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Gibberellic Acid and Presowing Chilling Increase Seed Germination of Indiangrass [*Sorghastrum nutans* (L.) Nash.]

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Abstract. **Following dry storage for 5 or 11 months (new and old seeds, respectively) at 5 °C, less than 10% of the seeds of Indiangrass germinated as determined by a standard germination test. We attempted to increase germination by subjecting seeds to dormancy-breaking treatments, including sodium hypochlorite soak (5.25% v/v NaOCl; 20 or 60 min), prechilling (5 °C for 2 weeks), gibberellic acid during germination (GA_3, 1000 mg·L^{-1}), and combinations thereof. Treatment with NaOCl increased the germination of non-prechilled seeds only when they were germinated in GA_3; a 60-min soak in NaOCl increased germination to 53% and 65% in new and old seeds, respectively. Prechilling increased germination to 65% and 47% in new and old seeds, respectively. Germination of new, prechilled seeds was increased further to 86% by either a 20-min soak in NaOCl or germination in GA_3. Germination of old, prechilled seeds was not promoted further by treatment with NaOCl, but was increased to 67% by germination in GA_3. Since NaOCl treatment alone failed to promote germination, we examined the effects on seedling emergence and growth of providing GA_3 at 1000 mg·L^{-1} during the 2-week prechilling period. While prechilling alone increased emergence to an average 34% for new and old seeds, prechilling with GA_3 increased emergence to 75% and 50% for new and old seeds, respectively. These treatments did not influence seedling shoot dry mass. Seed exposure to GA_3 during rather than after prechilling was more effective in promoting Indiangrass establishment.**

Indiangrass is a warm-season grass native to the plains and the Eastern United States. It has value as a forage crop (Cuomo et al., 1996) and may have value as a low maintenance, landscape perennial. Low seed vigor and seed dormancy, both characteristics of many warm-season grass species, can result in slow and inconsistent establishment of Indiangrass (Emal and Conard, 1973; Geng and Barnett, 1969). Afterripening can overcome Indiangrass seed dormancy. Cuokos (1944) found that storage of Indiangrass seed at room temperature for 25 months increased germination. Emal and Conard (1973) reported that germination of untreated seeds increased progressively after the first 7 months of storage.

Emal and Conard (1973) found that prechilling, soaking seeds in sodium hypochlorite [NaOCl, 6% (v/v) for 80 min], or germinating seeds on blotters moistened with GA_3 at 250 to 2000 mg·L^{-1} each increased Indiangrass germination, but they did not combine these treatments. Geng and Barnett (1969) reported that dehulling followed by prechilling increased germination in two of three Indiangrass cultivars, although hull removal increased the percentage of abnormal seedlings. The International Seed Testing Association (1985) recommends two weeks of moist prechilling at 5 °C (in 0.2% KNO_3) for Indiangrass seed prior to germination testing. Acid scarification, prechilling, and NaOCl treatments additively promoted germination of dormant, neoteric switchgrass (*Panicum virgatum* L.) seeds, but did not affect the germination of fully afterripened seeds (Haynes et al., 1997). Afterripening of Indiangrass seeds would be expected to reduce the efficacy of dormancy-breaking treatments.

Improved Indiangrass establishment in the landscape, whether sown directly or transplanted as plugs, would benefit the nursery and landscape industries. The purpose of this research was to test the efficacy of combining several dormancy-breaking treatments (NaOCl, prechilling, and GA_3) on the seed germination and seedling emergence of new and old seedlots of Indiangrass.

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Materials and Methods

Germination assay. Indiangrass seeds, harvested from one ecotype in Nebraska during Nov. 1995, were purchased from Jelitto Seed Co. (Schwarmstedt, Germany) during Apr. 1996. Half the seedlot was kept at 5 °C for 8 months from the time of receipt to the time of the test ("old seeds," actual age 13 months). The remaining seeds were used for seed increase. They were sown in 3 × 4 × 5.5-cm plug cells containing ProMix-BX (Premier Horticulture, Redhill, Pa.) and maintained in a 23 °C day/21 °C/night greenhouse under natural light (May to June). Seedlings were transplanted at 21 d after sowing into 3.8-L nursery containers filled with ProMix-BX. These plants, grown outdoors and isolated by at least 200 m from any nearby populations, received N weekly at 200 mg·L^{-1} as 20N–4.3P–8.3K (Peters General Peat-Lite fertilizer; Scotts-Sierra, Marysville, Ohio). Seeds from these plants were collected on 9 Sept. 1996, and stored at 5 °C for 5 months until used ("new" seeds). Thus, new and old seeds were stored for 5 and 13 months, respectively, before initiation of the germination assay.

Treatments consisted of two seed lots (new and old seeds) × three NaOCl soak durations (0, 20 or 60 min) × two prechilling durations (0 or 2 weeks) × two GA_3 concentrations (0 or 1000 mg·L^{-1}) for moistening germination blotters, in a randomized complete-block design. One box of 100 seeds was used for each treatment in each of four blocks.

For treatment in NaOCl, batches of 100 seeds were wrapped in paper towelling (Kimwipe; Kimberly-Clark Corp., Roswell, Ga.) then placed in a 38 × 10-mm propylene tissue capsule (HistoPrep; Fisher Scientific, Philadelphia). The capsules were submerged in 5.25% NaOCl (400 seeds per 100 mL NaOCl) and stirred at intervals. After 20 or 60 min, the NaOCl was poured from the beakers and the capsules were rinsed with distilled water. The seeds were removed from the capsules and towelling, then blotted dry. NaOCl-treated seeds (0, 20, or 60 min) were placed into 125 × 80 × 20-mm transparent polystyrene boxes (Stewart Plastics, Croydon, England) containing two layers of germination blotters (Seed Germination Blotters No. 385; Seedburo Co., Chicago). The blotters were saturated with 0.2% KNO_3, and seeds were prechilled in darkness for 2 weeks at 5 °C (International Seed Testing Association, 1985). Prechilled seeds then were washed from the polystyrene boxes into a sieve and rinsed with distilled water. For comparison with prechilled seeds, non-prechilled seeds were soaked in NaOCl and rinsed as described above after the 2-week prechilling was complete.

Seeds for each treatment combination were placed in a germination box containing two layers of germination paper soaked in distilled water or GA_3 at 1000 mg·L^{-1} (ProGibb Plus 2X; Abbott Laboratories, N. Chicago, Ill.). Seeds were germinated at 20/30 °C (8 h dark/16 h light; International Seed Testing Association, 1985). Germinated seeds (radicle protrusion) were counted and removed daily for 14 d. Final germination percentage (FGP) at 14 d after sowing, and its angular transformation (arsin $\sqrt{\%}$), were calculated and the transformed data were subjected to analysis of variance (ANOVA).

Emergence assay. New and old seeds were prechilled as described for the germination assay except that GA_3 at 0 or 1000 mg·L^{-1} was added to the 0.2% KNO_3 blotter moistening solution. Non-prechilled seeds were not treated

with GA_3. Four replications (50 seeds per replication) were used for each treatment.

Seeds were sown in 18 × 13.5 × 6-cm plastic trays (Kord 601 market packs; E.C. Geiger, Harleysville, Pa.) filled with Redi-Earth (Scotts-Sierra). The trays were watered, then seeds were sown into four 1-cm-deep furrows and covered with 1 cm of Redi-Earth. Treatments (trays) were arranged in randomized block design on the greenhouse bench. The greenhouse was set at 25 °C day/21 °C night (10.5- to 11.2-h photoperiods) under natural light (Feb.–Mar.).

Emergence (visible coleoptile) was counted daily. Final emergence percentage (FEP, and its arsin $\sqrt{\%}$ transformation), and days to 50% of FEP (E_{50}) and from 10% to 90% of FEP (E_{10-90}) were calculated. Twenty days after sowing, seedlings were cut at the growth medium surface, dried at 65 °C for 3 d, and weighed to determine shoot dry mass (SDM). SDM and emergence variables were subjected to one-way (six treatments) ANOVA.

Results and Discussion

Germination assay. New or old seeds that received no treatment had FGPs of <10 (Table 1) compared to >70 FGP for pretreated seeds, indicating that most seeds were dormant. Our storage of seeds at 5 °C rather than at a higher temperature may have reduced the afterripening response of the old seeds, since Emal and Conard (1973) found that percentage germination of nontreated Indiangrass seeds was progressive after the first 7 months of storage at room temperature.

Contrary to the results of Emal and Conard (1973), germination was not promoted by soaking seeds in NaOCl for up to 60 min (Table 1). Gibberellic acid treatment alone increased FGP of old, non-prechilled seeds from 6% to 34%, but had no effect on the germination of new seeds (Table 1). Soaking both new and old non-prechilled seed in NaOCl, followed by placement on blotters moistened with GA_3, increased germination; FGP of old seeds was 58 after a 20-min soak in NaOCl; that of new seeds was 53 after a 60-min soak. Thus, the promotive effect of GA_3 on germination of non-prechilled, dormant seeds was enhanced by first soaking the seed in NaOCl, with the old seed requiring less soak time than new seed to achieve a similar percentage germination. This additive effect of NaOCl and GA_3 in promoting germination also was observed in wild oats (*Avena fatua*) by Hsaoi and Quick (1985). While these workers found that seeds afterripened for a longer period had a reduced requirement for exogenous GA_3 in breaking dormancy, we found that older seed responded better to the NaOCl soak. Hsaoi and Quick (1985) suggested that NaOCl exerted a scarification effect, resulting in increased sensitivity to GA_3.

Indiangrass "seed" is a fertile spikelet containing a fertile and an infertile floret, each enclosed by a membranous lemma, and both in turn enclosed within two leathery glumes (Hitchcock and Chase, 1951). We observed some bleaching (whitening) of the glumes after a 20-min exposure to NaOCl, but pronounced bleaching after 60 min. While the caryopsis could be seen through the covering structures of old seeds after a 20-min exposure, the caryopsis of new seeds was not visible even after 60 min. This increased visibility of the caryopsis through the covering structures of old seeds may indicate greater scarification, resulting in greater penetration of GA_3, and thus greater germination of old than of new, non-prechilled seeds (Table 1). Haynes et al. (1997) noted that corrosion of the lemmas of NaOCl-treated (5.25%, 15 min) *Panicum virgatum* seeds was associated with increased germination. "Dehulling" increased the germination of Indiangrass (Geng and Barnett, 1969), *Stipa viridula* Trin. (Frank and Larson, 1970), and *Avena fatua* L. (Hsiao and Quick, 1984). These researchers suggested that hull removal allowed greater penetration of water and oxygen into the seed and removed a physical barrier to radicle emergence. We observed some deformation and bleaching of radicles from old, but not new, seeds following 60 min of NaOCl treatment, indicating greater sensitivity of old seeds to NaOCl.

Prechilling increased germination of seeds not treated with NaOCl, but to a greater extent in new than in old seeds (Table 1). Emal and Conard (1973) found 2 weeks of prechilling increased Indiangrass germination, with the promotive effect lessening with seed age and afterripening. Had we extended prechilling to 4 weeks, germination might have increased further as noted by Emal and Conard (1973). The presence of gibberellic acid during germination further increased germination of old, prechilled seeds, but NaOCl had no effect. For the new, prechilled seeds, either a 20-min soak in NaOCl or GA_3 increased FGP to 84. In order to exceed the promotive effects of prechilling alone, new seeds required at least 20 min in NaOCl, or germination on blotters moistened with GA_3, and old seeds required germination in GA_3 (Table 1). Geng and Barnett (1969) also demonstrated an additive effect of combining prechilling with another treatment (dehulling) in promoting Indiangrass germination.

Emergence assay. Since NaOCl treatment alone was less effective than prechilling or GA_3 treatment (Table 1), we examined the effects of GA_3 at 1000 mg·L^{-1} during the 2-week prechilling period on seedling emergence and growth. The FEP of new and old seeds without prechilling was <10 (Table 2). While prechilling without GA_3 increased FEP to 34 for new and old seeds, prechilling on blotters moistened with GA_3 at 1000 mg·L^{-1} increased FEP to 75 and 50 for new and old seeds, respectively. The major effect of prechilling with GA_3 was increased emergence. This treatment produced slightly faster emergence (lower E_{50}), but had no effect on emergence asynchrony (E_{10-90}) or seedling shoot dry mass (Table 2). All seedlings appeared normal.

Compared with prechilling alone, exposing old seeds to GA_3 during, rather than after, prechilling resulted in similar increases (≈45%) in FGP (Table 1) and FEP (Table 2). For new, prechilled seeds, GA_3 applied during germina-

Table 1. Final germination percentage, and its angular transformation, of new and old *Sorghastrum nutans* seeds at 20/30 °C (8 h/16 h) following soaking in sodium hypochlorite (5.25% v/v NaOCl; 0, 20, or 60 min), prechilling (5 °C; 0 or 2 weeks) or germination in gibberellic acid (GA_3; 0 or 1000 mg·L^{-1}).

NaOCl soak duration (min)	Prechill (wk)	GA_3 during germination (mg·L^{-1})	Final germination: New seeds (%)	New seeds [deg.]	Old seeds (%)	Old seeds [deg.]
0	0	0	0	[1]	7	[15]
	0	1000	7	[15]	34	[36]
	2	0	65	[54]	47	[43]
	2	1000	86	[68]	67	[55]
20	0	0	2	[6]	8	[16]
	0	1000	18	[25]	58	[49]
	2	0	86	[68]	53	[47]
	2	1000	86	[68]	71	[58]
60	0	0	7	[16]	10	[19]
	0	1000	53	[47]	65	[54]
	2	0	80	[64]	51	[46]
	2	1000	84	[67]	61	[51]
Interaction $LSD_{0.05}$					[6]	
Significance for [deg.]						
Seed age (SA)	NS					
Sodium hypochlorite (SH)	***					
SA × SH	***					
Prechill (PR)	***					
SA × PR	***					
SH × PR	***					
SA × SH × PR	*					
GA3 (GA)	***					
SA × GA	***					
SH × GA	NS					
SA × SH × GA	*					
PR × GA	***					
SA × PR × GA	NS					
SH × PR × GA	***					
SA × SH × PR × GA	NS					

NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Table 2. Final emergence percentage (FEP), days to 50% of FEP (E_{50}), days between 10% and 90% of FEP (E_{10-90}) and seedling shoot dry mass (SDM) at 20 d after sowing new and old seeds of *Sorghastrum nutans* subjected to prechilling (2 weeks, 5 °C), or gibberellic acid (GA_3, 1000 mg·L^{-1}) during prechilling.

	Seed treatment						
Seed age	Prechilling	GA_3 during prechilling (mg·L^{-1})	FEP %	FEP [deg.]	E_{50} (d)	E_{10-90} (d)	SDM (mg/shoot)
New	–	0	5	[10]	5.8	1.1	19.5
	+	0	35	[36]	4.9	1.9	21.7
	+	1000	75	[60]	4.5	1.8	20.1
Old	–	0	10	[18]	5.7	2.6	15.4
	+	0	34	[35]	5.3	2.7	16.4
	+	1000	50	[45]	4.6	2.8	5.7
1-way $LSD_{0.05}$:				[4]	0.3	0.6	4.2
Significance							
Seed age (SA)				NS	NS	***	***
Seed treatment (ST)				***	***	NS	NS
SA × ST				***	*	NS	NS

NS, *, **, ***Nonsignificant or significant at $P < 0.05$, 0.01 or 0.001, respectively.

tion increased FGP by 31% (Table 1), while GA_3 applied during prechilling increased FEP by 114% (Table 2). Thus, treatment during prechilling was more effective in promoting establishment.

Our results indicated that prechilling Indiangrass seeds in 0.2% KNO_3 containing GA_3 at 1000 mg·L^{-1} for 2 weeks at 5 °C markedly increased seedling emergence but had little effect on seedling growth. Although prechilling with GA_3 may not be practical for the grower, it may be a valuable seed treatment for the seed tester and supplier.

Literature Cited

Cuokos, C.J. 1944. Seed dormancy and germination in some native grasses. J. Amer. Soc. Agron. 36:337–345.

Cuomo, G.J., B.E. Anderson, L.J. New, and W.W. Wilhelm. 1996. Harvesting frequency and burning effects on monocultures of three warm season grasses. J. Range Mgt. 49:157–162.

Emal, J.G. and E.C. Conard. 1973. Seed dormancy and germination in Indiangrass as affected by light, chilling, and certain chemical treatments. Agron. J. 65:383–385.

Frank, A.B. and K.L. Larson. 1970. Influence of oxygen, sodium hypochlorite, and dehulling on germination of green needlegrass seed (*Stipa viridula* Trin.). Crop Sci. 10:679–682.

Geng, S. and F.L. Barnett. 1969. Effects of various dormancy reducing treatments on seed germination and establishment of *Sorghastrum nutans* (L.) Nash. Crop Sci. 9:800–803.

Haynes, J.G., W.G. Pill, and T.A. Evans. 1997. Seed treatments to improve seed germination and seedling emergence of switchgrass (*Panicum virgatum* L.). HortScience 32:1222–1226.

Hitchcock, A.S. and A. Chase. 1951. Manual of the grasses of the United States. USDA Misc. Publ. No. 200. U.S. Govt. Printing Office, Washington, D.C. 2nd ed.

Hsiao, A.I. and W.A. Quick. 1985. Wild oats (*Avena fatua* L.) seed dormancy as influenced by sodium hypochlorite, moist storage and gibberellin A3. Weed Res. 25:281–288.

International Seed Testing Association. 1985. International rules for seed testing. Seed Sci. and Technol. 13:299–355.