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Enhancement of Canola Seed Germination and Seedling Emergence at Low Temperature by Priming

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ABSTRACT

Some seedlots of canola (*Brassica napus* L. and *B. campestris* L.) have low percentage germination and poor seedling vigor. The effect of priming canola on both seed germination and seedling emergence was investigated in controlled environment cabinets. Germination was investigated using petri dish assays, whereas emergence was studied by sowing seeds into pots containing a sandy loam soil. Seed germination and seedling emergence from soil at 10°C was enhanced for several cultivars of both species due to priming. Time to 50% germination and emergence was also reduced. A *B. campestris* cv. Goldrush seedlot, which showed low germination in preliminary studies, was found to be particularly responsive to priming and, therefore, was used in subsequent studies to optimize the technique. The optimal priming time varied with temperature. Excellent responses occurred with priming for 14 to 16 h at 23°C or 60 h at 10°C. Temperature during priming had little effect on percentage germination, emergence percentage, or time to 50% germination or emergence. Seeds primed for 16 h at 23°C or for 60 h at 10°C initiated seedling emergence at 10°C 4 d earlier than nonprimed seeds. Seedling emergence of primed seeds was 73% compared with 31% for the nonprimed seeds. The benefits of priming on both percentage emergence and time to 50% emergence were reduced if the seeds were primed under anaerobic conditions. In addition, the leachate from primed seeds was inhibitory to both percentage germination and time to 50% germination, particularly at 10°C. The results obtained from this study indicate that seed priming has potential for improving seed germination and subsequent seedling establishment of canola seedlots with low germination, with low vigor, or when seeds are planted in cool (<10°C) soils.

LOW SOIL TEMPERATURES in the spring delay and reduce seedling emergence, particularly in small seeded crops such as canola (*Brassica* spp.). In the main canola production areas of western Canada, the mean soil temperature at a depth of 5 cm ranges from 5 to 13°C from 5 to 25 May (Environment Canada, 1984). The optimal temperature for canola seed germination is 15 to 20°C (Kondra et al., 1983). Suboptimal temperatures reduce both the percentage and rate of germination and seedling emergence (Blackshaw, 1991; Livingston and de Jong, 1990; Kondra et al., 1983).

Controlled hydration of seeds for a given period followed by dehydration to approximately the original moisture content is termed priming. Primed seeds often germinate and emerge more rapidly and in greater synchrony than nonprimed seeds, particularly under suboptimal temperature and moisture conditions (Bennett et al., 1992; Bradford, 1986). Researchers have reported that priming increased seedling stand and yield of parsley [*Petroselinum crispum* (Miller) Nyman ex A.W. Hill], carrots (*Daucus carota* L.), celery (*Apium graveolens* L.) and tomatoes (*Lycopersicon esculentum* Miller) (Alvarado et al., 1987; Bradford, 1986; Heydecker and Coolbear, 1978; Rennick and Tiernan, 1978). Osburn and Schroth (1989) found osmopriming (priming in the presence of an osmotic agent) of sugar beet (*Beta vulgaris* L.) seed reduced damping off caused by *Pythium ultimum* Trow.

To our knowledge, priming has not been reported for canola. The objectives of this study were to determine if priming had a beneficial effect on canola seed germination and seedling emergence at suboptimal temperature conditions and to determine the optimal conditions for priming canola seeds using a seedlot that displayed low germination.

MATERIALS AND METHODS

Seed Priming and Re-Drying

Seeds of three *B. campestris* L. cv. (Goldrush, Tobin, and Parkland), and six *B. napus* L. cv. (Legend, Profit, Delta, Samurai, Westar, and Garrison) from production in 1991 and 1992 crop years were obtained from Pioneer Hi-Bred, Georgetown, ON, Canada (courtesy of Dr. W. McNab) and Agriculture Canada, Saskatoon, SK, Canada (courtesy of Dr. K. Downey). Priming was conducted at ≈23°C for up to 20 h and at 10°C between 0 to 84 h. Sixty seeds of each cultivar were imbibed on one layer of Whatman no. 1 filter paper moistened with 3 mL of distilled water and placed in a 9-cm petri dish. The dishes were sealed with parafilm and placed at either 10 or 23°C for the desired time. Each treatment was replicated three times. The seeds were primed in the absence of light because preliminary experiments demonstrated light had no effect on the results. To minimize germination during redrying, free water was removed from the seeds by centrifugation at 4600 g for 5 min at 4°C immediately after priming. Centrifuged seeds were then dried for 3 d at 23°C to a constant seed weight. The effect of anaerobic conditions on priming was determined by immersing seeds completely in 1 mL of double distilled water in sealed 1.5-mL tapered tubes held at 23°C for 16 h or at 10°C for 60 h. The seeds were centrifuged and dried as described above. Nonprimed control seeds were also centrifuged as described above. The dry primed and control seeds were stored at −20°C.

Germination Test

Sixty seeds were placed on one layer of Whatman no. 1 filter paper moistened with 3 mL distilled water in a 9-cm petri dish. The dishes were sealed with parafilm and placed at either 10 or 23°C in the dark. The number of seeds exhibiting radicle emergence was recorded every day until germination ceased. The test was replicated three times for each treatment and cultivar.

To establish the presence of a germination inhibitor, the leachate from seeds primed for 16 h at 23°C was collected, passed through a 0.2-μm filter, and made up to 3 mL with double distilled water. The leachate (3 mL) was added to both primed (16 h at 23°C) and nonprimed (control) seeds as described above. Each treatment contained 60 seeds per petri

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Table 1. Mean percentage germination and time to 50% germination ($T_{50}G$) at 10°C for seven canola cultivars harvested in 1991 and 1992, primed at 23°C for 16 h.

Species and cultivar	Germination				$T_{50}G$			
	1991		1992		1991		1992	
	Nonprimed	Primed	Nonprimed	Primed	Nonprimed	Primed	Nonprimed	Primed
	%				d			
B. napus								
Legend	97.8	98.9^{ns}	96.7	97.8^{ns}	2.2	1.4***	3.0	1.7***
Profit	96.1	96.7^{ns}	97.2	97.8^{ns}	2.4	1.6***	2.7	1.8***
Delta	72.8	91.7***	87.8	92.8*	3.2	2.0***	2.9	1.7***
Westar	93.8	98.9*	90.0	94.4*	2.6	1.4***	4.1	1.9***
B. campestris								
Tobin	91.1	95.5*	81.1	91.7***	2.5	1.6***	3.0	1.9***
Parkland	93.3	97.2^{ns}	78.3	86.7*	2.6	1.6***	2.7	2.2*
Goldrush	54.9	84.4***	92.8	96.7^{ns}	4.8	2.4***	2.0	1.5*

ns, *, **, *** Not significant or significant compared with nonprimed control seeds at the 0.05, 0.01, and 0.001 level of probability based on *t*-test. Percentage data were arcsin transformed before analysis.

dish and was replicated three times. Percentage germination was determined at either 10 or 23°C as described above.

Seedling Emergence Experiment

Seeds were primed at either 10°C for 60 h or at 23°C for 16 h, either aerobically (petri dishes) or anaerobically (sealed microfuge tubes). Prior to seeding, the soil was watered and pre-equilibrated for 12 h at 10°C. The primed and nonprimed seeds were sown at a depth of 10 mm in a sandy loam soil in 15 by 12 cm plastic pots. Five replicates of 300 seeds of each cultivar were used for each treatment. The pots were placed in a controlled environment chamber at 10°C day/8.5°C night, 14-h photoperiod at 60% relative humidity (hereafter referred to as emergence at 10°C) in a completely randomized design. Water pre-equilibrated to 10°C was added every 3 d to the trays on which the pots were placed. The cumulative total of seedling hypocotyl emergence was recorded daily for 28 d.

Statistical Analysis

Germination or emergence was calculated as a percentage of the number of seeds sown. The time to 50% germination ($T_{50}G$) or 50% emergence ($T_{50}E$) of the total number that germinated or emerged was determined from the following equation:

$$T50 = T1 + ((N + 1)/2 - N1)(T2 - T1)/(N2 - N1)$$

Table 2. Mean percentage emergence and time in days to 50% emergence ($T_{50}E$) for seven canola cultivars at 10°C day/8.5°C night and 14-h photoperiod, primed at 23°C for 16 h.

Species and cultivar	Emergence		$T_{50}E$	
	Nonprimed	Primed	Nonprimed	Primed
	%		d	
B. napus				
Delta	74.7	79.2*	8.3	7.1**
Samurai	64.0	70.6*	8.0	7.2*
Westar	68.6	74.2^{ns}	9.3	7.9**
Garrison	77.5	78.3^{ns}	8.4	7.3*
B. campestris				
Tobin	56.4	57.8^{ns}	8.2	7.5*
Parkland	33.3	56.7***	9.2	7.2***
Goldrush	31.1	72.7***	14.3	9.8***

ns, *, **, *** Not significant or significant compared with nonprimed control seeds at the 0.05, 0.01, and 0.001 level of probability based on *t*-test. Percentage data were arcsin transformed before analysis.

where N was the total number of seeds germinated or emerged and $N1$, $N2$, the total number of seeds germinated or emerged at time $T1$, $T2$, where $N1 < (N + 1)/2 < N2$ (Coolbear et al., 1984). The results were expressed as the means and standard errors calculated from the replicates using the Statistic Analysis System (SAS Institute, 1987). The final germination or emergence percentage and time to 50% germination or emergence data (Tables 1, 2, and 3) were subjected to *t*-test analysis using the SAS TTEST procedure (SAS Institute, 1987). The percentage emergence and time to 50% emergence data reported in Table 4 were analyzed by Duncan's multiple range test using the SAS GLM procedure (SAS Institute, 1987). Percentage data were arcsin transformed before analysis; actual percentages are shown.

RESULTS

Priming seeds at 23°C for 16 h increased the percentage germination at 10°C in all cultivars tested except for Legend, Profit, the 1991 seedlot of Parkland, and the 1992 seedlot of Goldrush (Table 1). The $T_{50}G$ was reduced by about 1 d for all cultivars. Priming also resulted in increased emergence percentage at 10°C in two of four cultivars of *B. napus* (Delta and Samurai) and two of three cultivars of *B. campestris* (Parkland and Goldrush) and a reduced $T_{50}E$ in all canola cultivars (Table 2). A *B. campestris* cv. Goldrush (1991) seedlot

Table 3. Mean percentage germination and time to 50% germination ($T_{50}G$) of primed (16 h, 23°C) and nonprimed *Brassica campestris* cv. Goldrush seeds at 10 and 23°C after addition of seed leachate.

Treatment	Germination medium	Germination	$T_{50}G$
		%	h
		23°C	
Nonprimed	Water	85.0	34.6
	Leachate	77.8^{ns}	35.7^{ns}
Primed	Water	94.4	15.9
	Leachate	90.5^{ns}	20.3**
		10°C	
Nonprimed	Water	54.9	114.5
	Leachate	39.9*	131.4*
Primed	Water	84.4	81.4
	Leachate	65.6*	95.5*

ns, *, ** Not significant or significant compared with water treated seeds at the 0.05, 0.01, and 0.001 level of probability based on *t*-test. Percentage data were arcsin transformed before analysis.

Table 4. Mean percentage seedling emergence and time to 50% emergence ($T_{50}E$) for *Brassica campestris* cv. Goldrush seeds at 10°C under various priming conditions.

Priming condition	Emergence†	$T_{50}E$
	%	d
60 h at 10°C		
aerobic	72.7a‡	9.8c
anaerobic	47.2b	12.2b
16 h at 23°C		
aerobic	74.0a	10.0c
anaerobic	49.6b	13.0b
Nonprimed control	31.1c	14.3a

† Cumulative emergence after 28 d at 10°C.
‡ Values within a column followed by the same letter are not significantly different at the 0.05 level of probability, based on Duncan's multiple range test. Percentage data were arcsin transformed before analysis.

with low percentage germination was particularly responsive to priming and was used in subsequent experiments to determine the optimum priming conditions.

Priming seeds of Goldrush (1991) canola from 2 to 16 h at 23°C dramatically increased percentage germination at 10°C with a near linear effect for the first 14 h (Fig. 1). Priming at 10°C also dramatically increased percentage germination at 10°C with the largest effect occurring within the first 12 h of priming and germination gradually increasing to maximum at 60 h (Fig. 2). The $T_{50}G$ decreased linearly during the first 14 h as time of priming increased at 23°C (Fig. 1). The $T_{50}G$ for seeds primed at 10°C did not decrease until after 36 h of priming and then decreased from 4.4 to 2.9 d for seeds primed for 48 h or longer (Fig. 2). If the priming time at 23 and 10°C exceeded 16 and 72 h, respectively, germination percentage decreased (Fig. 1 and 2).

When seeds were germinated at 23°C, priming (either at 23 or 10°C) had no significant effect on the final germination percentage (data for seeds primed at 23°C shown in Table 3). In contrast, the $T_{50}G$ at 23°C was reduced more than half (35 h nonprimed vs. 16 h primed) regardless of the priming temperature (Table 3).

The leachate from primed seeds had no effect on percentage germination at 23°C, but at 10°C, percentage germination was reduced from 55 to 40% for the control seeds and 85 to 66% for the primed seeds (Table 3). The leachate had no effect on the $T_{50}G$ of nonprimed control seeds germinated at 23°C but increased the $T_{50}G$ from 16 to 20 h for the corresponding primed seeds. When germinated at 10°C, the leachate increased $T_{50}G$ by 17 and 14 h for the nonprimed and primed seeds, respectively (Table 3).

The cumulative seedling emergence from seeds primed for 16 h at 23°C or at 10°C for 60 h is presented in Fig. 3. Seeds primed under aerobic conditions initiated emergence 2 and 4 d earlier compared with seeds primed anaerobically and the nonprimed control seeds, respectively. Final percentage emergence of aerobically primed seeds was ≈73% compared with 47% for those primed anaerobically and 31% for the nonprimed control seeds (Table 4). The $T_{50}E$ at 10°C for aerobically primed seeds at either 23 or 10°C was 4 d less than for the nonprimed control seeds. In contrast, the $T_{50}E$ for anaerobically primed seeds was only 1.8 d less than the nonprimed seeds. Thus, aerobic priming increased percentage emergence and reduced $T_{50}E$ in a canola seedlot with low germination.

DISCUSSION

In this study, priming induced rapid and uniform germination of canola seed and rapid emergence of canola seedlings, particularly at low temperatures. The critical factors for the priming of canola seeds were the duration of the priming period and aeration during priming. Adegbuyi et al. (1981) and Heydecker and Gibbins (1978) reported that overpriming of parsley, celery, and herbage grass seeds increased the time to 50% germination in comparison to the optimal time. Similar results were noted with the Goldrush seeds; i.e., germination de-

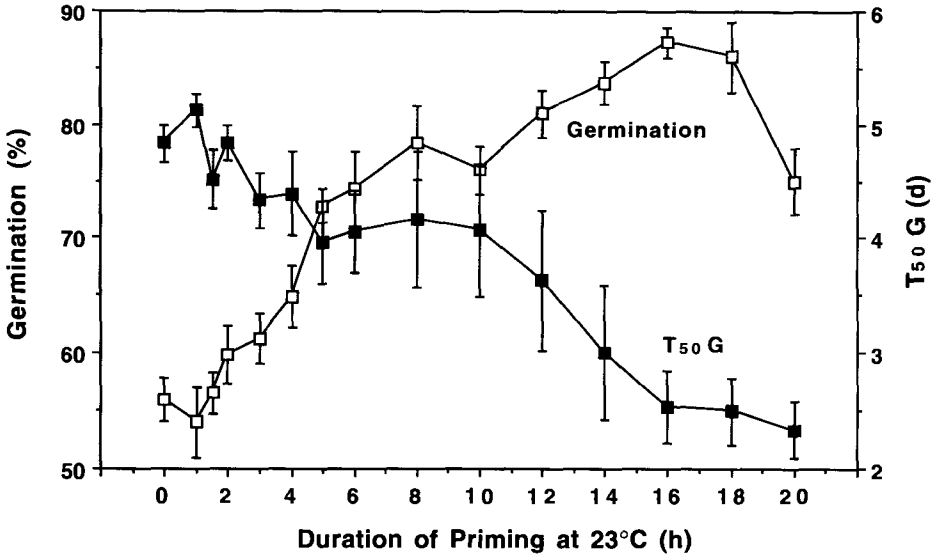

Fig. 1. Effect of duration of priming at 23°C on percentage germination and time to 50% germination ($T_{50}G$) of seeds of canola cv. Goldrush (1991) at 10°C. Vertical bars indicate standard errors.

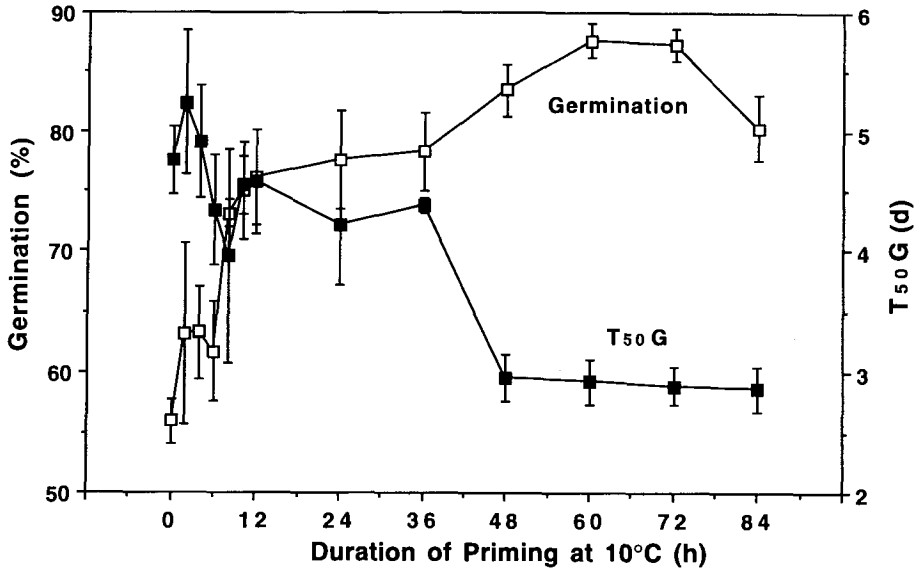

Fig. 2. Effect of duration of priming at 10°C on percentage germination and time to 50% germination ($T_{50}G$) of seeds of canola cv. Goldrush (1991) at 10°C. Vertical bars indicate standard errors.

creased when seeds were primed for longer than 16 h at 23°C or 60 h at 10°C (Fig. 1 and 2).

Heydecker et al. (1973) found that 20, 15, and 10°C were equally effective for the priming of onion seeds; however, a longer priming period was required at the lowest temperature. Onion (*Allium cepa* L.) seed primed at 10°C exhibited the highest rate of germination. Our data indicated that the priming temperature had no effect on the final germination percentage of canola seeds, but the optimal priming time at 10°C was 44 h longer in comparison to priming at 23°C.

The effect of priming has been partially explained by effective invigoration. Effective invigoration is the initiation of those metabolic events that normally occur during imbibition and that are subsequently fixed by drying (Hanson, 1973). The results of this study indicate that effective invigoration may be occurring during canola seed priming. We are currently studying the effect of priming on the induction of a cell division specific gene encoding the proliferating cell nuclear antigen (Markley at al., 1993) and the genes encoding the enzymes isocitrate lyase and malate synthase (Comai et al., 1989), which are involved in seed storage product metabolism.

In addition to initiating metabolic events, priming may also leach germination inhibitors from the seeds. The possibility that the beneficial effects of priming may be due to removal of germination inhibitors has been suggested previously (Heydecker and Coolbear, 1978). Removal of seed leachate reduced the germination time and increased germination percentage in carrots (Pill and

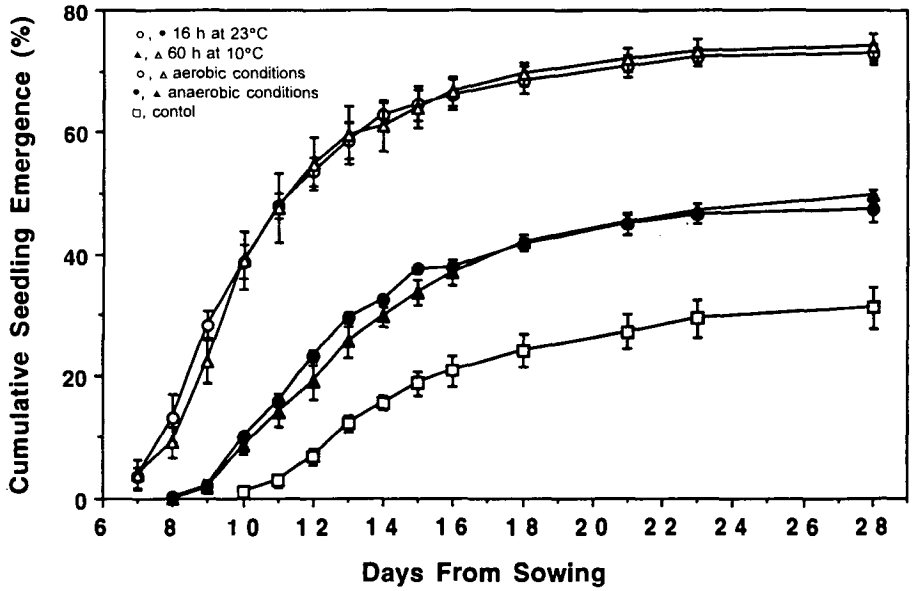

Fig. 3. Seedling emergence of canola cv. Goldrush in a sandy loam soil at 10°C. The seeds were primed for either 16 h at 23°C or for 60 h at 10°C under either aerobic or anaerobic conditions or nonprimed (control). Vertical bars indicate standard errors.

Finch-Savage, 1988) and celery (Furutani et al., 1985), indicating the possible presence of a germination inhibitor in the seed leachate. The canola cultivar Goldrush apparently contains an inhibitor that is leached out during priming. Interestingly, this inhibitor was more effective in inhibiting seed germination at 10°C than at 23°C. One possible reason is that the inhibitor is metabolized or degraded at the warmer temperature.

The possibility of a germination inhibitor could also explain the beneficial effects observed after a short priming period at either temperature, particularly if the inhibitor is water soluble. It has been demonstrated that during the imbibition of barley (*Hordeum vulgare* L.) seed under aerobic conditions, the level of abscisic acid, a natural inhibitor of germination, decreases rapidly and germination occurs shortly thereafter. However, if the seeds are imbibed under anaerobic conditions, abscisic acid is not degraded and the seeds do not germinate (Yamada, 1985).

These results indicate that seed priming techniques have the potential to improve canola seed germination and subsequent seedling emergence under low soil temperature conditions. All cultivars of *B. napus* and *B. campestris* tested displayed an increased percentage germination or emergence and/or a reduced time to 50% germination or emergence (Tables 1 and 2) when primed. The beneficial effect of priming was most pronounced in a seedlot with low percentage germination. Therefore, seed priming shows promise for improving germination and emergence of canola seedlots with low percentage germination or low vigor and for enhancing emergence at suboptimal soil conditions.

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