Genic Analysis of Heading Time by Aid of Close Linkage with Blast Resistance in Rice

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Heading time determines the regional adaptability of rice cultivars and is one of the primary breeding objectives to which breeders pay great attention. Many researches for genetical analysis of this trait have been conducted on cultivated rice.[7]

The variation of heading time in a hybrid population generally shows continuous distributions under natural field conditions, even when heading time is controlled by a small number of major genes. Such continuous variations make the genetical analysis difficult. The close linkage of the heading locus with marker genes may facilitate the genetical analysis of the complicated distributions, when the variation of heading time is traced by the segregation of the marker genes.

The authors have succeeded in the genic analysis for the heading locus of the linkage group I by the aid of the close linkage with a blast resistance gene that has been introduced from indica rice cultivars into Japanese ones, and found that the alleles of the locus mainly control the heading time of cultivated rice.[8,9,10]

Discovery of close linkage between heading time and blast resistance

Some indica rice cultivars showed resistance to a number of races of blast fungus (*Pyricularia oryzae* Cav.) prevalent in Japan.[2,4] Backcross breeding method was employed to introduce their blast resistance into Japanese cultivars. In the backcross generations seedlings were inoculated with the blast fungus strain Ken 53–33, which is one of the most virulent strains to Japanese cultivars, and resistant plants were selected for the following backcrossing.

A BC_4F_2 population from a cross between a Malaysian cultivar Morak Sepilai and an early-maturing Japanese cultivar Fujisaka 5 were measured for heading time in the field. The variation of heading time showed a clear-cut trimodal distribution. The BC_4F_3 progenies were tested for resistance to Ken 53–33 and the genotypes of BC_4F_2 plants were estimated. Homozygous resistant plants were largely late, heterozygous plants were largely medium, and homozygous susceptible ones were exclusively early in this hybrid population (Fig. 1).

This type of association between blast resistance and heading time was observed also in other indica-japonica crosses. Table 1 shows the close relationship between the two characters in the F_2 populations of crosses between two susceptible, early-maturing Japanese cultivars and six resistant, late-maturing indica ones. Indica cultivars used were Morak Sepilai (Malaysia), Kontor (Malaysia), TKM 1 (India), ADT 10 (India), Leuang Tawng (Thailand) and Chao Leuang (Thailand). In these six crosses the frequency of plants resistant to Ken 53–33 was high in late-maturing groups but low in early-maturing ones. The monogenic segregation ratio was obtained for both characters. The homozygous F_3 lines resistant to Ken 53–33

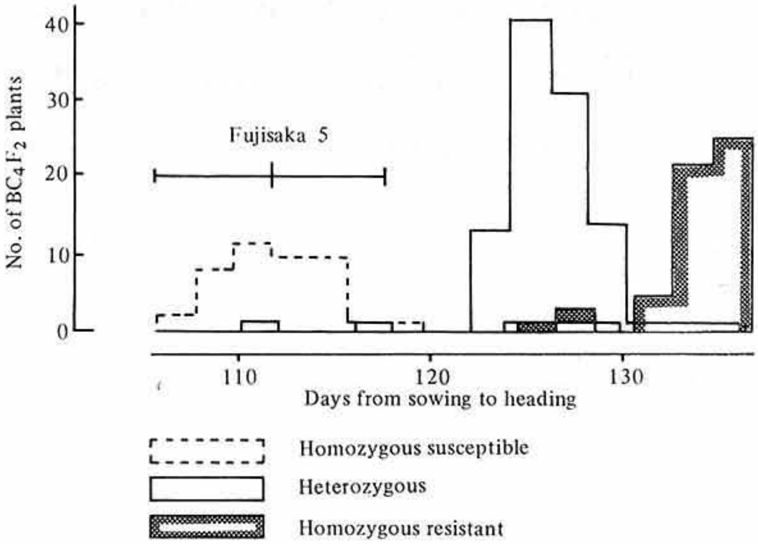

Fig. 1. Frequency distribution for heading date and resistance to the blast strain Ken 53-33 in the Morak Sepilai × Fujisaka 5 BC$_4$F$_2$ population.

Table 1. Segregation for resistance to the Ken 53-33 strain of blast fungus and maturity in the F$_2$ populations of six indica-japonica crosses

Backcross combination	Origin of indica	Generation	No. of F$_2$ plants				Recombination %
			Susceptible		Resistant		
			Early	Late	Early	Late	
Morak Sepilai × Fujisaka 5	Malaysia	BC$_4$F$_2$	48	2	2	184	1.7±0.8
Fujisaka 5 × Kentor	Malaysia	BC$_4$F$_2$	70	5	7	225	3.9±1.1
(Norin 8 × TKM 1)BC$_4$F$_6$ × Fujisaka 5	India	F$_2$	65	5	11	332	4.8±1.2
Ohtori × ADT 10	India	BC$_4$F$_2$	53	5	3	207	3.6±1.3
Fujisaka 5 × Leuang Tawng	Thailand	BC$_3$F$_2$	51	10	8	155	8.1±1.9
Chao Leuang × Fujisaka 5	Thailand	BC$_3$F$_2$	48	4	4	138	4.1±1.6

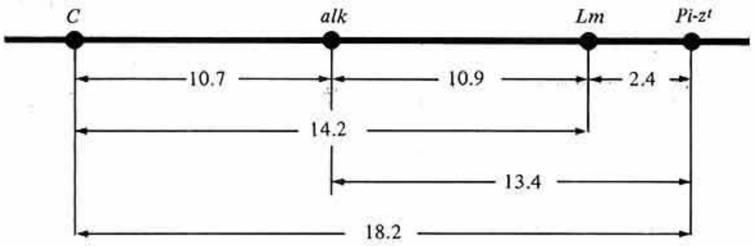

Fig. 2. A proposed arrangement of the four genes tested in the Morak Sepilai × Fujisaka 5 BC$_4$F$_2$ population. The numbers are the recombination values.

became completely susceptible to Ken 53-33-*zt*$^+$, a mutant strain virulent to the resistance gene *Pi-z*^{t}. From this result the blast resistance in these crosses was found to be controlled by *Pi-z*^{t} that had been transmitted by indica cultivars.[4] The linkage analysis in Table 1 confirmed that the gene for heading time in the six crosses is closely linked to *Pi-z*^{t}

Table 2. **Segregation for resistance to the Ken 53–33 strain of blast fungus in the F_3 families of the crosses between Fujisaka 5, Norin 8 and Toride 1**

Cross	No. of F_3 lines			χ^2 (1 : 2 : 1)	P
	Suscept.	Segregating	Resist.		
Toride 1 × Fujisaka 5	45	97	47	0. 174	.95—.90
Toride 1 × Norin 8	54	85	52	2. 350	.50—.25
Fujisaka 5 × Norin 8	203	0	0	—	—

and belongs to the same locus. The heading locus was designated as *Lm*. The average recombination value was estimated to be 3.5± 0.5%.

The Morak Sepilai × Fujisaka 5 BC_4F_2 population showed segregations for the apiculus coloration gene *C* and the alkali reaction gene *alk* as well as for $Pi\text{-}z^t$ and *Lm*. These two genes, belonging to the linkage group I[3,6], are also found to be linked to $Pi\text{-}z^t$ and *Lm*, as shown in Fig. 2.

In another experiment it was confirmed that the *Lm* locus controls the photoperiod sensitivity.[8]

Genic analysis for heading time by aid of close linkage

The F_2 population of the cross between an early-maturing cultivar Fujisaka 5 and a medium-maturing Norin 8 showed a unimodal, continuous distribution for heading time under natural field conditions (Fig. 3-A). It is impossible to identify the genotypes of the two parents for heading time from this continuous variation. In place of Norin 8 a medium-maturing line Toride 1, which was developed from the cross Norin 8 × TKM 1 by four repeated backcrosses with Norin 8 as a recurrent parent and had the $Pi\text{-}z^t$ gene for blast resistance derived from TKM 1[4], was crossed with Fujisaka 5. Toride 1 had the same agronomic characteristics as Norin 8. The two cultivars headed at the same time, and the variation of heading time in the F_2 population of a cross between them was small (Fig. 3-B), indicating that they have the identical genetic constitution for heading time.

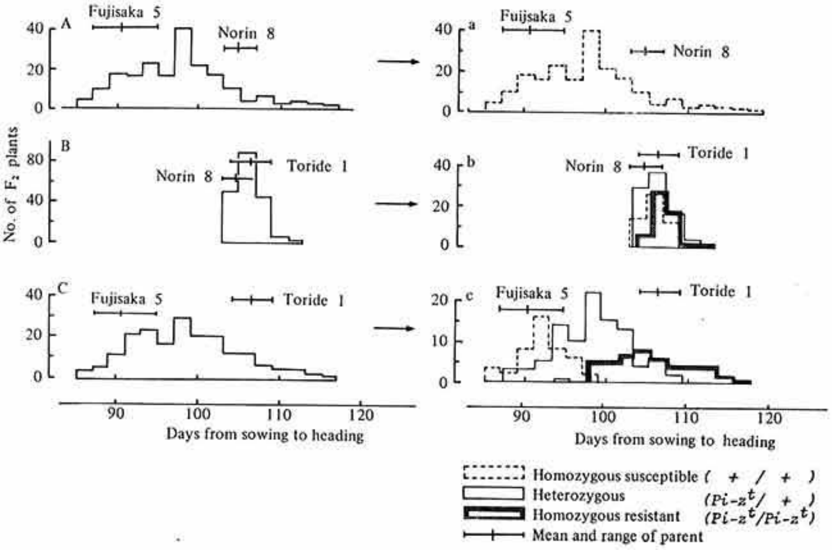

Fig. 3. Frequency distribution for heading date and resistance to the Ken 53–33 strain of blast fungus in the F_2 populations of the cross between Fujisaka 5, Norin 8 and Toride 1.

Table 3. Mean and variance of heading time (days to heading) of each genotype for the $Pi\text{-}z^t$ gene in the F_2 populations of the crosses between Fujisaka 5, Norin 8 and Toride 1

Cross	Overall		(+/+)		($Pi\text{-}z^t$/+)		($Pi\text{-}z^t/Pi\text{-}z^t$)		F†
	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	
Toride 1 × Norin 8	106.6	2.047	106.6	2.162	106.4	1.932	107.0	1.961	3.01
Toride 1 × Fujisaka 5	99.1	37.566	92.9	7.584	98.8	19.989	105.7	23.696	103.28**
Fujisaka 5 × Norin 8	98.0	44.730	—	—	—	—	—	—	—
Fujisaka 5	90.9	2.252							
Norin 8	105.3	0.403							
Toride 1	107.0	1.867							

†: $F=\frac{\text{Between-groups variance}}{\text{Within-group variance}}$

** Significant at 1% level.

The variation of heading time in the F_2 population of the cross Toride 1 × Fujisaka 5 showed a unimodal, continuous distribution like the Fujisaka 5 × Norin 8 cross (Fig. 3-C). The F_3 line from each F_2 plant was tested for resistance to Ken 53–33 (Table 2) and the F_2 genotype for the $Pi\text{-}z^t$ gene was estimated. Homozygous susceptible (+/+) plants were early, heterozygous ($Pi\text{-}z^t$/+) plants were medium, and homozygous resistant ($Pi\text{-}z^t/Pi\text{-}z^t$) were late (Fig. 3-c). The heading time of the three genotypes for $Pi\text{-}z^t$ was significantly different (Table 3). Since $Pi\text{-}z^t$ is linked to *Lm*, the differences in heading time were resulted mainly from the segregation of the alleles of the *Lm* locus.

In view of the experiments described above, it was proved that the same alleles of *Lm* mainly govern the variation of heading time in the cross Fujisaka 5 × Norin 8. The genotypes of Fujisaka 5 and Norin 8 were denoted by Lm^e/Lm^e and Lm^n/Lm^n, respectively.

Geographical cline with respect to the *Lm* locus

Using the above-mentioned method, the genetical analysis for heading time of the early- and late-maturing Japanese representative cultivars was made. Koshihikari and Shiranui were taken as examples. Koshihikari is a leading cultivar that has been grown widely in the temperate region since the release in 1956 due to its excellent eating quality and earliness. A late-maturing cultivar Shiranui was released in 1964 for the growing in southern Japan.

Two lines, an early-maturing "ER" and a late-maturing "LR", were used as testers. ER and LR were developed from a cross Morak Sepilai × Fujisaka 5 by four-time backcrosses of Fujisaka 5 as a recurrent parent, and had the $Pi\text{-}z^t$ gene. The F_2 population of the cross LR × ER formed a trimodal distribution for heading time, and the early-, the medium- and the late-maturing groups had the same heading time as ER, F_1's and LR, respectively (Fig. 4-A). The fit to a 1:2:1 ratio suggests that the early and the late alleles of the *Lm* locus resulted in the 15-day difference of heading time between ER and LR. Since ER and Fujisaka 5 similarly responded to different daylengths (Fig. 5), they are supposed to have a common allele of the *Lm* locus. Lm^e/Lm^e and Lm^u/Lm^u were given to the genotypes of ER and LR, respectively. Fig. 4 shows frequency distributions for heading time in the F_2 populations of the crosses between Koshihikari and Shiranui, and ER and LR, that were partitioned by the three genotypes for $Pi\text{-}z^t$.

The variation of heading in the F_2 of Koshihikari × ER was small and there is no significant difference among the three genotypes for $Pi\text{-}z^t$ (Fig. 4-b), indicating that Koshihikari carries the Lm^e allele. On the other hand, the continuous distribution of heading time in the cross Koshihikari × LR was divided into three groups by the $Pi\text{-}z^t$ genotypes. Homozygous resistant ($Pi\text{-}z^t/Pi\text{-}z^t$) plants were largely late, heterozygous ($Pi\text{-}z^t$/+) plants were largely

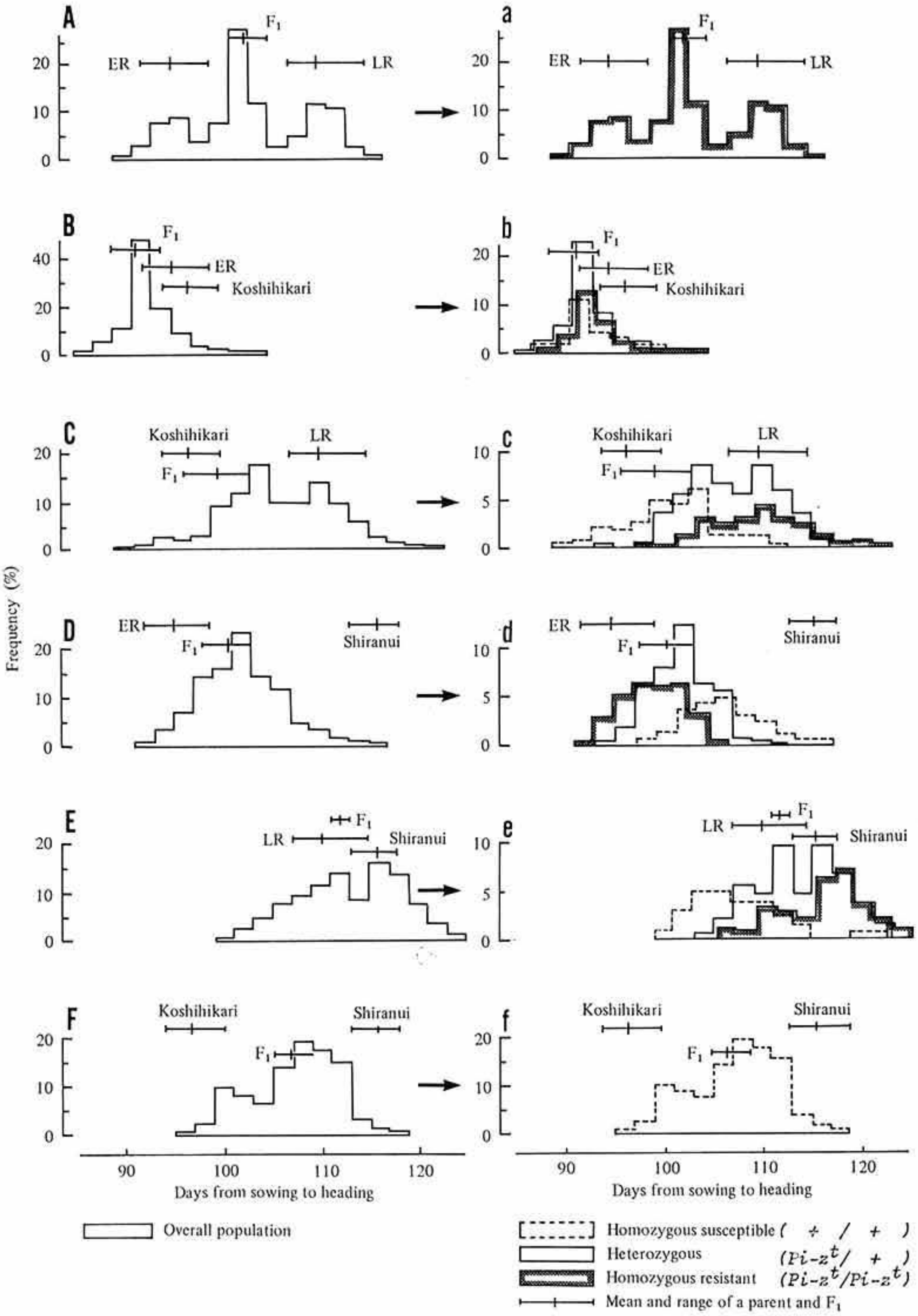

Fig. 4. Frequency distribution for heading date and resistance to the blast strain Ken 53-33 in the F_2 populations.

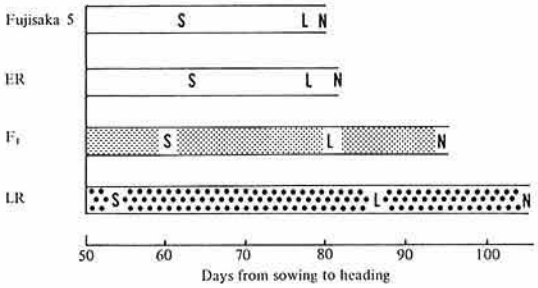

Fig. 5. Heading dates of Fujisaka 5, ER, LR and their F_1(ER × LR) plants under different daylengths. S, L, and N denote the heading date under the 9 hour-, 14 hour-, and natural daylength, respectively.

medium, and homozygous susceptible (+/+) ones were exclusively early (Fig. 4-c). It is clear that a gene pair, Lm^{e} and Lm^{u}, governs most of the variation in this cross.

In the crosses ER × Shiranui and Shiranui × LR also, the three genotypes for $Pi\text{–}z^{t}$ showed the significantly different heading time. In the cross Shiranui × LR, homozygous resistant plants were late and homozygous susceptible plants were early (Fig. 4-e), although the resistant parent LR headed earlier than the susceptible parent Shiranui. This contrary tendency reveals that Shiranui is homozygous for the Lm^{s} allele controlling earlier heading than Lm^{u}, but has the lateness gene of a locus other than *Lm*, resulting in late heading of Shiranui. These two gene pairs could be independently combined in the F_2 population, but the *Lm* locus mainly determines the variation.

The F_2 population of the cross Koshihikari × Shiranui showed a bimodal frequency distribution of heading (Fig. 4-F). The segregation in a 1 early : 3 late ratio, when separated at the 103th day, indicated the contribution of the Lm^{e} and Lm^{s} alleles to the variation as might be expected, based on the assumption that Koshihikari has such late allele as Shiranui with respect to the locus different from *Lm*.

As for other Japanese cultivars the remarkable differences in heading time between the genotypes for $Pi\text{–}z^{t}$ in the F_2 populations were also observed in the cases of the early-maturing cultivars with LR and of the late-maturing cultivars with ER, indicating that the early cultivars have early alleles and the late cultivars have late alleles of the *Lm* locus.

The genes for heading time linked with apiculus color have been detected widely not only in japonica but also in indica rice cultivars. Chandraratna[1] reported that the gene *Se* for photoperiod sensitivity was linked with the apiculus color gene *ap* with the recombination value of 17.3% in several indica crosses, and Kudo[3] also found that the linkage between the photoperiod sensitivity gene *Rs* and the apiculus color gene *C* with the recombination value of 21.0% in a indica-japonica cross. The value 14.2% between *C* and *Lm* in the present study was similar to these two values. Supposedly *Lm* would be identical with or allelic to *Se* and *Rs*. Taking these facts into consideration, it is considered that the *Lm* locus is one of the important loci controlling heading time of cultivated rice and it has differentiated multiple alleles to make rice plants adapt to various daylengths ranging widely from south to north latitude.

Oka[5] found latitudinal clines on the photoperiodic responses among the cultivars from various Asian countries, and thought that genes controlling photoperiodic responses are always under a high pressure of selection. The differentiation of the *Lm* alleles might have played an important role in the ecological distribution of rice cultivars.

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