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The Phenotypic Fluctuation Factor for Male Sterility in A1 Male-Sterile Lines of Sorghum (*Sorgum bicolor* Moench)

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Abstract

The present study was conducted to clarify the factors shaping the extent of male sterility in A1 male-sterile lines of sorghum (*Sorgum bicolor* Moench). Eleven male-sterile lines (A-line) with A1 cytoplasm were grown both on a university farm during crop season and in growth chambers controlled under three different temperatures during the period from the 3rd to the 10th leaf stages in 1999. Based on the degree of seed and pollen fertility in artificial self-pollinated plant, A-lines were classified into 4 groups. Six cultivars of Group-1, 'MS79,' 'CK60,' 'Martin,' 'Reliance,' 'Wheatland' and 'Tx624,' showed stable male-sterility in all experimental conditions examined. 'MS138' of Group-2 showed variable sterility under minimum temperatures above 24℃, and 'Tx3048' of Group-3 showed variable sterility under maximum temperatures above 26.5℃ at flower initiation stage. Three cultivars of Group-4, 'Redbine58,' 'Redlan' and 'Westland,' showed variable sterility with restoration of pollen fertility in the field condition. In male-sterile sorghum lines like 'MS138' and 'Tx3048,' a physiological condition between early vacuolated pollen stage and engorged pollen stage is considered to be critical for conversion from sterile to fertile.

Key Words: A1 cytoplasm, Cytoplasmic male sterility, Sorghum, Temperature.

Introduction

Cytoplasmic male sterility (CMS) is currently desired for the production of F_1 hybrid sorghum (*Sorghum bicolor* Moench) seed, since manual emasculation is impractical (Stephens and Holland, 1954). Among several sources of CMS in sorghum, A1 cytoplasm is the major male-sterile cytoplasm derived from 'Milo' (Pring et al., 1982; Pring et al., 1995; Xu et al., 1995). The male-sterile lines (A-lines) with A1 cytoplasm are the most important lines for production of F_1 hybrid seeds in Japan as well as in the USA (Schertz and Ritchey, 1978; Tarumoto, 1971). However, the A-lines that ensured their sterility at Nishinasuno (National Grassland Research Institute, Tochigi, Japan; latitude 36° 55' N; Daily mean temperature (DMT) of July 21.8℃) sometimes showed unstable male sterility at Higashi-Hiroshima (Hiroshima Pref. Agriculture Research Center, Hiroshima, Japan; latitude 34° 25' N; DMT of July 24.8℃). A similar fluctuation of their male sterility was observed at Sakai (Osaka Prefecture University, Osaka, Japan; latitude 34° 35' N; DMT of July 26.4℃), assuming that the temperature stimulus in the duration of flower development causes phenotypic fluctuation for sterility in A1 male sterile lines. The conversion from male fertility to male sterility, due to a low night temperature, was known in several sorghum genotypes (Brooking, 1979). In rice, thermo-sensitive genetic male-sterile lines were derived by conversion from male fertility to male sterility either by daily mean temperature above 24-26℃ (Latha et al., 2004) or by

maximum temperature higher than 30℃ (Lopez et al., 2003). However, the conversion from male sterility to male fertility has not been known in sorghum varieties except for in these cytoplasmic male-sterile lines (Adachi, 2000; Tarumoto and Oizumi, 1967; Tarumoto et al., 2000). Therefore, in order to clarify the factors of fluctuation of male-sterility, the seed and pollen fertility of A-lines with A1 cytoplasm was examined for sorghum genotypes grown in an experimental field and in growth chambers under different temperatures on the campus of Osaka Pref. University in 1999.

Materials and Methods

Eleven A-lines with A1 cytoplasm (Table 1) whose cytoplasmic type was determined as A1 (Adachi 2000) for study on the stability of male sterility and eleven counterpart maintenance lines (B-line) as a control were used in this study. These lines were grown on a university farm (latitude 34˚33' N) during the 1999 cropping season from June 10 to September 20. The field condition (FC) was 29.3℃ average maximum temperature, 21.9℃ average minimum temperature and 25.6℃ daily mean temperature (DMT) during a period from the 3rd to the 10th leaf stages (June 23 to July 28) and flower development duration. Eleven A-lines were grown in three growth chambers controlled under different day/night temperatures (Table 2) during a period from the 3rd to the 10th leaf stages although they were maintained outdoors before and after temperature treatment. The growth chambers were maintained at 24℃/15℃ (day/night) as a low temperature condition (LTC, 19.7℃ DMT) at 24℃/24℃ as a medium temperature condition (MTC, 24℃ DMT) and

Table 1. Male-sterile lines (A-line) with A1 cytoplasm and their origin.

A-lines	Origin and Organization introduced
MS79	IS2830A&B, Purdue Univ., USA
CK60	Combine kafir 60A&B, Texas A&M Univ., USA
Martin	MartinA&B, Iowa State Univ., USA
Reliance	RelianceA&B, Iowa State Univ., USA
Wheatland	Wheatland A&B, Iowa State Univ., USA
Tx624	Tx624A&B, Texas A&M Univ., USA
MS138	932233A&B, Purdue Univ., USA
Tx3048	Tx3048A&B, Texas A&M Univ., USA
Redbine58	Redbine58 A&B, Iowa State Univ., USA
Redlan	Redlan A&B, Texas A&M Univ., USA
Westland	Westland A&B, Texas A&M Univ., USA

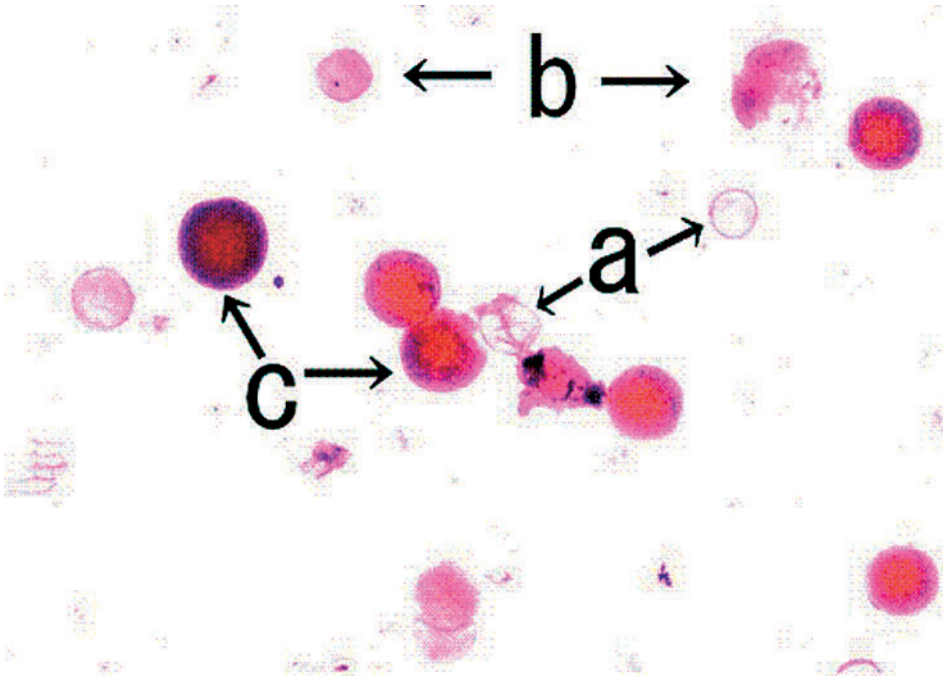

Fig. 1. The pollen observed at early vacuolated microspore stage (a), vacuolated pollen stage (b) and engorged pollen stage (c). The pollen reached at engorged stage (c) is classified into fertile.

Table 2. Average of maximum and minimum temperatures in a field at Sakai (Osaka Pref. University, Osaka, Japan) during the period between third and tenth leaf stages in 1999, and day temperature (5:30 – 18:00) and night temperature (18:00 – 5:30) in three growth cabinets in 1999.

Experiment	Maximum or day temperature	Minimum or night temperature	Daily mean temperature
Field condition (June 23 to July 28)	29.3	21.9	25.6
33℃/24℃ cabinet	33.0	24.0	28.7
24℃/24℃ cabinet	24.0	24.0	24.0
24℃/15℃ cabinet	24.0	15.0	19.7

at 33℃/24℃ as a high temperature condition (HTC, 28.7 DMT) to reveal seed and pollen fertility. The panicles of A-lines in all experiments and those of B-lines in FC were covered by paper bags before flowering and debagged three weeks after flowering, and then self-pollinated panicles were harvested after ripening. The percentage of seed fertility was determined for at least 200 spikelets per replicate. The pollen was collected from anthers one day before dehiscent and was stained with 0.5 % aceto-carmine solution after fixation by 70% ethanol. The pollen which achieved engorged pollen stage (Christensen *et al*. 1972) was considered to be fertile. The other pollen before early vacuolated microspore stage or vacuolated pollen stage was treated as sterile (Fig. 1). The percentage of pollen fertility was determined by the evaluation of at least 100 pollens per replication.

Results and Discussion

Seed fertility and pollen fertility of A-lines in four experimental conditions and B-lines in FC are shown in Table 3. The percentages of seed and pollen fertility in B-lines were 65 to 93% and 54 to 99%, respectively, which indicates that B-lines expressed their normal fertility as maintenance lines. No seed was set in A-lines, 'MS79,' 'CK60,' 'Martin,' 'Reliance,' 'Wheatland' and 'Tx624' by artificial self-pollination under all four conditions. However, 'MS138' showed 19% seed set in MTC (day/night, 24℃/24℃) and 21% seed set in HTC (33℃/24℃), 'Tx3048' showed 15% seed set in HTC and 13% seed set in FC (29.3℃/21.9℃), and 'Redbine58,' 'Redlan' and 'Westland' respectively showed 7%, 7% and 29% seed set in FC, while no seed set was shown in the other experimental conditions. In A-line, 'MS79', 'Reliance', 'Wheatland' and 'Tx624' had no pollen fertility under all the four conditions. However, 'CK60,' 'Martin,' 'Redbine58' and 'Westland' showed 5%, 7%, 16% and 61% pollen fertility in FC, respectively. 'MS138' showed 75% pollen fertility in MTC and 78% pollen fertility in HTC, 'Tx3048' showed 36% in HTC and 67% in FC, and 'Redlan' showed 12% in MTC and 57% in FC despite 0% pollen fertility in other experiments. In the experiments, a relatively high pollen fertility was observed either at a night temperature above 24℃ in MTC and HTC or at a day temperature above 29.3℃ in HTC and FC, however, their seed fertility was considerably lower than the expected seed fertility from their pollen fertility in three conditions. Based on the results, A-lines can be classified into 4 groups, Group-1, Group-2, Group-3 and Group-4 (Table 4). 'MS79,' 'CK60,' 'Martin,' 'Reliance,' 'Wheatland' and 'Tx624' were classified into Group-1 by their stable male-sterility in various experimental conditions except for low pollen restoration of 'CK60' and 'Martin' in FC. 'MS138' was classified into Group-2 due to its moderate seed setting under minimum temperatures above 24℃, and 'Tx3048' was classified into Group-3 due to its peculiar seed setting under maximum temperatures above 29.3 ℃ during a period from the 3rd to 10th leaf stages that corresponded to flower initiation of pollen development stages. 'Redbine58,' 'Redlan' and 'Westland' are classified into Group-4 by their seed setting with high pollen restoration that occurred only in FC.

Sixteen A-lines including 8 common lines in this study were evaluated for their seed fertility

Table 3. Percentage of seed fertility in self-pollination by paper bag and percentage of pollen fertility tested after stained with acetocarmine solution.

A-lines	Seed fertility				Pollen fertility			
	24℃/15℃	24℃/24℃	33℃/24℃	Field	24℃/15℃	24℃/24℃	33℃/24℃	Field
MS79	0	0	0	0	0	0	0	0
CK60	0	0	0	0	0	0	0	5
Martin	0	0	0	0	0	0	0	7
Reliance	0	0	0	0	0	0	0	0
Wheatland	0	0	0	0	0	0	0	0
Tx624	0	0	0	0	0	0	0	0
MS138	0	19	21	0	0	75	78	32
Tx3048	0	0	15	13	0	0	36	67
Redbine58	0	0	0	7	0	0	0	16
Redlan	0	0	0	7	0	12	0	57
Westland	0	0	0	29	0	0	0	61

The samples resulted were collected from
plants grown in growth cabinets of 24℃/15℃, 24℃/24℃ and 33℃/24℃, and a field in 1999.

in the field and greenhouse of Chugoku National Agricultural Experiment Station, Fukuyama, Japan (latitude 34° 27' N) . In the fields during the summer of 1965 (DMT of July 25.6℃), the percentage of seed fertility of 'Reliance,' 'Redbine58' and 'Westland' was 13.9, 3.1 and 0.5%, respectively, although the other 13 A-lines were completely sterile (Tarumoto and Oizumi, 1967). In the greenhouse in summer and winter of 1965, all the eleven A-lines were completely sterile (Tarumoto, 1971). In FC of Fukuyama in 1965 and Sakai in 1999, 'Redbine 58' and 'Westland' usually showed variable male sterility, while 'Reliance' showed it only at Fukuyama in 1965. In the greenhouse at Fukuyama and in a growth cabinet at Sakai, 'Redbine58' and 'Westland' commonly showed stable sterility, although the cause for the fluctuation of male sterility in 'Redbine58' and 'Westland' was unknown. However, relatively high pollen fertility was observed in all A-lines belonging to Group-2 and Group-3 as well as in three A-lines of Group-4. Even though the influence of minimum and/or maximum temperature to male sterility is unknown in sorghum cultivars, the temperature stimulus is considered to be related to phenotypic fluctuation of male sterility in Group-4, especially in 'Redbine58' and 'Westland.' Clearly the fluctuation of male sterility was related to minimum temperatures above 24℃ in 'MS138'(Group-2) and maximum temperatures higher than 29.3℃ in 'Tx3048'(Group-3). Our results (Table 3 and Fig. 1) imply that relatively high pollen fertility is associated with the development up until the engorged pollen stage in high temperatures, and a small amount of pollen probably would rend and scatter. From the comparisons of gene expression in starch biosynthesis in developing pollen between fertile and male-sterile sorghum lines, Datta et al. (2001) indicated that tapetum and pollen developments proceed normally up to the starch filling stage in fertile and male sterile lines, and that pollen abortion occurs in the late stage of pollen development. In male-sterile corn, Lee et al. (1980) emphasized that since pollen development proceeds in a very late stage even in full sterile S lines, pollen abortion may be more easily averted through physiological or by environmental interactions. Thus, in certain male-sterile sorghum lines like 'MS138' and 'Tx3048,' the environmental condition during the early vacuolated pollen stage and the engorged pollen stage is considered to be critical for conversion from sterile to fertile. However, the observation of pollen is limited to revealing the above conversion, thus more anatomical studies are necessary for clarifying the relationship between environmental condition and pollen fertility.

Expression of male sterility can be satisfactory explained in Group-1 by the interaction between cytoplasm and nuclear-gene (Singh

Table 4. The relationship between level of male sterility and temperature during the period of flower development in seed fertility, and the classification of A-lines according to the relationship.

Group (A-lines)	Level of male sterility	Temperature condition[1)]
Group 1 (MS79, CK60, Martin, Reliance Wheatland, Tx624)	stable	no concern
Group 2 (MS138)	variable	Temperatures > 24.0
Group 3 (Tx3048)	variable	DMT>25.6 AMXT>29.3
Group 4 (Redbine58, Redlan, Westland)	variable	DMT>25.6 AMXT≦29.3

[1)] DMT: Daily mean temperature, AMXT: Average of maximum temperature

and Hadley, 1961; Stephens and Holland, 1954), but this explanation is unsatisfactory in Group-2, Group-3 and Group-4 (Table 3). No differentiation of cytoplasmic type among eleven A-lines used in this study was confirmed by gene-specific PCR analysis on four mitochondrial m-RNA genes (COX2, COX3, COB and ORF25) (Adachi 2000 and Tarumoto et al., 2000) as was made known in Bailey-Serres et al. (1986). Since no structural modification exists among A1 cytoplasm in eleven A-lines, the presence of a modifier gene is assumable for the factor shaping the interaction between nuclear gene and A1 cytoplasm. Therefore, genes that fluctuate their relationship between sterile gene and A1 cytoplasm by high minimum and/or maximum temperature should be examined by further study.

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