditions (Boucaud and Unger, 1976) points towards the possibility that kinetin level might be a limiting factor under stressed conditions and explaining why exogenous application of kinetin resulted in increased germination. The enhancement of germination by kinetin under stressed conditions could be due to enhanced uptake of water either due to increased membrane permeability or to the internal concentration of osmotically active solutes (Kaufmann and Ross, 1970). It has been reported that the degradation of starch and accumulation of reducing sugars was enhanced in germinating wheat endosperm by kinetin and the accumulation of reducing sugars would increase the gradient for osmotic entry of water (Boothby and Wright, 1962). Further experiments need to be conducted for possible physiological mechanisms of aging repair.

References

Boothby D, Wright STC (1962) Effect of kinetin and other growth regulators on starch degradation. Nature 196:389-390.

Boucaud J, Unger IA (1976) Hormonal control of germination under saline conditions of three halophyte taxa in geneus Suaeda. Physiol Plant. 36:197-200.

Clerkx EJM, Vries HBDc, Ruys GJ, Groot PG, Koornneef M (2003) Characterization of *green seed*, and enhancer of *abi3-1* in Arabidopsis that affects seed longevity. Plant Physiol 132:1077-1084.

Dell'Aquila A, Tritto, V (1991) Germination and biochemical activities in wheat seed following delayed harvesting, ageing and osmotic priming. Seed Sci

Technol. 19:73-82.
Dickson MH (1980) Genetic aspects of seed quality. Hortic Sci 15: 771-774.
Elias SG, Copeland, LO (2001) Physiological and harvest maturity of canola in relation to seed quality. Agron J 93:1054-1058.
Finkelstein R, Gampala SSL, Rock CD (2002) Abscisic acid signaling in seeds and seedlings. Plant Cell Suppl 14: S15-S45.
Kalpana R, Madhava Rao KV (1997) Nucleic acid metabolism of seeds of pegeonpea (Cajanus cajan) cultivars during accelerated aging. Seed Sci Technol 25:293-301.
Kaufmann MR, Ross KJ (1970) Water potential, temperature and kinetin effects on seed germination in soil and solute system. Am J Bot 57:413-419.
Koornneef M, Léon-Kloosterziel KM, Schwartz SH, Zeevaart JAD (1998) The genetic and molecular dissection of abscisic acid biosynthesis and signal transduction in Arabidopsis. Plant Physiol Biochem 36: 83-89.
Leung J, Giraudat J (1998) Abscisic acid signal transduction. Annu Rev Plant Physiol Plant Mol Biol **49:** 199-222
McDonald MB (1999) Seed deterioration: physiology, repair and assessment. Seed Sci Technol **27:** 177-237.
McDonald MB, Phaneendranath BR (1978) A modified accelerated aging seed vigor test for soybean. J. Seed Technol 3:27-37.
Moore SH, Halloin JM (2000) Genetic resistance to seed deterioration. *In* SH Moore, RW Yaklich, eds, Genetic Improvement of Seed Quality, Ed 31, Vol XVII. Crop Science Society of America, Madison, WI, pp 21-37.
Petruzzeli L (1986) Wheat viability at high moisture content under hermetic and aerobic storage conditions. Ann. Bot. 58:29-265.
Sharma AD, Thakur M, Rana M, Singh K (2004) Effect of plant growth hormones and abiotic stresses on germination, growth and phosphatase activities in sorghum bicolor (L.) Mench seeds. African J. Biotch **3:**308-312.