Priming with Salt Solutions Improves Germination of Pansy Seed at High Temperatures

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Additional index words. osmoconditioning, thermoinhibition, inorganic salts, *Viola ×wittrockiana*

Abstract. Priming regimes were evaluated on pansy seeds (*Viola ×wittrockiana* Gam. 'Crystal Bowl Sky Blue') in high-temperature germination tests. Priming regimes evaluated included CaCl_2 , MgCl_2 , K_2HPO_4 , Na_2HPO_4 , NaCl , KCl , KNO_3 , Na_2SO_4 , and polyethylene glycol 15,000 (PEG_{15,000}) at -1.0 and -2.0 MPa, for 3, 6, or 9 d at 23°C . Primed and nonprimed control seeds were then germinated at 25, 30, or 35°C . Total percent germination of nonprimed control seeds was significantly less at 35°C than at 25°C . Seeds primed with CaCl_2 at -1.0 MPa for 3 d at 23°C had significantly higher germination at 35°C than all other priming regimes tested, including aerated PEG₈₀₀₀ at -1.0 MPa for 7 d at 15°C . Seed respiration, measured by O_2 uptake, during germination of seeds primed with CaCl_2 was higher than for control seeds or those primed with PEG₈₀₀₀. Priming pansy seed with CaCl_2 at -1.0 MPa for 3 d at 23°C was effective in increasing seedling emergence and for reducing the time of emergence in summer greenhouse studies.

One of the most important bedding plants produced by greenhouse growers is pansy. In warm regions of the United States, pansy is one of the major ornamental plants used for fall and winter landscaping. The conditions present during the hot months of summer cause pansy seeds to exhibit undesirable germination characteristics, such as wide variation in time from sowing to emergence and poor total germination. Imbibed pansy seed placed at 30°C or above results in high-temperature-induced thermoinhibition that causes low total germination, similar to that observed in spinach (*Spinacia oleracea* L.) (Atherton and Farooque, 1983).

Seed priming (osmoconditioning) has been used to improve vegetable and ornamental seed performance by increasing the speed of germination as well as improving germination uniformity (Bradford, 1986; Heydecker, 1977). Priming can also help overcome environmental stresses, such as germination at extremely high temperatures (Atherton and Farooque, 1983; Cantliffe, 1981). In bedding plants, dusty miller (*Senecio cineraria* DC.) and pansy seeds

primed in aerated solutions of PEG₈₀₀₀ had significantly higher germination rates at supraoptimal temperatures than nonprimed seeds (Carpenter, 1990; Carpenter and Boucher, 1991).

While inorganic salt solution priming has been used for many years to improve vegetable seed germination (Heydecker and Coolbear, 1977), little work has been reported on ornamental seed. Recent studies showed that salt solution priming significantly improved the germination of several species of herbaceous perennials (Fay et al., 1994; Samfield et al., 1991). In our laboratory, we have found salt solutions easier to handle and to remove from seeds than other priming ingredients, such as polyethylene glycol (PEG). We feel this is advantageous to growers who may wish to do their own in-house seed priming. Our objectives of this research then were to 1) screen several priming solutions containing inorganic salts for improving germination and emergence of pansy seed at supra-optimum temperatures, and 2) compare salt solution priming with current priming recommendations using PEG.

Materials and Methods

Germination in laboratory. 'Crystal Bowl Sky Blue' pansy seeds were used for all germination studies. Fifty seeds were placed in 9-cm petri dishes containing various priming solutions and incubated in darkness for 3, 6, or 9 d at 23°C . Treatment solutions included CaCl_2 , MgCl_2 , K_2HPO_4 , Na_2HPO_4 , NaCl , KCl , KNO_3 , Na_2SO_4 , with PEG_{15,000} serving as a comparison nonsalt solution. Treatment controls consisted of nontreated dry seeds. All solutions were initially adjusted to pH 7.0 with 1.0N HCl and the osmotic potential adjusted to either -1.0 MPa or -2.0 MPa at 23°C using a

Wescor 5100C Vapor Pressure Osmometer (Wescor Co., Logan, Utah). Petri dishes containing two pieces of Whatman #1 filter paper were filled with 3.5 mL of each solution to partially submerge the seeds. Solutions were changed every day to maintain a constant osmotic potential of the treatments. After priming, seeds were rinsed thoroughly in distilled water and surface-dried on filter paper for 24 h at 23°C . The mucilage layer around the seeds was not removed since seeds were separated and were easily removed from the filter paper. Preliminary experiments in our laboratory with 'Crystal Bowl Sky Blue' and in others with 'Majestic Giant Yellow' pansy seeds (Carpenter and Boucher, 1991) also showed that removal of the layer using a 24% KOH soak did not significantly affect germination (data not shown). After drying, treated and nontreated seeds were germinated in the dark in 9-cm petri dishes containing moistened Whatman #1 filter paper placed in incubators at either 25, 30, or 35°C . Radicle emergence to >2.0 mm was scored as germination and was recorded at 24-h intervals for 10 d. Total germination percentage (G) and mean emergence time (days to 50% of final germination, T_{50}) were recorded using the method of Furutani et al. (1985). Treatments were arranged in a randomized complete-block design with three replications (blocks) per treatment combination and data were tested using analysis of variance (ANOVA).

Germination in growth chamber and greenhouse. In a separate study to compare nonsalt priming using aerated solutions (Carpenter and Boucher, 1991) with salt solution priming as described above, three replications of 25 seeds each were primed at 15°C for 7 d in 150-mL beakers containing an aerated solution of -1.0 MPa PEG₈₀₀₀. For comparison, three 25-seed replications were additionally primed in petri plates containing -1.0 MPa CaCl_2 for 3 d at 23°C . After priming, seeds were rinsed and dried as described above with nontreated, dry seeds serving as controls.

The three 25-seed replications were sown in 406 size ($1.5 \times 1.5 \times 2.0$ cm) plug cells containing Sunshine LP5 plug medium (SunGro Horticulture, Bellevue, Wash.) and germinated either in plant growth chambers held at 25, 30, or 35°C or in the greenhouse during April and July (mean daily highs of 27 and 35°C , respectively). Plant growth chambers were lighted 12 h daily with cool-white fluorescent and incandescent lamps providing $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux while maximum light intensities in the greenhouse were $600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in April and 750 in July. Seeds were covered with 2 mm of screened Sunshine LP5 medium and germinated with clear plastic covers over the trays. The medium and inside of the cover were lightly misted with distilled water. Plug trays were arranged in a completely randomized design. Germination counts were made at the same hour each day in greenhouses and plant growth chambers for those seedlings that emerged through the medium surface. The final emergence and T_{50} were calculated 15 d after sowing. Data were tested using ANOVA.

Received for publication 6 May 1996. Accepted for publication 18 Dec. 1996. Support in part by U.S. Dept. of Agriculture—Competitive Grant No. 93-37100-8922 to BGC. We wish to thank Sakata Seed Co. for providing us with seed. Use of trade names does not imply endorsement of products named nor criticism of similar products not mentioned. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

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Respiration of pansy seeds after priming. Pansy seeds were primed (as before) in either CaCl_2 or PEG_{8000} solutions and prepared for germination. Respiration rates were measured with a Gilson Differential respirometer at 35 °C using standard methods. Gas exchange rates were measured on 0.5 g of dried seeds. Seeds were placed in the gas exchange flask on filter paper placed in the bottom of the vial. The filter paper inside the glass vial insured even wetting of the seeds with the distilled water between respiration readings. The glass vials with seeds were incubated in a Linaire Environmental Chamber in darkness at 35 °C. Oxygen use was determined by treating half of each treatment with a 10% KOH solution placed in wells separate from the distilled water, to absorb carbon dioxide. Respiration measurements were taken initially and recorded every 30 min for 2 h. After measuring respiration, the glass vials were returned to the chamber at 35 °C. The entire procedure was repeated every 24 h for 4 d.

Results and Discussion

Germination in laboratory. The percentage of nonprimed seeds and primed seeds of 'Crystal Bowl Sky Blue' that germinated (G) was significantly lower at 30 or 35 °C than at 25 °C (Table 1). This decrease in G would be expected for most seeds placed under less than optimum germination temperatures. Seeds primed at -1.0 MPa had significantly better G and needed fewer days to reach 50% of final germination (T_{50}) at all temperatures vs. those primed at -2.0 MPa (data not shown). The particular priming solution and the duration of the treatment were also significant factors affecting G and T_{50} . There was, however, a significant interaction between the duration of priming and the priming solution for G and T_{50} . While some treatments were best primed for 3 d, others resulted in better germination after longer priming durations.

The maximum beneficial effects of priming strongly depend on osmoticum, osmotic potential, and treatment duration. The literature suggests that priming solutions with relatively low osmotic potentials (-2.0 MPa) can be detrimental to the seed (Bradford, 1986). In pansy seeds, this appears to be the case, especially under long priming durations. The higher salt concentrations needed to achieve low osmotic potentials may cause greater ionic and osmotic stress, and, therefore, damage, than lower concentrations.

While increasing temperatures resulted in poorer germination, there was significant interaction in G between temperature and priming solution (Table 1). In some cases, nonprimed seeds had higher G at all temperatures vs. primed seeds, indicating the toxic effect of certain priming solutions. Priming did, however, generally reduce T_{50} vs. nonprimed seed. The germination delay in nonprimed seed, especially at unfavorable temperatures, may have been caused by several factors, including slow enzyme activation rate and inhibition of cellular expansion (Cantliffe et al., 1984; Heydecker 1977). From this study,

Table 1. Total germination percentage (G) and days to 50% final germination (T_{50}) of 'Crystal Bowl Sky Blue' pansy seed nonprimed or primed 3, 6, or 9 d with -1.0 MPa priming solution at various germination temperatures in the laboratory.

Priming agent	Temp (°C)	Seed response					
		Duration of priming (days)					
		3	6	9	3	6	9
		G (%)			T_{50} (days)		
None	25	86			4.6		
	30	78			5.0		
	35	42			5.1		
CaCl_2	25	76	78	80	2.9	2.7	2.6
	30	73	75	66	3.2	3.3	2.7
	35	81	52	29	3.5	3.9	3.9
MgCl_2	25	80	82	60	3.2	3.4	2.7
	30	66	68	46	3.3	3.4	4.0
	35	36	56	36	5.3	3.5	4.3
K_2HPO_4	25	64	88	85	4.6	3.6	2.6
	30	47	62	50	5.5	3.5	2.7
	35	30	45	30	4.9	4.0	3.3
Na_2HPO_4	25	74	70	66	4.4	3.3	3.2
	30	30	60	34	3.5	2.9	2.9
	35	31	33	23	5.3	3.4	4.4
NaCl	25	80	68	59	3.1	2.8	3.1
	30	55	56	48	4.0	2.9	3.1
	35	26	55	21	5.0	3.4	4.0
KCl	25	70	60	62	2.9	2.4	3.2
	30	59	63	42	4.3	3.3	2.7
	35	20	23	39	5.9	2.9	3.4
KNO_3	25	46	72	47	3.8	3.1	2.7
	30	30	56	42	5.3	3.6	3.7
	35	14	30	20	6.5	5.3	4.5
Na_2SO_4	25	70	76	46	4.2	3.2	4.0
	30	42	46	32	4.0	3.2	4.0
	35	10	20	18	7.2	4.3	4.5
$\text{PEG}_{15,000}$	25	75	---	---	4.2	---	---
	30	36	---	---	3.9	---	---
	35	28	---	---	4.7	---	---
Significance		G	T_{50}				
Priming solution (PS)		***	***				
Temperature (T)		***	***				
Priming days (PD)		***	***				
PS × T		*	NS				
PS × PD		**	**				
T × PD		NS	NS				
PS × T × PD		NS	*				

^zSeeds germinated during priming.

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively, using analysis of variance. Data are the means of three 50-seed replications.

the best priming treatment to enhance both G and T_{50} at excessively high temperatures was 3 d of priming with CaCl_2 . Priming with $\text{PEG}_{15,000}$ was not effective for this cultivar. After 3 d of priming with $\text{PEG}_{15,000}$, seeds began to germinate during priming, indicating an inappropriate concentration of PEG (Bradford, 1986; Carpenter and Boucher, 1991). The poor results with $\text{PEG}_{15,000}$ contrast with other studies in our laboratory using *Capsicum annuum* L. where we have seen better germination and less anaerobic metabolism using $\text{PEG}_{15,000}$ vs. PEG_{8000} (data not shown).

Germination in growth chamber and greenhouse. Seeds primed in CaCl_2 had markedly improved percent seedling emergence at 35 °C vs. nonprimed seeds (Table 2). Priming did not, however, improve percent emergence at either 25 or 30 °C, although the salt-primed seeds did germinate faster than the nonprimed controls. Emergence counts and rates in plug trays were lower than germination in the laboratory (Table 1) at all temperature regimes, as would be expected with the variability in moisture holding and aeration that one may find in

each particular plug cell. While germination and seedling emergence was significantly better at 35 °C with CaCl_2 as compared to nonprimed seed, it would probably be unacceptable for commercial production, and may only be useful where seeds are being germinated in a very warm environment.

Priming with PEG_{8000} had a negative effect on seedling emergence, especially at temperatures >30 °C (Table 2). The lower total percent emergence and longer T_{50} of PEG-primed seeds may be attributed to lower O_2 availability in PEG_{8000} (Mexal et al., 1975) or to decreased seed hydration (Alvarado and Bradford, 1988). Fay et al. (1994) reported that priming *Rudbeckia fulgida* Ait. in PEG_{8000} rather than KNO_3 generally resulted in lower total germination percentages and longer mean time of germination.

The poor results with PEG_{8000} compared to other similar work (Carpenter and Boucher, 1991) may be due to differences in pansy varieties, drying procedure, and overall priming technique. While PEG_{8000} was effective for 'Majestic Giant Yellow' (Carpenter and

Boucher, 1991), it was not an appropriate priming solution for 'Crystal Bowl Sky Blue.' Compared to salts, PEG₈₀₀₀ solutions are very viscous and may act as a barrier to gas exchange. The seeds could have been coated with PEG during the priming experiment, thereby affecting gas exchange. In earlier research in our laboratory, significant increases in anaerobic metabolism were found in seed primed in PEG, suggesting that the availability of oxygen is limited (Smith and Cobb, 1991). Oxygen concentration, like the availability of water, will strongly affect the rate of seed germination.

In greenhouse experiments, pansy seed germination was severely affected by heat stress. Both primed (CaCl₂) and nonprimed seeds had much poorer emergence for July (<35%) than April (≈84%) sowings. Emergent seedlings in July had poor vigor compared to those that emerged in April, likely due to the extreme temperatures reached in July (sometimes reaching 37 °C). However, primed seeds did germinate faster and more uniformly than the controls. The respective T₅₀ values for April were 5.4 and 7.5 d and for July they were 7.4 and 12.4 d, both differences being significant at $P \leq 0.05$.

Results in this study followed similar trends as the growth chamber studies, with the beneficial effects of priming appearing only at higher temperatures. Cantliffe et al. (1984) reported that priming with salts was effective in improving *Lactuca sativa* L. germination at 35 °C. In this study, priming with salt did reduce some of the negative effects of high temperature on 'Crystal Bowl Sky Blue' germination. For this cultivar, bedding plant growers should expect an improvement in G and T₅₀ by using salt-primed seed only under high temperatures. However, the thermoinhibition may never be completely overcome; primed seed sown in July did not improve germination to that of nonprimed seed sown in April.

Respiration of seeds after priming. The early stages of germination for all seeds were accompanied by an increase in respiratory rate (Fig. 1). The respiration rates of seeds primed with -1.0 MPa CaCl₂, redried, and germinated in distilled water were significantly higher ($P \leq 0.01$) than either PEG₈₀₀₀ primed seeds or nonprimed seeds. These findings agree with results of Smith and Cobb (1991) who reported that respiration rates of *Capsicum annuum* L. seeds primed for 12 and 18 d in a -0.90 MPa solution and germinated in distilled water were significantly higher than nonprimed seeds or those primed with lower osmotic potentials. After 4 d, radicle protrusion through the testa was visible only on seeds primed with CaCl₂, indicating an increase in nutrient reserve mobilization and metabolic activity (Bewley and Black, 1994) vs. nonprimed or PEG₈₀₀₀ primed seeds.

In conclusion, these studies indicate that select salt solutions can be used to improve pansy seed germination at supraoptimal tem-

Table 2. Effect of priming solution on total percent seedling emergence and days to 50% of final seedling emergence (T₅₀) of 'Crystal Bowl Sky Blue' pansy seed at various germination temperatures. Seeds were germinated in plug trays placed in growth chambers.

Priming agent	Seed response					
	Germination temp (°C)					
	25	30	35	25	30	35
	Emergence (%)			T ₅₀ (days)		
CaCl ₂	55 a ^z	59 a	52 a	8.9 a	8.6 c	8.3 b
PEG ₈₀₀₀	50 b	32 b	17 c	9.2 a	9.5 b	9.5 ab
None	68 a	60 a	35 b	9.7 a	10.3 a	9.3 a

^zMean separation within columns for each variable using Duncan's multiple range test at $P \leq 0.05$.

Fig. 1. Respiration of whole 'Crystal Bowl Sky Blue' pansy seeds that were primed with CaCl₂ (○—○) and PEG₈₀₀₀ (●—●) solutions. Dry, nonprimed seeds germinated in deionized water (■—■) were used as controls. Error bars, where larger than symbol size, indicate ± standard error of the mean.

peratures, however, seed performance in plug trays may not be as good as in laboratory tests. Bedding plant producers wishing to use this technology should experiment with several pansy cultivars before selecting an appropriate treatment.

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