Priming Improves Germination and Emergence of Combine-harvested *Amaranthus cruentus* L. Seeds

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Abstract. Hand-harvested and threshed grain amaranth seeds stored for 6.5 years and combine-harvested and threshed seeds (cylinder speed $26.4 \text{ m}\cdot\text{s}^{-1}$) stored for 9.5 years were subjected to several osmotic priming treatment. The selected priming treatment (-1.25 MPa polyethylene glycol at 15°C for 10 days) increased percent radicle emergence of hand-harvested seeds and mechanically damaged, combine-harvested seeds and resulted in germination rates that were at least as high as those achieved with other priming treatments. In an incubator test, priming increased percent radicle emergence of hand-harvested seeds only at 15°C ; however, it increased percent radicle emergence of combine-harvested seeds at 15 and 35°C . Priming also increased radicle emergence rate, but this response was more pronounced and exerted over a wider temperature range for the older, lower-vigor, combine-harvested seeds than for the younger, higher-vigor, hand-harvested seeds. In a greenhouse test, hand-harvested seeds had a higher percentage of normal seedlings and a lower percentage of abnormal seedlings than combine-harvested seeds. Priming had no effect on these variables. As a result of priming, normal seedling emergence rate and shoot fresh weight were higher from combine-harvested seeds than from hand-harvested seeds, such that values of these variables for primed, combine-harvested seeds were at least equal to those for nonprimed, hand-harvested seeds. Thus, the invigorating effect of priming was more pronounced for the lower-vigor, mechanically damaged, combine-harvested seeds than for the higher-vigor, hand-harvested seeds.

Amaranth is a dicotyledonous C_4 species that produces edible cereal grain and leaves that are valuable as a food and feed resource (Saunders and Becker, 1984). Amaranth has been characterized as a "new crop that is in its adolescence" (Kauffman and Weber, 1988), although it was a basic food of the New World in pre-Columbian times (National Research Council, 1984; Vietmeyer, 1981).

Although most grain amaranth is hand-harvested, combine-harvesting and threshing is required in the United States for this crop to be economically competitive with other grain crops. Mechanical harvesting research of grain amaranth, initiated in 1980 at the Rodale Research Center in Kutztown, Pa., lead to recommended settings and modifications for differ-

ent models of combine-harvesters to minimize grain losses (Weber et al., 1989). Amaranth seed is particularly vulnerable to mechanically induced damage because the embryo encircles the perisperm in one plane (Irving et al., 1981). Krishnan et al. (1994) established that the percentage of normal amaranth seedlings decreased and the percentage of abnormal seedlings increased as threshing cylinder linear speed increased from 8.1 to $30.7 \text{ m}\cdot\text{s}^{-1}$. Although no mechanical damage was apparent at $8.1 \text{ m}\cdot\text{s}^{-1}$, the seedcoat and endosperm were damaged at 12.8 and $22.4 \text{ m}\cdot\text{s}^{-1}$. The damage extended to the embryos at $30.7 \text{ m}\cdot\text{s}^{-1}$. That study, however, did not examine radicle emergence rate or seedling growth.

Germination of a seed lot is assessed by standard germination tests in which the number of seeds capable of producing normal seedlings under optimal conditions are recorded (International Seed Testing Association, 1985). There is no distinction between strongly or weakly terminable seeds. Poor seedling emergence may be associated with low vigor, resulting in the failure to complete the postgerminative-preemergence stage of growth (Doneen and McGillivray, 1943).

Osmotic seed priming is a presowing treatment in which seeds are exposed to an external water potential low enough to restrict germi-

nation and yet permit pregerminative physiological and biochemical activities (Bradford, 1986). Primed seeds may have a higher germination percentage and rate than nonprimed seeds, particularly under adverse seed-bed conditions such as low (Pill and Finch-Savage, 1988; Szafrrowska et al., 1981) or high temperature. (Carpenter, 1989; Carpenter and Boucher, 1991) and decreased water availability (Frett and Pill, 1989). There is some controversy, however, regarding the merits of priming with respect to existing seed vigor. Szafrrowska et al. (1981) noted that priming invigorated low-vigor carrot (*Daucus carota* L.) seeds more than high-vigor seeds, but Matthews and Powell (1986) suggested that priming can be of little benefit to low-vigor seeds.

In this study, we established the optimal priming conditions for hand- or combine-harvested and threshed grain amaranth seeds after extended storage (6.5–9.5 years) and assessed germination and seedling emergence responses of primed and nonprimed seeds.

Materials and Methods

General. 'RRC 1041' grain amaranth plants, grown at the Cheyenne Gap Amaranth farm in Luray, Kan., were harvested in Oct. 1982 using a combine (6620; John Deere, Moline, Ill.) equipped with a 56-cm rasp bar cylinder operated at $26.4 \text{ m}\cdot\text{s}^{-1}$ linear speed (900 rpm) and a grated concave clearance of 7.9 mm in the front and 4.8 mm in the back. Hand-harvested and threshed seeds of the same cultivar, not available in 1982, were harvested from the same farm in Oct. 1985. Between the time of harvesting or threshing and germination testing, the seeds were stored in sealed plastic bags at 20 to 27°C .

Germination of these seeds was tested in Mar. 1992. Four 100-seed replications of the combine-harvested and threshed seeds and hand-harvested and threshed seeds were placed in $125 \times 80 \times 20$ -mm transparent polystyrene boxes containing two layers of germination paper (Seed Germination Blotters no. 385; Seedburo Co., Chicago) that were moistened with 15 ml of half-strength Hoagland solution (Hoagland and Amen, 1950). They were maintained at constant 20°C in darkness. Germinated seeds (radicles visible) were counted daily and removed from the boxes. From these data, maximum percent radicle emergence and days to 50% of maximum percent radicle emergence (G_{50}) were calculated. Maximum percent radicle emergence and G_{50} values (mean $\pm$ SD) of the combine-harvested seeds were $34\% \pm 9\%$ and 3.6 ± 0.1 days, respectively, and values for hand-harvested seeds were $86\% \pm 4\%$ and 2.7 ± 0.1 days, respectively. Thus, the older combine-harvested seeds had lower germinability and vigor than the hand-harvested seeds.

Optimal priming treatment. Hand- and combine-harvested seeds were primed for 5, 10, 15, or 20 days in solutions of polyethylene glycol 8000 (PEG) with nominal water potentials of -0.50 , 0.75 , -1.00 , or -1.25 MPa at 15°C (Michel and Kaufmann, 1973). The prim-

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ing treatments were performed in boxes on papers (as previously described) moistened with 15 ml of PEG solution. Four replicate boxes each contained 100 seeds. Following priming, seeds were rinsed in demineralized water, allowed to dry at 21°C in 34% relative humidity for 1 week, then transferred to boxes containing two layers of germination paper moistened with 15 ml of half-strength Hoagland solution. Priming treatments were scheduled so that all germination tests (15°C in darkness) started at the same time. We recorded the number of seeds germinated (radicles first visible) daily until the numbers stabilized, and from these data, we calculated the maximum percent radicle emergence (and angular transformation) and G_{50} and subjected them to analysis of variance.

Germination temperature. Combine- and hand-harvested seeds were primed (–1.25 MPa PEG at 15°C for 10 days), rinsed, and dried as previously described. The germination of the primed seeds and nonprimed, combine- and hand-harvested seeds was tested at 15, 25, or 35°C in half-strength Hoagland solution. Five replications of 100 seeds of the 2 (harvest method) $\times$ 2 (priming treatment) factorial were arranged in a completely randomized design within incubators set at one of the three temperatures. Data collection and analyses were as previously described, except that data from each temperature were analyzed separately.

Seedling emergence in greenhouse. Primed seeds, prepared using methods previously described, and nonprimed, combine- and hand-harvested seeds were sown into five 0.5-cm-deep $\times$ 12-cm-long furrows in proprietary peat-based medium (Pro-Mix BX; Premier Brands, New Rochelle, N. Y.) contained in 17 $\times$ 12 $\times$ 6-cm plastic flats. The sown seeds were covered with 0.5 cm of Redi-Earth (W.R. Grace and Co., Cambridge, Mass.). The 2 $\times$ 2 factorial was arranged in randomized complete blocks with 100 seeds in each of eight replications (flats). The study was conducted in a greenhouse under natural light (June 1992) with day/night ranges of 16–20°C/12–14°C. Flats were sprinkle-irrigated daily. Seedling emergence (hypocotyl first visible) was counted daily. From these data, maximum percent seedling emergence and days to 50% of maximum percent seedling emergence (E_{50}) were calculated. Fourteen days after sowing, the percentages of abnormal (with cracked or deformed hypocotyls and cotyledons) seedlings and of final (normal) seedling emergence were assessed. Shoots of all normal seedlings were cut at the growth medium surface 14 days after sowing, and their fresh weights were determined. Data were subjected to analysis of variance.

Results and Discussion

Optimal priming treatment. Excessive germination occurred during priming at –0.5 MPa, so this treatment was excluded from statistical analysis. All priming treatments decreased G_{50} relative to that of nonprimed seeds, resulting in an average 2.6-day reduction for hand-harvested seeds and an average 4.5-day reduc-

tion for combine-harvested seeds (Table 1). G_{50} was affected less by water potential than by priming duration, with G_{50} generally increasing quadratically with increasing days of priming. Water potential had little effect on G_{50} when priming was <10 days. With ≥ 15 days of priming, the G_{50} of combine-harvested seeds decreased as water potential decreased.

Priming increased radicle emergence from 65% in nonprimed seeds to an average 80% in hand-harvested seeds and from 16% to an average 33% in combine-harvested seeds (Table 1). Exceeding 10 days of priming did not increase percent radicle emergence of hand-harvested or combine-harvested seeds; 20 days of priming resulted in significantly lower percent radicle emergence of hand-harvested seeds than more brief priming. Priming combine-harvested seeds in –1.25 MPa PEG for 10 days resulted in higher percent radicle emergence than other priming conditions. This treatment was selected for use in subsequent experiments because it increased percent radicle emergence of nonprimed seeds 1.7- and 2.9-fold in hand-harvested and combine-harvested seeds, respectively, and resulted in radicle emergence rates that were at

least as high as those achieved with other priming treatments.

Germination temperature. Priming increased the radicle emergence rate of hand- and combine-harvested seeds at 15 and 25°C and of combine-harvested seeds at 35°C. The G_{50} decrease due to priming was greater as germination temperatures decreased in combine-harvested seeds than in hand-harvested seeds. For example, G_{50} at 15°C was reduced by 7 days in combine-harvested seeds and by 2.5 days in hand-harvested seeds. Priming only increased percent radicle emergence of hand-harvested seeds at 15°C; nonprimed, hand-harvested seeds had $\geq 90\%$ radicle emergence at 25 and 35°C (Table 2). Priming increased percent radicle emergence of combine-harvested seeds by 7% at 15°C and by 18% at 35°C. The percent radicle emergence increase due to priming at 35°C (51% to 69%) could be of practical value if it could be retained under field conditions for seeds stored several years.

Increased germination rate and percentage at low temperatures due to priming have been reported for carrot (Pill and Finch-Savage, 1988; Szafirowska et al., 1981). The greater

Table 1. Maximum radicle emergence (MRE; percentage and angular transformation [AT]) and days to 50% maximum radicle emergence (G_{50}) of combine-harvested and threshed and hand-harvested and threshed amaranth seeds at 15°C in half-strength Hoagland solution following priming at 15°C in polyethylene glycol for various periods and water potentials.

Water potential (–MPa)	Days of priming	G_{50}		MRE (%) [AT°]	
		Harvesting method			
		Hand	Combine	Hand	Combine
Nonprimed seeds		6.7	7.6	65 [53]	16 [24]
$\pm$ SD ^y		± 0.5	± 1.1	± 9 [±5]	± 24 [±5]
0.75	5	1.6	2.8	80 [64]	35 [36]
	10	1.8	2.7	80 [63]	32 [34]
	15	2.4	3.9	86 [68]	24 [28]
	20	2.9	4.2	68 [55]	26 [30]
1.00	5	1.8	2.9	81 [64]	35 [36]
	10	1.4	2.7	82 [65]	37 [37]
	15	2.2	2.9	83 [65]	33 [35]
	20	2.7	3.7	75 [60]	35 [36]
1.25	5	1.9	3.0	84 [68]	31 [34]
	10	2.0	2.5	87 [69]	47 [44]
	15	2.5	2.9	87 [69]	33 [35]
	20	2.6	3.3	74 [60]	34 [36]
LSD _{0.05}		0.4		[7]	
Significance					
Harvesting method (HV)		***		***	
Water potential (WP)		***		**	
HV $\times$ WP		NS		NS	
Days of priming (DP)		***		**	
HV $\times$ DP		NS		**	
WP $\times$ DP		***		NS	
HV $\times$ WP $\times$ DP		NS		NS	
WP linear		***		**	
WP quadratic		NS		NS	
DP linear		***		**	
DP quadratic		***		**	
HV $\times$ WP linear		*		NS	
HV $\times$ WP quadratic		NS		NS	
HV $\times$ DP linear		NS		NS	
HV $\times$ DP quadratic		NS		NS	
Nonprimed vs. primed seeds		***		*	

^aAT = $\text{Arcsin} \times \sqrt{\%}$, in degrees.

^yn = 4.

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

improvement in radicle emergence rate with priming lower-vigor, combine-harvested seeds than with priming higher-vigor, hand-harvested seeds agreed with the observations of Szafirowska et al. (1981). They indicated priming benefited low-vigor more than high-vigor carrot seeds. At 35C, priming increased the rate and percentage of radicle emergence of combine-harvested seeds but not of hand-harvested seeds (Table 2). Increased germination percentage and rate at high temperatures in response to seed priming were noted in pansy (*Viola x wittrockiana* Gains.) (Carpenter and Boucher, 1991) and in salvia (*Salvia splendens*, F. Sellow ex Roem & Schutt) (Carpenter, 1989).

Priming exerted no discernible effect on seed morphology as revealed by scanning electron microscopy. However, combine-harvested seeds threshed at a cylinder linear speed of 26.4 m·s⁻¹ exhibited extensive mechanical injury that extended to the embryo (Krishnan et al., 1994), which encircles the perisperm (Irving et al., 1981). Although priming cannot reverse mechanical injury, it can invigorate seeds through a combination of processes (Pill, 1994) that may include the following: cellular repair and improved membrane integrity; decreased seed exudation and concomitant decreased growth of pathogenic organisms; enhanced mobilization of seed protein, lipid, and starch as a result of activation or synthesis of key enzymes; osmotic adjustment and increase in radicle turgor; advanced embryo development; weakened restraining tissues around the radicle; and increased potential for oxidative phosphorylation and adenosine triphosphate accumulation.

Seedling emergence in greenhouse. Seedling emergence from combine-harvested seeds was slower than from hand-harvested seeds, but priming reduced the E₅₀ of combine- and hand-harvested seeds so that the E₅₀ of primed, combine-harvested seeds was equal to that of nonprimed, hand-harvested seeds (Table 3). The percentage of normal seedlings was 3.3 times higher from hand-harvested seeds than from combine-harvested seeds, but priming did not increase the percentage of normal seedlings from either combine- or hand-harvested seeds.

The average percentage of normal seedlings obtained (Table 3) from hand-harvested seeds (84%) and combine-harvested seeds (34%) was lower than the respective averages of 93% and 53% radicle emergence values achieved in incubators at 25 and 35C (Table 2). The greater percentage of combine-harvested seeds than of hand-harvested seedlings that failed to complete the postgerminative-preemergence stage of growth reflected the lower vigor of the older and more damaged combine-harvested seeds. The higher percentage of abnormal seedlings from combine-harvested seeds (8.9%) than from 3-year-younger, hand-harvested seeds (2.6%) provided further evidence of the reduced vigor of the combine-harvested seeds (Table 3). Priming did not reduce the percentage of abnormal seedlings because mechanical injury cannot be reversed.

The increased speed of seedling emergence from combine-harvested seeds as a result of priming revealed that priming invigorated the mechanically damaged seeds (International Seed Testing Association, 1985). Higher shoot fresh weights of combine-harvested and hand-harvested seeds, as a result of priming (Table 3), provided further evidence for the invigorating effect of seed priming. This invigoration was more pronounced in combine-harvested seeds (75% higher shoot weight) than in hand-harvested seeds (27% higher shoot weight). Seedling shoot fresh weights from primed, combine-harvested seeds were equal to those from primed, hand-harvested seeds and were higher than those from nonprimed, hand-harvested seeds.

Our results show that the optimal osmotic priming treatment for amaranth seeds is -1.25 MPa polyethylene glycol(15C,10 days) because this treatment increased percent radicle emergence of hand-harvested and mechanically damaged, combine-harvested seeds and resulted in radicle emergence rates that were at least as high as those achieved with other priming treatments. In an incubator test, priming increased percent radicle emergence of hand-harvested seeds only at 15C, but percent radicle emergence of combine-harvested seeds increased at 15, 25, and 35C. Priming also resulted in faster germination but was more

pronounced and was exerted over a wider temperature range for the older, lower-vigor, combine-harvested seeds than for the higher-vigor, hand-harvested seeds. Although priming had no effect on the percentages of normal or abnormal seedlings in a greenhouse test, values for these variables were lower from hand-harvested than from combine-harvested seeds. Priming resulted in a greater increase in seedling emergence rate and shoot fresh weight in combine-harvested than in hand-harvested seeds. Values of these variables for primed, combine-harvested seeds were at least equal to those of nonprimed, hand-harvested seeds. Thus, the invigorating effect of priming was more pronounced for the lower-vigor, combine-harvested seeds than for the higher-vigor, hand-harvested seeds. The invigorating effect of priming also may be of benefit to seeds younger than the 9.5 years in this study. Likewise, priming may be more beneficial for combine-harvested seeds threshed at cylinder linear speeds lower than our 26.4 m·s⁻¹.

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Table 2. Maximum radicle emergence (MRE; percentage and angular transformation [AT]) and days to 50% maximum radicle emergence (G₅₀) at 15, 25, or 35C in half-strength Hoagland solution of combine- or hand-harvested and threshed amaranth seeds that were nonprimed or primed (-1.25 MPa, 10 days, 15C).

Harvest method (HV)	Seed treatment (ST)	Germination temp (°C)					
		15		25		35	
		G ₅₀	MRE	G ₅₀	MRE	G ₅₀	MRE
		(days)	(%) [AT ²]	(days)	(%) [AT ²]	(days)	(%) [AT ²]
Hand	Nonprimed	5.2	63 [52]	1.5	92 [73]	0.6	93 [75]
	Primed	2.7	70 [57]	0.6	90 [72]	0.5	95 [77]
Combine	Nonprimed	10.6	14 [22]	2.3	55 [48]	1.5	51 [45]
	Primed	3.6	21 [27]	0.6	52 [46]	0.6	69 [56]
LSD _{0.05}		2.2	[4]	0.3	[4]	0.2	[6]
Significance							
	HV	***	***	***	***	***	***
	ST	***	**	***	NS	***	***
	HV × ST	**	NS	***	NS	***	*

²AT = Arcsin × √%, in degrees.

ns, ***, ** *Nonsignificant or significant at P ≤ 0.001, 0.01, or 0.05, respectively.

Table 3. Maximum normal and abnormal seedling emergence (percentage and angular transformation [AT]), days to 50% of maximum percentage of normal seedling emergence (E₅₀), and normal seedling shoot fresh weight 14 days after sowing primed (-1.25 MPa, 10 days, 15C) or nonprimed, combine-harvested and threshed and hand-harvested and threshed amaranth seeds in the greenhouse.

Harvest method (HV)	Seed treatment (ST)	E ₅₀ (days)	Maximum emergence				Normal shoot
			Normal seedlings		Abnormal seedlings		fresh wt
			(%)	[AT ²]	(%)	[AT ²]	(mg/shoot)
Hand	Nonprimed	2.6	84	[67]	2.8	[8.1]	106
	Primed	1.9	85	[68]	2.3	[7.4]	135
Combine	Nonprimed	3.6	31	[34]	7.8	[16.0]	79
	Primed	2.5	36	[37]	9.9	[17.9]	138
LSD _{0.05}		0.3		[8]		[4.6]	23
Significance							
HV		***		***		***	NS
ST		***		NS		NS	***
HV × ST		*		NS		NS	*

²AT = Arcsin × √%, in degrees.

ns, ***, ** *Nonsignificant or significant at P ≤ 0.001 or 0.05, respectively.

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