Barrenness and Changes in Tassel Development and Flowering Habit of Hybrid Maize Associated with Low Air Temperatures

Taku Hayashi¹, Tsukasa Makino¹, Narichika Sato¹ and Kenzaburo Deguchi²

(¹Hokkaido Research Organization Konsen Agricultural Experiment Station, Nakashibetsu 086-1135, Japan; ²Hokkaido Research Organization Animal Research Center, Shintoku 081-0083, Japan)

Abstract: Severe barrenness of maize (Zea mays L.) occurred in 2003 in eastern Hokkaido (Konsen region), one of the coldest areas in Japan. In many fields, tassels with few or no spikelets were observed in the cultivar widely grown in this region. The anthesis date was delayed 7 days from the silking date in the cultivar in the field at Konsen Agricultural Experiment Station (KAES). In July, when the tassels were developing at the 6- to the 9-leaf stage, the air temperature and sunshine hours were much lower in 2003 than in the average year. Therefore, we hypothesized that the barrenness resulted from these specific climatic conditions in 2003. To examine this assumption, we applied low temperature treatment (10ºC for 7 days) to the plants of the cultivar widely grown in this region in 2003 at around the 6- to 9-leaf stage in a greenhouse at KAES. Tassels were formed at the 7-leaf stage, and developed but they did not reach their full-size at the 8-leaf stage. The tassel length was shorter when the plants were subjected to the low temperature treatment, especially at the 8-leaf stage. The days from silking to anthesis was increased by the treatment in 2 of the 3 test years. The present results suggest that the cultivar is sensitive to the low air temperature at the 8-leaf stage for the development of tassels and may result in barrenness.

Key words: Barrenness, Hybrid cultivar, Low temperature, Tassel development.

Maize (Zea mays L.) is one of the most important crops in the world. Although maize is originally adapted to a hot climate, the growing area is spreading widely even in a cool summer region (Shaw, 1988). Konsen region located in eastern part of Hokkaido, the most northern island of Japan, is one of the main centers of dairy farming, where maize is widely cultivated for feeds. In summer, however, a cool wind and fog are brought into Konsen region from the Pacific Ocean, resulting in a low air temperature and low radiation.

In 2003, a very cool summer year, severe barrenness occurred in farmers' fields in Konsen region (Fig. 1). In the main cultivar, whole crop dry matter yield decreased to 50% of normal years (courtesy of local agricultural extension centers). For continuation of cultivation of maize in this region, it is important to clarify the cause of the barrenness.

Barrenness can occur due to many causes, such as excessive planting density (Sass and Loeffel, 1959; Stinson and Moss, 1960), deficiency of assimilates after pollination due to shortage of radiation (Andrade et al., 2000; Sato et al., 2001), water deficiency during silking period (Hall et al., 1982; Schoper et al., 1986), insects damage (Sendo et al., 1995), shortage of pollen scattering (Uribelarrea et al., 2002; Westgate et al., 2003), decreased pollen viability due to high temperatures during pollination (Herrero and Johnson, 1980), and elongation of the anthesis-silking interval (Wooley et al., 1962; Bassetti and Westgate, 1994).

To the best of our knowledge, however, there is no report on the barrenness associated with low temperatures in commercial cultivars.

In the present study, we first analyzed the relation between the climatic conditions and the maize growth in Konsen region in 2003. Then, we explain the effect of low temperature on tassel development and flowering behavior in the greenhouse at Konsen Agricultural Experimental Station (KAES).

Materials and Methods

1. Field observation in 2003

The main cultivar in Konsen region in 2003 was 39B29 (Pioneer Hi-Bred, Iowa, USA) which is a dent × flint hybrid, and its relative maturity is 75 days. In 2003, we collected the data on planting date and planting density of
this cultivar in farmers' fields where severe barrenness was observed from local agricultural extension centers. We also recorded the date of anthesis and silking for the cultivar 39B29 in the field at KAES (43°33′ N, 144°59′ E, 50 m above sea level). The data obtained in the field of KAES in 2001 and 2002 on cultivar 39B29 were also used to analyze the data in 2003. The anthesis-silking interval (ASI) was determined as the days from silking to anthesis.

In the field of KAES, the plants were sown on 16 May in 2001, and 22 May in 2002 and 2003 with a 4-row plot (rows 4 m long spaced 0.72 m apart, hill distance 0.18 m) with 4 replications. Matured manure of 40 ton ha⁻¹ was applied before ploughing. Chemical fertilizer consisting of 8 g of N, 21 g of P₂O₅ and 6 g of K₂O per square meters were applied at sowing time, and of 4 g of N and 4 g of K₂O per square meters were additionally applied at the 9-leaf stage as a topdressing application. Climatic data was collected from Automated Meteorological Data Acquisition System (AMeDAS) at KAES (Japan Meteorological Agency, 2009).

2. Greenhouse experiments

To clarify the cause of barrenness observed in 2003 in Konsen region, we analyzed the effects of low temperature and short sunshine hours in a greenhouse at KAES for 3 years.

1) Plant materials and cultural conditions

Cultivar 39B29 was grown in a greenhouse (floor space: 9 m × 5 m). Three seeds were planted in a Wagner pot (1/2000 a) filled with a mixture of surface soil at KAES and peat moss (approximately 7 : 3 in volume) on 19 May in 2004, 13 August in 2009 and 5 May in 2010. At the sowing time, chemical fertilizers of 14 g of N, 10 g of P₂O₅ and 14 g of K₂O per m² were applied. In addition, after the low temperature treatment described below, top dressing chemical fertilizers of 5 g of N and 4 g of K₂O per m² were applied. Fourteen to 18 days after emergence, the plants were thinned to one plant per pot. The air temperature in the greenhouse was maintained above 20°C. Plants were watered adequately so as not to subject them to water stress.

2) Low temperature treatment

When the plants in the greenhouse expanded the sixth, seventh, eighth, or ninth leaf, 4 pots each were moved to air temperature-controlled rooms (Koito Industries,
Identification of leaf stage in the present study is shown in Fig. 2. In the room, the temperature was maintained at 10ºC and the solar radiation was reduced to 30% of greenhouse, to make similar environmental conditions as in July of 2003 in Konsen region. After 7 days, the plants were brought back to the greenhouse. Control plants in 4 pots were grown in the greenhouse throughout the experimental period.

(3) Measurements
The leaf stage at the start of each low temperature treatment (LS), and the dates of anthesis and silking were recorded for each plant. The anthesis date was determined as the date when more than one spikelet in the tassel extruded anther, and the silking date as the date when at least one pistil extruded from its ear. The ASI was calculated as in the field observation. In 2010, the tassel development at the seventh and eighth-leaf stage was observed by dissecting 2 spare plants. After the anthesis, the length of tassel with spikelets was measured to evaluate the effect of low temperature on the tassel development. The relative tassel length (RTL) was calculated as the ratio of tassel length for low temperature-treated plant relative to that of the control plants.

Table 1. Sunshine hours and precipitation for 10 d span accumulations at the pre-flowering season of maize.

| Year | June First | June Middle | June Last | July First | July Middle | July Last | August First | August Middle | August Last |
|------|------------|-------------|-----------|------------|-------------|-----------|--------------|----------------|-------------|
| 2003 | 67         | 47          | 31        | 48         | 9           | 20        | 13           | 19             |
| *Average* | 37         | 32          | 34        | 31         | 26          | 28        | 29           | 35             |
| Precipitation (mm) | 2003 | 36         | 47          | 73        | 9           | 97        | 48           | 187           | 20         | 26         | 35         | 61         |
| *Average* | 34         | 33          | 33        | 34         | 46          | 49        | 36           | 35             | 47         | 61         |

Average year data are averages of 1979 – 2000 for precipitation and 1986 – 2000 for sunshine hours (Japan Meteorological Agency, 2009).

Yokohama, Japan. Floor space: 2.3 m × 2.6 m).

Table 2. Date of anthesis and silking, and anthesis-silking interval (ASI) in the field of Konsen Agricultural Experiment Station.

| Year | Date of anthesis | Date of silking | ASI (d) |
|------|------------------|----------------|---------|
| 2001 | 24 August        | 22 August      | 2 ± 0.82 a |
| 2002 | 30 August        | 30 August      | 0 ± 0.25 a |
| 2003 | 28 August        | 21 August      | 7 ± 0.41 b |

ASI: (date of anthesis)–(date of silking), values are the means ± S.E of 4 replications. Values followed by the same letters are not different at the 1% confidence level by Tukey’s HSD test (n = 4).

3. Statistical analysis
For the data in the field and the greenhouse experiments, statistical analysis were performed with R version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results
1. The climatic conditions and the maize growth in the field in 2003
The climatic conditions in 2003 are shown in Fig. 3 and Table 1. Daily mean air temperature dropped to nearly 10ºC at the end of June. Thereafter, it was around 15ºC, but was again 10ºC in the end of July. In addition, in the
middle of August, it dropped to nearly 10°C. Daily sunshine hours was also very short from the middle to the end of July (20% of average year). The climatic conditions in summer of 2003 were characterized as the cool and cloudy July.

In farmers’ fields where severe barrenness occurred, maize plants were sown at the planting density of 8 – 9 plants m\(^{-2}\) in the end of May in 2003. Maize plants expanded 6 – 9 leaves in the cool and cloudy July of 2003. Anthesis and silking occurred at the end of August. Then, many tassels with few or no spikelets were observed (Fig. 4). In the test field at KAES, ASI was longer in 2003 than in the other years (Table 2). In 2001, anthesis occurred 2 days later than silking (2 ASI). In 2002, anthesis was 6 days later than in 2001, but silking occurred at the same date as anthesis, resulting in 0 ASI. In 2003, anthesis was 4 days later than in 2001, but silking occurred at almost the same date as in 2001, resulting in 7 ASI.

2. Greenhouse experiments

Greenhouse experiments were conducted at KAES for 3 years to examine the effect of low air temperature and low solar radiation from the 6- to 9-leaf stages on the tassel development. Tassel length was 6 mm at the 7-leaf stage and 20 mm at the 8-leaf stage (Fig. 5). Fig. 6 shows the changes in RTL in relation to LS. RTL was the shortest when the plants were subjected to low temperature at the 8-leaf stage. Even in 2009, although the sowing date was delayed to August, the low temperatures at the 8-leaf stage decreased RTL. A quadratic regression curve fitted to the relation between LS and RTL was significant in each year. Photos of the tassels treated at the 8-leaf stage in 2004 and 2009 are shown in Fig. 7.

Table 3 shows the effects of low temperature treatment on the anthesis and silking dates. In the treated plants, compared to the control plants, the anthesis delayed 4–8 days, while the silking delayed less. ASI tended to be longer in the treated plants than in the control plants in all years. The difference in ASI between the treated plants and the control plants was significant in 2009 and 2010. In addition, ASI varied with LS in 2010.

Discussion

Barrenness has been reported to occur due to excessive planting density (Sass and Loeffel, 1959; Stinson and Moss, 1960). The planting density in the farmers’ fields in Konsen region where severe barrenness occurred in 2003 was 8 – 9 plants m\(^{-2}\) which is relatively higher than in the other regions in Hokkaido. However, in Konsen region,
Hayashi et al. — Barrenness in Hybrid Maize Associated with Low Temperatures

and Kanemasu, 1983), tassel development is supposed to occur in July in Konsen region. Therefore, we considered that the climatic conditions in July in 2003 affected the tassel growth and limited the pollen production. The climatic conditions in July in 2003 were characterized by the lower air temperature and lower solar radiation than in the average year. The minimum value of daily mean air temperature in July reached near 10ºC. In the inbred lines of maize, Bechoux et al. (2000) reported that the number of spikelets in plants maintained at 10ºC for 7 days at the 7.5-leaf stage, the starting period of spikelet development, decreased to one third of the control. Tranel et al. (2009) also showed that the total length of tassel branches was reduced to half of the control when plants were exposed to a temperature treatment of 17/6ºC (day/night) for 7 days, during the tassel branch and spikelet initiation. In the present greenhouse experiment, the plants treated with temperature of 10ºC and low solar radiation for 7 days around the 8-leaf stage showed much shorter tassel length at the anthesis than the untreated control plants. This result indicates that the low temperature at the 8-leaf stage will affect tassel growth in the commercial hybrid as in the inbred lines.

In the present greenhouse experiment, we also found that ASI of the plants treated at the 8-leaf stage was significantly longer than that of control plants in 2 of 3 years. This indicates that the low temperature treatment at the 8-leaf stage restricted the tassel development. Bassetti

The planting density in 2003 did not differ from the other years, and the farmers generally used vinyl-mulch for cultivation to enhance the plant growth which enables high planting density as high as 10 plants m$^{-2}$ (Nakui et al., 1995). Therefore, it is considered that the planting density was not the main reason for the barrenness occurred in 2003.

It is also reported that the deficiency of assimilates due to shortage of radiation after pollination can be a cause of barrenness (Andrade et al., 2000; Sato et al., 2001). In 2003, the radiation was much lower than in the average year during the period from the middle to end of August when the anthesis and silking occurred. However, the situation of the barrenness in 2003 appeared to be a failure of pollination (Fig. 1). As shown in Fig. 4, the restriction of tassel growth was significant in 2003. The tassel growth generally affects pollen production and pollination. Westgate et al. (2003) reported that seasonal pollen production less than 3000 pollen grains per silk limited the number of kernels per plant, and male fertility levels lower than 20% reduced grain yield. Uribelarrea et al. (2002) also reported that approximately two pollen grains per exposed silk were required to have 95% kernel set. Therefore, we assumed that the barrenness in 2003 was the result of shortage of pollen scattering induced by restricted tassel growth. Since tassel initiation is known to occur at approximately one third of the time between sowing and anthesis on the basis of growing degree days (Warrington and Kanemasu, 1983), tassel development is supposed to occur in July in Konsen region. Therefore, we considered that the climatic conditions in July in 2003 affected the tassel growth and limited the pollen production.

The climatic conditions in July in 2003 were characterized by the lower air temperature and lower solar radiation than in the average year. The minimum value of daily mean air temperature in July reached near 10ºC. In the inbred lines of maize, Bechoux et al. (2000) reported that the number of spikelets in plants maintained at 10ºC for 7 days at the 7.5-leaf stage, the starting period of spikelet development, decreased to one third of the control. Tranel et al. (2009) also showed that the total length of tassel branches was reduced to half of the control when plants were exposed to a temperature treatment of 17/6ºC (day/night) for 7 days, during the tassel branch and spikelet initiation. In the present greenhouse experiment, the plants treated with temperature of 10ºC and low solar radiation for 7 days around the 8-leaf stage showed much shorter tassel length at the anthesis than the untreated control plants. This result indicates that the low temperature at the 8-leaf stage will affect tassel growth in the commercial hybrid as in the inbred lines.

In the present greenhouse experiment, we also found that ASI of the plants treated at the 8-leaf stage was significantly longer than that of control plants in 2 of 3 years. This indicates that the low temperature treatment at the 8-leaf stage restricted the tassel development. Bassetti

---

### Table 3. Effects of the low temperature treatment on the days to anthesis and silking, and anthesis-silking interval (ASI).

| Test year | Treatment | Anthesis (DAS) | Silking (DAS) | ASI (d) |
|-----------|-----------|---------------|--------------|---------|
| 2004      | Control   | 56.8          | 56.5         | 0.3 ± 0.25 a |
|           | 6-leaf stage | 61.5          | 60.8         | 0.8 ± 0.48 a |
|           | 7-leaf stage | 62.5          | 61.8         | 0.8 ± 0.25 a |
|           | 8-leaf stage | 61.3          | 60.5         | 0.8 ± 0.25 a |
|           | 9-leaf stage | 60.5          | 59.8         | 0.8 ± 0.25 a |
| 2009      | Control   | 46.5          | 47.0         | −0.5 ± 0.65 b |
|           | 6-leaf stage | 53.3          | 50.5         | 2.8 ± 0.25 a |
|           | 7-leaf stage | 54.3          | 51.0         | 3.3 ± 0.48 a |
|           | 8-leaf stage | 54.0          | 50.0         | 4.0 ± 0.58 a |
|           | 9-leaf stage | 53.0          | 51.0         | 2.0 ± 0.71 a |
| 2010      | Control   | 60.8          | 61.3         | −0.5 ± 0.29 b |
|           | 6-leaf stage | 64.5          | 65.0         | −0.5 ± 0.65 b |
|           | 7-leaf stage | 66.0          | 65.5         | 0.5 ± 0.50 ab |
|           | 8-leaf stage | 67.3          | 65.3         | 2.0 ± 0.00 a |
|           | 9-leaf stage | 67.5          | 67.3         | 0.3 ± 0.48 ab |

Treatment indicates the starting period of the low temperature treatment. DAS: days after sowing. ASI: values are the means ± S.E of 4 replications. Values followed by the same letters are not different at the 5% confidence level by Tukey’s HSD test (n = 4). The data may contain rounding errors.
and Westgate (1994) reported that a 5-day delay of anthesis after silking (5 ASI) led to a failure of grain set. Therefore, the delay of anthesis will be another cause of the barrenness in 2003.

Based on these results, we considered that the low temperature and low solar radiation at the 8-leaf stage in 2003 restricted the growth of tassels and prolonged the days to anthesis.

Recently, maize breeders have begun to select and improve maize varieties with smaller tassel sizes and shorter anthesis-silking intervals to obtain reduced male dominance to optimize ear fertility (Duvick et al., 2004). Consequently, in Konsen region, the anthesis period tends to occur after the silking period in almost all of the new cultivars every summer. The selection strategy may make maize tassels less tolerant to the stress of low temperatures. In rice (Oryza sativa L.), the yield decreases when subjected to cooling treatment at the young microspore stage, and the reduction in yield was caused by failure of fertilization that was induced by reduction in engorged pollen grains (Hayase et al., 1969; Ito et al., 1969; Satake and Hayase, 1970; Satake, 1991). Further studies on pollen quality and quantity, and ear fertility in response to low temperatures in hybrid maize cultivars are needed to prevent the occurrence of barrenness due to the failure of pollination.

Acknowledgements

We express our appreciation to the staff of the Hokkaido Prefectural Nemuro Agricultural Extension Center and the Kushiro Agricultural Extension Center for their valuable assistance in the survey of farmers’ fields in 2003.

References

Andrade, F.H., Otegui, M.E. and Vega, C. 2000. Intercepted radiation at flowering and kernel number in maize. Agron. J. 92: 92-97.

Bassetti, P. and Westgate, M.E. 1994. Floral asynchrony and kernel set in maize quantified by image analysis. Agron. J. 86: 699-703.

Bechoux, N., Bernier, G. and Lejeune, P. 2000. Environmental effects on the early stages of tassel morphogenesis in maize (Zea mays L.). Plant Cell Environ. 23: 91-98.

Duvick D.N., Smith, J.S.C. and Cooper, M. 2004. Changes in performance, parentage, and genetic diversity of successful corn hybrids, 1930-2000. In C.W. Smith, J. Betran and E.C.A. Runge eds., Corn: Origin, History, Technology, and Production. John Wiley & Sons, Inc., New Jersey. 65-98.

Hall A.J., Villeva, F., Trapani, N. and Chimenti, C. 1982. The effects of water stress and genotype on the dynamics of pollen-shedding and silking in maize. Field Crop. Res. 5: 349-363.

Hayase, H., Satake, T., Nishiyama, I. and Ito, N. 1969. Male sterility caused by cooling treatment at the meiostic stage in rice plants. II. The most sensitive stage to cooling and the fertilizing ability of pistils. Proc. Crop. Sci. Soc. Jpn. 38: 706-711.

Herrero, M.P. and Johnson, R.R. 1980. High temperature stress and pollen viability of maize. Crop Sci. 20: 796-800.

Ito, N., Hayase, H., Satake, T. and Nishiyama, I. 1969. Male sterility caused by cooling treatment at the meiotic stage in rice plants. III. Male abnormalities at anthesis. Proc. Crop. Sci. Soc. Jpn. 39: 60-64.

Japan Meteorological Agency 2009. Climatic data retrieval. [Online]. http://www.data.jma.go.jp/obd/stats/etrn/index.php (accessed February 2, 2009).

Nakui, T., Nonaka, K., Hara, S. and Shinoda, M. 1995. The effect of plastic mulch on the growth of corn plants and their TDN yield of silage in the Tokachi district. Res. Bull. Hokkaido Natl. Agric. Exp. Stn. 161: 73-80.

Sass, J.E. and Loeffel, F.A. 1959. Development of axillary buds in maize in relation to barrenness. Agron. J. 51: 484-486.

Satake, T. and Hayase, H. 1970. Male sterility caused by cooling treatment at the young microspore stage in rice plants. V. Estimations of pollen developmental stage and the most sensitive stage to coolness. Proc. Crop. Sci. Soc. Jpn. 39: 468-473.

Satake, T. 1991. Male sterility caused by cooling treatment at the young microspore stage in rice plants. XXX. Relation between fertilization and the number of engorged pollen grains among spikelets cooled at different pollen developmental stages. Jpn. J. Crop. Sci. 60: 523-528.

Sato, H., Koizuma, K. and Enoki, H. 2001. Variability of barrenness degree of maize at Sapporo in 1999. J. Hokkaido Soc. Grassl. Sci. 35: 14-21.

Shaper, J.B., Lambert, R.J. and Vasitas, B.L. 1986. Maize pollen viability and ear receptivity under water and high temperature stress. Crop Sci. 26: 1029-1033.

Sendo, S., Suzuki, K. and Miyoshi, T. 1995. Effects of aphid infestation on corn ear growth and its varietal difference. J. Hokkaido Soc. Grassl. Sci. 29: 51-54.

Shaw, R.H. 1988. Climate requirement. In G.F. Sprague and J.W. Dudley eds., Corn and Corn Improvement Third Edition. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Wisconsin. 609-611.

Sittson Jr., H.T. and Moss, D.N. 1960. Some effects of shade upon corn hybrids tolerant and intolerant of dense planting. Agron. J. 52: 482-484.

Tranel, D., Knapp, A. and Perdomo, A. 2009. Chilling effects during maize tassel development and the lack of compensational plasticity. Crop Sci. 49: 1852-1858.

Uribelarrea, M., Cárdeno, J., Otegui, M.E. and Westgate, M.E. 2002. Pollen production, pollination dynamics, and kernel set in maize. Crop. Sci. 42: 1910-1918.

Warrington, I.I. and Kanemasu, E.T. 1983. Corn growth response to temperature and photoperiod. I. Seedling emergence, tassel initiation, and anthesis. Agron. J. 75: 749-754.

Westgate, M.E., Lizaso, J. and Batchelor, W. 2003. Quantitative relationships between pollen shed density and grain yield in maize. Crop. Sci. 43: 934-942.

Woolley, D.G., Baracco, N.P. and Russel, W.A. 1962. Performance of four corn inbreds in single-cross hybrids as influenced by plant density and spacing patterns. Crop. Sci. 2: 441-444.

* In Japanese with English abstract.
** In Japanese with English summary.
*** In Japanese.