Inheritance of a Thermo-sensitivity Gene Controlling Flower Initiation in Sorghum

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The existence of a sorghum (*Sorghum bicolor* Moench) thermo-sensitivity gene has been proposed to account for the acceleration of flower initiation when plants are exposed to a temperature lower than 20°C. Four varieties and their F₁, F₂, F₃ and BC₁F₁ populations were evaluated for days to emergence of flag leaf (DEFL) under the field condition of a long daylength (>13 h) and temperatures over 20°C for at least 40 days after sowing. The F₁ hybrids, Hiromidori (Norin Ko-2) : 390 (early) × Regs.Hegari (late) and Natsuibuki (Norin Ko-9) : MS175 (late) × Daikoukaku (early), showed the same flowering response as Regs.Hegari and MS175 which show a delay in DEFL under temperatures over 20°C, suggesting the presence of a dominant thermo-sensitivity gene. In two different F₂ populations, the segregation of early and late plants for DEFL fitted in with the expected ratio of 1 : 3, indicating that a single dominant gene was controlling the phenotype. The inheritance mode was confirmed in two different BC₁F₁ populations, where the expected ratio of 1 : 1 (early : late) was observed in the segregation of BC₁F₁ of 390 × F₁ and F₁ × Daikoukaku. As expected, no segregation was observed in BC₁F₁ populations of F₁ × Regs.Hegari and MS175 × F₁. These results suggest that the sorghum thermo-sensitivity for flower initiation is controlled by a monogenic dominant gene of late over early, and the symbol *TT* is assigned to the genotype in which flower initiation is accelerated by the exposure to a temperature lower than 20°C.

Key Words: flowering, flower initiation, F₁ hybrid, *Sorghum bicolor*, thermo-sensitivity gene, photoperiod sensitivity gene.

Introduction

Sorghum is a short day plant originating in tropical Africa. The varietal differentiation of sorghum associated with its adaptation to temperate environments and/or from low to high-altitudes has involved changes in their flowering re-

sponse for successful reproduction and grain production. In sorghum, the natural mutations from photoperiod-sensitive to insensitive types and/or from thermo-neutral to thermo-sensitive types are considered to be genetic factors for changing the flowering response.

Sorghum lines adapted to temperate/sub-tropical climates in the USA showed a flowering response of 45 to 100 days to flowering and contained four maturity genes, *Ma*₁ to *Ma*₄, that were proposed to explain the varietal flowering response (Quinby 1967). Miller *et al.* (1968) reported that dominant genes of *Ma*₁ and *Ma*₂ controlled photoperiod sensitivity and the critical photoperiod was more than 13 h. Almost all sorghums cultivated in Japan are F₁ hybrids produced by crosses between male-sterile grain sorghum and another sorghum such as hegari, sudangrass and sorgo, all of these sorghums have a temperate adaptation (Tarumoto 1971). The flowering response of these F₁ hybrids can be elucidated on the basis of maturity genes as described by Quinby (1967) and Miller *et al.* (1968), although variation still existed. The days to heading of those F₁ hybrids is almost intermediate of their parents and was less than that of 100-day Milo, the latest genotype with *Ma*₁*Ma*₁*Ma*₂*Ma*₂*Ma*₃*Ma*₃*Ma*₄*Ma*₄ (Tarumoto 1971).

Two exceptions, however, are observed. One is observed in the hybrids between male-sterile grain sorghum and hegaries, like Hiromidori (390 × Regs.Hegari). Their flowering response was similar to that of Combine hegari (Quinby *et al.* 1973), but no clear genetic explanation has been made in Quinby *et al.* (1973) as well as in Tarumoto (1967, 1971) using the maturity genes of Quinby (1967). The other is in new hybrids, Tentaka and Kazetachi, between grain sorghum lines with very early maturity and photoperiod insensitivity. The flowering response of Tentaka and Kazetachi, which showed strong photoperiod sensitivity and very late maturity (Kasuga *et al.* 1992, Tarumoto *et al.* 1997), can not be explained by the maturity genes of Quinby (1967). To account for the observed flowering response for the above two kinds of hybrids, Tarumoto *et al.* (2000) proposed that a thermo-sensitivity gene (*T*) and a photoperiod sensitivity gene (*D*) were present and that *tt* displayed recessive epistasis to *DD*. From this hypothesis, the following predictions can be made; a) flower initiation of F₁ hybrids (*Ttdd*) between male-sterile grain sorghum (*ttdd*) and hegaries (*TTdd*) will be accelerated in association with reduction in total leaf number by exposure to minimum temperatures lower than 20°C (i.e., the opposite phenomenon will be over

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20°C); b) flower initiation of Tentaka and Kazetachi with the *TtDd* will be induced by short day (less than 12.5 h).

In order to study the inheritance of the sorghum thermo-sensitivity gene (*T*) proposed by Tarumoto *et al.* (2000), we evaluated the days to emergence of flag leaf (DEFL) of F_1 , F_2 , F_3 and BC_1F_1 from crosses between late and early flowering varieties under the field condition of a long day-length and minimum temperatures over 20°C for at least 40 days after sowing, as proposed by Adachi *et al.* (1999) and Tarumoto *et al.* (2000).

Materials and Methods

To study the inheritance of a sorghum thermo-sensitivity gene (Tarumoto *et al.* 2000), we used 390, Regs.Hegari, MS175, Daikoukaku, Hiromidori and Natuibuki as parental varieties (Table 1). Their genes predicted for thermo-sensitivity and photoperiod sensitivity, and our previous data of days to emergence of flag leaf (DEFL, hereafter) and days to flower initiation (DFI) were noted in Table 2 for better understanding of the flowering response of parental varieties. The two F_2 populations were produced by self-pollination of Hiromidori and Natuibuki in 1996 and 1998. Two F_3 lines with early maturity and five F_3 lines with late maturity were derived from F_2 populations of Hiromidori through selection and self pollination in 1997. Two BC_1F_1 populations were produced from crosses of 390 × Hiromidori and MS175 × Natuibuki using male-sterile lines, and another two BC_1F_1 were from crosses of Hiromidori × Regs.Hegari and Natuibuki × Daikoukaku using hand-emasculated female parents in 1998–1999.

DEFL has proved to be a better trait than days to heading or flowering for evaluating the response to flower initiation in sorghum and that the planting under field conditions of a long daylength and minimum temperatures over 20°C for at least 40 days after sowing at Sakai is suitable for evaluating the flowering response in sorghum, especially for

classification into varieties having different genes to flower initiation, such as Hiromidori and Tentaka (Adachi *et al.* 1999, Tarumoto *et al.* 2000). Hiromidori, its parents, F_2 and seven F_3 derived from Hiromidori were planted on July 10 in 1998, and all the varieties in Table 1, two F_2 derived from Hiromidori and Natuibuki and four BC_1F_1 were planted on July 3 in 2000, to evaluate the flowering response under the above suitable field condition at Sakai. DEFL in F_2 , F_3 and BC_1F_1 were classified into early or late relative to DEFL of the corresponding parental varieties and segregating pattern in F_2 .

Results and Discussion

During the 68-day growing period from July 3 to September 10 at Sakai, Osaka Prefecture University experimental field (latitude 34°35' N), daylength, including civil twilight, ranged from 14.37 h to 13.00 h, and from September 19 (77th day after sowing) it had decreased further to a shorter daylength of less than 12.5 h. The average maximum temperature was 32.2°C (ranging from 24.4 to 34.9°C) between

Table 1. Plant materials used and their origin

Variety	Origin and organization of introduction
390 (1) ¹⁾	Texas Blackhull Kafir 390A & B, Texas A & M Univ., USA
Regs.Hegari (2)	Hegari, Texas A & M Univ., USA
Hiromidori (F_1 : 1 × 2)	Sorghum Norin 1, Hiroshima Agr. Exp. Sta., Japan
MS175 (3)	932233A & B (Purdue No.601570 & 601571), Purdue Univ., USA
Daikoukaku (4)	Kaoliang Chohin 237, Nagano Animal Ind. Exp. Sta. Japan
Natuibuki (F_1 : 3 × 4)	Sorghum Norin 9, Nagano Animal Ind. Exp. Sta., Japan

¹⁾ Figures in parentheses show experimental numbers of maternal varieties.

Table 2. Genes predicted for thermo-sensitivity (*T*) and photoperiod sensitivity (*D*), and DEFL (days to emergence of flag leaf) and DFI (days to flower initiation) of the present materials observed in our previous studies

Variety	Genes ¹⁾ predicted	DEFL ²⁾ grown for 15 h DL at temperatures of			DFI ³⁾ grown for 12.5 h DL at temperatures of	
		35/25°C	30/20°C	30/15°C	25/25°C	30/20°C
390	<i>ttdd</i>	69	66	55	43	41
Regs.Hegari	<i>TTdd</i>	85	72	45	53	35
Hiromidori	<i>Ttdd</i>	87	82	52	49	39
MS175	<i>TTdd</i>	85	70	44	46	26
Daikoukaku	<i>ttdd</i>	42	42	43	—	—
Natuibuki	<i>Ttdd</i>	82	75	47	—	—

¹⁾ Cited from Tarumoto *et al.* (2000) and MS Thesis of Iwahara (2001). *T* for thermo-sensitivity and *D* for photoperiod sensitivity.

²⁾ Cited from Yanase *et al.* (2000) and MS thesis of Iwahara (2001), in which varieties were planted and grown under 15 h daylength (DL) at temperatures of 35/25 (day/night °C), 30/20 and 30/15°C in growth chambers. Data indicated that flower initiation of F_1 hybrids (*Ttdd*) and varieties (*TTdd*) is accelerated by exposure to night temperatures lower than 20°C.

³⁾ Cited from MS Thesis of Maeji (2001), in which varieties were planted and grown under 12.5 h daylength at temperatures of 25/25 (day/night °C) and 30/20°C in growth chambers. Data showed that flower initiation of F_1 hybrids (*Ttdd*) and varieties (*TTdd*) is accelerated by exposure to night temperatures lower than 20°C even under 12.5 h DL within a critical daylength.

July 10 and September 10 in 1998 and 33.1°C (ranging from 28.0 to 35.3°C) between July 3 and September 10 in 2000, and the average minimum was 25.1°C (ranging from 20.1 to 29.2°C) in 1998 and 25.9°C (ranging from 21.7 to 28.7°C) in 2000, respectively. A minimum temperature below 20°C was not evident until September 27 in 1998 and September 25 in 2000. Therefore, the suitable field conditions of long daylengths (>13 h) and high minimum temperatures (>20°C) for evaluating flowering response were performed until September 10 of 62th day after sowing in 1998 and 68th day after sowing in 2000.

In the case of Hiromidori (F_1 : 390 × Regs.Hegari), DEFL of 390, Regs.Hegari and Hiromidori were 48, 65 and 68 in 1998, and 45, 75 and 78 in 2000, respectively (Table 3). Because of the above field condition, DEFL of Regs.Hegari and Hiromidori was delayed by exposure to minimum temperatures over 20°C, and they were evaluated as late maturity. A segregating pattern for the DEFL in F_2 population grown in 2000 is shown in Fig. 1a. Since 390 (Texas Black-hull Kafir 390) and Regs.Hegari are considered to carry certain maturity genes described by Quinby (1967) in addition to thermo-sensitivity gene, the segregation was not clearly classified, especially in later DEFL. However, F_2 progenies for DEFL were classified roughly into two groups, one earlier group less than 60 DEFL and the other later group over 60 DEFL in 2000, and also in 1998, into two groups: one earlier group less than 58 DEFL and the other later group over 58 DEFL. The segregation gave an expected ra-

Fig. 1. Segregation for DEFL (days to emergence of flag leaf) in F_2 population derived from Hiromidori (a) and Natuibuki (b) in 2000.

Note) P_1 : 390, P_2 : Regs.Hegari, P_3 : MS175, P_4 : Daikoukaku
 $\triangle$: DEFL of F_1 , $\blacktriangle$: DEFL of parental varieties

tio of 1 : 3 (early : late) using 58 DEFL in 1998 and 60 DEFL in 2000 as the point of discrimination between early and late (Table 3). In 1998, two F_3 lines, E-1 and E-2, showed early

Table 3. DEFL (days to emergence of flag leaf) of parental varieties included Hiromidori, and segregation pattern for DEFL in F_2 , F_3 and backcross populations of Hiromidori during the summer of 1998 and 2000

Variety and/or population	Total number	No. of plants segregated into		Expected ratio (early : late)	χ^2	P
		early	late			
<u>1998:</u>		(early < 58) ²⁾	(late > 58)			
390	13	13(48) ²⁾	0			
Regs.Hegari	15	0	15(65)			
Hiromidori	18	0	18(68)			
F ₂	298	81	217	1 : 3	0.756	0.25–0.50
F ₃ ¹⁾ E-1	16	16	0	no segregation		
E-2	63	63	0	no segregation		
L-1	74	19	55	1 : 3	0.018	0.80–0.90
L-2	86	24	62	1 : 3	0.387	0.50–0.70
L-3	101	30	71	1 : 3	1.191	0.25–0.50
L-4	78	17	61	1 : 3	0.427	0.50–0.70
L-5	12	0	12	no segregation		
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<u>2000:</u>		(early < 60) ²⁾	(late > 60)			
390	19	19(45) ²⁾	0			
Regs.Hegari	16	0	16(75)			
Hiromidori	22	0	22(78)			
F ₂	266	56	210	1 : 3	2.210	0.10–0.25
BC ₁ F ₁ (390 × F ₁) ¹⁾	185	84	101	1 : 1	2.560	0.10–0.25
BC ₁ F ₁ (F ₁ × RH) ¹⁾	146	0	146	no segregation		

¹⁾ F_3 lines: E-1 and E-2 were derived from F_2 plants segregated into early DEFL, and L-1 to L-5 from F_2 plants segregated into late DEFL. Those F_2 were self-pollinated by covering individual panicles with paper bags. BC_1F_1 (390 × F_1) were derived from a cross between 390 and Hiromidori. BC_1F_1 (F_1 × RH) were derived from a cross between hand-emasculated Hiromidori and Regs.Hegari.

²⁾ Figures in parentheses show DEFL.

Table 4. DEFL (days to emergence of flag leaf) in parental varieties included Natuibuki, and segregation pattern for DEFL in F₂ and backcross populations of Natuibuki during the summer of 2000

Variety and/or population	Total number	No. of plants segregated into		Expected ratio (early : late)	χ^2	P
		early	late			
2000:		(early < 46) ²⁾	(late > 46)			
MS175	30	0	30(62) ²⁾			
Daikoukaku	23	23(34)	0			
Natuibuki	25	0	25(64)			
F ₂	209	58	151	1 : 3	0.834	0.25–0.50
BC ₁ F ₁ (MS175 × F ₁) ¹⁾	96	0	96	no segregation		
BC ₁ F ₁ (F ₁ × DK) ¹⁾	173	80	93	1 : 1	0.977	0.25–0.50

¹⁾ BC₁F₁ (MS175 × F₁) were derived from the cross between MS175 and Natuibuki. BC₁F₁ (F₁ × DK) were derived from a cross between hand-emasculated Natuibuki and Daikoukaku.

²⁾ Figures in parentheses show DEFL.

DEFL without segregation, four F₃ lines, L-1 to 4, segregated into an expected ratio of 1 : 3 (early : late), and F₃-L-5 were late DEFL without segregation. In 2000, as expected, BC₁F₁ of 390 × F₁ segregated into an expected ratio of 1 : 3 (early : late), and no segregation was observed in BC₁F₁ of F₁ × Regs.Hegari.

In the case of Natuibuki (F₁: MS175 × Daikoukaku), DEFL of MS175, Daikoukaku and Natuibuki were 62, 34 and 64, respectively (Table 4). Because of the above field condition, DEFL of MS175 and Natuibuki was delayed also by a minimum temperature over 20°C, and they were evaluated as late maturity. A segregating pattern for DEFL in the F₂ population of Natuibuki grown in 2000 is shown in Fig. 1b. Since MS175 (932233) and Daikoukaku are also considered to carry certain maturity genes described by Quinby (1967) in addition to thermo-sensitivity gene, the segregation was not clearly classified, also especially in later DEFL. However, F₂ progenies of Natuibuki for DEFL were classified roughly into two groups, one earlier group less than 46 DEFL and the other later group over 46 DEFL. The segregation gave an expected ratio of 1 : 3 (early : late) at the point of 46 DEFL to discriminate early from late (Table 4). As expected, in BC₁F₁ of F₁ × Daikoukaku, a segregation ratio of 1 : 1 (early : late) was observed, and no segregation was observed in BC₁F₁ of MS175 × F₁. These results indicate that the thermo-sensitivity gene is a monogenic dominant of late over early (Table 3 and Table 4).

The results presented here for DEFL of Regs.Hegari (*TTdd*), Hiromidori (*Ttdd*), MS175 (*TTdd*) and Natuibuki (*Ttdd*) prove that the delay of flower initiation in response to a high minimum temperature is caused by the function of dominant thermo-sensitivity gene associated with a recessive photoperiod sensitivity gene as proposed by Tarumoto *et al.* (2000). This evidence is particularly clear because of segregations in F₂, F₃ and BC₁F₁ for DEFL as shown in Table 3 and Table 4. The present study has identified the thermo-sensitivity gene proposed by Tarumoto *et al.* (2000), and it is inherited as a monogenic dominant of late over early flowering initiation. Thus, we propose the symbol of this thermo-sensitivity gene *T*.

By sowing in early July at Sakai, it has been possible to

characterise this gene as a dominant thermo-sensitivity gene under the field condition of a long daylength and minimum temperatures over 20°C. Also in reports of Yanase *et al.* (2000), Maeji (2001), Uozumi *et al.* (2001) and Uozumi *et al.* (2001), the varieties with a dominant thermo-sensitivity gene were characterized as delay of flower initiation associated with an increase of total leaf number by controlling night temperatures over 20°C under long daylengths in growth cabinets. The flowering response of Combine hegari and 100-day Milo (Quinby *et al.* 1973) is similar to that of Regs.Hegari, MS175, Hiromidori and Natuibuki in this study. In Quinby *et al.* (1973), however, a clear genetic and physiological explanation for the flowering response of Combine hegari and 100-day Milo was not done by maturity genes of Quinby (1967). According to Obata *et al.* (2002) and our unpublished data, it proves that Combine hegari, 60-day Milo, 90-day Milo and 100-day Milo should have a dominant thermo-sensitivity gene. Therefore, the flowering response of Combine hegari and 100-day Milo as reported by Quinby *et al.* (1973) would be elucidated using the thermo-sensitivity gene identified in this study.

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