Location of New Gene for Late Heading in Rice, *Oryza sativa* L. Using Interchange Homozygotes

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A near isogenic line T65-LH1 was developed from an upland rice variety R300 (Thailand) through eight successive backcrossing with Thaichung 65 (T65) as a recurrent parent. Previously unpublished study indicated T65-LH1 may harbor a recessive lateness gene which was likely located on the sixth chromosome. The present study was aimed at further identifying the lateness gene of this newly bred line by linkage study and allelism test. Linkage study was conducted using seven kinds of interchange homozygotes involved with the sixth chromosome. Individual interchange homozygous lines were crossed with T65-LH1, then, each F₁ population was backcrossed to T65-LH1. Segregations for heading times in all B₁F₁ populations showed early and late types fitted to the 1 : 1 expected ratio, suggesting that T65-LH1 carried a recessive lateness gene. Chi-square values for independence between the present gene and four breakpoints, 2-6, 6-7b, 6-7c and 6-10 were insignificant. However, those to three breakpoints, 4-6, 6-7 and 6-8 were significant at 1% level and the respective recombination values were estimated as 3.2%, 4.3% and 3.4%. This suggested the gene under study was located on the sixth chromosome. Subsequently, allelism test between the present gene and other three lateness genes, ef₂(t), ef₃(t) and ef₄(t) was carried out. Crosses of T65-LH1 and three testers having each of those lateness genes were made. Segregations for heading times in F₂ populations of those crosses fitted to the 9 : 3 : 3 : 1, 12 : 3 : 1 and 9 : 3 : 3 : 1 expected ratios. It suggested the present gene was independent from the lateness genes, ef₂(t), ef₃(t) and ef₄(t). In conclusion, the lateness gene harbored by T65-LH1 was a new gene and designated as ef₅. It was carried by the sixth chromosome.

Key Words: *Oryza sativa* L., heading time, lateness gene, interchange homozygote, linkage analysis, allelism test.

Introduction

Heading time of rice is an important agronomic trait which is responsible for seasonal and regional adaptability of cultivars (Chang et al. 1969, Sato and Hayashi 1985). Growing period from sowing to heading consists of the vegetative growth phase and reproductive phase. The duration of the later is almost constant across cultivars. Thus, heading time is chiefly determined by the vegetative growth phase which is subdivided into the basic vegetative phase (BVP) and photoperiod sensitive phase (PSP) (Chang et al. 1969).

More than 40 genes have been identified governing heading. However, most of them controlled the PSP (Chandraratna 1953, Yokoo and Fujimaki 1971, Poonyarit et al. 1989, Okumoto et al. 1991). For those governing the BVP period, as many as seven genes were reported. Among them, two genes were responsible for earliness and other five for lateness. An earliness gene Eᶠ₁, located on the tenth chromosome, accelerated heading time by about 15 days (Tsai and Oka 1966, Sato et al. 1988). Another earliness gene designated as Efx, located on the third chromosome and advanced flowering time by about 7 days (Sato et al. 1992, Sumi et al. 1998). Two out of five lateness genes, ef₁ and ef₁-h were the alleles of Eᶠ₁ (Tsai 1993, Nishida et al. 2002). While the other three genes, ef₂(t), ef₃(t) and ef₄(t) were known to be independent from each other and from Eᶠ₁ locus (Tsai 1985, 1986, 1991). Each of these three genes delayed heading by about 20 days, 11 days and 13 days, respectively.

In the tropics, hastened heading time brought about shortening of vegetative growth period of rice, eventually resulting in remarkable grain yield reduction, while delayed heading allowed it to have sufficient vegetative growth to produce higher grain yield and or biomass production (Kawano and Tanaka 1968, Akita 1989). Therefore, genes for late heading may play the key role for such concept and breeding of varieties carrying such genes would be important in rice varietal improvement program.

A late heading variety R300 originated from the upland ecosystem in Thailand was used as the initial female parent and crossed with the recurrent male parent Taichung 65 (T65), a well-known Japonica variety from Taiwan, to introduce the lateness gene(s) to T65. By 8 times of successive backcrossing the near isogenic line, T65-LH1, headed later than T65 about 20 days, was obtained. From the linkage...
study by using thirty-two interchange homozygotes it was found that T65-LH1 may carry a recessive lateness gene which was likely located on the sixth chromosome (data unpublished).

In the present study, further identification of the lateness gene of T65-LH1 by using seven kinds of interchange homozygotes involved with the sixth chromosome and allelism test with three lateness genes, \( ef_2 \), \( ef_3 \) and \( ef_4 \) were carried out.

**Materials and Methods**

**Linkage analysis**

Seven interchange homozygotes with genetic background of Taichung 65 (T65) (Table 3) carrying the interchange breakpoints of the sixth chromosome were used. Individual interchange homozygous lines (RT-lines) were crossed with T65-LH1. Each F\(_1\) population was backcrossed to T65-LH1 (RT-lines/T65-LH1//T65-LH1). The B1F\(_1\) populations, T65-LH1 and T65 were sown on the 3rd, February 1998. Seedlings at about 4 to 5-leaf age were transplanted, one seedling per hill, in the natural field with a 15 cm spacing. Fertilizers of 1.2 kg/a each of N, P\(_2\)O\(_5\) and K\(_2\)O were applied at a 5:3:2 ratio for the basal, the first (tillering stage) and second top-dressings (flowering time), respectively.

Heading times of main culms were examined. Segregations for chromosome types were determined by spikelet fertility, interchange heterozygotes were semi-sterile while interchange homozygotes and the normal plants were fertile. Recombination values between gene and interchange breakpoints were calculated by the product method (Joachim 1947).

**Allelism test**

Three testers, T65-ef2(t), T65-ef3(t) and T65-LH3 with genetic background of T65 were employed (Table 1). They carried each of the lateness genes, \( ef_2 \), \( ef_3 \) and \( ef_4 \). T65-LH1 was crossed with individual testers, then, \( F_1 \) plants were raised to obtain \( F_2 \) seeds. All \( F_2 \) populations, parental lines and T65 were sown on the 1st, August 2001. Seedlings at about 4 to 5-leaf age were transplanted, one seedling per hill, in the glasshouse with a 15 x 20 cm spacing. Fertilizers of 1.2 kg/a each of N, P\(_2\)O\(_5\) and K\(_2\)O were applied at a 5:3 ratio for the basal and the first top-dressing (tillering stage), respectively, while other 2:10 or 20% of that amount was reduced as to avoid lodging.

Heading times of main culms were examined. The segregation ratio for heading times in each \( F_2 \) population was determined basing on the valley of heading distribution and the ranges of heading time of parental lines. Generally, the \( F_2 \) plants segregated into T65 type, parental line types and very late type. The first group was the recombinant phenotypes distributed within the ranges of individual parental lines, and the third group was the recombinant phenotypes headed later than T65 and parental lines. When frequency distributions for heading times in \( F_2 \) plants were not clear enough to classify segregants into particular groups the progeny test was carried out. Both experiments were conducted at the experimental fields of the Faculty of Agriculture, University of the Ryukyu, Okinawa.

**Results**

**Linkage analysis**

Heading time of T65-LH1 is shown in Table 2. The growing period from sowing to heading of T65-LH1, under natural field condition, was about 137 days which was longer than that of Taichung 65 (T65) by more than 3 weeks. Results of gene and linkage analyses in B1F\(_1\) populations of the crosses RT-lines/T65-LH1//T65-LH1 are shown in Table 3. The heading times in all B1F\(_1\) populations segregated into early (T65) and late (T65-LH1) types fitted to the 1:1 expected ratios. This indicated T65-LH1 carried a recessive lateness gene. Chi-square values for independence between the present gene and four breakpoints, 2-6, 6-7b, 6-7c and 6-10 were insignificant. However, those to three breakpoints, 4-6, 6-7 and 6-8 were significant at 1% level with recombination values of 3.2%, 4.3% and 3.4%, respectively. Thus, the lateness gene of T65-LH1 was carried by the sixth chromosome.

**Allelism test**

Heading times of T65-LH1, testers and T65 grown under glasshouse field conditions are shown in Table 2. Generally, growing periods from sowing to heading of all lines in the first cropping season were longer than in the second cropping season. Heading times of T65-LH1 were about 119 and 106 days which were later than that of T65 by around 2 and 3 weeks in the first and second cropping seasons, respectively.

Segregation for heading times in \( F_2 \) of the crosses

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**Table 1. Near isogenic lines of Taichung 65 (T65) and donor of their lateness genes**

<table>
<thead>
<tr>
<th>Line</th>
<th>Gene</th>
<th>Backcrossed generationsup1</th>
<th>Donor of lateness gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>T65-ef2(t)</td>
<td>ef2(t)</td>
<td>7</td>
<td>T65 (X-rayed) (Tsai 1985, 1991)</td>
</tr>
<tr>
<td>T65-ef3(t)</td>
<td>ef3(t)</td>
<td>9</td>
<td>Inakupa (Philippines) (Tsai 1986, 1991)</td>
</tr>
<tr>
<td>T65-LH1</td>
<td>ef5</td>
<td>8</td>
<td>R300 (Thailand)</td>
</tr>
<tr>
<td>T65-LH3</td>
<td>ef4(t)</td>
<td>6</td>
<td>IR68 (IRRI) (Tsai 1991, Khun and Sato 2002)</td>
</tr>
</tbody>
</table>

sup1 Backcrossed to T65
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Table 2. Comparison of growing period from sowing to heading between Taichung 65 (T65) and its near isogenic lines

<table>
<thead>
<tr>
<th>Line</th>
<th>Growing period from sowing to heading</th>
<th>First cropping(^1)</th>
<th>First cropping(^2)</th>
<th>Second cropping(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T65</td>
<td></td>
<td>114.8</td>
<td>105.3</td>
<td>84.3</td>
</tr>
<tr>
<td>T65-ef2(t)</td>
<td></td>
<td>–</td>
<td>–</td>
<td>123.5** (39.2)</td>
</tr>
<tr>
<td>T65-ef3(t)</td>
<td></td>
<td>–</td>
<td>110.0* (4.7)</td>
<td>91.1** (6.8)</td>
</tr>
<tr>
<td>T65-LH1</td>
<td>137.8** (23.3)</td>
<td>119.2** (13.9)</td>
<td>106.0** (21.7)</td>
<td></td>
</tr>
<tr>
<td>T65-LH3</td>
<td></td>
<td>113.0** (7.7)</td>
<td>93.2** (8.9)</td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are the differences in days to heading from T65.
* and ** Significantly different at 5% and 1% level, respectively.

between T65-LH1 and the three testers is shown in Figure 1 and results of gene analysis in Table 4. When T65-LH1 was crossed with T65-ef2(t), the average number of days to heading of F\(_1\) plants was about 88.9 days which was close to that of T65 (about 84.3 days). Such plants were called as T65 type here (and so on). While F\(_2\) plants exhibited tetramodal distribution: T65 type, T65-LH1 type, T65-ef2(t) type and very late type (Fig. 1A). The average number of days to heading for each type was estimated as 86.4, 104.4, 125.5 and 135.5 days. The F\(_2\) segregation pattern fitted to a 9 : 3 : 3 : 1 expected ratio. It suggested the present gene was independent from gene \(ef2\)(t). In the cross between T65-LH1 and T65-ef3(t), F\(_2\) plants exhibited continuously trimodal distribution: T65 and T65-ef3(t) type, T65-LH1 type and very late type (Fig. 1B). The average number of days to heading for each type was 89.4, 104.4 and 111.6 days. The segregation pattern fitted well to a 12 : 3 : 1 expected ratio. Thus, the present gene was different from gene \(ef3\)(t). In addition, when both genes were combined, \(ef3\)(t) showed a dominant epistasis. Segregation for heading time in the cross between T65-LH1 and T65-LH3 is shown in Figure 1C. The average number of days to heading of F\(_1\) plants was around 88 days showing T65 type. F\(_2\) plants exhibited continuously tetramodal distribution: T65 type, T65-LH3 type, T65-LH1 type and very late type.

Fig. 1. Segregation for heading times in F\(_2\) populations of the crosses T65-LH1 and testers. Crossed lines are the ranges and means of heading times, arrows indicate the days on which individuals were grouped into separate classes, shaded area indicates the number of plants grouped after pedigree test.
Table 3. Genetic analysis for heading times in B1F1 populations of the crosses RT-lines/T65-LH1/T65-LH1

<table>
<thead>
<tr>
<th>Interchange homozygote</th>
<th>Break point</th>
<th>Number of plants</th>
<th>χ² value for heading time (1:1)</th>
<th>χ² value for independence (d.f. = 1)</th>
<th>Recombination value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT2-6-T65</td>
<td>2-6</td>
<td>96</td>
<td>118</td>
<td>103</td>
<td>87</td>
</tr>
<tr>
<td>RT4-6-T65</td>
<td>4-6</td>
<td>6</td>
<td>142</td>
<td>127</td>
<td>3</td>
</tr>
<tr>
<td>RT6-7-T65</td>
<td>6-7</td>
<td>8</td>
<td>223</td>
<td>198</td>
<td>11</td>
</tr>
<tr>
<td>RT6-7b-T65</td>
<td>6-7b</td>
<td>146</td>
<td>114</td>
<td>115</td>
<td>103</td>
</tr>
<tr>
<td>RT6-7c-T65</td>
<td>6-7c</td>
<td>68</td>
<td>88</td>
<td>70</td>
<td>77</td>
</tr>
<tr>
<td>RT6-8-T65</td>
<td>6-8</td>
<td>8</td>
<td>245</td>
<td>244</td>
<td>9</td>
</tr>
<tr>
<td>RT6-10-T65</td>
<td>6-10</td>
<td>85</td>
<td>83</td>
<td>88</td>
<td>71</td>
</tr>
</tbody>
</table>

****: Significant at 1% level.
F and S: Fertile and Semi-sterile, respectively.

Table 4. Genetic analysis for heading times in F2 populations from crosses between T65-LH1 and the three testers

<table>
<thead>
<tr>
<th>Cross</th>
<th>Number of plants</th>
<th>Total</th>
<th>Ratio</th>
<th>χ² value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T65</td>
<td>T65-ef2(t)</td>
<td>T65-LH3</td>
<td>T65-LH1</td>
</tr>
<tr>
<td>T65-LH1/T65-ef2(t)</td>
<td>180 (165.3)</td>
<td>52 (55.1)</td>
<td>40 (55.1)</td>
<td>22 (18.3)</td>
</tr>
<tr>
<td>T65-ef3(t)/T65-LH1</td>
<td>216 (219)</td>
<td>59 (54.7)</td>
<td>17 (18.2)</td>
<td></td>
</tr>
<tr>
<td>T65-LH3/T65-LH1</td>
<td>182 (171.5)</td>
<td>62 (57.1)</td>
<td>52 (57.1)</td>
<td>9 (19.0)</td>
</tr>
</tbody>
</table>

Values in parentheses are the expected number of plants estimated on segregation.

**1)** Recombinant phenotypes distributed within the range of T65 with average number of days to heading of 86.4 days.

**2)** Phenotypes distributed within the ranges of parental lines, T65-ef3(t), T65-LH3, T65-LH1 and T65-ef2(t) and the respective average number of days to heading for the later three types were estimated as 93.9, 104.4 and 125.5 days.

**3)** Recombinant phenotypes headed later than T65 and parental lines, and the average number of days to heading were estimated as 135.5, 111.6 and 115.1 days in F2 of crosses T65-LH1/T65-ef2(t), T65-ef3(t)/T65-LH1 and T65-LH3/T65-LH1, respectively.

**4)** Number of plants distributed in a combined range for T65 and T65-ef3(t) plants with average number of days to heading of 89.4 days.

and very late type. The average number of days to heading for individual types was 86.4, 93.9, 104.4 and 115.1 days. The segregation fitted to a 9:3:3:1 expected ratio. Consequently, the present gene was located at different locus from that of ef4(t).

**Discussion**

Growing period from sowing to heading of T65-LH1 which was significantly longer than that of Taichung 65 (T65) varied largely between natural and glasshouse fields, and between the first and second cropping seasons. Such differences were also observed in T65 and testers, T65-ef2(t), T65-ef3(t) and T65-ef4(t). This indicated that heading time of rice fluctuated in association with environmental conditions of crop cultivation. The natural daylength at Nishihara, Okinawa varied from 13 hours 50 minutes to 11 hours 50 minutes and it was found that such daylength did not affect the number of days to heading of T65 and other lines carrying genes governing the basic vegetative phase (BVP) but did the temperature of cultivated field (Sato 1987). Thus, such condition in Okinawa showed nearly optimal for the measurement of cultivars’ BVP.

Delay of heading time in T65-LH1 was suggested to be controlled by a recessive gene. Linkage analysis suggested that the present gene was located on the sixth chromosome since it was linked closely to three breakpoints on this chromosome. Five genes for lateness regulated the BVP, ef1 (Tsai 1993), ef1-h (Nishida et al. 2002), ef2(t) (Tsai 1985, 1991), ef3(t) (Tsai 1986, 1991) and ef4(t) (Tsai 1991). Out of them, ef1 and ef1-h were carried by the tenth chromosome. Thus, the present gene differed from the two lateness alleles. While other three genes, ef2(t), ef3(t) and ef4(t) were known to differ from each other but their crucial chromosomal locations remained unidentified. We found that the present gene was independent from the three lateness genes. The earliness gene Ef1 detected by Tsai and Oka (1966) was identified to locate on the tenth chromosome (Sato et al. 1988) and Ef2x was existed on the third chromosome (Sato et al. 1992, Sumi et al. 1998). Based on our results, the present gene was independent from the two earliness genes. Among genes regulated photoperiod sensitive phase, on the other hand, only Sel on the sixth chromosome was known to affect on the BVP (Chandraratna 1953, Yokoo and Fujimaki 1971, Yokoo and...
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