

Improved Germination and Modified Imbibition of *shrunken-2* Sweet Corn by Seed Disinfection and Solid Matrix Priming

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Abstract. Generally, sweet corn cultivars (*Zea mays* L.) carrying the *shrunken-2* (*sh2*) gene have lower germination and seedling vigor than normal or sugary (*su*) cultivars. Seeds of *sh2* 'How Sweet It Is' (HSII) and 'Crisp N'Sweet 711' (CNS-711) were imbibed for 6 hours. Rapid water uptake, higher seed leakage, and fungal infection were found in HSII, the lower germinating cultivar. Imbibition rate and leakage conductivity were reduced in both cultivars during the first 5 hours at 5C as compared with 25C. Sodium hypochlorite was an effective seed disinfectant. When the seeds were primed with sodium hypochlorite via solid matrix priming (SMP), germination under stressful conditions (soilcold test) was significantly improved in both cultivars. Primed seeds had significantly lower imbibitional rates and leakage conductivity than nonprimed seeds. The superior germination measured in primed and disinfected seeds was possibly due to the lower imbibitional rate and reduced seed fungal infection.

The incorporation of the mutant *sh2* gene in sweet corn has greatly improved sweet corn eating quality (Garwood et al., 1976). The *sh2* supersweet hybrids have high levels of sugar in the endosperm and excellent postharvest sugar retention (Laughnan, 1953). However, germination and seedling vigor are often poor, especially under stress conditions (Styer et al., 1980). Small endosperm (Warm, 1980), susceptibility to seed- and soil-borne diseases (Piecarka et al., 1978), and dysfunction in reserve mobilization (Styer and Cantliffe, 1983) have been reported as causes of reduced seed viability and seedling vigor.

Imbibition, or seed hydration, is the first step in germination. Rapid water uptake can negatively affect germination (Powell and Mathews, 1978). If water absorption is reduced during the early stages of imbibition, tissues develop in an organized manner, allowing sufficient time for membrane rearrangement and thus possibly reducing imbibition injury (Woodstock and Tao, 1981). Throughout imbibition, seeds lose a wide variety of carbohydrates, mineral nutrients, proteins, and organic acids (Nordin, 1984). Leakage can provide essential nutritive substances for fungi to develop on and around the seed (Schroth and Cook, 1964). Berger and Wolf (1974) reported that poor stand in *sh2* sweet corn was associated with seed rot and damping off. *Fusarium moniliforme* (Sheldon) penetrated *sh2* corn kernels via small cracks in the pericarp, where the pathogen localized between the pericarp and aleurone layer, and eventually moved into the endosperm and embryo (Styer and Cantliffe, 1983). Sodium hypochlorite has been used as a seed disinfectant in pepper (Fieldhouse and Sasser, 1975) and corn (Schoen and Kulik, 1977). Similarly, seedlings of 'Iochief' and 'Earlivee' sweet corn were less infected with *F. moniliforme* after sodium hypochlorite seed disinfection (Anderegg and Guthrie, 1981).

Seed priming has been a successful presowing seed treatment to improve the rate and uniformity of seed emergence under stress conditions. SMP controls seed hydration by the physical and osmotic characteristic of a solid matrix carrier (Kubik et al., 1989). The biochemical mechanisms involved in osmotic priming treatments are not entirely understood (Bradford, 1986).

Minimal research has been conducted on the seed membrane system during and after priming.

The objectives of this investigation were to 1) analyze the relationship among seed imbibition, leachate characteristics, and germination of *sh2* sweet corns during SMP treatments, 2) characterize the fungal infection of *sh2* sweet corn seeds, and 3) attempt to effectively control fungi with nonsynthetic fungicide disinfection. A combination of priming treatment with seed disinfection was evaluated as a method to improve *sh2* sweet corn germination and seedling vigor.

Materials and Methods

Plant material. Seeds of *sh2* sweet corn cultivars HSII and CNS-711 were obtained (Crookham Seed Co., Caldwell, Idaho) without chemical treatment. HSII (115 seeds per 10 g) is a white kernel hybrid that has poor emergence under stressful field conditions. CNS-711 (105 seeds per 100 g) has yellow kernels and has had good emergence under field conditions (Parera and Cantliffe, 1990).

Imbibition and seed leachate. Fifty seeds were imbibed in 50 ml of distilled water for 6 h at 25C to correlate germination, seed imbibition, electrolyte conductivity and potassium, and total sugar concentrations in the leachate. Also, 50 seeds were imbibed in distilled water at 5C and 25C to determine the effect of temperature on imbibition rate, electrolyte conductivity, and characteristics of the leachate.

Imbibition was defined as an increase in fresh weight. Changes in fresh weight were measured every hour after seed surface blotting and expressed as a percentage of fresh weight. Conductivity was measured at room temperature ($25 \pm 1C$) with a conductivity meter (Lecto Mho-Meter, Lab Line Instruments, Melrose Park, Ill.). Data were expressed as micromho per gram of seed. Potassium concentration was determined by a Perkin Elmer/232 flame spectrophotometer (Chapman and Pratt, 1961) and expressed as parts per million per gram of seed. Total sugar was analyzed by a calorimetric phenol method (Dubois et al., 1956). One milliliter of seed leachate was diluted in 250 ml of distilled water, and 1 ml of phenol (80%) was added to 2 ml of diluted sample, glucose standard, or distilled water (blank). After

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Abbreviations: CNS-711, 'Crisp N' Sweet 711'; HSII, 'How Sweet It Is'; *sh2*, *shrunken-2*; SMP, solid matrix priming.

mixing, 5 ml of sulfuric acid was added. The tubes were mixed again and placed in a water bath (25C) for 15 min. Absorbance was read at 490 nm in a Beckman DU-20 spectrophotometer (Beckman Instruments, Irvine, Calif.) and expressed as grams of sugar per 100 ml. Total soluble sugar content of dry seeds was determined by grinding a 5-g sample of seeds in a Virtis homogenizer (Virtis, Gardiner, N.Y.), mixing it with 8.5 ml of ethanol (95%) in a Mason jar, and then homogenizing it 1 min with a blender at high speed (Styer, 1982). Samples were sealed, placed in boiling water for 20 min to stop enzyme activity, and stored at - 20C overnight to precipitate insoluble materials. Each sample was filtered through Whatman no. 1 qualitative filter paper in a Buchner funnel (Fisher Scientific, Pittsburgh). Soluble sugars of the samples were assayed calorimetrically as described above.

Germination tests. The rolled-towel germination tests (AOSA, 1983) were used to correlate germination with imbibition and seed leachate and to evaluate seed disinfection methods. Fifty seeds were placed on each of three moist, nontoxic germination papers (Anchor Paper Co., St. Paul, Minn.). Papers were rolled, placed in plastic containers (21.5 × 32.5 × 5.5 cm), and incubated in a dark germinator at 25 ± 1C for 7 days. The SMP treatments also were evaluated through a cold soil germination test (AOSA, 1983). Twenty seeds were sown in a plastic container (18.7 × 12.5 × 9 cm), filled with 2.5 cm of Arredondo fine sand soil (loamy, siliceous, hyperthermic Grossarenic Palenundult) from a field in which corn had been grown the previous two seasons. The soil was compacted and another 2.5 cm was placed on top of the seeds. The soil was adjusted to 70% of its water-holding capacity. The containers were sealed and incubated at 10C for 7 days, then transferred to 25C for 4 days. Seedlings with leaves 2 mm in length above the soil were classified as emerged.

Seed disinfection. Two hundred seeds of each cultivar were enclosed in cheesecloth bags and soaked for 15, 30, or 60 min in a 0.05% or 0.5% solution (v/v) of sodium hypochlorite plus Tween-20, or in hot (45C) or cold (control; 25 ± 1C) tap water. After each treatment, the seeds were rinsed (15 sec) three times with tap water and air-dried [25 ± 1C, 45% relative humidity (RH)] for 1 h before the germination or incubation experiments. To determine the level of pathogenic infection on and in the seed before and after seed treatment, four seeds of each cultivar and treatment were plated in an acidified potato-dextrose agar (APDA). The plates were incubated at 25C for 10 days under continuous fluorescent light (5000 lux).

Solid matrix priming. Seeds (3 g) were mixed with 6 g of calcined clay (Emathlite, Mid-Florida Mining, Lowell, Fla.), and 2.5 ml of distilled water (HSII) or 2 ml (CNS-711) in a closed container (Filtunit, Nalgene, Rochester, N.Y.). The containers were rotated continuously at 0.22 rpm and incubated at 5C for 6 h, then transferred to 25C for 24 h. After 30 h of incubation, 2 ml (HSII) or 1.5 ml (CNS-711) of water or sodium hypochlorite (0.05%) was added and seeds were incubated an additional 15 h. After treatment the seeds were dried to the initial moisture content (6%) in a chamber at 25 ± 1C and 45% RH. The rate of imbibition and leakage conductivity after priming was determined by soaking 10 seeds in 25 ml of distilled water at 25C, and measured as described above.

Statistical analysis. All experiments were conducted as a randomized complete-block design with each treatment replicated four times. Percentage data were analyzed as square root arcsin transformations.

Results and Discussion

HSII had significantly lower germination in a rolled towel test, and HSII seeds contained more soluble sugar than seeds of CNS-711 (Table 1). After a 6-h soak in distilled water at 25C, HSII seeds imbibed water more rapidly, had more K and total sugar in the leachate that had greater electrolyte conductivity than that of seeds of CNS-711. The high levels of total soluble sugar in seeds could contribute to more rapid water uptake via greater water potential gradients. Imbibition damage was more severe in a rapidly imbibing cultivar of dwarf bean seed than one that imbibed water more slowly (Powell et al., 1986). Rapid imbibition may induce disruption of cell membranes (Powell and Matthews, 1978). Alteration in cell membrane structure caused by a rapid water uptake in seeds of HSII may have led to the high concentration of electrolytes found in the leachate.

Standard germination was negatively correlated with imbibition rate (6-h soak at 25C) ($r = -0.97$), leakage conductivity ($r = -0.76$), K concentration ($r = -0.75$), and total sugar levels in the seed leachate ($r = -0.76$). In contrast, there was a high positive correlation between 6-h imbibition and 1) electric seed leachate conductivity ($r = 0.99$), 2) K ($r = 0.98$), and 3) seed total soluble sugar content ($r = 0.97$). The stronger negative correlation measured between a standard germination test and seed leachate electrical conductivity confirmed the confidence of the method as an effective indicator of seed germination in sweet corn (Waters and Blanchette, 1983). Imbibition (Fig. 1) and leachate conductivity (Fig. 2) in both cultivars were significantly reduced when the seeds were soaked at 5C rather than at 25C. However, leachate conductivity was only significantly different between temperatures during the early stage of imbibition. Reduced water viscosity at the lower temperature (Vertucci, 1989) could have reduced the rate of imbibition and seed leachate.

The fungi detected by the APDA incubation test included *Fusarium* spp., *Rhizopus* sp., *Penicillium* spp., *Aspergillus* sp., and *Pythium* spp. (data not shown). These same pathogens were detected on *sh2* 'Florida Sweet' almost 20 years ago (Berger and Wolf, 1974). Fungal infection and development were more severe in HSII seeds, which had more crevices and cracks in the pericarp as seen in SEM micrographs (Parera, 1990), and this cultivar leaked more electrolytes than CNS-711. The seed characteristics of HSII may have created ideal conditions for fungal infection and development.

In both cultivars, seeds treated with sodium hypochlorite had significantly less or no fungal infection after incubation in APDA. Under standard germination conditions, seeds of CNS-711 treated with sodium hypochlorite (0.05% or 0.5%) or hot water did not

Table 1. Sweet corn standard germination, total seed soluble sugars, imbibition rate, and seed leachate characteristics after 6 h of imbibition at 25C.

Criterion	Cultivars	
	HSII	CNS-711
Germination (%)	76	97*
Total soluble sugars (g/100 ml)	0.184	0.077**
Imbibition (% FW)	95	66**
Leachate conductivity (μmhos/g seed)	98	35**
Potassium (ppm/g seed)	42	13**
Leachate sugar (g/100 ml)	0.318	0.069**

***Significant F test at $P = 0.05$ or 0.01 , respectively.

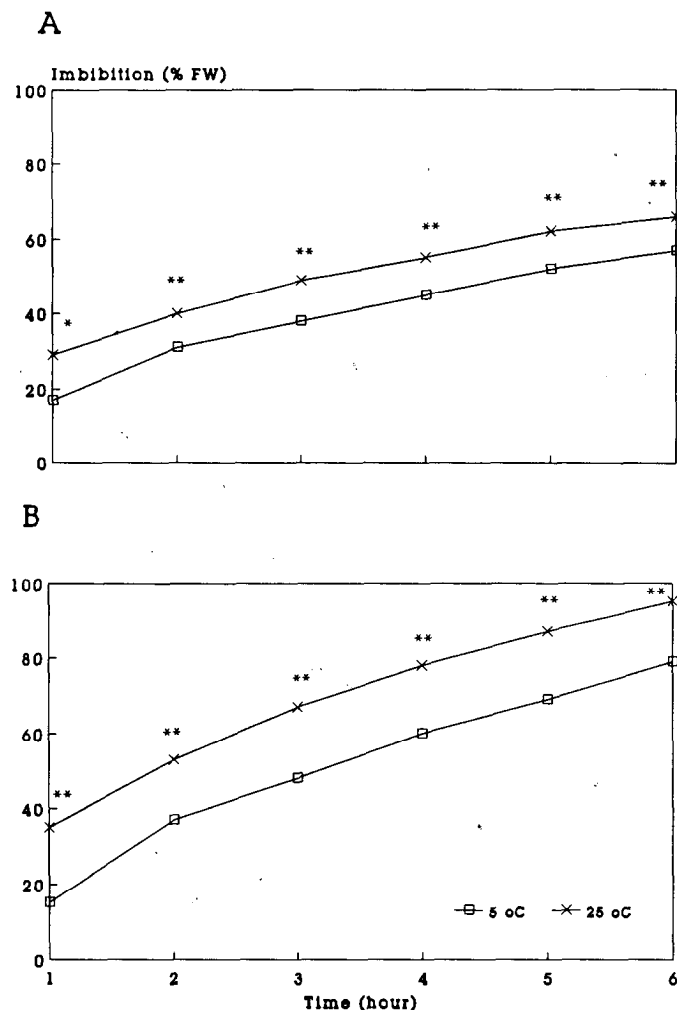


Fig. 1. Imbibition (50 seeds per 50 ml water) in CNS-711 (A) and HSII (B) sweet corn at 5 and 25C. ^{*},^{**} F test nonsignificant or significant at $P = 0.01$, respectively.

have an improved germination percentage over the control (Table 2). However, standard germination in a rolled-towel test of HSII was significantly improved in seeds treated with 0.05% sodium hypochlorite (Table 2). Hot water (45C) or sodium hypochlorite at 0.5% significantly reduced germination compared with the control. Germination was also significantly lower when the seeds were soaked 60 rather than 15 or 30 min. These results suggest that seed characteristics affected treatment effectiveness. The germination of HSII, which had a higher imbibition rate, was severely reduced by the higher concentration of sodium hypochlorite, longer soak time (60 min), or hot water.

To slow the rate of water uptake during the SMP treatments, a mix of seeds and clay was incubated at a cooler temperature (5C) during the first 6 h of imbibition. The entire mixture was transferred to 25C for periods up to 45 h so that *sh2* sweet corn germination could proceed normally. A significant reduction in imbibition rate during the first 6 h was measured between seed incubated in SMP mix at 5 and 25C (data not shown). There was no significant interaction between cultivar and treatment in the germination tests. When SMP was combined with a 0.05% sodium hypochlorite solution, germination in a standard or a cold test was significantly increased (Table 3). The SMP + 0.05% sodium hypochlorite treatment had a 3-fold increase in emergence compared with the control in the cold test. Sodium

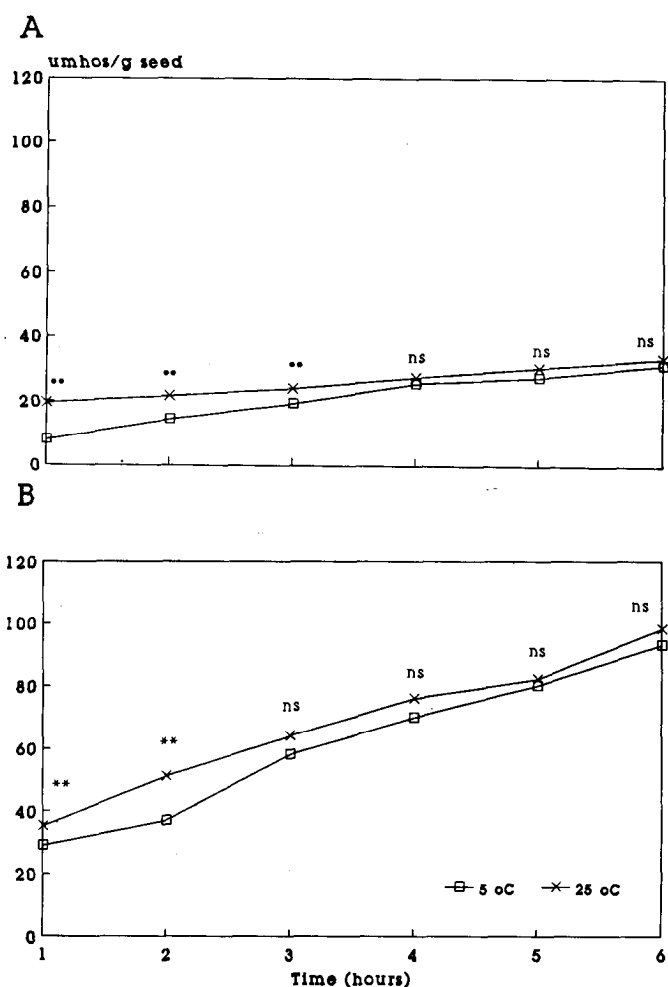


Fig. 2. Leachate conductivity (50 seeds per 50 ml water) in CNS-711 (A) and HSII (B) sweet corn at 5 and 25C. ^{*},^{**} F test nonsignificant or significant at $P = 0.01$, respectively.

Table 2. Effect of seed disinfection with sodium hypochlorite (NaOCl) and soaking time on percent standard germination of two *sh2* sweet corn cultivars.

Variable	Cultivars	
	CNS-711	HSII
Seed treatment ^a	Germination (%)	
NaOCl, 0.05%	98	86
0.5%	97	70
Water, hot	96	66
cold	96	80
LSD _(0.05)	2	6
Soaking time (min) ^b		
15	98	78
30	97	77
60	96	68
LSD _(0.05)	2	7

^aData pooled over soaking time.

^bData pooled over seed treatments.

hypochlorite or SMP alone did not significantly improve emergence compared with the control in the cold soil test. In the rolled-towel test, germination following the sodium hypochlorite (0.05%) treatment was similar as with the combination of SMP and disinfection (Table 3).

Imbibition and seed leachate conductivity were lower for primed

Table 3. Sweet corn standard germination and cold test results, after various priming and seed disinfection treatments.

Seed treatment	Germination (%) ^z	
	Standard	Cold test
SMP + NaOCl (0.05%)	88	37
SMP	66	18
NaOCl (0.05%)	90	8
Control	65	10
LSD _(0.05)	16	10

^zData pooled over two cultivars (CNS-711 and HSII).

Table 4. Sweet corn seed imbibition percentage after 6 h at 25C, and seed leachate electrical conductivity in primed and control seeds.

Criterion	Seed treatment [†]	
	Primed	Control
Imbibition (%)	41.7	46.4*
Conductivity (μmhos/g seed)	72.8	96.5*

[†]Data pooled over two cultivars (CNS-711 and HSII).

*Significant F test at P = 0.05.

(SMP + 0.05 sodium hypochlorite) than for control seeds after a 5-h soak in distilled water at 25C (Table 4). There was no interaction between cultivar and seed treatment.

The significant improvement in seed emergence observed by the combination of SMP and seed disinfection might be attributed to: 1) the decreased water uptake rate by primed seeds avoids early imbibitional injury; 2) lower levels of seed leachate, indicative of improved membrane reorganization, reduced substrate availability for fungal growth. An effective reduction of seed pathogen levels was achieved by the incorporation of sodium hypochlorite to the priming treatment after the seeds were partially hydrated for 6 h at low temperature (5 C). The short hydration treatment preceding exposure to pathogens appeared to be an effective method for reducing pathogens localized both on the seed surface and within the seed or in seed crevices.

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