

# Genetic Analysis of Heading Characters in Foxtail Millet (*Setaria italica* (L.) P. Beauv.) Using the Progeny from the Cross between the Two Diverse Strains, Gai 53 and Kuromochi

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## Introduction

Heading time is an important character highly associated with the regional adaptability of crop plants. Much varietal difference in heading time has been reported in many crops such as rice and soybean<sup>3)</sup>. Genetic systems bringing about varietal difference have been studied in detail in these crops. Foxtail millet also has much varietal difference in heading time<sup>9, 11, 12, 13, 21, 22, 24, 25)</sup>. To our knowledge, however, there has been no report on the genetic system controlling heading time in foxtail millet. In the present study, genetic analysis of heading time in foxtail millet was performed using the progeny from the cross between two strains differing in heading time.

## Materials and Methods

### Plant material

Kuromochi (KM) and Gai 53 (G53) were selected from our collection<sup>21)</sup>. KM is a native cultivar of Kagoshima Prefecture, Japan, and was collected in 1950. This cultivar has a late heading trait and it shows anthocyanin pigmentation at the base. G53 is a strain collected in Leningrad, Russia, in 1965. This strain has an early heading trait. It does not show anthocyanin pigmentation at the base.

### Estimation of photoperiodic response

KM and G53 were sown every month, and were grown in a greenhouse on the Experimental Farm of the Faculty of Agriculture, Kagoshima University, Kagoshima, Japan (31° 30'N) under natural daylength condition. Then heading date was recorded for each plant. Sowing dates were the 20th of every month from April 2000 to March 2002. Six seeds from each strain were sown in field soil in a pot 15cm in diameter and 18cm in depth. The soil was sterilized by microwave treatment (high output for thirty minutes) using a microwave oven (NE-1021 Type: National, Osaka, Japan) to avoid contamination of the soil with seeds of other foxtail millet cultivars or weeds. Seedlings were thinned to one per pot a week after sowing. Two weeks after sowing, HYPONex (mixed

fertilizer; HYPONex, Osaka, Japan) solution (0.25 %) was supplied at a rate of 25 ml per pot every week as an additional fertilizer. Five replications were provided for each strain of each month. Heading date (days to heading: DH) of the main culm was recorded. The air temperature in the greenhouse was automatically controlled to be between 20 and 25 °C by ventilation and heating. However, it was above 25°C when the outside temperature was above 25°C in spring to early fall.

### Estimation of basic vegetative growth (BVG) and photoperiod sensitivity (PS)

The period from germination to flower initiation in rice, a short-day plant well studied on heading characters, comprises the photoperiodically insensitive phase called the basic vegetative growth (BVG) period and the subsequent photoperiodically sensitive phase<sup>1)</sup>. Many methods have been proposed to estimate the degree of BVG and photoperiod sensitivity (PS)<sup>14, 16, 20)</sup>. A simple but popular index to estimate the degree of BVG is DH under 10h daylength (an optimum short daylength), and that for estimating the degree of PS is the difference between DHs under long daylength and those under 10h daylength<sup>5, 7, 17, 26)</sup>. Since foxtail millet is also a short-day plant, these indices were adopted for foxtail millet in the present study. Since natural daylength in the period from May to August could be regarded as long daylength (see **Results**), the DHs of both strains grown along with the F<sub>2</sub> population from the cross between them, which was sown on July 20, 2000, under natural daylength and 10h daylength conditions (see **Genetic analysis**), were used for the estimation of BVG and PS.

### Genetic analysis

#### F<sub>2</sub> generation

G53 was crossed with KM. G53 was emasculated by soaking the panicle in hot water at 45°C for 8 minutes (Fukunaga, personal comm.). Then the pollen of KM was shed on the panicle of G53. Anthocyanin pigmentation at the node was used as a marker trait to ascertain whether true hybrids were obtained or not because it is known to be conferred by dominant genes. The F<sub>2</sub> population derived from the cross between G53 and KM was subjected to genetic analysis for heading time as follows. It was grown under two daylength conditions, namely, natural daylength condition and short daylength condition (10h) in the above-mentioned greenhouse. The short daylength condition was realized by covering the plants with blackout curtains from 18:00 to 8:00. Three seeds were sown in the sterilized field soil in a pot 15cm in diameter and 18cm in depth. The sowing date was June 20, 2000. Seedlings were thinned to one per pot a week after sowing. The number of F<sub>2</sub> plants examined was 180 under the natural daylength condition and 138 under the short daylength condition. Ten plants of each parental strain were grown under both conditions. Two weeks after sowing, HYPONex (mixed fertilizer; HYPONex, Osaka, Japan) solution (0.25 %) was supplied at a rate of 25 ml per pot every week as an additional fertilizer. Positions of pots were changed at random twice a week under each condition in order to minimize the environmental variance. DHs of main culms were recorded for each plant. Panicles of the main culm were bagged to avoid outcrossing.

#### F<sub>3</sub> generation

A total of 118 F<sub>3</sub> lines derived from selfing of randomly chosen F<sub>2</sub> plants grown under the natural daylength condition were examined for DH. One hundred seeds per F<sub>3</sub> line were sown in the sterilized field soil in a plastic pot 12 cm in diameter and 10cm in depth. Seedlings were grown in the above-mentioned greenhouse until two weeks after sowing date. Then, they were transplanted to the Experimental Farm of the Faculty of Agriculture, Kagoshima University, Kagoshima, Japan (31°

30°N). Fifty plants per line were transplanted in a row on a ridge. The interval between ridges was 60 cm, while that between plants on a ridge was seven cm. Parental strains were planted in the same way with three replications located at random. Heading date was recorded for each plant. The sowing and transplanting dates were June 27 and July 9, 2001, respectively.

## Results

### Estimation of photoperiodic response

Natural daylength in Kagoshima is longest in June and shortest in December (Fig. 1). The DHs of KM fluctuated according to the natural daylength: plants experiencing summer solstice showed longer DHs while those grown in fall and winter (relatively short daylength conditions) showed shorter DHs. On the other hand, the DHs of G53 were stable irrespective of natural daylength (Fig. 2). These results suggested that KM is more strongly photoperiod sensitive than G53. It is known

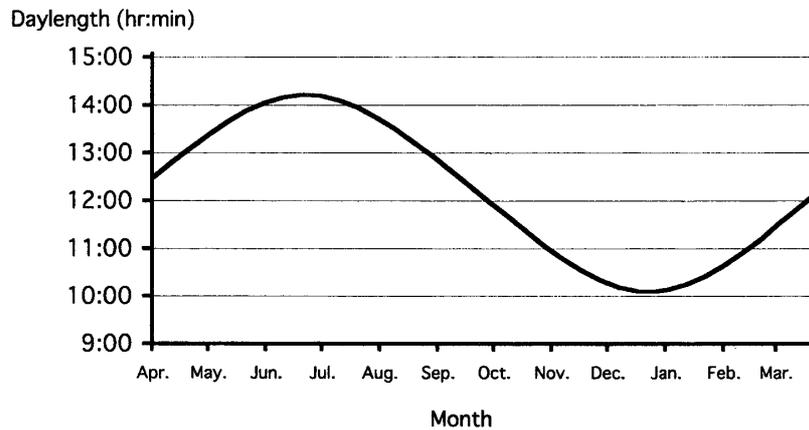


Fig. 1. Fluctuation of natural daylength in Kagoshima (30° 30'N), Japan.

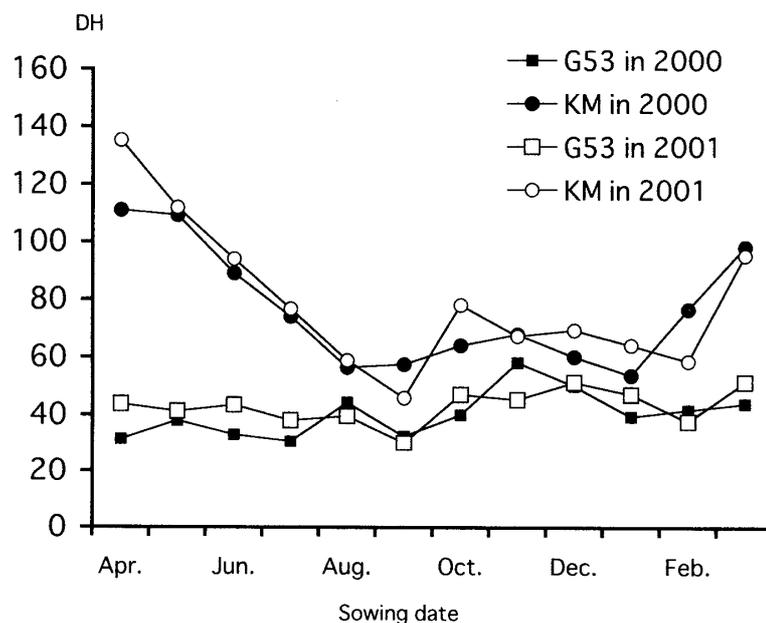


Fig. 2. Days to heading (DH) of KM and G53 sown on different dates. The two cultivars were sown on the 20th of every month from April 2000 to March 2002. The Japanese academic year was adopted in this figure.

that low temperature delays heading in foxtail millet<sup>13)</sup>. The relatively large DH value of G53 sown in Oct. to Jan. might be due to low temperature in late fall and winter.

### Estimation of basic vegetative growth (BVG) and photoperiod sensitivity (PS)

From Figs. 1 and 2, the natural daylength in the period from May to August could be regarded as long daylength. Therefore, the BVG and PS of KM and G53 were estimated using plants grown with the  $F_2$  population. Average DHs of KM and G53 under the short daylength condition were 35.3 and 26.9, respectively, while those of KM and G53 under the natural daylength condition were 89.9 and 33.1, respectively. Therefore, the BVG and PS of KM were estimated to be 35.3 and 44.6, respectively, while those of G53 were estimated to be 26.9 and 6.2, respectively. These results indicate that KM has larger BVG and PS than G53.

### Genetic analysis using $F_2$ population

Fig. 3 shows the frequency distributions of DHs in the  $F_2$  population from the cross between G53 and KM under the natural daylength condition and under the short daylength condition. Under

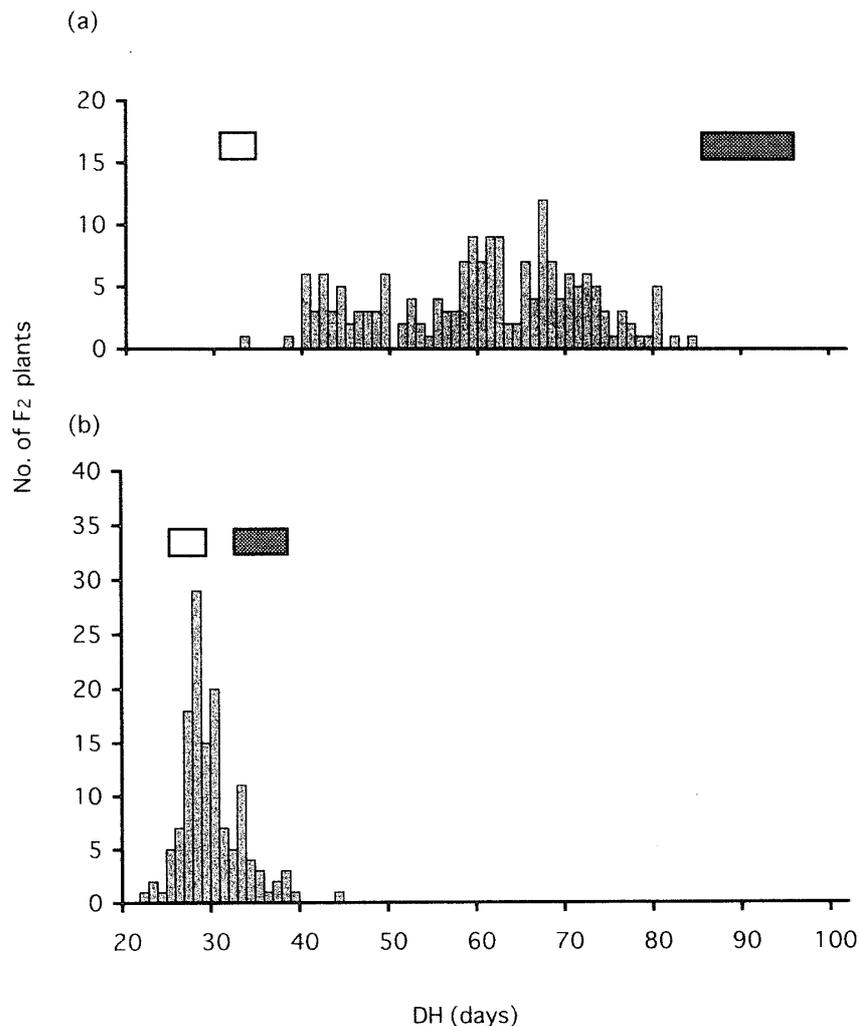


Fig. 3. Frequency distributions of days to heading (DH) for  $F_2$  population derived from the cross between G53 and KM. (a): natural daylength condition, (b): 10h-daylength condition. The open box and the shaded box indicate the ranges of G53 and KM, respectively.

the short daylength condition, the distribution was continuous with no distinct gaps to divide the population into several groups. Many of the F<sub>2</sub> plants headed within the range of the early parent G53 while few of them headed within the range of or later than the late parent KM. Many transgressive segregants appeared toward early heading while only two transgressive segregants toward late heading were observed. From these results, three conclusions were drawn. (1) The BVG period is controlled by many genes with small effect in this population. (2) The short BVG period (early heading) gene is dominant over the long BVG period gene (late heading) at most of the loci. (3) KM shows long BVG, but carries short BVG period gene(s) whose effect is inhibited by other gene(s).

Under the natural daylength condition, the F<sub>2</sub> population showed a continuous distribution with no transgressive segregants. Few plants headed within the ranges of parental strains. Both genes controlling BVG and those controlling PS were segregating in this population, but variance in DHs was obviously larger under natural daylength than under 10h daylength. Therefore, the distribution of DHs mainly reflected the segregation of genes controlling PS. There seemed a breakpoint around 50 days, with the F<sub>2</sub> population divided into 48 early and 132 late plants. This ratio fitted a monogenic segregation of 1:3 ( $\chi^2 = 0.6056$ , d.f. = 1, P = 0.60). Hence, the difference in DHs under natural daylength between KM and G53 seemed to be mainly controlled by a single gene with large effect.

### Genetic analysis using F<sub>3</sub> lines

Table 1 shows the distributions of DHs of representative F<sub>3</sub> lines under the natural daylength condition. If one major gene was segregating in this cross, F<sub>3</sub> lines could be classified into three groups, that is, one comprising lines fixed for early heading, another one comprising lines fixed for late heading, and the other one comprising lines whose DHs were distributed between the parental strains. However, F<sub>3</sub> lines showed continuous variation. These results indicated that no major gene controlling PS was segregating in this cross, and that the variation in DHs in this cross was under polygenic control. Habitability in standard unit<sup>23)</sup>, which was the correlation coefficient between DHs of F<sub>2</sub> plants and average DHs of their respective F<sub>3</sub> lines, was a highly significant 0.88. This indicated that variation in DHs in this cross was controlled by genes rather than environment.

### Discussion

Takei and Sakamoto<sup>24)</sup> classified foxtail millet strains into three types according to heading response to daylength. Type I comprises early maturing strains indifferent to daylength condition. This type is represented by strains from areas of high latitude such as Europe, Central Asia and the northern part of East Asia. Type II comprises strains with very long BVG, and is represented by strains from low latitudinal areas such as Halmahera Is., Indonesia and Luzon Is., Philippines. Type III comprises late maturing strains grown in areas at a latitude of around 30° N. This type is represented by strains sown in summer in Kyushu Is. and Shikoku Is., Japan. There were some intermediate strains between these types. These findings suggested a relationship between the heading response to daylength and the geographical distribution that determines the growing season in traditional cultivation practices of foxtail millet. Judging from the collection sites and heading characters, KM and G53 are thought to belong to Type III and Type I, respectively. Japonica rice cultivars planted in Japan and Taiwan are classified into four types according to heading characters and geographic distribution. Cultivars planted at high latitudes (42-45°) in Japan have almost



complete photoperiod insensitivity and short BVG. Those planted at medium latitudes (36-41°) in Japan have medium PS and somewhat long BVG. Those planted at low latitudes (31-36°) in Japan have strong PS and short BVG. Those planted in Taiwan (22-25°) have weak PS and long BVG. The difference between types is mainly controlled by the gene combination at two PS loci, *E<sub>1</sub>* and *Se1*, and one BVG locus, *Eft*<sup>8, 18</sup>. Before starting the present study, we presumed that a few major genes controlled the difference in heading-time between the two strains. However, our experimental results unexpectedly indicated that the difference is under polygenic control. We plan to analyze different cross combinations between strains differing in heading time.

A number of genetic analyses of heading character have been performed in rice, and as many as 22 genes for heading-time have been identified<sup>10</sup>. Many DNA-marker-assisted quantitative trait loci (QTL) analyses of heading time have also been performed recently<sup>15, 27, 28</sup>. Some of the correspondences between major genes and QTLs for heading time have been identified<sup>6</sup>. Most cereals, including foxtail millet, have been shown to have similar genomes in terms of gene content and gene order, irrespective of chromosome number and genome size<sup>2, 4</sup>. Paterson et al.<sup>19</sup> showed that the locations of some QTL controlling heading-time character were conserved among rice, maize, and sorghum genomes. From these studies, it is thought that foxtail millet has as many genes controlling heading-time character as rice and other cereals, and that the locations of these genes on the foxtail millet genome correspond to those on other cereals' genomes. DNA markers will be of much help in analyzing the number and chromosomal location of the genes. Such analyses are being undertaken.

### Summary

Foxtail millet has much varietal difference in heading time. To our knowledge, however, there has been no report on the genetic system controlling heading time in foxtail millet. In the present study, genetic analysis of heading time in foxtail millet was performed using the progeny from the cross between two strains differing in heading time, Kuromochi (KM) and Gai 53 (G53). Response to photoperiod suggested that KM has greater basic vegetative growth (BVG) and photoperiod sensitivity (PS) than G53. The results of genetic analysis indicate that difference in BVG and PS between the two strains is under polygenic control. The geographical distribution of foxtail millet cultivars and the relationship between foxtail millet and other grasses were discussed from the viewpoint of genes controlling heading time.

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