Trisomic Analysis of a Lateness Gene ef2 in Rice, *Oryza sativa* L.

Leang Hak Khun¹, Keiji Motomura*², Seiichi Murayama¹, Shinichi Adaniya¹ and Akihiro Nose³

¹) Faculty of Agriculture, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan
²) Faculty of Agriculture, Saga University, 1 Honjo, Saga 840-8502, Japan

The lateness gene ef2(t), formerly designated as If-I, was induced by X-ray irradiation applied to a rice variety Taichung 65. The present study was performed to investigate the allelic relationship between ef2(t) and two earliness genes, Ef3 and Ef1, and the chromosomal location through trisomic analysis. Rice line T65-ef2(t) carrying gene ef2(t) was crossed with two tester lines, T65-ER-21 and T65-ER-1 harboring the earliness genes, Ef3 and Ef1, respectively and heading time in the F₂ plants was examined. F₂ plants of the cross T65-ef2(t)/T65-ER-21 showed a trimodal distribution: T65-ER-21 and T65 type, new type and T65-ef2(t) type. The F₂ segregation fitted to a 12:3:1 expected ratio. On the other hand, the F₂ plants of the cross T65-ef2(t)/T65-ER-1 exhibited a tetramodal distribution: T65-ER-1 type, T65 type, new type and T65-ef2(t) type, the segregation fitting to a 9:3:3:1 expected ratio. These results suggested that ef2(t) was non-allelic to both Ef3 and Ef1. Trisomic analysis was performed using seven Triplo lines carrying extra chromosomes 2, 4, 5, 7, 9, 10 and 12, respectively. The trisomics were used as maternal parent for crossing with F₁ plants (trisomics) was examined. F₂ population derived from a cross between T65-ef2(t) and Triplo 9 showed a typical trisomic segregation pattern, suggesting that ef2(t) was located on chromosome 9.

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

---

**Introduction**

Growth period from sowing to heading of rice includes the vegetative growth phase and the reproductive phase. The latter does not vary appreciably among rice varieties, while the former which is subdivided into the basic vegetative phase (BVP) and photoperiod-sensitive phase (PSP) is largely affected by genetic factors and environmental conditions (Chang et al. 1969, Hosoi 1981, Sato and Takahashi 1983).

In the tropics, accelerated heading time led to the shortening of the vegetative growth of rice, eventually resulting in remarkable grain yield reduction, while delayed heading allowed the crop to have sufficient vegetative growth to produce higher grain yield and/or biomass (Kawano and Tanaka 1968, Akita 1989). Therefore, it is very important to breed rice varieties carrying genes for late heading.

To date as many as eight genes controlling the BVP period have been reported. Of these, two dominant genes, Ef1 on chromosome 10 (Tsai and Oka 1966, Sato et al. 1988) and Ef3 on chromosome 3 (Sato et al. 1992, Sumi et al. 1998) were responsible for earliness, and other six recessive genes, ef1 (Tsai 1986, 1993), ef1-h (Nishida et al. 2002), ef2(t) (Tsai 1985, 1991), ef3(t) (Tsai 1986, 1991), ef4(t) (Tsai 1991) and ef5 on chromosome 6 (Khun and Sato 2002) for lateness. The lateness gene ef2(t) formerly designated as If-I was independent of the earliness gene Ef1. The Ef1 locus was known to carry two lateness alleles, ef1 and ef1-h, showing that ef2(t) was independent of both alleles. Moreover, ef2(t) was found to differ from other three lateness genes, ef3(t), ef4(t) and ef5. However, its allelic relationship with Ef3 and chromosomal location remained to be elucidated.

In the present study, we performed an allelism test of ef2(t) with Ef3 and with Ef1. Trisomic analysis was also conducted to reveal the chromosomal location of ef2(t).

**Materials and Methods**

**Plant materials**

The near isogenic line T65-ef2(t) was induced by X-ray irradiation applied to a rice variety Taichung 65 (T65) and found to harbor a lateness gene ef2(t) which delayed flowering time of rice by about three weeks. Many late heading lines were found to carry lateness alleles at this locus (Tsai 1985, 1991).

**Allelism test**

Two tester lines, T65-ER-21 and T65-ER-1 with the genetic background of T65 (carrying a lateness allele ef1) were used (Table 1). Each of them harbored the earliness genes, Ef3 (Sato et al. 1992, Sumi et al. 1998) and Ef1 (Tsai and Oka 1966, Sato et al. 1988). T65-ef2(t) was crossed with individual testers and F₁ plants from the two crosses were grown to obtain F₂ seeds. Both F₂ populations, parental lines and T65 were sown on August 2, 2002. Seedlings at about...
the 4 to 5-leaf age were transplanted on August 19, 2002 in a field located in the glasshouse, one seedling per hill at a 15 × 20 cm spacing. Fertilizers consisting of 1 kg/a each of N, P₂O₅ and K₂O were applied at the ratio of 5 : 3 : 2 for the basal, the first topdressing (tillering stage) and second topdressing (flowering time), respectively.

Heading time of the first emerged panicle was recorded. The segregation ratio of F₂ populations was determined based on the frequency distribution for heading time and the range (of heading time) of the parental lines. From F₂ plants of the cross T65-ef2(t)/T65-ER-1 (Fig. 1B), 14 individual plants distributed between the T65 type and the new type were subjected to a progeny test to identify the phenotypes belonging to the respective groups.

**Trisomic analysis**

Seven primary trisomics, namely Triplo 2, Triplo 4, Triplo 5, Triplo 7, Triplo 9, Triplo 10 and Triplo 12 (Iwata and Omura 1984, Iwata 1990) were employed. All the triplo lines were the isogenic lines of T65 and the notations corresponding to individual Triplo lines indicated the extra chromosome numbers, for example, Triplo 9 indicated the extra chromosome 9. Based on the morphological traits peculiar to each trisomic (Iwata et al. 1970, Khush et al. 1984), it was possible to distinguish the trisomics from the disomics so that the cytological analysis for the extra chromosome was not required. The trisomics were used as maternal parent for crossing with T65-ef2(t) and F₁ plants (trisomic plants) were grown to obtain F₂ seeds. The F₂ populations of the crosses Triplo lines/T65-ef2(t), parental lines and T65 were sown on July 30, 2003. Seedlings at about the 4 to 5 leaf age were transplanted on August 21, 2003 in a field located in a glasshouse. The number of seedlings per hill, spacing between hills and fertilizer application were similar to those described in the above experiment. In each F₂ population as well as Triplo line, the trisomics were transplanted separately from the disomics in order to avoid shading and/or competitive effects, as the trisomics were generally weaker and shorter than the disomics.

Heading time of the first emerged panicle was recorded and the goodness of fit of the observed segregation ratios to the expected ones was examined by the chi-square test. When the ef2(t) locus was located on the disomic chromosome, the expected segregation ratio in F₂ plants was 3 early (T65 type) : 1 late (T65-ef2(t) type). On the other hand, when ef2(t) was located on the trisomic chromosome and the transmission rate of x+1 gamete was 33.3%, for the disomics, the ratio of 8 early (T65 type) : 1 late (T65-ef2(t) type) was expected. As for the trisomics, two kinds of segregation ratios were expected; 44 early (trisomic type) : 1 late (T65-ef2(t) type) by chromatid segregation (Haldane 1930) and 35 : 1 by maximum equational segregation (Burnham 1962).

Both experiments, the allelism test and trisomic analysis, were conducted at the Faculty of Agriculture, University of the Ryukyus, Okinawa.

---

**Fig. 1.** Segregation for heading time in F₂ populations of the crosses T65-ef2(t) and testers. Crossed lines (——) denote the ranges and means of heading time, arrows indicate the days on which individuals were grouped into separate classes.

**Table 1.** List of research materials used and average number of days to heading

<table>
<thead>
<tr>
<th>Line</th>
<th>Genotype</th>
<th>Number of days to heading</th>
<th>Number of backcrossing</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>T65</td>
<td>ef1ef1Ef2Ef2efx</td>
<td>78.6</td>
<td></td>
<td>Taichung 65 (Ryukyus)</td>
</tr>
<tr>
<td>T65-ER-1</td>
<td>Ef1ef1Ef2Ef2efx</td>
<td>66.2</td>
<td>10</td>
<td>T1-10: A58 (Kokusyokuto-2); 208 (Japan)</td>
</tr>
<tr>
<td>T65-ER-21</td>
<td>ef1ef1Ef2Ef2Ef</td>
<td>74.5</td>
<td>8</td>
<td>Kokusyokuto-2 (Japan)</td>
</tr>
<tr>
<td>T65-ef2(t)</td>
<td>ef1ef1ef2ef2efx</td>
<td>114.2</td>
<td>7</td>
<td>T65 (irradiated)</td>
</tr>
</tbody>
</table>

1) Examined in 2nd cropping season in 2002.
2) Backcrossed to T65.
Results

Allelism test

Frequency distribution for heading time in the F2 plants of the crosses between T65-ef2(t) and the two testers, T65-ER-21 and T65-ER-1 is shown in Figure 1 and the results of the gene analysis are presented in Table 2. F2 plants obtained from the cross between T65-ef2(t) and T65-ER-1 harboring gene Efx showed a trimodal distribution: T65-ER-21 (Efx—Ef2—) and T65 (efxefEfxEf2—) type, new (Efx—ef2ef2) type and T65-ef2(t) (efxefxe2ef2) type (Fig. 1A). The average number of days to heading for each group was estimated at 76.9 days, 95.5 days and 113.1 days and the F2 segregation fitted to a 12:3:1 expected ratio (Table 2), suggesting that ef2(t) was independent of Efx. On the other hand, when T65-ef2(t) was crossed with T65-ER-1 carrying gene Ef1, the F2 plants exhibited a tetramodal distribution: T65-ER-1 (Ef1—Ef2—) type, T65 (ef1ef1Ef2—) type, new (Ef1—ef2ef2) type and T65-ef2(t) (ef1ef1ef2ef2) type (Fig. 1B). The average number of days to heading for the respective types was 67.0 days, 77.8 days, 86.5 days and 113.1 days and the F2 segregation fitted to a 9:3:3:1 expected ratio. Consequently, ef2(t) was independent of Ef1.

Trisomic analysis

Results of trisomic analysis for heading time in the F2 plants of the crosses between the Triplo lines and T65-ef2(t) are shown in Table 3. Each F2 population segregated into an early (Triplo line or T65) type and a late (T65-ef2(t)) type. In the F2 populations of six crosses: Triplo 2/T65-ef2(t), Triplo 4/T65-ef2(t), Triplo 5/T65-ef2(t), Triplo 7/T65-ef2(t), Triplo 10/T65-ef2(t) and Triplo 12/T65-ef2(t), the segregation ratio of the early and late classes in both disomic and trisomic parts fitted to the 3:1 expected ratio, respectively. However, in the F2 plants of the cross between Triplo 9 and T65-ef2(t), either disomics and trisomics did not fit to the 3:1 ratio, whereas the disomics fitted to a 8:1 ratio and the trisomics fitted to a 35:1 or 44:1 ratio. These results clearly suggested that the lateness gene ef2(t) was located on chromosome 9.

Table 2. Segregation for heading time in F2 populations of the crosses with T65-ef2(t)

<table>
<thead>
<tr>
<th>Cross</th>
<th>Number of plants</th>
<th>T65-ER-1 type</th>
<th>T65-ER-21 type</th>
<th>T65 type</th>
<th>New type</th>
<th>T65-ef2(t) type</th>
<th>Total</th>
<th>( \chi^2 ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T65-ef2(t)/T65-ER-21</td>
<td>228</td>
<td>40</td>
<td>16</td>
<td>284</td>
<td>4.526</td>
<td>(12:3:1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T65-ef2(t)/T65-ER-1</td>
<td>173</td>
<td>45</td>
<td>18</td>
<td>287</td>
<td>2.419</td>
<td>(9:3:3:1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figures in parentheses represent the expected ratios.

**1)** Parental phenotypes distributed within the ranges of T65-ER-21, T65-ER-1 and T65-ef2(t) and the respective average number of days to heading for the latter two types was estimated at 67.0 and 113.1 days, while that of the T65-ER-21 type could not be obtained.

**2)** Recombinant phenotypes distributed within the range of T65 with an average number of days to heading of 77.8 days.

**3)** Recombinant phenotypes headed later than T65-ER-1, T65-ER-21 and T65, and earlier than T65-ef2(t) and the average number of days to heading was estimated at 86.5 days and 95.5 days in the F2 of the crosses T65-ef2(t)/T65-ER-21 and T65-ef2(t)/T65-ER-1, respectively.

**4)** Number of plants distributed in a combined range for T65-ER-21 and T65 plants with an average number of days to heading of 76.9 days.

Table 3. Segregation for heading time caused by ef2(t) locus in F2 populations of the crosses between Triplo lines and T65-ef2(t)

<table>
<thead>
<tr>
<th>Cross</th>
<th>Type of F2 plant</th>
<th>Number of plants</th>
<th>( \chi^2 ) value for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Late</td>
<td>3:1</td>
</tr>
<tr>
<td>Triplo 2/T65-ef2(t)</td>
<td>Disomic</td>
<td>124</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Trisomic</td>
<td>83</td>
<td>37</td>
</tr>
<tr>
<td>Triplo 4/T65-ef2(t)</td>
<td>Disomic</td>
<td>136</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Trisomic</td>
<td>85</td>
<td>40</td>
</tr>
<tr>
<td>Triplo 5/T65-ef2(t)</td>
<td>Disomic</td>
<td>166</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Trisomic</td>
<td>61</td>
<td>24</td>
</tr>
<tr>
<td>Triplo 7/T65-ef2(t)</td>
<td>Disomic</td>
<td>131</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Trisomic</td>
<td>72</td>
<td>35</td>
</tr>
<tr>
<td>Triplo 9/T65-ef2(t)</td>
<td>Disomic</td>
<td>163</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Trisomic</td>
<td>106</td>
<td>1</td>
</tr>
<tr>
<td>Triplo 10/T65-ef2(t)</td>
<td>Disomic</td>
<td>163</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Trisomic</td>
<td>61</td>
<td>17</td>
</tr>
<tr>
<td>Triplo 12/T65-ef2(t)</td>
<td>Disomic</td>
<td>143</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Trisomic</td>
<td>81</td>
<td>22</td>
</tr>
</tbody>
</table>

** Significant at 1% level.
Discussion

The lateness gene ef2(t) detected by Tsai (1985, 1991) was subjected to further genetic analysis, such as allelism test and trisomic analysis.

Through the allelism test, we revealed that ef2(t) was independent of the earliness genes Ef1, a dominant gene on chromosome 3 (Sato et al. 1992, Sumi et al. 1998), and Ef1, an early locus on chromosome 10 (Tsai and Oka 1966, Sato et al. 1988). Tsai (1985) also pointed out that ef2(t) was different from Ef1. Thus, ef2(t) may not be located on chromosome 3 or 10. Evidently, through trisomic analysis, we found that ef2(t) was carried by chromosome 9 because the gene segregated into a trisomic manner in the F2 population of Triplo 9. Since the lateness genes, ef1 and ef1-h were allelic to Ef1 on chromosome 10 (Tsai 1993, Nishida et al. 2002) and ef3 was located on chromosome 6 (Khun and Sato 2002), based on our findings, ef2(t) was independent of those genes. In addition, according to Tsai (1986, 1991), ef2(t) was also independent of ef3(t) and ef4(t). On the other hand, since among the photoperiod-sensitive genes identified—for example, Se1 on chromosome 6 (Chandraratna 1953, Yoktoo and Fujimaki 1971), Ef1 on chromosome 7 (Yamagata 1984, Okamoto and Tanisaka 1997) and Hd6 on chromosome 3 (Yamamoto et al. 2000)—none was located on chromosome 9, it appears that ef2(t) was independent of those genes. Therefore, it was suggested that the designation of the present gene should be ef2.

The recombinant phenotypes observed in the cross T65-ef2(t)/T65-ER-21 were T65 (efxexf/ef2f2) and new (EfxfexfEf2f2). Since the number of days to heading of the former (76.9 days) was smaller than that of the latter (95.5 days) it appeared that Ef2 exerted a more pronounced effect on the acceleration of the flowering time than Efxf. The recombinant phenotypes observed in the cross T65-ef2(t)/T65-ER-1 were T65 (ef1ef1Ef2f2) and new (Ef1—efef2f2). As the number of days to heading of the first group (77.8 days) was smaller than that of the second one (86.5 days) it appeared that Ef2 exerted an even more pronounced effect on the advancement of flowering time compared to Ef1. On the other hand, due to the importance of plants having the Efxf—efef2f2 and Ef1—efef2f2 genotypes that could be utilized as testers in genetic analysis for the heading time trait in the near future, we selected and raised those phenotypes to obtain stable lines.

In rice, many trisomic analyses have been performed (Iwata et al. 1990, Hoshii et al. 1981, and Khush 1994, 1999), while for the heading time trait, only few studies have been reported (Okamoto and Tanisaka 1997, Nishida et al. 2002). In our case, the successful elucidation of the chromosomal location of ef2 was mainly due to the use of the F2 populations from the crosses between Triplo lines with a similar genetic background to that of T65 and T65-ef2(t) in which the phenotypic characteristic of heading time could be easily observed.

The availability of DNA markers and the genetic linkage map of rice provide a more direct and rapid technique for the determination of the chromosomal location of genes of interest. In the future, linkage analysis by using DNA markers and/or conventional marker genes should be performed in order to map ef2 on chromosome 9.

Acknowledgements

We thank Dr. Kuo-Hai Tsai, Department of Agronomy, National Chung Hsing University, Taiwan for providing the seeds of T65-ef2(t) used in the present study. We would like to express our condolences over the loss of Dr. Shigetoshi Sato, former professor of the Lab of Plant Breeding, Faculty of Agriculture, University of the Ryukyus.

Literature Cited


Sanchez, A.C. and G.S. Khush (1994) Chromosomal location of some marker genes in rice using the primary trisomics. J. Hered. 85:


