Improving the Field Emergence Performance of Super Sweet Corn by Sand Priming

Guangwu Zhao¹, Tailin Zhong¹ and Dongsong Zheng²

¹School of Agriculture and Food Science and Botanical Garden, Zhejiang Forestry College, Huan Cheng North Road 88, Lin’an 311300, Zhejiang, P. R. China; ²College of Physics and Electronic Information, Wenzhou University, Wenzhou 325027, Zhejiang, P. R. China)

Abstract: A priming method called sand priming was developed using sand as a priming solid matrix. The effect of sand priming on improving the field emergence performance of five super sweet corn cultivars was investigated. Sand priming significantly improved field emergence performance of all super sweet corn cultivars, and there was marked improvement by priming at 20°C for 24 hr. After sand priming at 20°C for 24 hr, field emergence percentage (FEP) of “Green Superman”, “Huatian 1”, “Yangtian 1”, “Mitian 8”, and “Chaotian 43” was increased by 52.1%, 37.5%, 38.0%, 40.9%, and 33.3%, respectively. Their field emergence speed (FES) was 2.3, 1.8, 2.0, 2.0, and 1.8 times of the control, respectively. To further elucidate the effect of sand priming on improving the field emergence performance of super sweet corn, we analyzed the membrane system integrity, α-amylase activity and protein content. Sand priming at 20°C for 24 hr improved membrane system integrity and α-amylase activity in all super sweet corn cultivars. Furthermore, sand priming at 20°C for 24 hr accelerated the degradation of embryo protein after 1 d germination in “Green Superman”.

Key words: α-amylase, Electrolyte leakage, Embryo protein, Field emergence performance, Sand priming, Super sweet corn.

Super sweet corn (Zea mays L.) is widely planted and is favored by consumers. However, seeds of Shrunken-2 (sh2) varieties such as Green Superman are less vigorous than the other sweet corn types due to reduced starch reserves for germination, cracked seed coats, and increased sugars which render the seed more susceptible to disease. Poor seedling establishment was the major obstacle in sweet corn production (Zhao et al., 2007). Therefore, how to improve seedling emergence has been extensively studied in super sweet corn.

Seed priming is a technique controlling hydration and drying that results in more rapid germination when the seeds are re-imbibed. Seed priming has been found to be a feasible technology to improve seedling emergence in some field crops such as cotton, common maize, rice and wheat (Murungu et al., 2004; Farooq et al., 2006). Sand is an excellent inert matrix, because it is inexpensive and easily to purchase. Evidence has emerged that sand priming significantly improved germination performance in fir and rice (Ma et al., 2003; Hu et al., 2005).

In the present experiment, a priming method was developed using sand as a priming solid matrix (sand priming). Field emergence percentage (FEP) and field emergence speed (FES) are vital indices for field performance assessment since they directly affect crop yield and quality (Zhao et al., 2007). Therefore, the effect of sand priming on improving the field emergence performance of super sweet corn was investigated. Many studies suggested that membrane system integrity, α-amylase activity, and embryo protein content were considered to be important indices of germination performance (Liu et al., 1999; Black and Bewley, 2000; Farooq et al., 2006). To further elucidate the effect of sand priming on the field emergence, we also analyzed the performance of super sweet corn, membrane system integrity, α-amylase activity, protein content. The aim of our study is to understand the conditions necessary to improve the field emergence performance of super sweet corn by sand priming.
Table 1. Field emergence performance of seeds subjected to sand priming at various temperatures and durations in five super sweet corn cultivars.

| Sand priming | Green Superman | Huatian 1 | Yangtian 1 | Mitian 8 | Chaotian 43 |
|--------------|----------------|-----------|------------|----------|-------------|
|              | FEP | FES | FEP | FES | FEP | FES | FEP | FES | FEP | FES |
| 15°C/12 hr   | 55.0 d | 28.0 c | 64.0 d | 34.0 cd | 55.0 c | 26.0 d | 51.0 d | 22.0 c | 65.0 d | 42.0 d |
| 15°C/18 hr   | 59.0 c | 30.0 bc | 67.0 cd | 35.0 c | 57.0 c | 30.0 bc | 53.0 cd | 25.0 bc | 67.0 cd | 45.0 c |
| 15°C/24 hr   | 61.0 c | 32.0 b | 69.0 c | 39.0 b | 60.0 bc | 32.0 b | 56.0 c | 27.0 b | 70.0 c | 47.0 b |
| 20°C/12 hr   | 60.0 c | 30.0 bc | 69.0 c | 38.0 bc | 59.0 bc | 29.0 c | 55.0 c | 25.0 bc | 70.0 c | 45.0 c |
| 20°C/18 hr   | 65.0 b | 35.0 b | 73.0 b | 40.0 b | 65.0 b | 32.0 b | 60.0 b | 28.0 b | 74.0 b | 48.0 b |
| 20°C/24 hr   | 73.0 a | 42.0 a | 77.0 a | 45.0 a | 69.0 a | 40.0 a | 62.0 a | 32.0 a | 80.0 a | 53.0 a |
| 25°C/12 hr   | 63.0 bc | 34.0 b | 72.0 b | 41.0 b | 62.0 bc | 33.0 b | 58.0 bc | 30.0 ab | 71.0 c | 50.0 ab |
| 25°C/18 hr   | 61.0 c | 31.0 bc | 69.0 c | 39.0 b | 60.0 bc | 30.0 bc | 56.0 c | 26.0 bc | 68.0 cd | 36.0 bc |
| 25°C/24 hr   | 57.0 cd | 26.0 c | 67.0 cd | 31.0 d | 56.0 c | 24.0 d | 52.0 d | 21.0 c | 64.0 d | 42.0 d |
| Control      | 48.0 e | 18.0 d | 56.0 e | 25.0 e | 50.0 d | 20.0 e | 44.0 e | 16.0 d | 60.0 e | 30.0 e |

Means of different treatments followed by the same letters are not significantly different at the 0.05 probability level according to Duncan’s LSR.

Materials and Methods

1. Seed materials
   Seeds of five widely planted super sweet corn cultivars “Green Superman”, “Huatian 1”, “Yangtian 1”, “Mitian 8”, and “Chaotian 43” that carries shrunken-2 (sh2) mutant endosperm were produced under the same environmental conditions in Jiuquan, Gansu province in China. This region is an important corn seed production base with average annual precipitation of no more than 100 mm. A distance of 400 m was used to avoid cross pollination. Seeds were harvested when the average moisture content was 25%, shelled with a thresher, dried to 9.0% moisture content by sun curing, and then stored under the conditions of 4ºC and 40% relative humidity (RH).

2. Seed treatments
   Fresh sand passing through a sieve with a mesh size of 0.8 mm was sterilized at 180ºC for 1 hr. After the sand was cooled, sterile water was added to make the ratio of water volume/sand weight 18.0% (v/w). Seeds were uniformly embedded in the wet sand and incubated at 15ºC, 20ºC and 25ºC for 12 hr, 18 hr, and 24 hr in darkness. Then, seeds were cleaned, re-dried to the initial seed moisture content (9.0%) at 30ºC for 48 hr in a drying cabinet (Tuopu, Hangzhou, China), and then stored under the conditions of 4ºC and 40% RH.

3. Field emergence test
   The field emergence test was conducted using a randomized complete block design on three replicates of 100 seeds for each hybrid. The plant and row spacing was 30 cm × 30 cm. Seeds were sown in soil 5 cm deep when the average temperature was 16.5ºC and the average relative water content was 72% at the sowing season in 2007. The number of emerged seedlings higher than 2 cm was recorded every day until no further emergence. FES was calculated when 50% FEP was reached.

4. Electrical conductivity test
   The electrical conductivity test was conducted on three replicates of 100 seeds for each treatment. Seeds were soaked in 250 mL distilled water at 20ºC for 8 hr, 16 hr, 24 hr, 32 hr and 40 hr. Electrical conductivity was then tested with a DDS-307A conductivity meter (Shanghai, China). The conductivity per gram of seed weight for each replicate was calculated according to the formula: EC (µS cm⁻¹ g⁻¹) = [Conductivity reading (µS cm⁻¹)−background reading] / Seed weight (g) of replicate.

5. Determination of α-amylase activity
   Three replicates of 1.0 g seeds for each treatment grown at 20ºC for 60 hr with an interval of 12 hr were hand-ground at 4ºC, mixed with 10 mL extraction buffer (20 mmol L⁻¹ sodium acetate, 1 mmol L⁻¹ CaCl₂, pH 5.5), and incubated at 4ºC for 1 hr with agitation. Then, the suspensions were centrifuged at 10000 r min⁻¹ for 10 min, and the supernatants were analyzed for α-amylase activity by the DNS method (Bernfeld, 1955).

6. Extraction, content determination and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of protein
   Seeds of “Green Superman” were incubated at 20ºC for 0–2 d. Then, 1.0 g detached embryos were ground with extraction buffer (0.25 M NaCl, 1% SDS, 1% 2-mercaptoethanol, 0.05 M sodium phosphate buffer pH 7.5). The homogenates were transferred to a 10 mL tube and centrifuged at 12000 r min⁻¹ for 15 min at 4ºC. The supernatants were then transferred to
another tube and centrifuged at 12000 r \text{ min}^{-1} \text{ for } 10 \text{ min at 4}^\circ\text{C}. Finally, the supernatants were collected and conserved at 4^\circ\text{C}. Protein standard curve was made by a series of bovine serum albumin (BSA) standard solution. An aliquot of 0.8 mL sample was mixed with 0.2 mL Coomassie Brilliant Blue G250 (CBB-G250). The OD value at 595 nm was determined with a 721W spectrophotometer (Shanghai, China). Protein content was confirmed by the standard curve. SDS-PAGE was used to separate proteins according to their size. Procedures for SDS-PAGE were: (1) making the gel and assembling the gel apparatus, (2) mixing protein samples with sample buffer containing SDS and heating the mixture at a high temperature, (3) loading samples and running the electrophoresis, and (4) fixing and staining the separated proteins with CBB-R250.

Results

1. Effect of sand priming on field emergence performance

Sand priming significantly improved field emergence performance of all super sweet corn cultivars (Table 1). Priming at 20ºC for 24 hr brought about marked improvement (Table 1). After priming at 20ºC for 24 hr, FEP of “Green Superman”, “Huatian 1”, “Yangtian 1”, “Mitian 8” and “Chaotian 43” was increased by 52.1%, 37.5%, 38.0%, 40.9% and 33.3%, respectively. FES of “Green Superman”, “Huatian 1”, “Yangtian 1”, “Mitian 8”, “Chaotian 43” was 2.3, 1.8, 2.0, 2.0, 1.8 times higher than that of the control, respectively.

At 15ºC and 20ºC, FEP and FES of all cultivars were improved with the increase of priming temperature. However, at 25ºC, they were decreased with the increase of priming time. When the priming time was 12 hr, FEP and FES of all cultivars were improved with the increase of priming temperature. However, when the priming time was 18 hr and 24 hr, FEP and FES increased initially, but then decreased with the increase of priming temperature.

2. Effect of sand priming on membrane system integrity

Sand priming at 20ºC for 24 hr decreased the electrolyte leakage in all super sweet corn cultivars irrespective of imbibition time (Fig. 1). Furthermore, the electrolyte leakage of seeds subjected to sand priming at 20ºC for 24 hr increased more slowly than the control with the during imbibition. To compare the effect of sand priming on electrolyte leakage among different cultivars, we performed regression analysis and established the regression equation between electrolyte leakage and imbibition time (Fig. 1). In the regression equation, the slope denoted electrolyte leakage rate. The electrolyte leakage rate of “Green Superman”, “Huatian 1”, “Yangtian 1”, “Mitian 8” and “Chaotian 43” subjected to sand priming at 20ºC for 24 hr was 53.2%, 25.3%, 11.6%, 18.0% and 41.9% lower than that of the control, respectively. The above results suggested that sand priming improved membrane system integrity.

3. Effect of sand priming on $\alpha$-amylase activity

Sand priming at 20ºC for 24 hr increased the $\alpha$-amylase activity in all super sweet corn cultivars at any germination time (Fig. 2). Furthermore, $\alpha$-amylase activity in seeds subjected to sand priming at 20ºC
for 24 hr increased during seed germination more rapidly than the control. To compare the effect of sand priming on $\alpha$-amylase activity among different cultivars, we performed regression analysis and established the regression equation between $\alpha$-amylase activity and germination time (Fig. 2). In the regression equation, the slope denoted the rate of increase in $\alpha$-amylase activity. The rate of increase in $\alpha$-amylase activity of “Green Superman”, “Huatian 1”, “Yangtian 1”, “Mitian 8” and “Chaotian 43”, respectively. The above results suggested that sand priming improved $\alpha$-amylase activity and accelerated the rate of increase in $\alpha$-amylase activity during seed germination.

4. Effect of sand priming on protein content of "Green Superman"

During 0–1 d germination, there were no obvious differences in embryo protein content between super sweet corn “Green Superman” seeds subjected to sand priming at 20°C for 24h and the control (Fig. 3). As shown in Fig. 4, embryo proteins (28.0, 32.6, 50.0 and 75.4 kDa) were all slowly degraded during 0–1 d germination in primed seeds and the control. Embryo
protein contents of primed seeds and the control declined by 18.8% and 17.6%, respectively, after 1 d germination compared with 0 day germination. During 1–2 d germination, it declined markedly in primed seed, but still slowly in the control. This obvious change was also seen in embryo protein degradation on the SDS-PAGE map (Fig. 4). From 1 to 2 d germination, embryo protein contents of the primed seeds and the control declined by 41.4% and 20.0%, respectively. The above results suggested that sand priming accelerated embryo protein degradation after 1 d germination in “Green Superman”.

Discussion

The present study showed that sand priming technology can improve the field emergence performance of super sweet corn as reported in rice. Hu et al. (2005) reported that sand priming improved germination percentage, germination index, seedling height, root length, number of root and root dry weight and so on. However, there is an obvious difference in the conditions for improving the emergence performance between super sweet corn and rice. Sand priming at 20°C for 24 hr was optimal for super sweet corn, but unsuitable for rice.

Many environmental factors can influence priming effects. McDonald (1999) considered that priming temperature was critical. In this study, we found that the field emergence performance was improved with the increase of temperature (15°C, 20°C, 25°C) when the priming time was 12 hr, but increased initially and then decreased with the increase of temperature when the priming time was 18 hr and 24 hr. However, Haigh et al. (1986) concluded that temperature (15°C, 20°C, 25°C) during priming had little effect on germination performances of onion seeds. Besides priming temperature, priming time was also pivotal (Bradford and Haigh, 1994). In this study, we found that the field emergence performance was improved with the increase of priming time (12 hr, 18 hr, 24 hr) at 15°C and 20°C, whereas they were decreased with the increase of priming time at 25°C. Furthermore, the most remarkable improvement occurred at the combination of 20°C and 24 hr. These findings suggest that different temperature and time must be evaluated for each crop species to determine which provides the best priming effects.

Black and Bewley (2000) considered that seed priming promoted the repair of cell membrane and the recovery of membrane system integrity resulting in the decrease in substance leakage in seeds. Our results showed that sand priming reduced electrolyte leakage, which was in accordance with those in wheat and soybean (Soltani et al., 2001; Mann et al., 2002). However, expect for “Chaotian 43”, the initial electrolyte leakages in “Green Superman” and “Mitian 8” were higher than those of the controls (Fig. 1). The result suggested that the primed seeds leaked some electrolytes during priming.

The activity of α-amylase is closely related to seed germination ability. Farooq et al. (2006) considered that the higher the α-amylase activity the higher would be metabolic activity in seeds, which indicated the higher germination ability. Skadsen (1993) reported that higher quality malting barley showed high α-amylase activity soon after germination, whereas a poor malting quality variety showed little α-amylase activity. In this study, sand priming improved α-amylase activity and accelerated the increase of α-amylase activity during seed germination. The results suggest that sand priming ensures the proper hydration which results in improved α-amylase activity that hydrolyzes the macro starch molecules into smaller sugars such as maltose for the utility during seed germination.

Interestingly, after sand priming at 20°C for 24 hr, the electrolyte leakage rate during imbibition was lowest, and the rate of increase in α-amylase activity during seed germination was highest in “Green Superman” among all super sweet corn cultivars (Figs. 1, 2). The results suggest that the electrolyte leakage and α-amylase activity change in parallel with the field emergence performance after 20°C/24 hr sand priming.

Our results suggest that sand priming improves seed vigor. A previous study showed that the content of embryo protein in high vigor seeds declined more rapidly than that in low vigor seeds during germination (Liu et al., 1999), which was consistent with our results in “Green Superman”. All these results suggest that it is crucial for seed germination to degrade embryo protein to supply energy for seed germination. Proteins were also synthesized as they were degraded during seed germination. Sand priming should lead to
the synthesis of some special proteins. Liu et al. (2000) found that seeds with high vigor synthesized more heat shock proteins during seed germination. Gallardo et al. (2001) conducted the proteomic analysis of Arabidopsis seed germination and priming by two-dimensional gels. They characterized some special proteins. Some of them were involved either in the imbibition process of the seeds (such as a WD-40 repeat protein) or in the seed dehydration process (such as cytosolic glyceraldehyde-3-phosphate dehydrogenase). Which special proteins were induced by sand priming in our study should be further investigated.

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