Benzyladenine in the Priming Solution Reduces Thermodormancy of Lettuce Seeds

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Summary. When lettuce seeds are imbibed and subjected to high temperature for periods of 72 h or more, dormancy known as thermodormancy is induced. Priming of three cultivars of lettuce (*Lactuca sativa* L.) seeds in 1% (w/v) K_3PO_4 for 20 h in the dark reduced thermodormancy. Addition of 100 mg 6-benzyladenine (BA)/liter to the priming solution increased germination in petri dishes at 35C in 'Green Lakes' from 65% in seeds that were primed without BA, to 92% when BA was added to the priming solution. In 'South Bay' these percentages were 24% and 86%, respectively. Seedling emergence was improved in other lots of 'Green Lakes' and 'Montello' using soilless mix.

Thermodormancy is an important problem in establishing stands of lettuce when seeds are sown under high soil temperatures. It can be circumvented by seed-priming (Cantliffe et al., 1981; Guedes and Cantliffe, 1977, 1980; Guedes et al., 1979). For the procedure to be successful commercially, the components of priming must be standardized as much as possible. These components include the type of soak solution (osmoticum) and its osmotic potential, aeration, temperature, light, soak duration, seed redrying procedures, seed storage, and seed quality (Cantliffe, 1983). For lettuce, many of these factors have been standardized, including soaking the seeds in aerated solutions of 1% (w/v) K_3PO_4 for 20 h in the dark (Cantliffe, 1981), followed by redrying the seeds at low temperature and relative humidity to retard radicle growth

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(Cantliffe et al., 1984). Perkins-Veazie and Cantliffe (1984) found that lettuce thermodormancy can be bypassed successfully by priming high-quality seeds, and that the priming was ineffective with aged or deteriorated seeds.

The upper temperature limit that initiates the onset of thermodormancy varies among cultivars (Thompson et al., 1979). Some cultivars are affected by temperatures slightly above 30C, while others germinate at 33C. Little or no lettuce seed germination occurs above 35C. This difference in temperature sensitivity has led to varied success of priming for overcoming thermodormancy.

Cytokinins reduced thermodormancy in lettuce, especially when seeds were germinated directly in cytokinin solutions (Sharples, 1973). When seeds were soaked in cytokinin and subsequently sown apart from the hormone, the treatment was generally ineffective (Smith et al, 1968). In our work, a cytokinin (BA) was added to the priming solution in an attempt to ensure high germination percentages of lettuce after the seeds were dried and imbibed at 35C, regardless of cultivar or seed source.

Lettuce seeds were primed under similar conditions throughout all experiments. BA (Abbott Laboratories, North Chicago, Ill.) was added as a treatment to the priming solution. Seeds were primed at 15C in aerated 1% (w/v) K_2PO_4 in the dark for 20 h. The seeds were placed in 200-mm test tubes with 20 ml of soak solution per gram of seeds. After priming, the seeds were washed with 100 ml of distilled water and surface moisture was removed by 2 to 3 min of suction. The seeds were dried immediately and stored at 5C, 45% RH.

Germination tests were conducted in incubators in the dark by placing 25 seeds on Whatman No. 3 filter paper in a 9-cm-diameter petri dish and adding 5 ml of distilled water followed by covering. Each treatment was replicated four times and most experiments were repeated at least twice. Germination, defined as visible radicle protrusion through the seedcoat and pericarp, was counted daily for 5 days. Average number of days to germination (ADG) was calculated according to the following formula:

$$ADG = (A_1T_1 + A_2T_2 + \dots + A_nT_n) / (A_1 + A_2 + \dots + A_n)$$

where: A = number of seeds germinating per day, T = time corresponding to A in days, and n = number of days to final count.

In the first experiment, 'Green Lakes' and 'South Bay' lettuce seeds were primed without BA, dried, then germinated at 10, 15, 25, 30, or 35C. In the second experiment with the same cultivars, BA was added to the priming solution in concentrations ranging from 0 to 500 mg. In the third experiment, 'Green Lakes' (a different seed lot from the above) and 'Montello' lettuce seeds were primed with or without 100 mg BA/liter, dried, then germinated in incubators at 15, 25, or 35C or in a 1 peat: 1 vermiculite (v/v) mix in growth chambers maintained at constant 15 or 35C.

Germination of 'Green Lakes' and 'South Bay' was high (98%), regardless of treatment, at 10, 15, 25, and 30C (data not shown). At 35C, only primed seeds germinated, however, values were low—52% for 'Green Lakes' and 36% for 'South Bay'. ADG at 10 or 15C was improved for both cultivars by priming (Table 1). At 25 and 30C, ADG was similar for control and primed seeds.

With 25 mg BA/liter priming solution, germination of 'Green Lakes' at 35C increased from 65% to 85%, but higher BA concentrations did not increase germination further (Fig. 1). Germination of 'South Bay' seeds at 35C was not increased by BA addition until ≥ 100 mg BA/liter was added to the priming solution. Subsequent additions of 150, 250, and 500 mg BA/liter further increased germination of this cultivar. Abnormal germination, manifested as thickened hypocotyls, was observed in seedlings primed at 250 or 500 mg BA/liter. Morphological abnormalities were not observed in seedlings primed in 100 mg BA/liter; thus, this concentration

was selected for the third experiment.

In a third experiment with different seed lots, percentage germination at 15 and 25C was unaffected by seed priming, with or without BA in the osmoticum (Table 2). At 35C, only primed 'Green Lakes' seed germinated, and the addition of BA to the priming solution increased germination significantly. Results with 'Montello' were similar. At 15C, seed priming decreased ADG, but at 25 or 35C, ADG was the same whether or not the seeds were primed.

When primed or nontreated seeds were sown in soilless mix in the growth chamber, emergence at 15C was unaffected by priming (Table 3). At 35C, percentage emergence of primed seed was low in both cultivars unless BA was used. ADS; at 15C was not affected by seed priming.

Seed priming effectively overcomes thermodormancy in lettuce both in the laboratory and the field (Cantliffe, 1981). In the past, the actual germination percentage of primed seeds achieved at 35C has varied, ranging from 50% to 80%. With the addition of BA to the priming solution, germination at 35C was generally $>90\%$, a germination percentage similar to that achieved at an ideal germination temperature (25C). Previous reports using cytokinin to improve germination of lettuce at high temperatures relied on either leaving the growth regulator in contact with the seed during germination (Sharples, 1973) or pregerminating the seed directly in cytokinin solutions at low temperature and then transferring the seed to the high temperature (Smith et al., 1968). The results from the former procedure were generally more favorable, while those from the latter usually led to $<50\%$ germination.

Germination at 35C in a soilless

Table 1. Effects of temperature and seed priming on average days to germination (ADG) by two lettuce cultivars.

Cultivar	Seed treatment	Temperature (°C)				
		10	15	25	30	35
		ADG (days)				
Green Lakes	Control	3.1	2.0	1.0	1.6	---
	Primed	2.4	1.1	1.0	1.4	1.0
		*	*	NS	NS	
South Bay	Control	3.1	2.0	1.1	1.8	---
	Primed	2.2	1.1	1.1	1.5	1.1
		*	*	NS	NS	

*NS Seed treatments within a temperature and cultivar significantly different or not significant at the 5% level (F test), respectively.

Fig. 1. Germination at 35C of 'Green Lakes' (GL) and 'South Bay' (SB) lettuce seeds in petri dishes after priming with 0 to 500 mg BA/liter of 1% K_3PO_4 . Mean separation within cultivars is by Duncan's multiple range test at the 5% level.

Table 2. Effects of priming with BA on germination and average days to germination of two lettuce cultivars at different temperatures.

Cultivar	Seed treatment ^a	Temperature (°C)					
		15	25	35	15	25	35
		Germination (%) ^b			ADG (days) ^b		
Green Lakes	Control	97 a	97 a	0 a	2.0 a	1.0	---
	Primed	94 a	100 a	76 b	1.3 b	1.0 a	1.0 a
	Primed + BA	94 a	99 a	90 c	1.0 c	1.0 a	1.0 a
Montello	Control	99 a	100 a	16 a	1.5 a	1.0 a	1.0 a
	Primed	100 a	96 a	60 b	1.3 b	1.0 a	1.0 a
	Primed + BA	99 a	99 a	91 c	1.0 c	1.0 a	1.0 a

^aPrimed = 1% K_3PO_4 (15C, 20 h, dark). Primed + BA = 100 mg BA/liter, 1% K_3PO_4 (15C, 20 h, dark).

^bMeans within a cultivar and temperature separated by Duncan's multiple range test, 5% level.

Table 3. Effects of priming with BA on emergence percentage and average days to emergence (ADE) of two lettuce cultivars at different temperatures in soilless mix in growth chambers.

Cultivar	Seed treatment	Temperature (°C)			
		15	35	15	35
		<i>Emergence (%)</i>		<i>ADE (days)</i>	
Green Lakes	Control	90 a	0 a ^z	5.6 a	---
	Primed	83 a	17 b	6.9 a	4.0 a
	Primed + BA	96 a	68 c	5.6 a	3.4 a
Montello	Control	96 a	4 a	6.5 a	4.3 a
	Primed	94 a	34 b	5.9 a	3.7 a
	Primed + BA	95 a	65 c	5.6 a	3.9 a

^{*}Means significantly different at the 5% level within temperatures, Duncan's multiple range test.

mix was improved 2-fold for 'Montello' and 4-fold for 'Green Lakes' when BA was added to the priming solution. Heydecker and Joshua (1977) observed reduced germination at 29C when kinetin-soaked lettuce seeds were germinated in a potting mix instead of

petri dishes. Under the conditions of the laboratory experiments, 100 mg BA/liter was sufficient for most lettuce seed lots to reach >90% germination at 35C, without any adverse effects on the seedling.

Lettuce seed priming appears to

be most effective when high-quality seeds are soaked at 15C in aerated osmotic solutions (salt or polyethylene glycol) at concentrations that prohibit radicle growth. The addition of cytokinin improves the overall effectiveness of the priming treatment and tends to reduce treatment variances due to seed source or cultivar.

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