2001

Temperature and Photoperiod Effects on Sterility in a Cytoplasmic Male-Sterile Soybean

M. B. Smith
Iowa State University

Harry T. Horner
Iowa State University, hth@iastate.edu

R. G. Palmer
United States Department of Agriculture

Temperature and Photoperiod Effects on Sterility in a Cytoplasmic Male-Sterile Soybean

Abstract
Manual cross-pollination to produce large quantities of hybrid soybean seed is difficult and time consuming. An environmentally stable sterility system is one of the necessary components to produce large quantities of hybrid seed. The objective of this study was to subject cytoplasmic male-sterile (CMS) BC5F1 plants, from a cross of a Chinese *Glycine max* wild-type soybean with a Chinese wild annual soybean *G. soja* (male parent) and controls, to a variety of different temperature and photoperiod treatments to test whether CMS is stable under various environmental conditions. Plants were grown in growth chambers under controlled temperature, photoperiod, and irradiance regimes until pod set, and then they were transferred to a glasshouse until they matured. Plants were evaluated for time of anthesis after photoperiod induction (13 h light/11 h dark) and fertility or sterility. Anther squash and pod set data showed that sterility of the CMS line was stable under all environmental conditions tested, whereas fertility-restored control plants remained fertile. Extreme environmental conditions led to delayed floral induction and/or stunted growth.

Disciplines
Agronomy and Crop Sciences | Botany | Plant Breeding and Genetics

Comments
This article is from *Crop Science* 41 (2001): 702, doi: 10.2135/cropsci2001.413702x.

Temperature and Photoperiod Effects on Sterility in a Cytoplasmic Male-Sterile Soybean

M. B. Smith, H. T. Horner,* and R. G. Palmer

ABSTRACT

Manual cross-pollination to produce large quantities of hybrid soybean seed is difficult and time consuming. An environmentally stable sterility system is one of the necessary components to produce large quantities of hybrid seed. The objective of this study was to subject cytoplasmic male-sterile (CMS) BC5F1 plants, from a cross of a Chinese *Glycine max* wild-type soybean with a Chinese wild annual soybean *G. soja* (male parent) and controls, to a variety of different temperature and photoperiod treatments to test whether CMS is stable under various environmental conditions. Plants were grown in growth chambers under controlled temperature, photoperiod, and irradiance regimes until pod set, and then they were transferred to a glasshouse until they matured. Plants were evaluated for time of anthesis after photoperiod induction (13 h light/11 h dark) and fertility or sterility. Anther squash and pod set data showed that sterility of the CMS line was stable under all environmental conditions tested, whereas fertility-restored control plants remained fertile. Extreme environmental conditions led to delayed floral induction and/or stunted growth.

Cytoplasmic Male Sterility is a male-sterile, maternally inherited condition in plants that usually results in complete male sterility while leaving female fertility unimpaired. CMS has been detected in more than 150 angiosperm species (Kaul, 1988), but CMS has been identified only a few times in soybean (Davis, 1987; Ding et al., 1998; Gai et al., 1995; Sun et al., 1997; Zhang and Dai, 1997). The soybean CMS systems described by these authors have not been exploited for commercial seed production, although soybean is a major crop on a worldwide scale and its global production is increasing annually. A functional CMS system, which would be a major step to commercial hybrid seed production with increased yield, selective increase of oil and protein quality, and lessened disease and insect susceptibility, would be desirable. A major challenge associated with developing a usable CMS system is that *Glycine* is almost exclusively self-pollinating. Usually by anthesis, pollination and fertilization already have occurred (Fujita et al., 1997). Nonetheless, outcrossing seems possible in some species of *Glycine*, especially where increased seed set has been correlated with an increased visit by efficient pollinators (Fujita et al., 1997; Schoen and Brown, 1991).

In practice, the degree of sterility of CMS plants often is less than 100%, depending upon the origin of the cross, that is, the farther apart the parental genomes, usually the greater the degree of male sterility (Shivanna and Sawhney, 1997). In contrast, plants resulting from cross-pollination of less-distant parental genomes (e.g., intraspecific crosses from geographically more similar regions) are more likely to be fertile. CMS also may occur spontaneously in a population (Shivanna and Sawhney, 1997), but it does not appear as a single-point gene mutation (Newton and Gabay-Laughnan, 1998). Also, CMS systems are susceptible to specific environmental changes of photoperiod and temperature, and often revert to partial or complete fertility (Janska and Mackenzie, 1993; McVetty, 1998).

Most CMS systems that have been investigated thoroughly involve complex mitochondrial genome rearrangements (Hanson, 1991). The available evidence suggests an incompatibility between the sporophytically inherited organelle genomes, the mitochondria and plastids, and the paternally inherited nuclear genome. This genetic disposition results mainly in mitochondrial dysfunction expressed in the tapetal cells of the anther tissues (Wise et al., 1999), and as a result, microsporogenesis and/or microgametogenesis is disrupted.

During plant development and growth, all quantitatively variable traits are adjusted to one another and are controlled by interaction of the entire system (Wallace and Yan 1998). Therefore, flowering in higher plants is an interdependent event and interacts with all other changeable genetic and environmental factors. In soybean, photoperiod-gene activity affects the partitioning of photosynthate between reproductive and vegetative tissues. Photoperiod and temperature modulate the extent of expression of the photoperiod-gene activity, and they thereby control the direction of photosynthate flow and the tendency to flower (Yan and Wallace, 1998).

In soybean, the critical photoperiod is defined as a set of minimum and maximum photoperiod values, above and below which the plants cannot be induced to flower (Hartwig 1970). However, these values are not absolute and can be modulated by temperature. Consequently, plants usually flower regardless of daylength after a genetically determined cumulative amount of photoperiod and temperature (Wallace and Yan, 1998).

An interspecific cross of a Chinese wild-type soybean [*Glycine max* (L.) Merr.] as female parent with a wild annual soybean *G. soja* (Siebold and Zucc.) from

M.B. Smith and H.T. Horner, Dep. of Botany and Bessey Microscopy Facility, Iowa State Univ., Ames, IA 50011-1020; R.G. Palmer, USDA-ARS, CICG Research Unit and Dep. of Agronomy and Zoology/Genetics, Iowa State University, Ames, IA 50011-1010. This is a joint contribution of the USDA-ARS, CICG Research Unit and Journal Paper no. J-18748 of the Iowa Agric. and Home Econ. Exp. Stn., Ames, IA 50011; Project no. 3352. A portion of this research was supported by a grant to H.H. and RAP from Pioneer Hi-Bred International, Inc., Johnston, IA. The mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA, Iowa State University, or Pioneer Hi-Bred International, Inc., and does not imply its approval to the exclusion of other products that may be suitable. Received 28 Aug. 2000. *Corresponding author (hth@iastate.edu).

Published in Crop Sci. 41:702–704 (2001).

Abbreviations: BPR, basic photoperiod regime; BTR, basic temperature regime; CMS, cytoplasmic male-sterile.

Kaifeng, China, was CMS (Sun et al., 1997). The purpose of this environmental study was to challenge the CMS A-line and two fertility-restored lines to various photoperiod and temperature regimes in order to test the stability of the CMS system.

MATERIALS AND METHODS

Design of Growth Chamber Treatments

Seeds of the BC5F1 CMS A-line, which resulted from an interspecific cross between *G. max* and *G. soja* (Sun et al., 1997), were obtained from Pioneer Hi-Bred International, Johnston, IA. The *G. soja* parent originated near 33° North latitude, and is approximately equivalent to a Mid IV maturity group (T.C. Kilen, personal communication, 1995). The *G. max* parent is from Gongzhuling, which is around 36.5° North latitude and about 100 m above sea level, and it is approximately equivalent to a Mid IV-V maturity group (T.C. Kilen, personal communication, 1995). Seed supply of the CMS A-line was limited because of the low success in making the cross-pollinations. A greenhouse fire at the Pioneer greenhouse in Johnston, Iowa, in June 1998 resulted in loss of the parent plants. No F_1 seeds were available for further studies.

Seeds were placed on wetted germination paper in an incubator at 25° C in the dark for ≈3 d. Seeds from two fertility-restored proprietary lines, PH1 and PH2 (Pioneer Hi-Bred International), were treated the same as the CMS A-line and served as controls. Young seedlings were transferred to black plastic pots (3.84 L or 7.68 L) that contained unsterilized soil mix (2 parts field soil:1 part sphagnum peat:1 part sand) and pots were placed into a growth chamber.

Irradiance inside the growth chambers ranged from 350 to 1500 μmol s^{-1} m^{-2} (range between heavily shaded areas and areas without any shade), depending upon the amount of vegetative growth intercepting the irradiance and the distance of plants from the source. The irradiance source was a standard 3:1 mixture of individual fluorescent and incandescent lamps. Temperature settings represent the average temperature inside the growth chamber as measured with minimum and maximum thermometers.

The variable experimental parameters (treatments) were photoperiod and temperature. Thirteen treatments were conducted. Three to six plants per genotype were used for each treatment. Treatments were done sequentially, from Treatment 1 (T1) to Treatment 13 (T13). All plants of the same genotype within each treatment behaved similarly. The only treatments that were repeated were T4 and T9, and T7 and T8. Dates of planting, photoinduction, and termination of all experiments were recorded.

At anthesis, fresh flowers were collected from the CMS A-line plants as well as from the fertility-restored control plants and fixed in 70% ethanol. Stamens were dissected from the flowers, and pollen grains were stained with aqueous I_2KI (iodine potassium iodide) solution to assess fertility or sterility (Jensen, 1962). After pod set occurred on the fertility-restored control plants, all plants, with or without pod set, were transferred to a glasshouse and observed for at least 4 wks more to monitor maturation.

Control Conditions

In each growth chamber treatment, two *G. max* restorer lines (PH1 and PH2) and cultivar Harosoy (treatments T7 and T8) were grown with the CMS A-line plants. A photoperiod regime with a minimum of 2 wks of long growing days (18 h light/6 h dark, followed by 1 wk of 16 h light/8 h dark, 1 wk of 14 h light/10 h dark, and a floral induction period of 13 h light/11 h dark) was determined optimal. This photoperiod regime will be designated as the basic photoperiod regime (BPR), and any variations are indicated. The flowering induction period that leads to floral initiation and anthesis is determined genetically, and it lies within a narrow and characteristic time span for each genotype. For values greater or less than this critical photoperiod, plants cannot be induced to flower by photoperiod, but they will flower when a cumulative value for all determinants, genetic and environmental, is reached. The temperature regime of 29/21 to 24°C has been considered optimal for soybean (Caviness and Fagala, 1973; Roberts et al., 1996), and was used as our basic temperature regime (BTR). The combination of both BPR and BTR will be referred to as standard conditions. During the stay in the growth chamber, and during the observation period in the glasshouse (after flowering), the following data were collected: fertility or sterility (evaluated by staining of pollen grains with I_2KI), and pod set on the mature plants.

RESULTS AND DISCUSSION

Throughout all treatments (T1 to T13; Table 1), the CMS A-line remained 100% male sterile. Phenotypically, the CMS A-line produced only vestigial dwarf

Table 1. Summary of 13 photoperiod and temperature treatments used to test a Chinese CMS soybean line and its two control fertility restored lines for maintenance of sterility and fertility.

	Temperature												
	Normal 29/24°C			High 32/24°C		Low 25/15°C		32/24°C‡		29/24°C‡		Temperature variation-§	Temperature variation-¶
Photoperiod†	CMS	Fertility-restored PH lines	Harosoy	CMS	Fertility-restored PH lines	CMS	Fertility-restored PH lines	CMS	Harosoy	CMS	Harosoy	CMS	CMS
1	T5#	T6	T7 T8	T5	T6	T4 T9	T9	T7	T7	T8	T8	T12	T13
2	T1	T1											
3				T2	T2								
4						T10	T10						
5						T3							
6	T11												

† Photoperiod 1 = 18h/6h (2 wk); 16h/8h (1 wk); 14h/10h (1 wk); 13h/11h (to end of experiment): Photoperiod 2 = 18h/6h (3 wk); 16h/8h (1 wk); 14h/10h (1 wk); 13h/11h (to end of experiment): Photoperiod 3 = 18h/6h (2 wk); 16h/8h (1 wk); 14h/10h (1 wk); 13h/11h (1 wk); 12.5h/11.5h (to end of experiment): Photoperiod 4 = 18h/6h (2 wk); 14h/10h (to end of experiment): Photoperiod 5 = 14h/10h continuous (continued to end of experiment): Photoperiod 6 = 18h/6h (4 wk); 16h/8h (1 wk); 14h/10h (1 wk); 13h/11h (to end of experiment).
‡ Meiotic heat shock of 38/24°C for 3d during meiosis.
§ Temperature variation = 29/24°C (2 wk); 35/19°C (1 wk); 40/15°C (to end of experiment).
¶ Temperature variation = 29/24°C (2 wk); 21/18°C (1 wk); 15/12°C (to end of experiment).
# T = treatment numbers.

pods, ≈1.2 to 1.9 cm long, the seeds of which were nonviable. This agrees with the results of Sun (personal communication, 1999). All anther squashes from the CMS A-line showed mostly unstained, collapsed pollen grains when stained with I_2KI, and they only occasionally displayed vacuolate pollen grains that contained a few starch granules that stained light brown. No dark blue to dark brown-staining pollen grains, typical for pollen from fertile anthers, were found.

In soybean (Acock and Acock, 1995; Roberts et al., 1996; Summerfield et al., 1985; Wallace and Yan, 1998), as well as in many other plants (Estrada and Mutschler, 1984; Izhar, 1975; Marshall et al., 1974; Rick and Boynton, 1967), the influences of photoperiod and temperature on plant growth, flowering, and pod set have been studied. The CMS A-line in this study, however, is a hybrid with one annual wild-type *G. soja* parent, and the responses of *G. soja* to photoperiod and temperature are less well known. The Pioneer proprietary fertility-restored lines seemed to respond within the parameters of any *G. max* genotype originating from ≈35° North latitude. Generally, the *G. soja* parent showed an overall delay of 1 wk in days to flowering. The lower day and night temperature in Treatments 3, 4, 9, and 10 hastened flowering in the control plants, but delayed flowering in the CMS A-line. The temperature shock (treatments 7 and 8) during meiosis failed to elicit a response in the CMS A-line or in Harosoy. The CMS A-line remained male sterile, and Harosoy remained male fertile.

Together, these treatments demonstrated the stability of the CMS A-line, which under the tested experimental conditions, did not revert to fertility. No pod set, other than vestigial pods, typical for CMS soybean, was observed. The fertility restored lines (controls) remained fertile under all experimental conditions tested.

ACKNOWLEDGMENTS

The authors thank Pioneer Hi-Bred International, Inc., for supporting this research, Drs. Richard J. Gladon and Daria H. Schmidt for their many helpful comments, and Ms. Christina Gallup for helping with the preparation of Table 1. The authors would like to also thank Tracey Pepper and John Mattila for technical help.

REFERENCES

Acock, M.C., and B. Acock. 1995. Photoperiod sensitivity during soybean flower development. Biotronics 24:25–34.

Caviness, C.E., and B.L. Fagala. 1973. Influence of temperature on a partially male-sterile soybean strain. Crop Sci. 13:503–504.

Davis, W.H. 1987. Process for forming seeds capable of growing hybrid soybean plants. U. S. Patent number 4 648 204. Date issued: 10 March.

Ding, D., Z. Cui, and J. Gai. 1998. Development and cytological features of the cytoplasmic-nuclear male-sterile soybean line NJCMS1A. Soybean Genet. Newsl. 25:34–35.

Estrada, S., and M.A. Mutschler. 1984. Temperature-influenced instability in a genic male-sterile common bean. J. Hortic. Sci. 19:401–402.

Fujita, R., M. Ohara, K. Okazaki, and Y. Shimamoto. 1997. The extent of natural cross-pollination in wild soybean (*Glycine soja*). J. Hered. 88:124–128.

Gai, J., Z.D. Cui, Z. Ren, and D. Ding. 1995. A report on the nuclear cytoplasmic sterility from a cross between two soybean cultivars. Soybean Genet. Newsl. 22:85–88.

Hanson, M.R. 1991. Plant mitochondrial mutations and male sterility. Annu. Rev. Genet. 25:461–486.

Hartwig, E.E. 1970. Growth and reproductive characteristics of soybeans [*Glycine max* (L.) Merr.] grown under short-day conditions. Trop. Sci. 12:47–53.

Izhar, S. 1975. The timing of temperature effect on microsporogenesis in cytoplasmic male-sterile petunia. J. Hered. 66:313–314.

Janska, H., and S.A. Mackenzie. 1993. Unusual mitochondrial genome organization in cytoplasmic male-sterile common bean and the nature of cytoplasmic reversion to fertility. Genetics 135:869–879.

Jensen, W.A. 1962. Botanical histochemistry. Principles and practice. W. H. Freeman and Company, San Francisco, CA.

Kaul, M.L.H. 1988. Male sterility in higher plants. Springer-Verlag, Berlin, Germany.

Marshall, D.R., N.J. Thomson, G.H. Nicholls, and C.M. Patrick. 1974. Effects of temperature and day length on cytoplasmic male sterility in cotton (*Gossypium*). Aust. J. Agric. Res. 25:443–447.

McVetty, P.B.E. 1998. Cytoplasmic male sterility. p. 155–182. *In* K.R. Shivanna and V.K. Sawhney (ed.) Pollen biotechnology for crop production and improvement. Cambridge University Press, Cambridge, UK.

Newton, K.J., and S.J. Gabay-Laughnan. 1998. Abnormal growth and male sterility associated with mitochondrial DNA rearrangements in plants. p. 365–381. *In* K. K. Singh (ed.) Mitochondrial DNA mutations in aging, disease and cancer. R. G. Landes Company, Georgetown, TX.

Rick, C.M., and J.E. Boynton. 1967. A temperature-sensitive male-sterile mutant of the tomato. Am. J. Bot. 54:601–611.

Roberts, E.H., A. Qi, R.H. Ellis, R.J. Summerfield, R.J. Lawn, and S. Shanmugasundaram. 1996. Use of field observations to characterise genotypic flowering responses to photoperiod and temperature: A soybean exemplar. Theor. Appl. Genet. 93:519–533.

Schoen, D.J., and A.H.D. Brown. 1991. Whole- and part-flower self-pollination in *Glycine clandestine* and *G. argyrea* and the evolution of autogamy. Evolution 45:1651–1664.

Shivanna, K.R., and V.K. Sawhney (ed.). 1997. Pollen biotechnology for crop production and improvement. Cambridge University Press, Cambridge, UK.

Summerfield, R.J., S. Shanmugasundaram, E.H. Roberts, and P. Hadley. 1985. Soybean adaptation to photo-thermal environments and implications for screening germplasm. p. 333–352. *In* S. Shanmugasundaram and E.W. Sulzberger (ed.) Soybean in tropical and subtropical cropping systems, Proc. of a Symp., Tsukuba, Japan, 26 Sept.–1 Oct., 1983. Fortune Printing Co., Ltd. Taiwan.

Sun, H., L. Zhao, and M. Huang. 1997. Cytoplasmic-nuclear male sterile soybean line from interspecific crosses between *G. max* and *G. soja.* p. 99–102. *In* B. Napompeth (ed.) Proc. World Soybean Res. Conf., 5th, Chiang Mai, Thailand. 21–27 Feb. 1994. Kasetsart University Press, Bangkok, Thailand.

Wallace, D.H., and W. Yan. 1998. Plant breeding and whole crop systems, improving adaptation, maturity, and yield. CAB International, Wallingford, UK.

Wise, R.P., C.R. Bronson, P.S. Schnable, and H.T. Horner. 1999. The genetics, pathology, and molecular biology of T-cytoplasm male sterility in maize. Adv. Agron. 65:79–130.

Yan, W., and D.H. Wallace. 1998. Simulation and prediction of plant phenology for five crops based on photoperiod × temperature interaction. Ann. Bot. (London) 81:705–716.