Inclusion of Ethylene Precursor into Priming Solution Improves Germination and Emergence of Narrow-leaved Purple Coneflower Seeds

Ahmet Korkmaz^{1, 3},
Mehmet Nuri Nas¹,
Nusret Ozbay¹, and
Iskender Tiryaki²

ADDITIONAL INDEX WORDS. *Echinacea angustifolia*, dormancy, stratification

SUMMARY. The effects of stratification and priming on germination and emergence performance of narrow-leaved purple coneflower (*Echinacea angustifolia*) seeds were investigated. Seeds were pre-chilled for 3 weeks at 4 ± 0.5 °C (39.2 ± 0.9 °F) in light or primed for 3 days at 20 ± 0.5 °C (68.0 ± 0.9 °F) in darkness in Nas and Read medium (NRM) or in 2% potassium nitrate (KNO_3) supplemented with 3 or 5 μM 1-aminocyclopropane carboxylic acid (ACC) or 500 $\text{mg}\cdot\text{L}^{-1}$ (ppm) or 1000 $\text{mg}\cdot\text{L}^{-1}$ gibberellic acid (GA_3). Following stratification and priming, seeds were subjected to germination and emergence tests at 25 ± 0.5 °C (77.0 ± 0.9 °F). Priming the seeds in NRM or KNO_3 containing 3 μM ACC gave the highest germination percentages with 78% and 80%, respectively. Stratification alone increased germination to 69% compared to nontreated seeds, which had the lowest germination percentage of 57%. Emergence was enhanced by priming seeds in the presence of 3 μM ACC (75%) compared to stratified seeds (62%), while nontreated seeds had the lowest emergence percentage

of 26%. These results indicate that priming in the presence of ACC might be an alternative to lengthy stratification treatments to break the dormancy and improve the germination and emergence of narrow-leaved purple coneflower seeds.

The genus *Echinacea* (family Asteraceae) is native to North America and consists of nine species, all of which are commonly referred to as purple coneflowers (McGregor, 1968). The three most common species are the common purple coneflower (*Echinacea purpurea*), the pale purple coneflower (*Echinacea pallida*), and the narrow-leaved purple coneflower. All of the species are perennials, with many flowered heads, and have medicinal properties (Schulthess et al., 1991). Purple coneflowers produce compounds that appear to possess strong anti-inflammatory and wound-healing activities and stimulate the immune system (Cox, 1998). Additionally, they are also grown for their ornamental values, with flower color ranging from white to rose-pink to dark red (Cox, 1998).

Purple coneflowers can be propagated by achenes (referred to here as seeds), division of off-shoots from crown (Feghahati and Reese, 1994); however, propagation by seeds is limited by embryo or seedcoat dormancy and usually requires light, scarification, or stratification (a period of cold-moist treatment) to increase germination (Baskin et al., 1992). All purple coneflowers have some degree of germination problem, narrow-leaved purple coneflower being the most difficult to germinate. Although stratification alone can break dormancy to some extent, there are conflicting results in the literature as to the required duration, which varied from 2 to 12 weeks (Parmenter et al., 1996). Baskin et al. (1992) reported that 12 weeks of stratification at 5 °C (41.0 °F) in the presence of light increased narrow-leaved purple coneflower germination percentage, while Li (1998) recommended only 4 weeks of stratification at 4 °C.

The use of ethylene has been reported to improve narrow-leaved purple coneflower germination by breaking the seed dormancy. Feghahati and Reese (1994) reported that a 2-week stratification treatment combined with ethephon (an ethylene-releasing compound) and continuous light, fol-

lowed by a 2-week germination period at 25 °C, could induce >90% seed germination. Sari et al. (1999) also demonstrated that germination rates of greater than 90% can be achieved when seeds are treated with either 1 mM ethephon or 2500 $\text{mg}\cdot\text{L}^{-1}$ GA_3 during cold stratification for 8 weeks.

Priming or osmoconditioning consists of the incubation of seeds in an osmoticum, usually a salt or polyethylene glycol (PEG) solution, in order to control their water uptake and prevent radicle protrusion (Bray, 1995). Common purple coneflower seeds primed in PEG for 10 d had greater rate, synchrony, and percentage of germination and emergence compared to non-primed seeds (Pill et al., 1994). Samfield et al. (1990) also reported that priming in phosphate buffer (50 mM) for 6 d improved germination rate and synchrony of common purple coneflower. Very little information, however, has been reported in the literature about using seed priming to improve germination of narrow-leaved purple coneflower. The objective of the present study was to investigate if combining seed priming with plant growth regulators would be an alternative method to stratification as a mean to improve germination and emergence of this species.

Materials and methods

Mature seeds of narrow-leaved purple coneflower, harvested in 2002, were purchased from Johnny's Selected Seeds Co. (Winslow, Maine). All the experiments reported in this study were carried out in Fall 2003 at Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey.

During the entire experiment to prevent subsequent pathogen growth, the seeds were disinfested in 1% (a.i.) sodium hypochlorite for 10 min. Following disinfestation, they were rinsed under running tap water for 1 min and surface dried by placing them between paper towels for 30 min at room temperature.

Single layers of seeds [2 g (0.07 oz)] were placed on double layers of Whatman #1 filter paper saturated with 10 mL (0.34 fl oz) distilled water in $10 \times 10 \times 4$ -cm ($3.9 \times 3.9 \times 1.6$ inches) covered transparent polystyrene germination boxes (Ater Plastik, Kocaeli, Turkey). The germination boxes were kept in an incubator at 4 ± 0.5 °C for 3 weeks under cool fluorescent lamps

¹Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Horticulture, Kahramanmaras, Turkey, 46060.

²Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Field Crops, Kahramanmaras, Turkey, 46060.

³To whom reprint requests should be addressed. E-mail address: akorkmaz@ksu.edu.tr

providing a continuous photosynthetic photon flux density (*PPFD*) of 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Moisture levels were maintained in the boxes by adding equal amounts of distilled water into each box as needed. Following stratification, the seeds were transferred to a growth chamber for germination and emergence tests.

Preliminary experiments were conducted to determine the length of the priming duration, and we found that priming in KNO_3 for 3 d was the optimal duration (data not shown) since seeds started to germinate in priming solution after 3 d. Seeds (2 g) were primed in 2% KNO_3 (-0.87 MPa) solution for 3 d at 20 ± 0.5 °C under cool fluorescent lamps providing a continuous *PPFD* of 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ containing one of the following: 3 or 5 μM ACC (precursor of ethylene), or 500 or 1000 $\text{mg}\cdot\text{L}^{-1}$ GA_3 (Sigma Aldrich, St. Louis, Mo.). Seeds were also primed in full-strength NRM (-0.21 MPa) (Nas and Read, 2004) containing no plant growth regulators. NRM was developed based on mineral and vitamin content of raw hazelnut (*Corylus avellana*) kernels for in vitro micropropagation of hazelnut. Nas and Read (2004) reported that a composition of minerals and vitamins in proportions similar to those found in the seeds provided an optimum culture medium for shoot growth. Therefore, in the current study NRM was used to explore if it could be an alternative priming solution enhancing vegetative growth in seeds (i.e., seed germination and emergence) compared to other known priming solutions. Seeds were placed in covered germination boxes on double layers of filter paper saturated with 8 mL (0.27 fl oz) of one of the priming agents, which were changed every day to maintain a constant osmotic potential. Following priming, the seeds from each box were washed in a sieve and rinsed under running tap water for 1 min and subjected to germination and emergence tests along with stratified and nontreated seeds.

Germination tests were conducted at 25 ± 0.5 °C in darkness. Seeds were placed on two layers of filter paper moistened with 3 mL (0.10 fl oz) of distilled water in covered 5.5-cm-diameter (2.17 inches) petri dishes. Treatments were arranged in a completely randomized design with four replications of 50 seeds. Radicle protrusion to 1 mm was scored as germination.

Seed germination was recorded daily until the numbers stabilized (for 10 d) and germinated seeds were removed. From the total number of seeds germinated, final germination percentage (FGP) and its angular transformation ($\arcsine\sqrt{\text{FGP}}$), days to 50% of FGP, and days between 10% and 90% of FGP were calculated (Murray et al., 1993). Time to 50% of FGP (G_{50}) is an inverse measure of germination rate, while time between 10% and 90% of FGP (G_{10-90}) is considered to be an estimate of the spread of germination, the inverse of germination synchrony.

For emergence test, 25 seeds from each treatment were planted at 1.0-cm (0.4 inch) depth in 15 × 4 cm (5.9 × 1.6 inches) (diameter × height) round plastic germination cups filled with peat-lite. The cups were watered and placed in a growth chamber at 25 ± 0.5 °C and under cool fluorescent lamps providing a *PPFD* of 40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for 14 h·d⁻¹ at the seedling level. The treatments were replicated four times and all cups were arranged in a randomized complete-block design in the growth chamber. The numbers of seedlings emerged (hypocotyl arc visible) were recorded daily until the number of emerged seedlings stabilized (for 14 d). Final emergence percentage (FEP) and its angular transformation ($\arcsine\sqrt{\text{FEP}}$), days to 50% of FEP (E_{50}), and days between 10% and 90% of FEP (E_{10-90}) were calculated. Shoots were cut at the medium surface and their fresh weights were determined 14 d after planting.

Germination and emergence data were tested by analysis of variance. Mean separation was performed by the Fisher's least significant difference at $P = 0.05$.

Results

Priming or stratifying narrow-leaved purple coneflower seeds significantly improved germination percentage at 25 °C compared to nontreated seeds (Table 1). Among the priming treatments involving plant growth regulators, only inclusion of ACC (3 or 5 μM) into KNO_3 solution further increased FGP, while adding GA_3 had no significant effect on FGP compared to KNO_3 alone. Priming in KNO_3 supplemented with 3 μM ACC (80%) and NRM (78%) were the only treatments to give significantly higher FGP than stratification (69%) and adding either concentration of GA_3 into the priming solutions resulted in similar FGP as stratified seeds. Nontreated seeds had the lowest FGP of all with 57%.

All treatments hastened the germination of narrow-leaved purple coneflower seeds compared to nontreated seeds (Table 1). Seeds primed in NRM had the fastest germination as indicated by the lowest G_{50} of 0.95 d, which was 1 d faster than stratified seeds and 3 d faster than nontreated seeds. Priming in the presence of either concentration of ACC and KNO_3 alone also led to faster germination than stratified seeds. Germination of nontreated seeds was very slow, requiring 4.04 d to reach G_{50} .

Table 1. Final germination percentage (FGP) and angular transformation of FGP (degrees), days to 50% of FGP (G_{50}) and days between 10% and 90% germination (G_{10-90}) of narrow-leaved purple coneflower seeds in darkness at 25 °C (77.0 °F) for 10 d following stratification at 4 °C (39.2 °F) for 3 weeks or priming for 3 d at 20 °C (68.0 °F) in Nas and Read medium (NRM) or 2% potassium nitrate (KNO_3) with various concentrations of 1-aminocyclopropane carboxylic acid (ACC) or gibberellic acid (GA_3).

Treatments ^a	FGP		G_{50} (d)	G_{10-90} (d)
	(%)	(degrees)		
KNO_3 + 3 μM ACC	80	64	1.39	2.19
KNO_3 + 5 μM ACC	77	61	1.43	1.93
KNO_3 + 500 $\text{mg}\cdot\text{L}^{-1}$ GA_3	69	57	1.84	1.68
KNO_3 + 1000 $\text{mg}\cdot\text{L}^{-1}$ GA_3	70	57	1.94	2.02
KNO_3	66	54	1.59	2.07
NRM	78	62	0.95	1.76
Stratification	69	57	1.97	2.95
Nontreated seeds	57	49	4.04	3.24
LSD _{0.05}		(4)	0.20	0.25
Significance		***	***	***

^a1 $\text{mg}\cdot\text{L}^{-1}$ = 1 ppm; LSD_{0.05} = Fisher's least significant difference at $P = 0.05$.

***Significant at $P \leq 0.001$.

All priming treatments significantly improved the germination synchrony of narrow-leaved purple coneflower seeds compared to stratified and nontreated seeds (Table 1). Priming in KNO_3 supplemented with $500 \text{ mg} \cdot \text{L}^{-1} \text{ GA}_3$ ($G_{10-90} = 1.68 \text{ d}$) and NRM ($G_{10-90} = 1.76 \text{ d}$) resulted in more synchronous germination than seeds primed in KNO_3 only ($G_{10-90} = 2.07 \text{ d}$), while inclusion of either concentration of ACC into KNO_3 did not further improve germination synchrony over KNO_3 alone. The least synchronous germination was obtained from nontreated seeds ($G_{10-90} = 3.24 \text{ d}$) followed by stratification ($G_{10-90} = 2.95 \text{ d}$).

Priming or stratification improved seedling emergence compared to nontreated seeds (Table 2). Among the priming treatments, the highest FEP was achieved when the seeds were primed in the presence of $3 \mu\text{M}$ ACC (75%), which was the only treatment with significantly higher FEP than the stratification alone (62%). Even though the FGP of nontreated seeds was 57% (Table 1), their FEP was quite low (26%).

All seed treatments improved emergence rate of narrow-leaved purple coneflower compared to nontreated seeds (Table 2). The fastest emergence rates, however, were obtained when the seeds were primed in the presence of 3 and $5 \mu\text{M}$ ACC added to the KNO_3 solution, as indicated by the lowest E_{50} values of 3.96 and 3.79 d, respectively. In addition to priming in the presence

of either concentration of ACC, seeds primed in the presence of $500 \text{ mg} \cdot \text{L}^{-1} \text{ GA}_3$ added to the priming solution emerged faster than stratified seeds. Priming the seeds in NRM and KNO_3 only resulted in similar emergence rates as stratification. Nontreated seeds emerged the slowest of all, requiring 8.01 d to reach E_{50} .

All treatments failed to improve the emergence synchrony (Table 2). Even though all seed treatments gave greater shoot fresh weights than nontreated seeds, priming in the presence of either concentration of GA_3 were the only treatments with significantly higher shoot fresh weight than stratification (Table 2).

Discussion

One of the basic obstacles in field production of purple coneflower is the poor and erratic seed germination. Baskin et al. (1992) found that fresh mature seeds of narrow-leaved purple coneflower germinated only 6% when they were placed under light and 2% when placed in darkness, but that a 12-week cold stratification treatment at 5°C broke dormancy. In another study, no germination was observed when narrow-leaved purple coneflower and pale purple coneflower were directly sown in the spring (Smith-Jochum and Albercht, 1988). Li (1998) recommended stratification of seeds in moist sand at 1 to 4°C (33.8 to 39.2°F) for 4 to 6 weeks. In the present study, we found that priming in KNO_3 supplemented with $3 \mu\text{M}$ ACC or NRM

for 3 d resulted in greater germination percentages compared to seeds stratified for 3 weeks. Among the priming treatments, however, priming in the presence of $3 \mu\text{M}$ ACC was the only treatment resulting in significantly higher final emergence percentages than stratification alone.

The mechanisms controlling seed dormancy in purple coneflower are not well understood. Embryo dormancy or dormancy imposed either mechanically or chemically by the pericarp, testa, or other surrounding tissues may be involved (Feghahati and Reese, 1994). The limitations to propagation of purple coneflowers are attributable, at least partially, to a semipermeable inner membranous seedcoat that may restrict water and oxygen passage to the embryo and prevent leaching of inhibitors from the cotyledons (Atwater, 1980).

Osmotic priming has improved seed germination in many plant species, with increased final germination percentage being the greatest benefit (Heydecker and Coolbear, 1977). Priming increased rates and percentages of germination (Samfield et al., 1991) and emergence of common purple coneflower seeds (Pill et al., 1994). Following priming, seeds have completed phase I (hydration) and II (lag phase) of germination, and only require a favorable water potential gradient for water uptake in order to begin radicle growth (Pill, 1995). During the process of priming, with water entering the seeds, endosperm loosening and hydrolysis of starch that causes synthesis of new proteins and enzymes are known to occur (Nascimento et al., 2001). Higher germination and emergence percentages and rates obtained from priming dormant seeds may have resulted from these positive effects of priming.

Several members of Asteraceae family, including narrow-leaved purple coneflower, require ethylene to break dormancy (Feghahati and Reese, 1994; Katoh and Esashi, 1975); however, the role of ethylene and its mechanism of action have not been fully elucidated. As with the length of the stratification duration, there is conflicting information in the literature on the length of ethylene treatment. Ethephon treatment accompanying prechilling of the seeds for 11 d in the light was found to be the most effective treatment for inducing germination (Macchia et al.,

Table 2. Final emergence percentage (FEP) and angular transformation of FEP (degrees), days to 50% of FEP (E_{50}), days between 10% and 90% emergence (E_{10-90}) of narrow-leaved purple coneflower seeds and shoot fresh weight of seedlings grown at 25°C (77.0°F) for 14 d following stratification at 4°C (39.2°F) for 3 weeks or priming for 3 d at 20°C (68.0°F) in Nas and Read medium (NRM) or 2% potassium nitrate (KNO_3) with various concentrations of 1-aminocyclopropane carboxylic acid (ACC) or gibberellic acid (GA_3).

Treatments ^z	FEP		E_{50} (d)	E_{10-90} (d)	Shoot fresh wt (mg/plant) ^y
	(%)	(degrees)			
$\text{KNO}_3 + 3 \mu\text{M}$ ACC	75	61	3.96	1.99	32.1
$\text{KNO}_3 + 5 \mu\text{M}$ ACC	66	55	3.79	1.91	34.8
$\text{KNO}_3 + 500 \text{ mg} \cdot \text{L}^{-1} \text{ GA}_3$	69	57	4.57	1.96	37.7
$\text{KNO}_3 + 1000 \text{ mg} \cdot \text{L}^{-1} \text{ GA}_3$	65	54	4.94	2.14	38.1
KNO_3	61	52	5.53	2.61	34.4
NRM	68	56	4.88	2.07	34.3
Stratification	62	52	5.22	1.99	31.7
Nontreated seeds	26	31	8.01	2.32	22.4
$\text{LSD}_{0.05}$		(7)	0.44	---	4.2
Significance		***	***	NS	***

^z1 $\text{mg} \cdot \text{L}^{-1} = 1 \text{ ppm}$; $\text{LSD}_{0.05}$ = Fisher's least significant difference at $P = 0.05$.

^y28,350 mg = 1 oz

ns, **Nonsignificant or significant at $P \leq 0.001$, respectively.

2001), while 4 weeks of stratification in the presence of ethephon was necessary to obtain higher germination rates (Sari et al., 1999).

In previous studies, ethylene treatment of seeds was always conducted with stratification, which lasted for weeks. The present study, however, revealed that priming the seeds for as short as 3 d in the presence of 3 μ M ACC was enough to obtain higher germination and emergence percentage than stratification alone. Therefore, it can be concluded from the results of this study that priming in the presence of ethylene or ethylene-like compounds (i.e., ACC) at a low concentration can be an alternative to weeks of stratification treatments to break the dormancy and improve the germination and emergence of narrow-leaved purple coneflower seeds. Moreover, it would also be interesting to explore if NRM supplemented with plant growth regulators (i.e., ACC) synergistically increases germination and would be an alternative priming agent to KNO₃.

Literature cited

- Atwater, B.R. 1980. Germination, dormancy and morphology of the seeds of herbaceous ornamental plants. *Seed Sci. Technol.* 8:523–573.
- Baskin, C.C., J.M. Baskin, and G.R. Hoffman. 1992. Seed dormancy in the prairie forb *Echinacea angustifolia* var. *angustifolia* (Asteraceae): After ripening pattern during cold stratification. *Intl. J. Plant Sci.* 153:239–243.
- Bray, C.M. 1995. Biochemical processes during the osmopriming of seeds, p. 767–789. In: J. Kigel and G. Galili (eds.). *Seed development and germination*. Marcel Dekker, New York.
- Cox, J. 1998. Purple coneflower (*Echinacea purpurea*). *Organic Gardening* 45:52–54.
- Feghahati, S.M.J. and R.N. Reese. 1994. Ethylene-, light-, and prechill-enhanced germination of *Echinacea angustifolia* seeds. *J. Amer. Soc. Hort. Sci.* 119:853–858.
- Heydecker, W. and P. Coolbear. 1977. Seed treatments for improved performance: Survey and attempted prognosis. *Seed Sci. Technol.* 5:353–425.
- Katoh, H. and Y. Esashi. 1975. Dormancy and impotency of cocklebur seeds. I. CO₂, C₂H₂, O₂ and high temperature. *Plant Cell Physiol.* 16:687–696.
- Li, T.S.C. 1998. *Echinacea*: Cultivation and medicinal value. *HortTechnology* 8:122–129.
- Macchia, M., L.G. Angelini, and L. Ceccerani. 2001. Methods to overcome seed dormancy in *Echinacea angustifolia* DC. *Scientia Hort.* 89:317–324.
- McGregor, R.L. 1968. The taxonomy of the genus *Echinacea* (Compositae). *Univ. of Kansas Sci. Bul.* 48:113–142.
- Murray, G., J.B. Swensen, and J.J. Gallian. 1993. Emergence of sugar beet seedlings at low soil temperature following seed soaking and priming. *HortScience* 28:31–32.
- Nas, M.N. and P.E. Read. 2004. A hypothesis for the development of a defined tissue culture medium of higher plants and micropropagation of hazelnuts. *Scientia Hort.* 101:189–200.
- Nascimento, W.M., D.C. Cantliffe, and D.J. Huber. 2001. Endo- β -mannanase activity and seed germination of thermosensitive and thermotolerant lettuce genotypes in response to seed priming. *Seed Sci. Res.* 11:255–264.
- Parmenter, G.A., L.C. Burton, and R.P. Littlejohn. 1996. Chilling requirement of commercial *Echinacea* seed. *N.Z. J. Crop Hort. Sci.* 24:109–114.
- Pill, W.G., C.K. Crossan, J.J. Frett, and W.G. Smith. 1994. Matric and osmotic priming of *Echinacea purpurea* (L.) Monech seeds. *Scientia Hort.* 59:34–44.
- Pill, W.G. 1995. Low water potential and pre-sowing germination treatments to improve seed quality, p. 319–359. In: A.S. Basra (ed.). *Seed quality*. Food Products Press, Binghamton, N.Y.
- Samfield, D.M., J. Zajicek, and B.G. Cobb. 1990. Germination of *Coreopsis lanceolata* and *Echinacea purpurea* seeds following priming and storage. *HortScience* 25:1605–1606.
- Samfield, D.M., J. Zajicek, and B.G. Cobb. 1991. Rate and uniformity of herbaceous perennial seed germination and emergence as affected by priming. *J. Amer. Soc. Hort. Sci.* 116:10–13.
- Sari, A.O., M.R. Morales, and J.E. Simon. 1999. *Echinacea angustifolia*: An emerging medicinal, p. 490–493. In: J. Janick (ed.). *Perspectives on new crops and new uses*. ASHS Press, Alexandria, Va.
- Schulthess, B.H., E. Giger, and T.W. Bauermann. 1991. *Echinacea*: Anatomy, phytochemical pattern, and germination of the achene. *Planta Medica* 57:384–388.
- Smith-Jochum, C. and M.L. Albercht. 1988. Transplanting or seeding in raised beds aids field establishment of some *Echinacea* species. *HortScience* 23:1004–1005.