Priming Improves High-temperature Germination of Pansy Seed

William J. Carpenter

Department of Environmental Horticulture, Institute of Food and Agricultural Sciences, Gainesville, FL 32611

Joseph F. Boucher

Department of Statistics, Institute of Food and Agricultural Sciences, Gainesville, FL 32611

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Abstract. The optimum conditions for priming pansy (*Viola × wittrockiana*) seeds were in aerated osmotic solutions of polyethylene glycol 8000 (PEG 8000) at – 1.0 MPa for 7 days at 15C. Priming at – 0.8 MPa and 15C caused 8% to 26% of seeds to germinate during 4 to 13 days of priming. Increasing numbers of abnormal seedlings developed when priming was extended beyond 7 days. Final germination percentages were better in laboratory studies at 35C for primed (51%) than nonprimed (10%) seeds. Removal of the mucilage released by the seed with 240 g KOH or 170 g NaOH/liter for 15 or 30 seconds during priming did not affect total germination percentages, but did improve seed handling. Primed seed had higher, faster, and more uniform germination than nonprimed seeds after sowing in growing medium in plant growth chambers or greenhouses.

Pansy is an annual or short-lived perennial widely grown as a bedding plant and occasionally produced for cut flowers. Pansies are cold-tolerant (Post, 1949) and popular for landscaping in regions with cool spring and summer nights, such as northern Europe and the northern regions of the United States. In warm regions of the Northern Hemisphere, pansies are used for fall and winter landscaping, which requires sowing seeds during the hot months. Imbibed pansy seed placed at 30C or above enter a high-temper-

ature-induced thermoinhibition stage that causes low total germination (unpublished data), similar to that in lettuce (Guedes and Cantille, 1980).

During seasons of high temperatures, most commercial producers germinate pansy seeds in 18 to 21C refrigerated rooms and later transplant the seedlings to production flats in evaporatively cooled greenhouses at reduced light intensities. Some producers directly sow seeds in "plug" trays in refrigerated rooms, with trays of seedlings later moved into production greenhouses.

Seed priming in osmotic solutions of PEG 8000 or K₂PO₄ and KNO₃ salts as osmotica improved the germination of impatiens (*Impatiens wallerana* Hook) (Simmonds, 1980), lettuce (*Lactuca sativa* L.) (Cantliffe, 1981), salvia (*Sahia splendens* F.) (Carpenter, 1989), and tomato (*Lycopersicon esculentum*) (Rumpel and Szudyga, 1978) at

excessively high temperatures. Priming also shortened emergence time, resulted in more uniform germination, and occasionally increased total germination of some seeds (Heydecker, 1977). No recommendations have been reported for priming pansy seed. The major objectives of this research were to develop procedures for priming pansy seed and to determine if priming enhances germination at sub- and supra-optimal temperatures.

Seed priming. Seeds of 'Majestic Giant Yellow' and 'Majestic Giant Red' pansy were primed in 80-ml test tubes containing aerated PEG 8000 solutions at – 0.8, – 1.0, or – 1.2 MPa for 4, 7, 10, or 13 days at 15C. The water potential of the PEG 8000 solutions was measured at 15C with a Wescor vapor pressure osmometer (Wescor Co., Logan, Utah). Following priming, seeds were washed

Table 1. Effect of germination temperature on criteria of germination' of pansy seed non-primed or primed 7 days with – 1.0 MPa PEG at 15C.

Seed treatment	Temp (°C)	Germination		
		G (%)	T ₅₀ (days)	T ₉₀ – T ₁₀ (days)
Nonprimed	10	75	11	15
	15	85	5	6
	20	90	4	4
	25	84	4	4
	30	56	4	5
	35	10	5	6
Primed	10	84	6	8
	15	87	4	5
	20	91	3	3
	25	88	3	3
	30	84	3	3
	35	51	4	5
Significance				
Temperature (T)		**	**	**
Treatment (TR)		**	**	**
T × TR		**	**	**

'G' = total germination percentage; T₅₀ = days to 50% germination; T₉₀ – T₁₀ = days elapsed between 10% and 90% germination.

**Significant at P = 0.01, data are the means of 400 seeds.

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Fig. 1. The effect of PEG 8000 concentration and priming duration at 15C on subsequent total germination percent (A), days to 50% germination (T_{50}) (B), and days elapsed between 10% and 90% germination ($T_{90} - T_{10}$) (C) of primed pansy seed germinated at 20C.

Fig. 2. Cumulative germination of seeds sown in plug trays in plant growth chambers at constant 25C (A), 30C (B), or 35C (C), comparing seed primed with -1.0 MPa PEG 8000 at 15C for 7 days with nonprimed seed of 'Majestic Giant Purple' pansy. The estimated values for total germination percent (G), days to 50% of final germination (T_{50}), and rate of increase (K) were obtained with the equation: % Germination = $G / (1 + e^{-k(\text{day} - T_{50})})$, and compared for primed and nonprimed seeds at each temperature using asymptotic 95% confidence intervals.

		25C	30C	35C
$\hat{G}$	Nonprimed	85 a	48 b	2 b
	Primed	92 a	69 a	35 a
$\hat{T}_{50}$	Nonprimed	8 a	8 a	9 a
	Primed	6 b	5 b	5 b
$\hat{K}$	Nonprimed	1.0 b	1.1 b	1.2 b
	Primed	1.6 a	1.6 a	1.8 a

with 100 ml of distilled water (DW), surface moisture was removed by 30 min of vacuum aspiration, and stored for 2 days at SC and

45% relative humidity (RH) before germination at 20C. Treatments consisted of four 100-seed replications, each germinated in 9-

Table 2. Total germination percentage of primed and nonprimed pansy seed after removal of the mucilaginous seed cover by chemical soaking treatment.

Seed treatment	Germination of 20C (%)			
	Chemical	Concn (%)	Soaking (min)	Non-primed / Primed
None	---	---	---	89 / 90
NaOH	17	0.25	86	88
	17	0.5	92	90
KOH	24	0.25	93	87
	24	0.5	90	85
NaOCl	1.3	3.0	91	92
	2.6	3.0	88	86
	3.9	3.0	76	53
Significance				
Tukey's HSD ($P = 0.05$)				7 / 8

cm petri dishes containing two Whatman No. 1 filter papers moistened with 5 ml of DW; seeds were germinated in the dark. Daily germination counts were made of seeds with visible radicle protrusion through the testa. Total germination percentage (G), mean emergence time [days to 50% of final germination T_{50} , using the formula of Furutani et al. (1985)], and span of emergence in days between 10% and 90% ($T_{90} - T_{10}$), obtained by probit analysis (Ingram and Buchanan, 1984), were calculated for this and subsequent studies. The experimental design was a randomized complete block arranged as a $4 \times 3 \times 2$ factorial, with data tested using an analysis of variance (ANOVA) and a multiple regression analysis.

Germination temperature. Laboratory studies compared the seed germination of 'Majestic Giant Yellow' after priming in aerated solutions of -1.0 MPa PEG 8000 for 7 days at 15C. Seeds were prepared, as described above, before germination in incubators at a constant 10, 15, 20, 25, 30, or 35C. Treatments consisted of four 100-seed replications. Daily counts were made of seeds with emerged radicles. The experimental design was a randomized complete block arranged as a 3×6 factorial, with G, T_{50} , and $T_{90} - T_{10}$ analyzed by ANOVA.

Removal of mucilaginous seed cover. 'Majestic Giant Yellow' pansy seeds were primed in aerated -1.0 MPa PEG 8000 solutions for 7 days at 15C, then washed with 100 ml of DW, and surface-dried. Treatments containing four 100-seed replications were placed in 240 g KOH or 170 g NaOH/liter for 15 or 30 sec or in 13, 26, or 39 g NaOCl/liter for 3 min (Robinson, 1983). Seeds were placed in 10-ml glass vials, the solution was added, and the container was sealed and shaken for the allotted period before removing the solvent by filtration through a screen. Seeds were rinsed immediately with DW for 5 min, air-dried on filter paper overnight, and germinated at 20C in petri dishes as previously described. The experimental design was a randomized complete block with data analyzed by ANOVA and Tukey's honestly significant difference test at $P = 0.05$.

Direct seeding. Seeds were primed in aerated solutions and prepared as described

Table 3. Effect of constant 25, 30, or 35C temperatures in plant growth chambers and 32C days/24C nights in greenhouse trials on G, T_{50} , and $T_{90} - T_{10}$ of pansy seed nonprimed or primed 7 days in PEG 8000 at -1.0 MPa and 15C.

Trial	Seed treatment	Temp (°C)	Germination ^a		
			G (%)	T_{50} (days)	$T_{90} - T_{10}$ (days)
Growth chamber	Nonprimed	25	83	8	5
		30	55	8	5
		35	3	---	---
	Primed	25	92	6	3
		30	72	6	4
		35	36	7	5
Greenhouse	Nonprimed	24/32	79	6	4
	Primed	24/32	91	5	4
Significance					
Temperature (T)			**	NS	*
Treatment (TR)			**	*	*
T × TR			**	*	NS

^aSee Table 1 for definition of terms.

NS, *, **Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively.

above. Treatments containing four 112-seed replications were sown in 392-cell "plug" trays containing moist Vergro Transplant Mix B (Vergro Co., Tampa, Fla.). Seeds were covered with 2 mm of screened medium and germinated with clear plastic covers over the trays. The inside of the cover and medium were syringed daily with DW to maintain a high RH. Plant growth chambers were held at 25, 30, or 35C, while the daily mean highs and lows in the greenhouse were 32 and 24C. Growth chambers were lighted 12 h daily with cool-white fluorescent and incandescent lamps providing $213 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ and $118 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ photosynthetic photon flux, respectively, and the maximum greenhouse light intensity was $455 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$. Germination counts were made at the same hour each day in greenhouses and plant growth chambers of those seedlings that emerged through the medium surface. The experimental design was a randomized complete block, with the data for G of primed and nonprimed seeds at 25, 30, or 35C plant growth chambers analyzed by nonlinear regression procedures using SAS (SAS Institute, 1985) proc nlin, with estimated values compared using the asymptotic 95% confidence intervals.

Germination responses were similar for 'Majestic Giant Yellow' and 'Majestic Giant Red' primed at each PEG 8000 concentration; therefore, data for both cultivars were combined in Fig. 1. No differences were found among blocks, so blocks were not included in the regression analysis. Analysis of the response to G resulted in a regression equation with four terms: a) linear x linear (PEG 8000 concentration x priming duration) interactive terms, b) a negative linear term for priming duration, c) linear, and d) quadratic terms for PEG 8000 concentration. A significant interaction existed at the -0.8 MPa priming level, where a decreasing trend in G paralleled an increase in priming duration from 4 to 13 days (Fig. 1A). At the -1.2 MPa priming concentration, G increased as the priming duration increased from 4 to 13 days. A trend of increasing G occurred as priming concentrations were in-

creased from -0.8 MPa to a maximum at -1.1 MPa, then declining to -1.2 MPa. As a result of the quadratic trend, no differences in G were found at -1.0 or -1.2 MPa for 10 and 13 days, but at 4 and 7 days priming, the G at -1.0 MPa was higher. Lower G resulted from -0.8 MPa than from -1.0 or -1.2 MPa concentrations at all durations of priming (Fig. 1A). Reduced G resulted at -0.8 MPa because of seed radicle emergence during priming. Elongation of the seed radicle during priming in -0.8 MPa osmoticum resulted in reduced G after seed drying and storage for 2 days. Germination of 8%, 17%, 21%, and 26% of seed occurred during priming for 4, 7, 10, and 13 days at 15C, respectively. These germination data were not included for -0.8 MPa in Fig. 1.

The analyses for days to T_{50} and $T_{90} - T_{10}$ showed trends similar to those for G. The days for both declined as the duration of priming increased (Fig. 1 B and C), with linear and quadratic responses with changes in PEG 8000 concentrations. The linear and quadratic trend lines for osmotic concentrations in days to T_{50} and $T_{90} - T_{10}$ progressively increased from -0.8 MPa to 1.2 MPa. The rates of increase were greater from -0.8 MPa to -1.0 MPa than from -1.0 MPa to -1.2 MPa (Fig. 1 B and C). The negative linear term for priming duration indicated that the days to T_{50} or $T_{90} - T_{10}$ will decrease as the priming duration increased from 4 to 13 days, regardless of the concentration of the osmotic solution.

The benefits from seed priming were found to vary with germination temperature. At 15 to 25C, the recommended temperature range for pansy germination, G was similar for primed and nonprimed seeds (Table 1). Nonprimed seed germination percentages decreased with temperatures above or below the 15 to 25C range, but G decreased less in primed treatments. This pattern resulted in primed seeds having significantly higher G at 10, 30, or 35C than nonprimed seeds. Seeds primed had 5 times the G at 35C of nonprimed seed (10% vs. 51%).

Seeds primed had lower T_{50} and $T_{90} - T_{10}$ than nonprimed seeds. Treatment differ-

ences were smaller at favorable germination temperatures of 20 to 30C and increased at 10, 15, or 35C. The germination delay of nonprimed seed at unfavorable temperatures may have resulted from slower reactivation of essential enzymes. Heydecker (1977) and Heydecker and Coolbear (1977) reported that hydration of seeds during priming enhanced the embryos' ability to synthesize protein and RNA, resulting in earlier germination even under unfavorable environments.

Soaking primed and nonprimed 'Majestic Giant Yellow' pansy seed in 170 g NaOH or 240 g KOH/liter for 15 or 30 sec or 13 to 26 g NaOCl/liter for 3 min removed most of the mucilaginous seed cover. The chemical concentrations and durations of treatments used, except 39 g NaOCl/liter for 3 min, had no effect on G (Table 2). Both reduced G, and the latter treatment injured the seedlings. No quantitative measurements of remaining seed mucilage were made, but seed handling and storage were improved. The problem of primed or imbibed pansy seeds being tightly bound together by the mucilage after drying was greatly reduced.

Seed primed in aerated PEG 8000 osmoticum at -1.0 MPa for 7 days at 15C and prepared and stored 3 days had higher G than nonprimed seeds when sown directly in plug trays and germinated in plant growth chambers or greenhouses (Table 3). Primed seeds germinated in plant growth chambers at constant 2.5 or 30C had 9% or 17%, respectively, higher G than nonprimed seed (Table 3). Primed seed sown directly and germinated in plant growth chambers required fewer days to T_{50} and $T_{90} - T_{10}$ than nonprimed seed. Primed seed T_{50} at 2.5 and 30C required 35% and 32% fewer days, respectively, than nonprimed seeds. The emergence spans ($T_{90} - T_{10}$) for primed seed at 25 and 30C were 34% and 12% shorter, respectively, than nonprimed seeds (Table 3). No differences were found between the cumulative germination curves for primed and nonprimed seed at 25C in plant growth chambers, but significantly larger total germination percentages for primed seed were found at 30 and 35C (Fig. 2). The periods of rapid germination occurring between days 5 and 8 at 25C became progressively decreased at 30C and 35C for primed and nonprimed seeds, but primed seeds continued to have higher G. Primed seed sown directly in growing medium and germinated in greenhouses at temperatures alternating between 32C days/24C nights had G 12% higher than nonprimed seed (Table 3). The T_{50} and $T_{90} - T_{10}$ for primed seed were 13% and 23% shorter, respectively, than for nonprimed seeds.

The results from the laboratory experiments showed that priming seeds for 7 days at 15C was best using PEG 8000 at -1.0 MPa. A higher incident of abnormal seedlings was found when priming was increased from 7 to 13 days. Only 2% of the seedlings were abnormal following priming for 7 days at 15C, and 9% and 16% after priming for 10 and 13 days, respectively. The major abnormality was a delay in or failure of the leaves to emerge from the seed. Bewley and Black (1986) report a similar condition can result when imbibed seeds

are maintained for extended periods under anaerobic conditions.

Pansies are cold-tolerant, but G of non-primed seed declined at constant 10C, and days to T_{50} and $T_{90} - T_{10}$ at 10 and 15C were significantly increased. Seed priming at 15C limited the reduction in G at suboptimal germination temperatures and significantly reduced the days to T_{50} and $T_{90} - T_{10}$. Although priming reduces the time required for germination, it cannot replace more favorable temperatures. These results are similar to those of O'Sullivan and Bouw (1984), who concluded that low-temperature germination and emergence of pepper (*Capsicum annum* L.) seed was accelerated by seed priming.

Nonprimed seeds had large reductions in G at constant 30 and 35C, with few imbibed seeds germinating at 35C. Foard and Haber (1966) reported that imbibed lettuce seeds that failed to germinate at 35C had high respiration rates and continued cell division. They believed high temperatures prevented the cell elongation needed for radicle protrusion to occur. Heydecker and Coolbear (1977) reported that seeds complete a high-temperature-sensitive phase during priming, thereafter permitting germination at otherwise unfavorable temperatures.

In our studies, although 36% to 51% of primed pansy seeds germinated at constant 35C, radicle lengths were reduced and emergent seedlings lacked the vigor necessary for commercial production. Comparing germination in plant growth chambers at constant 35C with greenhouses at daily 24 to 32C alternating temperatures showed that those seeds receiving alternating temperatures had significantly higher G and reduced T_{50} and $T_{90} - T_{10}$. The capacity of primed seed to achieve 91% G at alternating 24 to 32C, while maintaining adequate seedling vigor and quality, indicated the potential exists for germination of direct-seeded primed pansy seeds in greenhouses at some supra-optimal temperatures.

Literature Cited

- Bewley, J.D. and M. Black. 1986. Seeds: Physiology of development and germination. Plenum Press, London.
- Cantliffe, D.J. 1981. Priming of lettuce seed for early and uniform emergence under conditions of environmental stress. *Acta. Hort.* 122:29-38.
- Carpenter, W.J. 1989. *Salvia splendens* seed pre-germination and priming for rapid and uniform plant emergence. *J. Amer. Soc. Hort. Sci.* 114:247-250.
- Foard, D.E. and A.H. Haber. 1966. Mitosis in thermodormant lettuce seeds with reference to histological location, localized expansion, and seed storage. *Planta* 71:160-170.
- Furutani, S. C., B. H. Zandstra, and H.C. Price. 1985. Low temperature germination of celery seeds for fluid drilling. *J. Amer. Soc. Hort. Sci.* 110:153-156.
- Guedes, A.C. and D.J. Cantliffe. 1980. Germination of lettuce seeds at high temperature after seed priming. *J. Amer. Soc. Hort. Sci.* 105:777-781.
- Heydecker, W. 1977. Stress and seed germination: An agronomic view, p. 237-276. In: A.A. Khan (ed.). *The physiology and biochemistry of seed dormancy and germination*. North Holland Publishing Co., Amsterdam.
- Heydecker, W. and P. Coolbear. 1977. Seed treatments for improved performance-survey and attempted prognosis. *Seed Sci. Technol.* 5:353-425.
- Ingram, D.L. and D.W. Buchanan. 1984. Lethal high temperatures for roots of three citrus rootstock. *J. Amer. Soc. Hort. Sci.* 109:189-193.
- O'Sullivan, J. and W.J. Bouw. 1984. Pepper seed treatment for low-temperature germination. *Can. J. Plant Sci.* 64:387-393.
- Post, K. 1949. *Florist crop production and marketing*. Orange-Judd, New York.
- Robinson, T. 1983. *The organic constituents of higher plants*. Cordus Press, North Amherst, Mass.
- Rumpel, J. and I. Szudyga. 1978. The influence of pre-sowing seed treatments on germinations and emergence of tomato 'New Yorker' at low temperatures. *Sci. Hort.* 9:119-125.
- SAS Institute. 1985. *SAS user's guide: Statistics*. version 5 ed. SAS Institute, Cary, N.C.
- Simmonds, J. 1980. Increasing seedling establishment of *Impatiens wallerana* in response to low temperature or polyethylene glycol seed treatments. *Can. J. Plant Sci.* 60:561-569.