Korean J. Crop Sci. 47(5): 361~367(2002)

Effect of Harvest Time on Seed Quality of Silage Corn Inbreds and Hybrids

Suk Soon Lee*†, Sang Hee Yun*, Jung Moon Seo*, Hwang Kee Min, Si Hwan Ryu**, and Jong Yeol Park****

**Col. of Natural Resources, Yeungnam Univ. Gyeongsan, 712-749, Korea*
***Maize Exp. Stn, Gangwon-do Agri. Res. and Ext. Service, Hongchon, 200-939, Korea*

ABSTRACT : In order to determine the optimum harvest time for the seed production of inbreds and hybrids in silage corn, the ears of sib-pollinated 'KS5', 'KS7rhm', and 'Ga209' and cross-pollinated 'KS5' × 'KS6' (Suwon19), 'KS7 rhm' × 'KS117' (Suwonok), and 'Ga209' × 'DB544' (Kwanganok) were harvested at the one-week intervals from 4 to 10 weeks after silking. The optimum harvest time for the seed production for 'KS5', 'KS5' × 'KS6', 'KS7 rhm', and 'KS7rhm' × 'KS117' was 7 weeks after silking considering both emergence rate and plumule growth in cold test. Although earlier harvested seeds showed similar germination rate as the seeds harvested at the optimum time at 25°C, their emergence rate were lower in cold test. Seed weight and α-amylase activity of earlier harvested seeds were lower compared to those of seeds harvested at the optimum time, while leakage of total sugars and electrolytes were higher. However, the later harvested seeds showed lower germination rates at 25°C and emergence rates in cold test probably due to the lower α-amylase activity although they showed increased seed weight and reduced leakage of total sugars and electrolytes. In contrast, the emergence rate of 'Ga209' and 'Ga209' × 'DB544' in cold test increased up to 10 weeks after silking probably due to the increased seed weight and α-amylase activity and reduced sugar and electrolyte leakages during the germination. The cross-pollinated F1 hybrid seeds showed higher germination and emergence rates at 25°C and in cold test, and higher plumule growth and α-amylase activity compared to those of sib-pollinated inbreds.

Keywords : harvest time, silage corn, inbred, hybrid, seed production, cold test, sugars, EC, α-amylase, germination rate, and emergence rate

The determination of optimum harvest time is essential for the production of high quality seeds. The physiological maturity of corn seeds can be determined by 1) maximum seed dry weight, 2) the development of black layer in the base of seeds (Carter & Poneleit, 1973), 3) the number of days after silking, 4) seed moisture content (Brooking, 1990), 5) respiration rate of seeds (Knitte & Burris, 1976), etc.

†Corresponding author: (Phone) +82-53-810-2914 (E-mail) sslee@yumail.ac.kr <Received October 9, 2002>

Harvests of immature corn seeds have been resulted in increasing seed damages and reducing viability and seedling vigor (Reiss, 1944; Knitte & Burris, 1976; Styer *et al.*, 1980) as well as yield reduction (Brown, 1920). However, the effects of too late harvest on the germination and seedling vigor are not well documented. Knitte & Burris (1976) reported that late harvested seeds reduced root growth although germination rate and shoot growth were not adversely affected (Styer *et al.*, 1980).

In 2002, 81 M/T of hybrid corn seeds were produced in Korea and 264 M/T was imported to produce corn silage (National Seed Management Office, 2002). The price of imported seed was about 2-3 times higher compared to that of domestic hybrid seeds although silage yield potential of domestic and imported hybrid corn seeds was similar (Lee & Lee, 1987; Lee & Choi, 1990). Many farmers in Gangwon-do have had the experiences to produce hybrid corn seeds, but no research reports are effectively related to the optimum harvest time for the seed production of inbreds and hybrids under the Korean environmental conditions. Therefore, the optimum harvest time for the seed production of major silage corn hybrids and inbred lines developed in Korea was studied to stimulate the production of hybrid corn seeds.

MATERIALS AND METHODS

Inbred lines of corn hybrids of Suwon19 ('KS5' × KS6'), Suwonok ('KS7rhm' × 'KS 117'), and Kwanganok ('Ga209' × 'DB544') were planted in the same field on May 2, 2001 in Hongcheon, Gangwon-do. Four rows of female lines and two rows of male lines were planted alternatively at the 30 cm distance in 60 cm wide rows. All the tassels were covered with paper bags and silks were covered with plastic tubes before emergence. Seeds of female inbred lines were sib-pollinated and F1 hybrid seeds were cross-pollinated by hand.

Ear samples were taken from 4 to 10 weeks after silking at the one-week intervals and dried in a 35°C air-forced drier for two days. The ears were hand-shelled and stored in a -10°C freezer until they were used in various experiments.

The seed dry weight was averaged after measurement of the weight of randomly selected 100 dried seeds with three replications.

Moisture content of seeds at different harvest times was calculated as the following formula; Moisture content (%) = [(fresh weight-dry weight)/dry weight] × 100. Dry weight was measured after drying in an oven at the 105°C for 48 hours.

To measure the leakage of total sugars from the seeds, 20 seeds were soaked in 20 mL distilled water at 25°C for 24 hours. The leachate was filtered through a Whatman #42 filter paper. Then, 5 mL of the leachate was added slowly to the 10 mL of Anthrone reagent (2 g of Anthrone reagent was dissolved in 1,000 mL of 98% sulfuric acid) contained in a 20 mL test tube. The mixture was reacted in a boiling water bath for 7.5 minutes, cooled down rapidly in ice water, and left at room temperature for 15 minutes. Absorption of the sample solution was measured at the 630 nm in a spectrophotometer (UVIKON Spectrophotometer, Kontron, Italy) and total sugars were calculated as glucose equivalent (Yoshida *et al.*, 1972).

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

For germination test 30 seeds were planted on six layers of moistened paper towels in a 35×60×3 cm plastic box at 25°C for 7 days in the dark with three replications. To estimate the field emergence of the seeds, cold test was conducted in soil where corn was planted in the previous year and adjusted soil moisture to 70%. Thirty seeds were planted at the 2 cm depth and allowed to germinate at 10°C for 7 days and followed by at 25°C for 4 days. Germination and emergence rates were calculated according to AOSA (1990).

The α-amylase activity of seeds was measured by the method of Reiss (1994). Three seeds were soaked in water at 25°C for 7 days, dipped in liquid nitrogen, and ground in a mortar with a pestle. The ground sample was mixed in 10 mM cold citric acid-sodium citrate buffer and centrifuged for 20 minutes at 20,000 g to remove the starch grains, cell walls, mitochondria, and nuclei. One milliliter of the supernatant and 2 mL of the soluble starch buffer (0.05% starch in 0.05 M citric acid-sodium citrate buffer solution) were mixed for 20 minutes. Then, the α-amylase activity reaction was stopped by adding 7 mL of 1 N HCl and mixed. One milliliter of iodine solution (5 g KI and 0.35 g KIO_3 in 1 liter of 2 mM NaOH) was added to develop blue color. Absorbance of the sample solution was measured at 595 nm in a spectrophotometer (UVIKON Spectrophotometer, Kontron, Italy).

RESULTS

Seed dry weight

Seed dry weight of sib- and cross-pollinated inbreds are shown in Fig. 1. Generally 100-seed weight increased rapidly from 4 to 6 weeks after silking and leveled off or increased further through 7 or 8 weeks after silking depending on inbreds. The seed weight of sib-pollinated 'KS5' and 'KS7rhm' was higher than that of the cross-pollinated seeds, while that of sib-pollinated 'Ga209' was lower than cross-pollinated seeds. Genetically, the seed weight of sib- or cross-pollinated inbreds should be similar, thus the differences in seed weight between sib- and cross-pollinated inbreds might come from some environmental effects such as the number of seeds in an ear, soil fertility, soil moisture contents, etc.

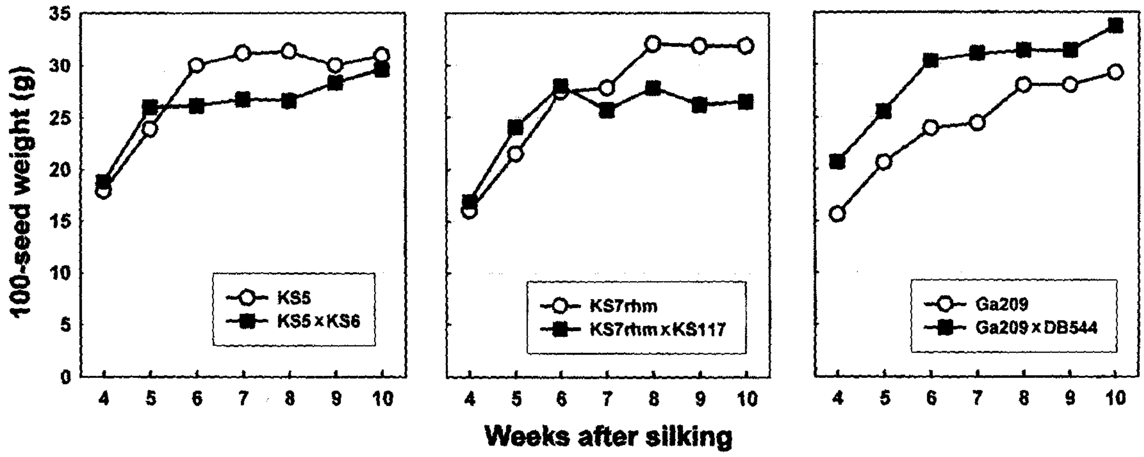

Fig. 1. Changes in 100-seed weight of three self- and cross-pollinated corn inbreds.

Seed moisture content

The changes in seed moisture content of sib- and cross-pollinated inbreds during the ripening stages are shown in Fig. 2. Four weeks after silking, the seed moisture content of all sib- and cross-pollinated inbreds ranged 53.7-59.8% and decreased continuously through 10 weeks after silking to get 20-30%. Seed moisture contents of cross-pollinated inbreds decreased more rapidly compared to that of sib-pollinated inbreds, especially in 'KS7rhm' × 'KS 117' (Suwonok) and 'Ga209' ×'DB544' (Kwanganok).

Among the inbreds the seed moisture content of sib- and cross-pollinated 'Ga209' decreased more slowly compared to two other inbreds. This slow decrease in seed moisture content may be due to the delayed leaf senescence of 'Ga209' and related to the continuous increase in seed dry weight through 10 weeks after silking as shown in Fig. 2.

Leakage of sugars from seeds in water

The leakage of total sugars from the seeds soaked in water is shown in Fig. 3. Four weeks after silking, seeds of sib-pollinated inbreds leaked more total sugars compared to their cross-pollinated seeds in all inbreds and the amounts of sugars leaked from the seeds differed depending on inbreds. The sugar leakage of sib- and cross-pollinated 'KS5' and 'KS7rhm' decreased rapidly through six weeks after silking and then leveled off, while that of sib-pollinated 'Ga209' decreased more slowly with seed maturity.

Leakage of electrolytes

To determine the leakage of electrolytes from the seeds soaked in water, the electrical conductivity (EC) of the seed soaking solutions was measured (Fig. 4). Four weeks after silking, the EC of the seed soaking solution of sib- and cross-pollinated 'KS7rhm' was greater compared to that of

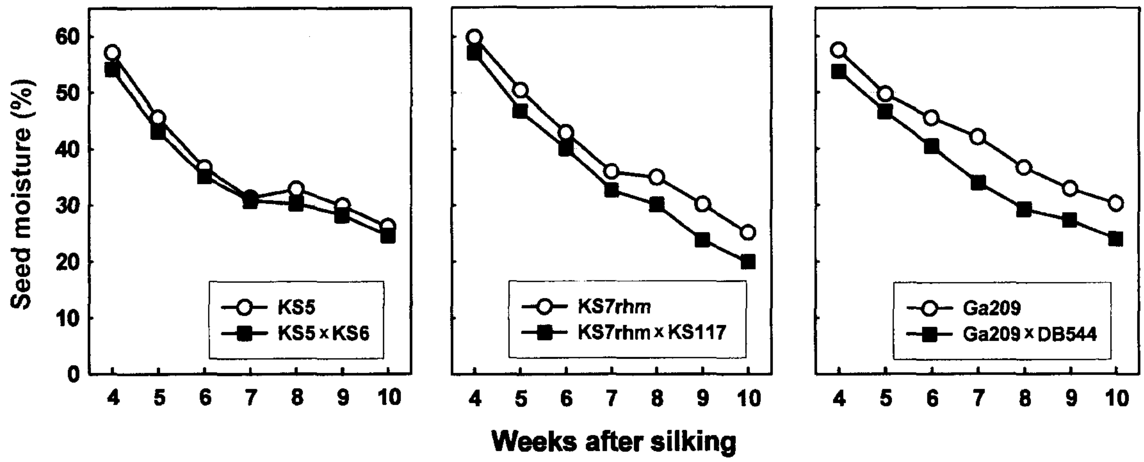

Fig. 2. Changes in seed moisture contents of three sib- and cross- pollinated inbreds.

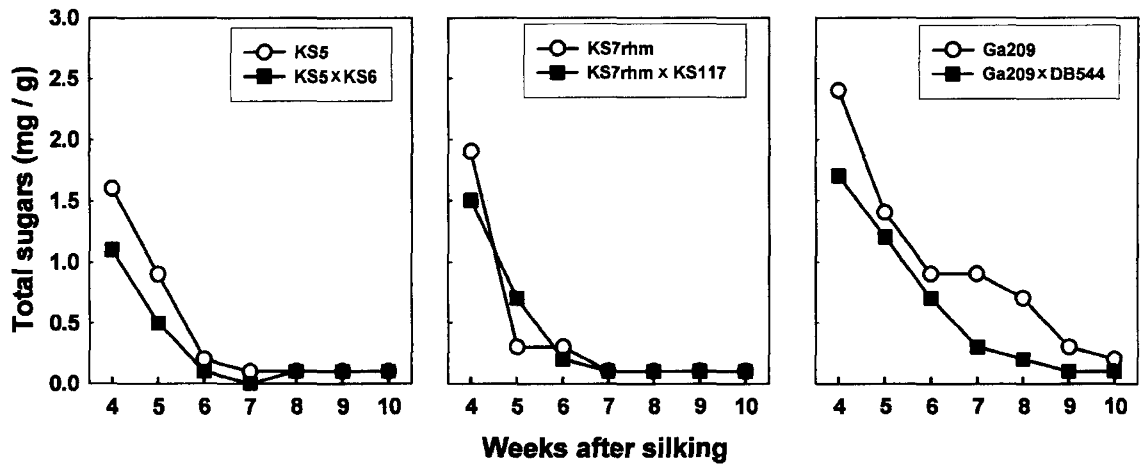

Fig. 3. Changes in total sugar leakage of seed soaking solution of three sib- and cross-pollinated inbreds.

two other inbreds. The EC decreased rapidly from 4 to 6 weeks after silking and then leveled off or decreased a little in all inbreds. The EC of seed soaking solution of sib-pollinated seeds was higher or similar to the cross-pollinated seeds in all inbreds.

Germination rates at 25°C

Germination rates of sib- and cross-pollinated seeds of three inbreds at 25°C are shown in Fig. 5. Generally the germination rates of cross-pollinated hybrid seeds were higher compared to those of the sib-pollinated inbreds at most harvest times although there were few exceptions. The germination rates of cross-pollinated 'KS5' (Suwon19) were over 96.7% at all harvest times and they were higher than those of sib-pollinated 'KS5' by 14.4-23.3%.

In contrast, the germination rates of cross-pollinated 'KS7rhm' (Suwonok) ranged 80-95%, while those of sib-pollinated 'KS7rhm' were similar to the cross-pollinated hybrid seeds from 4 to 7 weeks after silking, then they decreased as seeds matured.

The germination rates of 'Ga209' differed from those of 'KS5' and 'KS7rhm'. The germination rate of both sib- and cross-pollinated 'Ga209' increased up to 5-8 and 6-7 weeks after silking, respectively, then they decreased with seed maturity.

Emergence rates and plumule growth in cold test

Emergence rates: Emergence rates of seeds at the different harvest times of three sib- and cross-pollinated inbreds conducted in the cold test are shown in Fig. 6. Emergence rates of all cross-pollinated hybrid seeds were much higher than those of their own sib-pollinated maternal inbreds. Four weeks after the silking, the emergence rates of both sib- and cross-pollinated 'KS5' and 'KS7rhm' were less than 70% and they

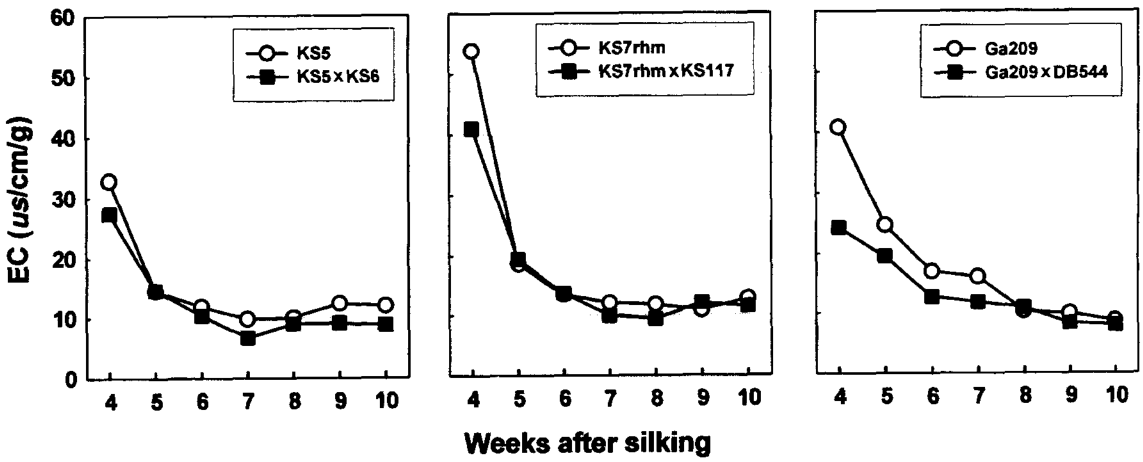

Fig. 4. Changes in electrical conductivity (EC) of the seed soaking solutions of three sib- and cross-pollinated inbreds.

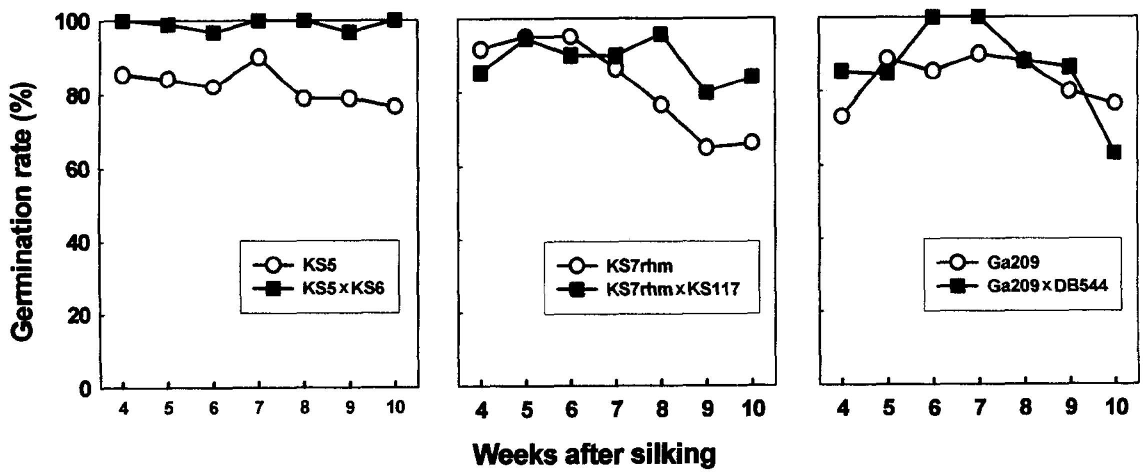

Fig. 5. Changes in germination rates of three sib- and cross-pollinated inbreds at 25°C.

increased rapidly to 6 and 7 weeks after silking, respectively and decreased with further maturity. In contrast, the emergence rates of sib- and cross-pollinated 'Ga209' increased continuously with maturity until 10 weeks after silking.

Plumule growth: The plumule growth of seeds at the different harvest times of three sib- and cross-pollinated inbreds conducted in the cold test is shown in Fig. 7. Four weeks after silking, the plumule length was extremely shorter compared to that of other harvest times in all sib- and cross-pollinated seeds. However, plumule length was similar from 5 to 10 weeks after silking. The plumule length of all cross-pollinated seeds was much higher compared to that of their own maternal sib-pollinated inbreds.

α-amylase activity

The α-amylase activity of the seeds at the different harvest times of three sib- and cross-pollinated inbreds is shown in Fig. 8. The α-amylase activity of cross-pollinated seeds of 'KS5' and 'Ga209' was much higher compared to that of their own maternal inbreds, while 'KS7rhm' was similar. The -amylase activity of all sib- and cross-pollinated inbreds increased with maturity from 4 to 6 or 8 weeks after silking depending on inbreds and then decreased.

DISCUSSIONS

The optimum harvest time for the seed production of inbred and hybrid corn should be determined considering both field emergence rate and subsequent plant growth. In this experiment the optimum harvest time for 'KS5', 'KS5' × 'KS6'(Suwon19), 'KS7rhm', and 'KS7rhm' × 'KS117' (S uwonok) was 7 weeks after silking considering both emergence rate and plumule growth in cold test. At the optimum harvest time the moisture content of seeds ranged 30-35% and it was similar to other reports (Curtis, 1980). Although seeds harvested between 4 and 6 weeks after silking showed

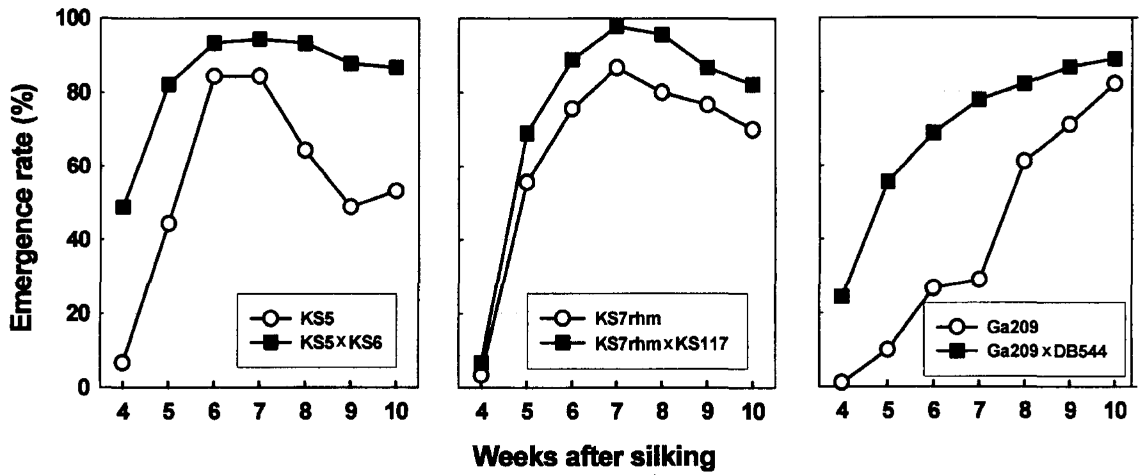

Fig. 6. Changes in emergence rates of three sib- and cross-pollinated inbreds in cold test.

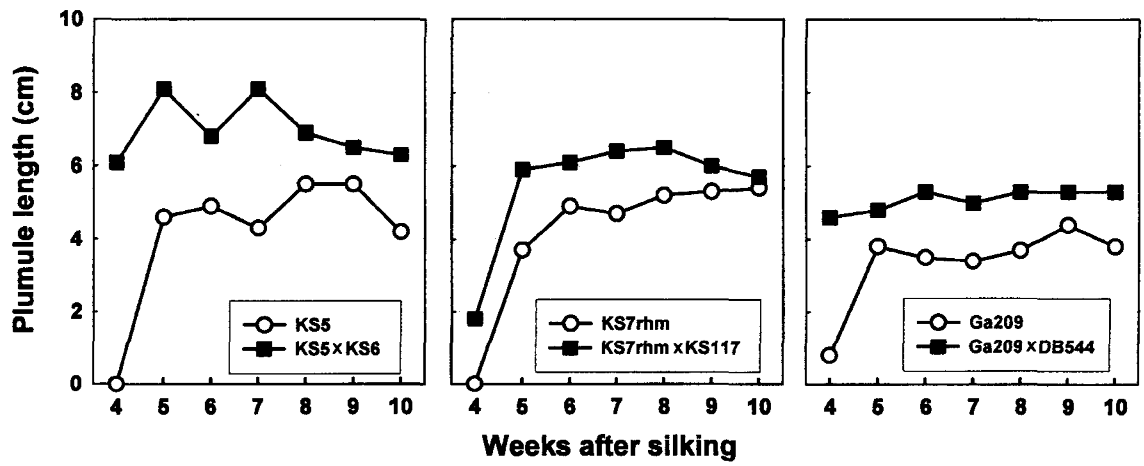

Fig. 7. Changes in the plumule length of three sib- and cross-pollinated inbreds in cold test.

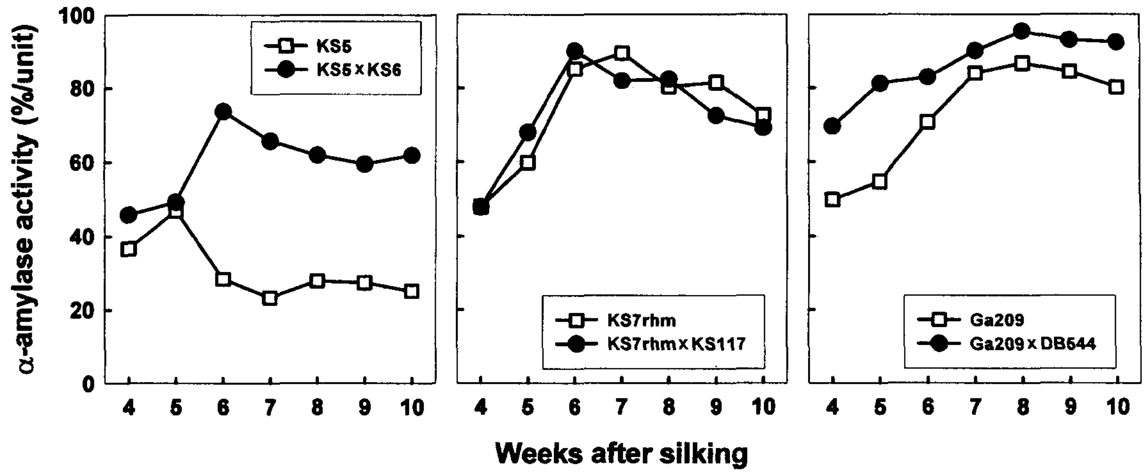

Fig. 8. Changes in α-amylase activity of three sib-and cross-pollinated inbreds.

similar germination rates as those at optimum harvest time (7 weeks after silking) at 25°C (Fig. 5), the emergence rates of some sib- and cross-pollinated inbreds were significantly low in cold test (Fig. 6). These immature seeds accumulated less dry matter (Fig. 1) and leaked more total sugars (Fig. 3) and electrolytes (Fig. 4), and showed a lower α-amylase activity (Fig. 8). Therefore, the seedlings might not be emerged after germination due to the lack of metabolic energy available for the embryo growth or might be decayed due to pathogenic microorganisms stimulated by leakage of cytoplasmic materials such as sugars (Fig. 3) and other substances.

In contrast, lower germination rate at 25°C and lower emergence rate in cold test of seeds harvested later than 8 weeks after silking are complicate to understand. The seeds harvested fully matured accumulated similar or more dry matter than the seeds harvested 7 weeks after silking (Fig. 1) and leaked less total sugars (Fig. 3) and electrolytes (Fig. 4). However, the lower α-amylase activity might be one of the reasons for the decreased germination and emergence rates. Knittle & Burris (1976) showed that late harvested seeds reduced root growth although germination rate at 25°C and shoot growth were not affected.

However, sib-pollinated and cross-pollinated 'Ga209' (Kwanganok) showed different results from those of 'KS5' and 'KS7 rhm'. Emergence rates in cold test increased continuously up to 10 weeks after silking in both sib- and cross-pollinated seeds (Fig. 6). The continuously increased 100-seed weight (Fig. 1) and α-amylase activity (Fig. 8) would support the increased emergence rates with maturity of seeds. Also, the slowly decreased seed moisture content and total sugar and electrolyte leakages may support the longer ripening period of the inbreds because of a slower senescence of leaves at the late maturing stage.

ACKNOWLEDGMENTS

The authors thanks the Research and Development Promotion Center for Agriculture and Forestry for its financial support.

REFERENCES

Association of Official Seed Analysis (AOSA). 1990. Rules for seed testing. *Proc. Assoc. Off. Seed Anal.* 60 : 27-76.

Bennett, M. A. and L. Waters, Jr. 1988. Seed maturity, seed size, and seed hydration effects on the seed quality of a corn inbred. *J. Amer. Soc. Hort. Sci.* 113(3): 348-353.

Brooking, I. R. 1990. Maize ear moisture during grain filling and its relation to physiological maturity and grain drying. *Field Crops Res.* 23(1) : 55-68.

Carter, M. W. and C. G. Poneleit. 1973. Black layer maturity and filling period variation among inbred lines of corn (*Zea mays* L.). *Crop Sci.* 13 : 436-439.

Daynard, T. B., J. W Tanner. and W. G Duncan. 1971. Duration of the grain period and its relation to grain yield in corn (*Zea mays* L.). *Crop Sci.* 11 : 45-48.

Knittle, K. H. and J. S. Burris. 1976. Effects of seed maturation on subsequent seeding vigor in maize. *Crop Sci.* 16 : 851-855.

Lee, S. S. and S. J. Choi. 1990. Forage productivity of corn and sorghum hybrids in rice black-streaked dwarf virus prevalent area. *J. Korean Grassl. Sci.* 10(1) : 42-47.

Lee, S. S. and J. M. Lee. 1987. Productivity of silage corn hybrids in rice-black streaked dwarf virus prevalent region. *J. Korean Grassl. Sci.* 7(3) : 140-145.

Reiss, C. 1994. Amylase: Enzyme assay. In Experiment in Plant Physiology. Prentice Hall, Englewood Cliffs, New Jersey, USA. p. 9-13, 261-262.

Styer, R. C., D. J. Cantliffe, and L. C. Hannah. 1980. Differential seed and seedling vigor in *shrunken-2* compared to three other genotypes of corn at various sages of development. *J. Amer. Soc. Hort. Sci.* 105(3) : 329-332.

Wann, E. V. 1980. Seed vigor and respiration of maize seeds with different endosperm genotypes. *J. Amer. Soc. Hort. Sci.* 105(1) : 31-34.

Yoshida, S., D. A. Forno, J. H. Cock, and K. A. Gomez. 1972. Determination of sugar and starch in plant tissue. *In* Laboratory Manual for Physiological Studies of Rice (2nd ed.). IRRI. Los Banos, Philippines. p. 70.