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Effect of Seed Priming on Quality Improvement of Maize Seeds in Different Genotypes

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ABSTRACT: In Korea, production of super sweet corn has been economically feasible and is substituting for traditional sweet corn due to better flavor in recent years. Major limiting factors for super sweet corn production are low field emergence and low seedling vigor. The optimum water potential (WP) for the priming of normal and aged seeds of dent, sweet (*su*) and super sweet (*sh2*) corns was studied to improve low seed quality. Seeds were primed at 0, -0.3, -0.6, -0.9, and -1.2 MPa of polyethylene glycol (PEG) 8000 solution at 15 °C for 2 days. Priming effects differed depending on the type of corn, seed quality, and WP of PEG solution. Although WP of priming solution did not influence the emergence rate of extremely high quality normal dent corn seeds, it reduced time to 50% emergence (T50) and increased plumule weight. In contrast, the emergence rate of aged field corn was improved by seed priming at 0 MPa and plumule weight and α-amylase activity was enhanced. The optimum WP for both normal and aged sweet and super sweet corn seeds was between -0.3 and -0.6 Mpa. At the optimum WP emergence rate, α-amylase activity, and content of DNA and soluble protein increased, while T50 and leakage of total sugars and electrolytes reduced.

Keywords: dent corn, sweet corn, super sweet corn, seed priming, sugar leakage, electrolyte leakage, DNA, soluble protein, emergence rate, α-amylase activity, cold soil test

Commercial production of super sweet corn in Korea started in 1990s and traditional sweet corn is gradually substituting for super sweet corn in recent years. Kernels of super sweet corn have 2 ~ 3-folds more sugar content over sweet corn at the "roasting ear" stage (Lee *et al.*, 1999; Seo *et al*, 2002) and maintain higher sugar level for longer period after harvest (Lee *et al*, 1987) mainly due to a slower conversion of sugars to starch. Since the quality of fresh market sweet corn is closely related to sugar content, super sweet corn allows additional time to transport and store the corn with superior quality and reduces the need for refrigeration after harvest.

The problems of super sweet corn production are poor field emergence and seedling growth under the suboptimal temperature and high soil moisture conditions in Korea. Although the field emergence rate of super sweet corn of commercial hybrids ranged from 79.9 to 98.2 % when planted at optimum planting date under the black plastic mulch (Seo *et al.*, 2002), much lower emergence rate is expected when planted early for higher market price. In fact, some imported seeds were not distributed to farmers due to a low germination rate. To maintain an optimum plant population, farmers sow 2 or 3 seeds in a hill and remove extra plants at the 3-leaf stage; this wastes expensive seeds and requires extra labor.

Germination of seeds and subsequent seedling growth are directly related to the yield of crops. The poor seed quality of super sweet corn comes from genetic variation (Seo, *et al.*, 2002), improper seed maturity (Lee, 2000), cell membrane damages during imbibition (Chern & Sung, 1991), mechanical damages from threshing and planting machines (Peterson *et al.*, 1995), seed deterioration during the storage (Chang & Sung, 1998), leakage of sugars and electrolytes during the germination (Wann, 1986), and infection of pathogens (Hartz & Caprile, 1995).

The low quality of super sweet corn seeds can be improved by priming (Murray, 1990), matriconditioning (Seo *et al.*, 2003), presoaking (Saota *et al.*, 1987), hardening (Bennett & Waters, Jr., 1987) and GA treatment (Sanwo & Demason, 1994). Also, seed treatment of fungicides and inoculation of antagonistic microorganisms (Hartz & Caprile, 1995) improve indirectly field emergence rate especially under the suboptimal environmental conditions.

The effects of priming on super sweet corn seeds differed depending on the WP of the solution, duration, aeration, genotype, and seed quality. When priming was effective, it improved the germination rate and uniformity of seed germination and reduced emergence time in many crops under stress conditions (Bradford, 1986). In super sweet corn seeds, the observed improvements were attributed to priming-induced quantitative changes in biochemical contents of the seeds and improved membrane integrity, and to the enhanced α- and β-amylase activities, free sugars, DNA and RNA contents (Sung & Chang, 1993).

In this study, we investigated the optimum WP for the

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seed priming of normal and aged seeds of three corn types and the effects of priming on the emergence, α-amylase activity, leakage of total sugars and electrolytes in soaking solution, and changes in DNA and soluble protein content during the seed priming.

MATERIALS AND METHIDS

Plant materials

Effects of seed priming were studied for normal and artificially aged seeds of three corn types, dent corn, sweet corn (*su*), and super sweet corn (*sh2*). The dent corn was a single cross hybrid (cv. Suwon19) bred in Korea and seeds were produced at the Maize Experiment Station, Ganwon-do Agricultural Research and Extension Service in Hongcheon, Korea in 2001. Seeds of sweet corn were a double cross hybrid between Early Sunglow (♀) and Golden Cross Bantam70 (♂) and those of super sweet corn were a double cross hybrid between Fortune (♀) and Xtrasweet 82 (♂) produced on the Yeungnam University Agricultural Research Farm in Gyeongsan, Korea in 2001.

To make low quality seeds, normal seeds were artificially aged at 95% relative humidity (RH) in a 50 ± 0.1 °C growth chamber for 15 days. RH was maintained in a sealed plastic box containing a glycerol/water solution with specific gravity of 1.03 according to the method of Forney & Bandl (1992). Seeds were taken out from the aging box daily and stored in a freezer at -12 °C after drying at room temperature A germination test was conducted in moist paper towels according to the Association of Official Seed Analyst (AOSA, 1990). Aged seeds of which germination rate was about half of the normal seeds were made by aging dent and sweet corns for 10 days and super sweet corn for 3 days according to the results of the preliminary experiment.

Seed priming

To find out the optimum water potential for the priming of different corn types, 30 seeds were placed on moist paper towels saturated with 0, -0.3, -0 6, -0 9, and -1.2 MPa PEG 8000 solutions at 15 ± 0.1 °C for 2 days in plastic trays sealed with Parafilm to prevent water loss. After priming, the seeds were washed with running tap water for 1 minute, then removed water with a paper towel, and dried at room temperature. The seeds were stored in a freezer at -12 °C WP of PEG solutions was made by the equation of Mitchel (1983);

Ψ (MPa) = 0.129 $(PEG)^2T$ – 14 $(PEG)^2$ – 0.4 (PEG),

where PEG = g/1000 mL, T = °C.

Emergence rate, T50, and Plumule weight

Emergence rate, time to 50 % emergence (T50), and plumule weight of normal and aged seeds primed at various WP of PEG solutions were observed in warm and cold soil tests Thirty seeds were planted in a soil with 70% moisture contained in a 35 × 60 × 3 cm plastic tray and allowed to germinate at 25 ± 0.1°C or in a cold soil at 10 ± 0.1°C for 7 days followed by 25 ± 0.1°C for 7 days in a growth chamber (Growth Chamber HB-301LP, Hanbaeck Scientific Co., Korea). Emergence rate was measured according to AOSA (1990). Time to 50 % germination (T50) was calculated according to the following formula suggested by Taylor (2000):

$$T50 = t_i + \frac{(N/2 - n_i)}{n_j - n_i} \times (t_j - t_i).$$

Where N is the final number of germinants, n*i* and n*j* are the cumulative numbers of seeds germinated by adjacent counts at times t*i* and t*j*.

Plumule was cut just above the ground and dry weight was measured after drying in an oven at 80 ± 0.1 °C for 48 hours

Leakage of sugars and Electrolytes from seeds

To measure the leakage of total sugars, 20 seeds were soaked in 20 mL triple distilled water at 25 °C for 24 hours. The leachate was filtered through a Whatman #42 filter paper. Then, 10 mL of 0.2% Anthrone reagent in 98% sulfuric acid was added slowly to 5 mL of the leachate, mixed well, immediately heated in boiling water for 7.5 minutes, cooled in ice water, and left at room temperature for 15 minutes as in the method employed by Lee *et al.* (1995). Absorbance of the sample solutions was measured at 630 nm with a spectrophotometer (UVIKON Spectrophotometer, Kontron, Italy) and total sugars were calculated in glucose equivalents.

To measure leakage of electrolytes, 25 seeds were soaked in 75 mL of triple distilled water at 20 °C for 24 hours. Electrical conductivity (EC) of solution was measured using an EC meter (MC126 Conductivity Meter, Switzerland) according to the AOSA (1990).

α-amylase activity

The α-amylase activity of the seeds was measured by the method designed by Reiss (1994). Three seeds were soaked in distilled water at 25 °C for 7 days, dipped in liquid nitrogen, and ground in a mortar with a pestle. Ground samples were mixed in 10 mM cold citric acid-sodium citrate buffer solution and centrifuged for 20 minutes at 20,000 g to

remove the starch grains, cell walls, mitochondria, and nuclei. We mixed 1 mL supernatant and 2 mL of the soluble starch buffer solution (0.05 % starch in 0.05 M citric acid-sodium citrate buffer solution) for 20 minutes. Then, the α-amylase activity was stopped by adding 7 mL of 1 N HCl to the supernatant and buffer solution and mixed together We added 1 mL of iodine solution (5 g KI and 0.35 g KIO3 in 1,000 mL of 2 mM NaOH) to the mixture to develop a blue color. Absorbance of sample solutions was measured at 595 nm with a spectrophotometer (UVIKON Spectrophotometer, Kontron, Italy). The α-amylase activity was expressed as the percentage of starch lost.

DNA and Protein Analysis

Seeds were ground in a cyclone mill (1093 Cyclotec Sample Mill, Foss Tecator, Sweden) to pass a 100-mesh screen. For both DNA and protein analyses, 0.05 g of ground sample was put in a 1.5-mL e-tube and 600 μL of 2% CTAB buffer (100 mM Tri-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTBA in 1,000 mL water) previously warmed to 65 °C was added and incubated in a 65 °C water bath for 20 min with frequent agitating. Then, 600 μL of chloroform and isoamyl alcohol solution mixed with 24 : 1 ratio was added and shaken in a shaker (Orbital Shaker, Finemould Precision Ind., Co., Korea) at 130 ~ 150 rpm for 15 minutes. Then, 200 μL of supernatant was transferred to 1.5-mL e-tube, 2 μL of RNase (M610A, Promega, USA) was added, reacted at 37 °C for 30 minutes, and washed with 99 and 70% ethanol in order. The residue was dissolved in 200 μL TE buffer (10 mM Tri-HCl, 1 mM EDTA in 1,000 mL water) and the absorbance of sample was measured at 260 nm for DNA and

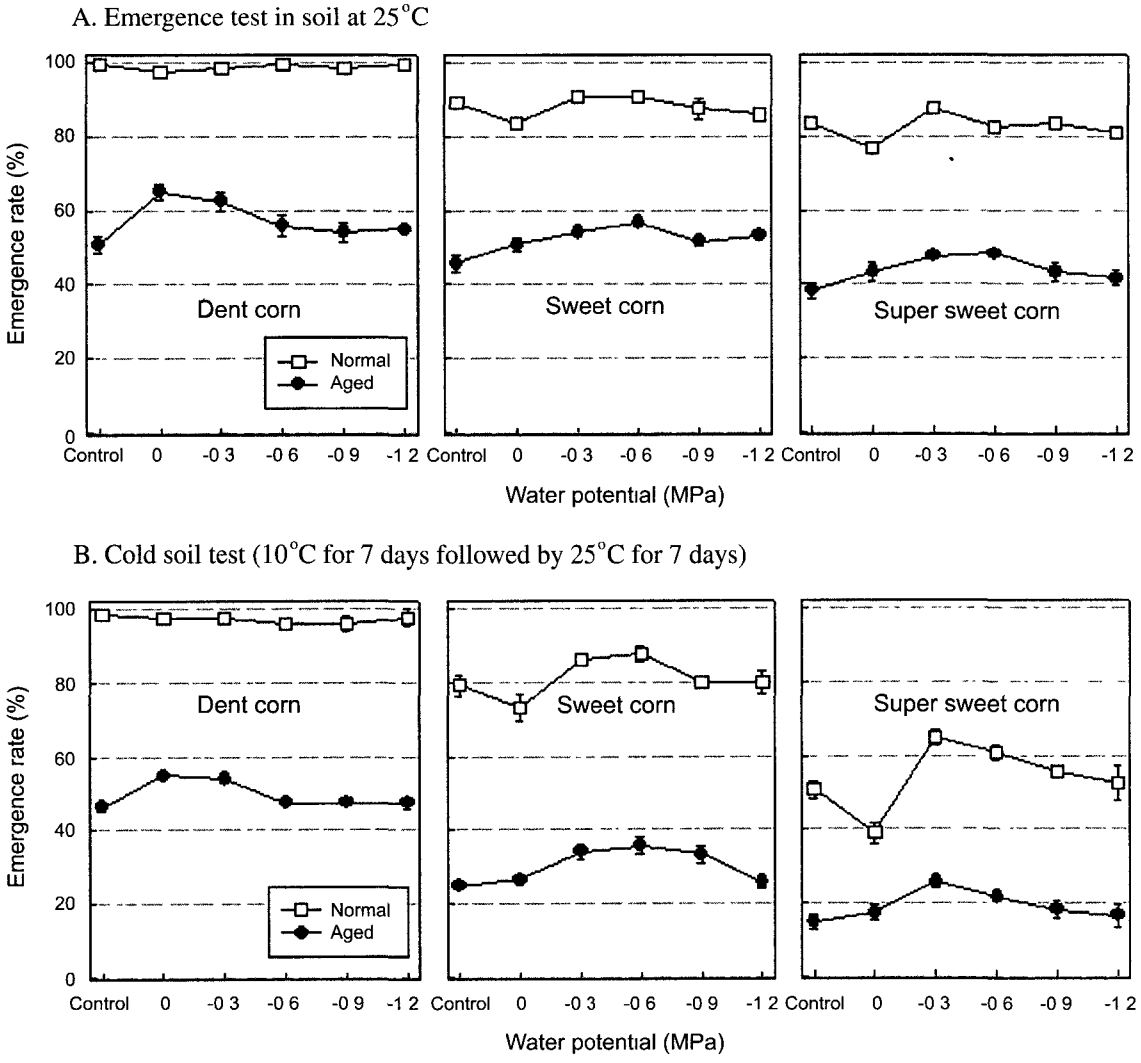

Fig. 1. Emergence rate of normal and aged dent, sweet, and super sweet corn seeds primed at different WP and germinated in soil at 25°C (A) and cold soil test (B). Control seeds were not primed Vertical line bars indicate standard error.

at 280 nm for protein with a spectrophotometer (UVIKON Spectrophotometer, Konton, Italy) according to the method of Nam & Ahn (1999).

RESULTS AND DISCUSSION

Emergence rate of primed seeds

Emergence rate of different quality seeds of three corn types are shown in Fig 1. Emergence rate of unprimed normal dent corn seeds was almost 100% and it remained high regardless of the WP of priming solutions when germinated both at 25°C (Fig. 1-A) and in cold soil test (Fig. 1-B), while priming of aged seeds at 0 and -0.3 MPa PEG solutions increased emergence rate significantly both at 25°C and in cold soil test.

Emergence rate of normal sweet and super sweet corn seeds primed at 0 MPa (distilled water) was lower than that of unprimed control seeds by 10 ~ 15 % when germinated both at 25°C and in cold soil test. However, the emergence rate of normal sweet and super sweet corn seeds increased with decreasing WP up to 0.3 or -0.6 MPa, and then decreased with further decrease in WP of PEG solutions.

In contrast, priming of aged sweet and super sweet corn seeds increased emergence rate even at 0 MPa and the optimum WP for priming was between 0.3 and 0.6 MPa. Chern & Sung (1991) reported priming of super sweet corn seeds at 0.3 MPa of PEG solutions could alleviate imbibition injury by retarding fast water absorption.

Time to 50 % emergence (T50)

T50 of primed dent corn, sweet and super sweet corn seeds germinated in moist paper towels at 25 °C and in cold soil test are shown in Table 1. Generally, T50 was similar among the corn types although T50 of dent corn was slightly shorter than those of sweet and super sweet corn and T50 of normal seeds was slightly shorter than that of aged seeds in each corn type. Generally, T50 value was shortest at the optimum WP of PEG solution for emergence rate, but the difference was small.

Table 1. The number of days to emergence (T50) of normal and aged dent, sweet, and super sweet corn seeds primed at different WP and germinated at 25°C and in cold soil test. (Unit, days)

Type of corn	WP (MPa)	25°C		Cold soil test†	
		Normal seed	Aged seed	Normal seed	Aged seed
Dent corn	Control‡	3.5 a§	3.6 a	9.0 a	9.4 a
	0	3 1 b	3.5 b	8.8 bc	9.2 abc
	-0 3	2 8 b	3.5 b	8.9 ab	9.1 bcd
	-0.6	3.3 a	3.5 b	8.7 c	9.3 ab
	-0 9	3 4 a	3.5 b	8 9 ab	9.0 cd
	-1 2	3.4 a	3.5 b	8.9 ab	8.9 d
Sweet corn	Control‡	3 7 ns	4.9 a	9 6 a	10.1 a
	0	3.5	4.4 c	9 3 b	9.3 c
	-0 3	3.4	4 5 bc	9 3 b	9 2 c
	-0.6	3.6	4.5 bc	9.2 bc	9.7 b
	-0 9	3.7	4 6 b	9 0 c	10 0 a
	-1.2	3.7	4 6 b	9 3 b	10.1 a
Super sweet corn	Control‡	3.5 ab	4 4 a	9.7 a	10 4 ns
	0	3.5 a	3.8 c	9.4 b	9.5
	-0 3	2.8 d	3 9 c	9.4 b	9.8
	-0 6	3.2 c	4 2 b	9.4 b	9.5
	-0 9	3.4 ab	4.1 b	9.1 c	10.0
	-1 2	3 3 bc	4.3 ab	9 4 b	10.1

†Cold soil test, 7 days at 10 °C followed by 25 °C for 7 days
‡Control seeds were not primed.
§Means within a column for a given corn type followed by the same letter are not significantly different at the 5% level by Duncan's New Multiple Range Test (DNMRT).

Plumule dry weight

The plumule dry weight of seeds primed at different WP of PEG solution is shown in Table 2. The plumule dry weight was greater in the order of super sweet corn < sweet corn < dent corn both in moist paper towel at 25 °C and in cold soil test. Dry weight of normal seeds was much greater compared to that of the aged seeds in all corn types.

In both normal and aged dent corn seeds, WP of PEG solution did not affect the plumule dry weight when tested at 25 °C, while the plumule dry weight of seeds primed in PEG solution was higher than that of unprimed seeds in cold soil test. However, the effects of WP on plumule dry weight of sweet and super sweet corn seeds were inconsistent, but generally plumule weight was highest at the optimum WP for emergence rate of -0.3 or -0 6 MPa.

Leakage of sugars and Electrolytes from seeds

The leakage of total sugars of normal and aged seeds primed at various WP is shown in Fig. 2. The leakage of total sugars were greater in the order of dent corn < sweet corn < super sweet corn. Both normal and aged dent corn seeds leaked very little sugars regardless of the WP of PEG solution.

The aged sweet corn seeds primed in PEG solution leaked a little more total sugars than that of normal seeds. Both normal and aged sweet corn primed at 0 MPa leaked less total sugars than unprimed seeds and leakage of sugars tended to increase as the WP of PEG solution decreased.

Both normal and aged super sweet corn seeds leaked significant amounts of total sugars and aged seeds leaked much more sugars than normal seeds at the comparable WP of PEG solution. The optimum WP to reduce leakage of total sugars was -0.6 MPa.

As a measure of electrolytes leakage to soaking water, EC of seed soaking solution is shown in Fig. 3. The EC of the seed soaked solution was greater in the order of dent corn < sweet corn < super sweet corn. Also, Wann (1986) showed that super sweet corn seeds leaked more electrolytes compared to those of sweet corn and waxy corns.

In dent corn EC of aged seeds was slightly higher than that of normal seeds. Priming of both normal and aged seeds reduced EC of soaking solution compared to unprimed seeds, while EC of seed soaking solution of all primed seeds was similar In contrast, the EC of seed soaking solution of

Table 2. Plumule dry weight of normal and aged dent, sweet, and super sweet corn seeds primed at different WP and germinated at 25°C and in cold soil test. (Unit, mg/plant)

Type of corn	WP (MPa)	25°C		Cold soil test†	
		Normal seed	Aged seed	Normal seed	Aged seed
Dent corn	Control‡	54.6 ns	32 2 ns	55.5 b§	35.7 cd
	0	58 3	31.1	61.0 a	33.7 d
	-0.3	54 2	34 1	62.6 a	39 8 a
	-0.6	57.8	36 1	59.9 a	36 4 bc
	-0.9	52.8	36 7	60.0 a	38 3 ab
	-1.2	61.8	33.7	62 2 a	37 3 bc
Sweet corn	Control‡	34.0 c	22.5 c	38 7 ns	25 1 d
	0	38.9 ab	24.7 bc	39.9	28 0 c
	-0.3	41.9 a	30.5 a	42.1	31.7 a
	-0 6	38 5 ab	30.5 a	40 5	30.6 ab
	-0 9	35 1 bc	29.7 a	37.2	29.5 b
	-1 2	37.2 bc	27 7 ab	40.8	29.7 b
Super sweet corn	Control‡	18.2 ns	11 6 ns	23.9 b	14.1 b
	0	17 5	11.9	16 2 c	12 0 c
	-0.3	22.3	11.6	26 2 ab	15 4 ab
	-0.6	17.6	12 2	26 0 ab	16 0 a
	-0.9	19.8	13.0	27 2 a	14 8 ab
	-1.2	20.6	10.4	24.0 b	14.2 b

†Cold soil test, 7 days at 10°C followed by 25°C for 7 days
‡Control seeds were not primed.
§Means within a column for a given corn type followed by the same letter are not significantly different at the 5% level by DNMRT

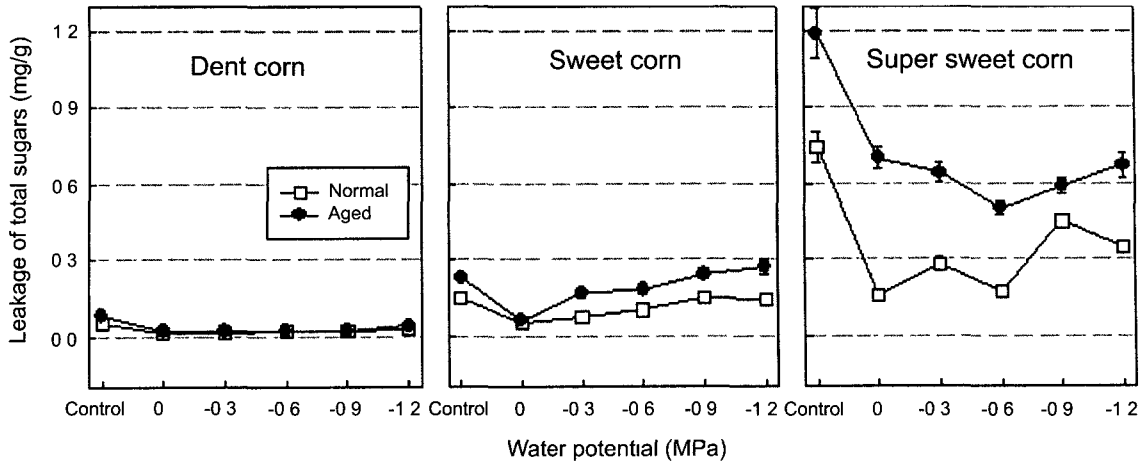

Fig. 2. Leakage of total sugars from normal and aged dent, sweet, and super sweet corn seeds primed at different WP and soaked in water. Control seeds were not primed. Vertical line bars indicate standard error

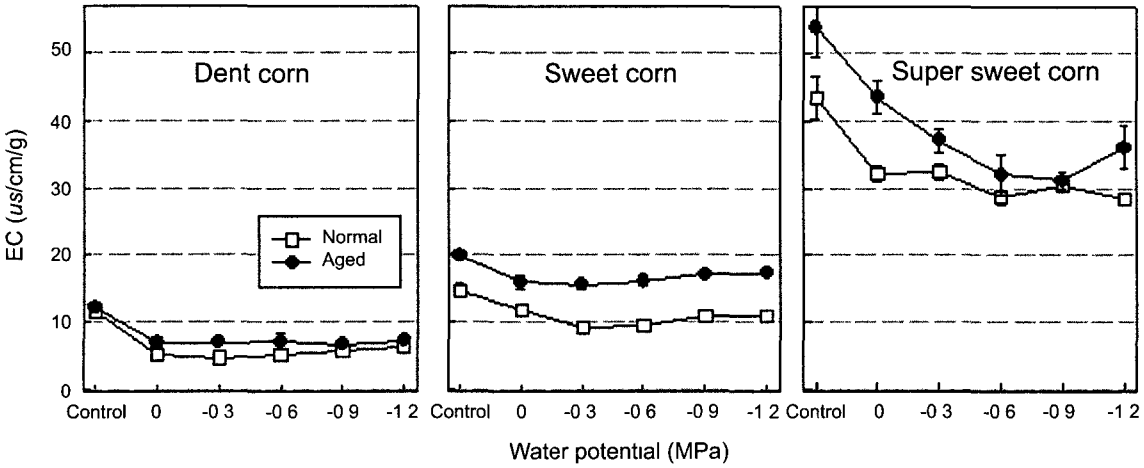

Fig. 3. Leakage of electrolytes from normal and aged dent, sweet, and super sweet corn seeds primed at different WP and soaked on water. Control seeds were not primed Vertical line bars indicate standard error.

aged sweet and super sweet corn seeds was much higher compared to that of normal seeds. The optimum WP of PEG solution to minimize EC of sweet corn was -0.3 MPa and -0.6 MPa for super sweet corn. Increased EC in aged seeds could be caused by loss of plasma membrane integrity (Abdul-Baki & Anderson, 1972) and the damages of membranes could be partially repaired during the seed priming.

α-amylase activity

The α-amylase activity of dent, sweet and super sweet corn seeds primed at various WP is shown in Fig. 4. The α-amylase activity was greater in the order of sweet corn < dent < super sweet corn and that of normal seeds was greater than that of aged ones in all corn types. Similar results were observed by Seo *et al.* (2003) in corn seeds matriconditioned at different water potentials.

In dent corn, the α-amylase activity of seeds primed at 0 MPa was much higher than that of unprimed control seeds, while it decreased with further decrease in WP of priming solution in both normal and aged seeds. However, seeds of sweet and super sweet corn showed highest α-amylase activity at WP of priming solutions of -0.3 and -0.6 MPa in both normal and aged seeds.

DNA and Soluble protein

DNA and soluble protein contents of dent, sweet, and super sweet corn seeds primed at various WP are shown in Table 3. DNA and soluble protein contents were greater in the order of super sweet corn < dent < sweet corn. Normal seeds had much higher DNA and soluble protein contents

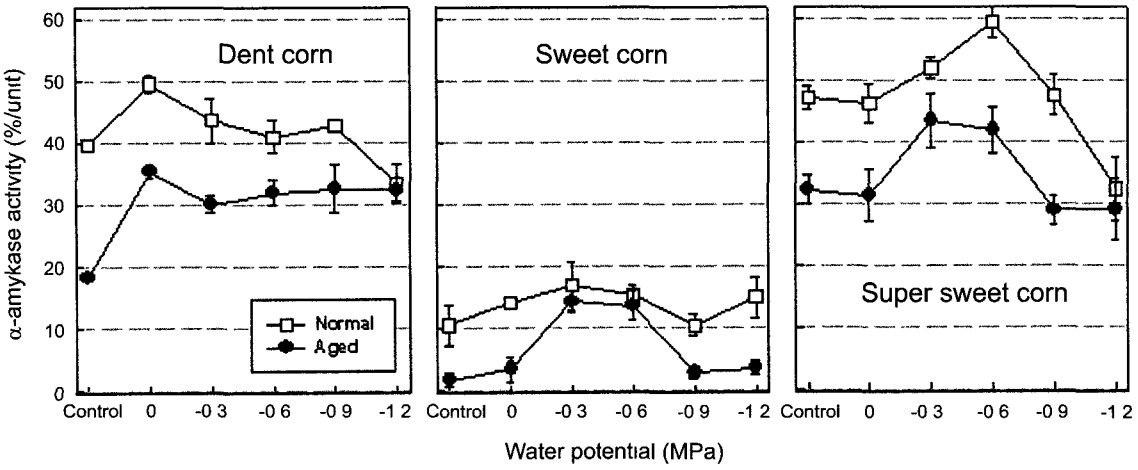

Fig. 4. The α-amylase activity of normal and aged dent, sweet, and super sweet corn seeds primed at different WP. Control seeds were not primed. Vertical line bars indicate standard error.

Table 3. DNA and protein contents of normal and aged dent, sweet, and super sweet corn seeds primed at different WP.

Type of corn	WP (MPa)	DNA (μg/g)		Soluble protein (mg/g)	
		Normal seed	Aged seed	Normal seed	Aged seed
Dent corn	Control†	37.2 d‡	23.0 d	2.2 b	1 7 bc
	0	51 5 a	27.9 a	2.8 a	1.9 a
	-0.3	44.4 b	25.7 b	2.3 b	1.8 ab
	-0.6	41.5 bc	24 0 c	2.2 b	1 6 c
	-0.9	42 8 bc	25.2 b	2.2 b	1.6 c
	-1.2	39 8 cd	24 8 bc	2.3 b	1.6 c
Sweet corn	Control†	164.0 c	95.9 c	9.4 b	8.4 cd
	0	173.3 ab	103 3 ab	10.3 a	7.2 e
	-0.3	178.0 a	105.0 a	10.6 a	8.9 ab
	-0.6	177.9 a	101.6 ab	10.6 a	9.1 a
	-0.9	175 0 a	97.8 bc	10.9 a	8 5 bc
	-1.2	168 2 bc	95.0 c	10.3 a	7 9 d
Super sweet corn	Control†	28 0 b	17.0 c	1 7 c	0.8 b
	0	29 1 a	19 2 ab	2.2 a	1 0 a
	-0 3	28.9 a	19 2 ab	1 9 b	1.0 ab
	-0 6	29.1 a	19 9 a	2 0 ab	1.0 a
	-0 9	29.2 a	19.2 ab	2.1 ab	1.0 ab
	-1 2	28.2 a	18.0 bc	2 0 ab	0.8 b

†Control seeds were not primed.
‡Means within a column for a given corn type followed by the same letter are not significantly different at the 5% level by DNMRT.

and similar results were observed by Seo *et al.* (2003) in corn seeds matriconditioned at different water potentials.

In dent corn, DNA and soluble protein contents were highest when seeds were primed at 0 MPa WP regardless of seed quality, and they tended to decrease with further decrease in WP. In sweet and super sweet corn, DNA and soluble proteins content were highest at WP between -0.3 and -0.6 MPa.

CONCLUSION

Effects of seed priming differed depending on corn type,

seed quality, and WP of priming solutions. Emergence rate of normal dent corn was extremely high (almost 100 %) and was not affected by priming at any WP (Fig. 1), while seed priming enhanced germination speed a little (Table 1) and increased plumule weight (Fig. 2) probably due to advanced germination processes during the priming. However, emergence rate of aged dent corn seeds was low and it must be related to the reduced DNA synthesis at the early stage of germination (Cruz-Garcia *et al*, 1995) In this experiment, the improved emergence rate of aged dent corn seeds primed at 0 MPa seems to be related to a high α-amylase activity (Fig. 4) and increased DNA and soluble protein contents (Table 2 & 3).

The emergence rate of unprimed normal sweet and super sweet corn seeds ranged 50-87 % and priming enhanced emergence rate at any WP of PEG solution except normal seeds primed at 0 MPa (Fig 3). The optimum WP for priming of sweet and super sweet corn seeds was between 0.3 and 0.6 MPa At the optimum WP the higher emergence rate could be the results of reduced leakage of total sugars and electrolytes, increased DNA and soluble contents, and α-amylase activity.

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