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Germination Behavior of Five Genotypic Different of Immature Vegetable Corn Seed and in Response to Hydro-Priming

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ABSTRACT

During the mission of corn germplasm collection, collectors, in some cases, inevitably collect immature seeds because frequent visits to the collection sites are not possible. Therefore, the objectives of this study were find out the possibility of collecting seed from fresh ear vegetable corns and find out the effect of hydro priming on their seed germination behaviors. Five vegetable corn inbred lines were harvested at soft dough stage (R_4-R_5) or 21 days after silking and air dried to 13% moisture. Measurements were made on kernel dry weight, endosperm dry weight, embryo dry weight, and endosperm and embryo ratio. Then seeds were primed in water for 0, 3, 6, 9, 12, 15, and 18 hours, followed by surface drying and immediate sowing. The treatments were laid out in 5 (inbred lines) × 7 (priming durations) factorial combinations in a completely randomized design with three replications. The results indicated that kernel dry weight of inbred lines with shrunken-2 (*sh*$_2$) gene or brittle (*bt*) gene was significantly lower than that of inbred lines with sugary (*su*) or waxy (*wx*) genotype. The endosperm/embryo dry weight ratio was also low in the high-sugar lines due primarily to their small endosperm. Differences among inbred lines for seed germination were primarily due to kernel and endosperm size, and the interactions between inbred line and priming duration were also significant for all characters. The hydro-priming application in this study did not enhance seed germination and seed vigor, but it enhanced the time to germination, indicating that the early germination was archived when hydro-priming was applied. Therefore, there was the possibility of the collection the germplasm at soft dough stage (edible stage), and hydro-priming can be used to enhance the time of germination.

Keywords: germplasm collection, seed priming, seed quality, soft dough stage, sweet corn, waxy corn

INTRODUCTION

Landrace varieties are important germplasm sources for plant breeding. For vegetable corn, these varieties are conserved and maintained by smallholder growers. The erosion of germplasm *in situ* has been occurred rapidly because the adoption of improved varieties by corn growers. Therefore, collection of landrace varieties is necessary for future use. During the mission of corn germplasm collection, collectors, in some cases, inevitably collect immature seeds because frequent visits to the collection sites are not possible. In addition to germplasm collection, the harvest of immature seed may be necessary for rapid generation advance in breeding programs or when environmental conditions are not favorable such as drought or flooding conditions.

Corn seeds reached physiological maturity at 32-60 days after silking depending on varieties, environments and their interaction (Hillson and Penny 1965; Ngamprasitthi *et al*. 2008; Sawatdikarn 2008: Siri *et al.* 2008). At this stage, the seeds archived its maximum dry weight and seed quality (Copeland and McDonald 2001). Immature seeds, in contrast, are much lower in seed quality and germination compared to mature seeds.

Seed priming has been applied to improve seed performance during germination and seedling early growth (Bennett and Waters 1987; Clark *et al*. 2001; Harris 2007; Dezfuli *et al.* 2008). The general purpose of seed priming is to hydrate partially the seeds to a point that germination processes are initiated but not completed. Most priming treatments involve imbibing seeds with restricted amounts of water to allow sufficient hydration and advance of metabolic processes but preventing the protrusion of the radicle. Seed priming can be accomplished through different methods such as hydro-priming (soaking in water), osmo-priming (soaking in osmotic solutions such as polyethylene glycol, potassium salts, e.g., KCl, K_2SO_4), solid matrix priming, and using plant growth regulators (PGRs) (Dezfuli *et al.* 2008). Hydro-priming is most commonly used because this technique minimizes the use of chemicals and avoids discarding materials that can be undesirable and incompatibles with the environment (McDonald 2000).

Seed priming is performed mostly in aiming to improve the germinability of deteriorated seed. For immature seed, seed priming might increase seed quality e.g. germination percentage, germination index, time to 50% germination (T_{50}), mean germination times (MGT), vigor index, seedling growth rate, and seedling dry weight, and optimum duration seed priming should be identified for immature seeds of vegetable corn with different types of endosperms.

The questions underlying this research are whether or not seed priming can improve these parameters under laboratory conditions and what optimum priming durations for different types of endosperms are. The objectives of this study were compare germination behaviors of immature seeds of vegetable corn with different types of endosperms and to evaluate the effects of hydro-priming treatments on seed germination behavior of fresh ear harvested corns.

MATERIALS AND METHODS

Plant materials and field management

To assess the priming effects on germination parameters, five vegetable corn inbred lines representing three groups of vegetable corn (waxy corn ('101*wx*' and '216*wx*'), sweet corn ('101*su*') and super sweet corn ('101*bt*' and '216*sh*$_2$')) were selected for this study. These lines are promising inbreds in the pipeline of breeding program at the Plant Breeding Research Center for Sustainable

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Plants were grown under field conditions with optimum agronomic practices for commercial production. Ears were harvested at soft dough stage (R_4-R_5) or 21 days after silking. The fresh ears of five inbred lines were dried to 13% moisture content at room temperature in a shelter with ceiling fans. Dry kernels were removed from cobs manually.

Kernel characters evaluated

For seed characters, ten kernels were used for each sample, and the samples were replicated three times. Endosperm and embryo were separated from the kernels and pericarps were discarded. Endosperm and embryo were oven-dried at 80°C for 24 hrs or until constant weight, and endosperm dry weight, embryo dry weight and endosperm/embryo ratio were determined.

Separated dry seed samples were used for determining kernel dry weight. Ten kernels were used for each sample and the samples were replicated three times. The samples were incubated in an oven using the procedures described previously for embryo and endosperm dry weight. After oven-dry, kernel dry weight was determined.

Hydro-priming experiment

Seeds of five inbred lines were primed in distilled water for seven priming durations of 0, 3, 6, 9, 12, 15, and 18 hrs, followed by surface drying. Fifty seeds from each of the 35 treatment combinations were sowed in sand boxes and the experiment was replicated three times. The seeds were germinated at average temperatures of 32/26°C day/night and average natural light intensity of 3665 lumen m^{-2} with photoperiod of 13 hrs.

The seedlings were evaluated as germination percentage (%), germination index, time to 50% germination (T_{50}), mean germination times (MGT), vigor index, seedling growth rate, and seedling dry weight.

The time to 50% germination (T_{50}) was calculated according to the following formula:

$$T_{50} = t_i + \frac{\left[(N/2) - n_i\right](t_i - t_j)}{n_i - n_j}$$

where N is the final number of germination and n_i, n_j cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

The germination index (GI) was calculated by following formula:

$$GI = \frac{\text{No. of germination seed}}{\text{Days of first count}} + \ldots + \frac{\text{No. of germination seed}}{\text{Days of final count}}$$

Mean germination time (MGT) was calculated based on the following equation:

$$MGT = \frac{\sum Dn}{\sum n}$$

where n is the number of seed, which were germinated on day D, and D is number of days counted from the beginning of germination.

The vigor index was calculated according to following formula:

Vigor index (VI) = [seedling length (cm) × germination percentage]

The seed ling growth rate (SGR) was calculated by following formula:

$$SGR = \frac{\text{Seedling dry weight (mg)}}{\text{No. of normal seedling}}$$

Final germination percentage (%), seedling length (cm), and seedling dry weight (mg) was recorded after 7 days of planting.

Statistical analysis of data

Data were subjected to analysis of variance depending on the experimental designs. For seed characters, the analysis of variance followed a completely randomized design (CRD), and seed germination behaviors followed a 5 × 7 factorial experiment in CRD with three replications (Roger 2003). Treatment means were compared using least significant test (LSD) at 5% level of probability. Pearson correlations were studied between seed parameters and seed germination parameter and correlation coefficients were determined (Roger 2003). All calculations were accomplished using MSTAT-C software package.

RESULTS AND DISCUSSION

Significant differences among inbred lines were observed for kernel dry weight, endosperm dry weight, embryo dry weight and endosperm/embryo ratio (**Table 1**). Waxy genotype had the highest kernel dry weight followed by sugary genotype, brittle genotype and shrunken-2 genotype, respectively. The differences in endosperm dry weight among inbred lines followed a similar pattern of those for kernel dry weight. The correlation coefficient between these characters was positive and highly significant (0.93, $p \leq 0.01$) (**Table 2**). The results might indicate that endosperm weight determines kernel size (Wann 1980; Styer and Cantiliffe 1984).

Embryo dry weight and endosperm/embryo ratio did not follow the patterns of kernel dry weight and endosperm dry weight (**Table 1**). These characters were dependent on lines rather than dependent on types of endosperm. The correlations between embryo dry weight with kernel dry weight and endosperm dry weight were also not significant (**Table 2**), indicating that embryo size was not dependent on endosperm size. The line 101*su* had the highest embryo dry weight, but it also had the lowest endosperm/embryo ratio. The line 216*wx*, in contrast, had intermediate embryo dry weight, but it had the highest endosperm/embryo ratio because it had the highest endosperm dry weight.

Differences in seed characters might cause differences in seedling behaviors when these seeds were germinated in laboratory. Wann (1980) founded that the kernel dry weight and endosperm dry weight of the high sugar genotypes (shrunken-2 and brittle) were less than sugary and normal genotype, while the embryo dry weight and endosperm/embryo ratio were dependent on the genotypes rather than the kernel dry weight and endosperm dry weight. Moreover, Styer and Cantiliffe (1984) also founded that the normal genotype had the highest kernel dry weight and endosperm dry weight, followed by the sugary, brittle, and shrunken-2, respectively.

Analysis of variance revealed that duration of priming significantly affected germination percentage (%), germination index, time to 50% germination (T_{50}), mean of germination times (MGT), vigor index, seedling growth rate, and seedling growth rate, but did not significantly affect seedling length (**Table 3**). Differences among inbred lines were also observed for all characters evaluated, and the interactions between inbred line and priming duration were also significant for all characters. The results indicated that there

Table 1 Kernel dry weight, endosperm dry weight, embryo dry weight, and endosperm and embryo ratio from 5 inbred lines.

Inbred Lines	Kernel DW (mg)	Endosperm DW (mg)	Embryo DW (mg)	End: Emb Ratio
101*wx*	83.11	62.08	13.44	4.61
101*su*	71.58	48.94	16.45	3.04
101*bt*	63.12	47.51	11.96	3.98
216*wx*	91.55	73.68	12.69	5.90
216*sh*$_2$	52.44	38.78	8.51	4.57
$LSD_{0.05}$	7.79	9.78	3.32	1.19

All weights are expressed as mean per kernel

Mean separation within columns by least significant test (LSD) at 5% level of probability

Table 2 Correlation between the seed parameters and seed germination behaviors of five inbred lines

	EnDW		EmDW		En:Em		%G		GI		T_{50}		MGT		VI		SGR		SL	
SDW	0.93	**	0.48	ns	0.42	ns	0.92	**	0.89	**	-0.69	**	-0.78	**	0.94	**	0.04	ns	0.83	**
EnDW			0.25	ns	0.67	**	0.85	**	0.83	**	-0.66	**	-0.73	**	0.89	**	-0.01	ns	0.82	**
EmDW					-0.53	*	0.44	ns	0.37	ns	-0.11	ns	-0.26	ns	0.38	ns	0.34	ns	0.31	ns
En:Em							0.40	ns	0.42	ns	-0.48	ns	-0.41	ns	0.46	ns	-0.19	ns	0.40	ns
%G									0.99	**	-0.85	**	-0.94	**	0.99	**	-0.14	ns	0.74	**
GI											-0.89	**	-0.97	**	0.98	**	-0.25	ns	0.73	**
T_{50}													0.96	**	-0.83	**	0.47	ns	-0.44	ns
MGT															-0.92	**	0.40	ns	-0.60	*
VI																	-0.19	ns	0.83	**
SGR																			-0.14	ns

SDW; seed dry weight, EnDW; endosperm dry weight, EmDW; embryo dry weight,
En: Em; endosperm/embryo ratio, %G; germination percentage, GI; germination index,
T_{50}; time to 50% germination, MGT; mean germination times, VI; vigor index,
SGR; seedling growth rate, and SL: seedling length
*: high significant, **: highly significant, ns: not significant

Table 3 Analysis of variance of the seed germination behaviors on 5×7 factorial combinations in a completely randomized design with three replications.

Source	df.	Mean Square															
		% Germination		Germination Index		T_{50} (days)		Mean Germination Time		Vigor Index		Seedling Growth Rate (mg/plant)		Seedling Length (cm.)			
Priming Time	6	1,175.4	**	82.8	**	0.87	**	0.356	**	836,691	**	918.0	**	17.25	ns		
Variety	4	14,287.8	**	3378.1	**	1.89	**	1.099	**	27,700,000	**	2323.6	**	688.91	**		
Time x Variety	24	129.2	**	22.7	**	0.17	**	0.032	**	308,395	**	671.4	**	58.48	**		
Error	68	12.3		2.3		0.05		0.005		75,506		102.5		20.08			
CV (%)		6.19		7.11		5.42		1.28		13.62		9.78		13.22			

**: highly significant.
ns: not significant

were differential responses of inbred lines to seed priming, and therefore identification of optimum priming times was difficult and still unresolved in this study.

Effects of priming duration on seed germination behaviors (germination percentage (%), germination index, time to 50% germination (T_{50}), mean germination times (MGT), vigor index, seedling growth rate, and seedling length) are shown in **Fig. 1**. The inbred lines with waxy endosperm showed consistently high germination percentage and the inbred line with sugary gene show intermediate germination percentage (**Fig. 1A**). Germination percentages of brittle and shrunken-2 genotypes were lowest, and the interactions between priming duration and inbred lines were evident between shrunken-2 and brittle genotypes only.

Similar to germination percentage, brittle and shrunken-2 genotypes has the lowest germination index that indicates the lowest germination speed (**Fig. 1B**). The sugary genotype had intermediate germination index, and the inbred lines with waxy endosperm germinated more rapidly than sugary genotype. The behaviors of inbred lines for germination index were similar to those for vigor index. The results indicated that germination speed was dependent on seed vigor.

These results indicated that directly germinated seeds (control) seemed to have higher germination percentage than primed seeds, and the germination percentages were generally reduced with priming duration. The possible explanation could be attributed to the loss of sugar during imbibitions because immature seeds have higher sugar content than mature seeds. However, this assumption was not tested in our study. Level of maturity would also be an important factor influencing priming success. Welbaum and Bradford (1991) clearly demonstrated in muskmelon that higher germination percentage was obtained from mature seeds, and the seeds harvested at 60 days after anthesis were more responsive to osmopriming than seeds harvested after 40 days.

Seed priming reduced germination time in all inbred lines. In general, the longer time was used to prime seeds the shorter time was used for germination. Shrunken-2 and brittle inbred lines took longer time to germinate than waxy inbred line especially for un-primed seeds. The reductions of time to germinate as affected by seed priming were evident for both days to 50% germination and days to mean germination time.

The results showed that the waxy genotype had the highest germination percentage, germination index, and vigor index, and it also had the earliest germination. These could be due to its high kernel dry weight and endosperm dry weight. The positive and significant correlation coefficients between seed dry weight and endosperm dry weight with germination percentage, germination index and vigor index all so supported the results (**Table 2**). These results indicated that the germination percentage, germination index and vigor index of each inbred lines were depended on their seed dry weight and endosperm dry weight. However, seed dry weight and endosperm dry weight were negatively correlated with time to 50% germination and mean germination times, indicating that the early germination was archived from the higher seed dry weight and endosperm dry weight.

The data suggested that differences in the vigor and seed germination behaviors might be closely related to weight of endosperm. Wann (1980) obtained significant difference in seedling growth between different genotypes in a standard germination test and concluded that the small endosperm of high-sugar genotype is primarily responsible for their poor seed and seedling vigor. Styer and Cantiliffe (1984) also founded that the germination was lower in shrunken-2 genotype than the brittle, sugary and normal genotype, indicating that the poor seed vigor of *sh*$_2$ is directly related to a starch deficient endosperm which cannot sustain early seedling growth. The lower starch content results in a collapsed and shrunken seeds with low endosperm to embryo ratio.

The effect of seed priming on seedling growth rate is genotype-dependent as there was no common pattern for all inbred lines in response to seed priming even in inbred lines with the same type of endosperm. The responses in most inbred lines were fluctuated across priming times. For example, the line 101*su* seemed to reduce seedling growth from priming 0 to 15 hrs priming, but it increased seedling growth rate at 18 hrs priming. In contrast to 101*su*, the line 101*bt* had sharp reduction in seedling growth rate at 18 hrs

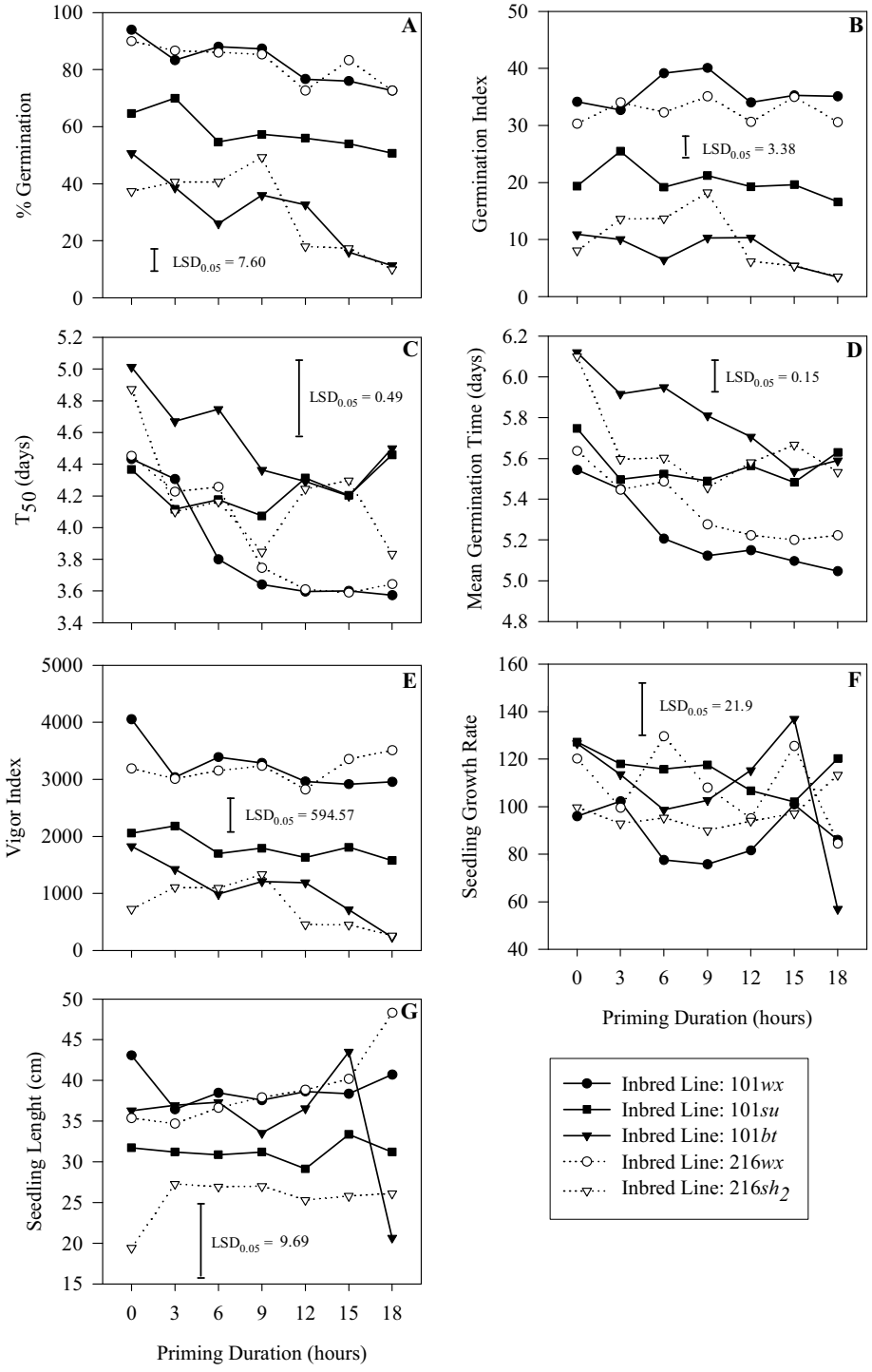

Fig. 1 Effect of hydro-priming duration on the germination behaviors of five inbred lines.

priming. However, the consistent increase in seedling growth rate was found in the line 216sh_2.

For seedling length, the consistently increasing effect was observed in inbred lines 216sh_2 and 216*wx*, whereas the effect of seed priming in other inbred lines was either inconsistent or decreasing. The seedling length was highly fluctuated in the lines 101*bt*, showing sharp decrease at 18 hrs priming.

CONCLUSION

Kernel dry weight and endosperm dry weight contributed to the differences in seed germination behaviors of the inbred lines when harvested at immature stage. The inbred lines with high kernel dry weight and endosperm dry weight performed better than the inbred lines with low kernel dry weight and endosperm dry weight. The highest seed germination parameters were obtained from waxy genotype followed by sugary, brittle, and shrunken-2 genotype. The hydro-priming in all inbred lines did not enhance the germination percentage, germination index, and vigor index, while it did enhance time to 50% germination, and mean germination times, indicating that the application of hydro-priming promoted early germination. The results also showed that vegetable corn seeds harvested at immature stage had acceptable germination, and seed priming could reduced germination time, but seed priming did not increase germination percentage. This information will be helpful for breeders and germplasm collectors who sometimes may harvest the corn at immature stage.

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