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Inhibiting Gene for a Late-Heading Gene Responsible for Photoperiod Sensitivity in Rice (*Oryza sativa*)

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***Abstract*—**Two *indica* varieties, IR36 and 'Suweon 258' ("S") are middle-heading in southern Japan. 36U, also middle-heading, is an isogenic line of IR36 carrying *Ur1* (Undulate rachis-1) gene. However, late-heading plants segregated in the F_2 population from the F_1 of S × 36U, and so did in the following generations. The concerning lateness gene is designated as *Ex*. From the F_8 generation, isogenic-line pair of early-heading and late-heading lines, denoted by "E" (ex/ex) and "L" (Ex/Ex), were developed. Genetic analyses of heading time were conducted, using F_1s and F_2s among L, E, S and 36U. The following inferences were drawn from the experimental results: 1) L, and both of E and 36U harbor *Ex* and *ex*, respectively; 2) Besides *Ex*, S harbors an inhibitor gene to it, i.e. *I-Ex* which is a novel finding of the present study. 3) *Ex* is a dominant allele at the *E1* locus.

***Keywords*—**Basic vegetative phase, heading time, lateness gene, photoperiod-sensitive phase.

I. Introduction

HEADING time is an important agronomic trait which is related with regional and seasonal adaptability in rice [16], [21]. The growing period from sowing to heading consists of the vegetative growth phase and reproductive phase. The duration of the reproductive phase is almost constant across varieties. Thus, heading is chiefly determined by the vegetative growth phase which is subdivided into the basic vegetative phase and photoperiod-sensitive phase [16].

More than 40 genes concerning heading time have been reported. However, most of them control photoperiod-sensitive phase [7], [8], [12], [19]. Photoperiod-sensitive phase is partially determined by the critical daylength (the longest daylength at which floral initiation takes place). Varieties with a shorter critical daylength initiate panicle formation later in summer season in Japan.

In rice, there are two important loci concerning photoperiod sensitivity, viz. *Se1* and *E1* [9]-[11], [18]. Regarding the *Se1* locus on chromosome 6, a photoperiod insensitivity allele *Se1-e* is widely distributed among varieties grown in the northeast region of Japan, while a photoperiod-sensitive allele *Se1-n* is harbored by middle and late heading varieties in the southwest region [11]. A higher photoperiod-sensitive allele *Se1-u* compared with *Se1-n* is carried in an *indica* variety 'Morak Sepilai' [10], [11]. *Se1-u* delays heading by about 30 days compared with *Se1-e*. Regarding the *E1* locus on chromosome 7 [18], a photoperiod-sensitive allele *E1* delays heading by 19 to 26 days compared with its non-photoperiod-sensitive allele *e1* [5].

Suweon 258 (hereafter "S") is a Tongil-type short-culm variety developed in Korea [1]. IR36 is a typical improved variety possessing rather early maturity in subtropics. These *indica*-type varieties can be regarded as middle-heading in southern Japan. However, late-heading plants segregated in the F_2 population from the F_1 of S × 36U. 36U is an isogenic line of IR36 carrying *Ur1* (Undulate rachis-1 gene). In F_3 and later generations, both late-heading and middle-heading plants appeared in a progeny population from a plant having heading time intermediate between late and middle. According to Trung et al. [15], an incompletely dominant gene for lateness caused the variation of late and early heading in the F_8 generation of the same cross. From the F_8 generation, an isogenic-line pair of late and early lines were developed, which were denoted by "L" and "E", respectively (Murai unpublished). Accordingly, either S or 36U should harbor the lateness gene, although either of S and 36U is middle-heading. Genetic analyses were conducted, by using F_1s and F_2s among L, E, S and 36U. On the basis of the obtained data, it is tried to verify that S harbors not only the lateness gene but also an inhibitor gene for this gene. Additionally, it was examined whether the lateness gene is an allele at the *Se1* locus or not by the use of F_2 of L × a late-heading line carrying a highly photoperiod-sensitive *Se1* allele.

II. Materials and Methods

A. Isogenic Lines

The above-mentioned lateness gene is designated as *Ex* in the present study. The maternal parent S is one of Tongil-type varieties which had been developed by the cooperation between Korea and IRRI (International Rice Research Institute). IR36 is a semi-dwarf variety developed at IRRI, which had been broadly grown in South East Asia in 1980s [14]. The paternal parent 36U is the isogenic line of IR36 carrying *Ur1* (Undulate rachis-1) gene, which was developed

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after 15 backcrosses by IR36 for the initial F_1 of IR36 × N-55 (*Ur1*-carrying line). The heading-time characteristic of 36U is almost the same as that of IR 36. In each of the F_2 to F_7 generations originating from the F_1 of S × 36U (2001 to 2006), plants having heading times intermediate between late and middle (the *Ex/ex* genotype) were selected for raising the next generation. In 2007, 37 F_8 plants from a F_7 plant were grown, and one of the latest plants and one of the earliest ones were selected to obtain an isogenic-line pair ("L" and "E"). L headed 20.8 days later than E in a paddy field condition in 2012 (see Table I).

B. Days to Heading under Short-Photoperiod Condition

Sterilized Seeds of L, E, S and 36U were soaked in water at 27ºC for 2 days. Cylindrical pots with an inside diameter of 15.6 cm were used. A commercial soil for rice seedling containing chemical fertilizers was taken into each pot by 250 ml with a rate of N 0.12, P_2O_5 0.19, K_2O and MgO 0.03 g/pot. A slow-release coated fertilizer ECOLONG® 180 type (Chisso Asahi Fertilizer Co., Ltd.), about 3% of each element of which was readily available, was applied on the bottom of each pot at a rate of 0.54 g/pot for each of N, P_2O_5 and K_2O, to retain fertilizer effect after panicle differentiation. Six seeds were sown circularly just inside of each pot. This dense-planting cultivation was adopted to produce many main-culm panicles from limited number of pots. The growth was conducted in an indoor-type growth chamber, illuminated by both fluorescent lamps and electric bulbs at about 600μmol PAR m^{-2} s^{-1} from 9:00 a.m. to 7:00 p.m. (10 hours), which was set at 23ºC through day and night. All of the tillers except the main shoots were removed 54 days after sowing. Heading date was recorded when panicle emerged from its flag leaf sheath.

C. F_1 and F_2 Populations

Crosses were made between parental lines (S × 36U), between parental lines and isogenic-line pair (S × E, S × L, 36U × E and 36U × L), and between isogenic lines (E × L). All of the six F_1s and five F_2 populations except that of E × L were grown in a paddy field in 2012.

D. Field Experiment

Sterilized seeds of S, 36U, L, E, the six F_1s and the five F_2s were sown in plastic trays on 30th April 2012, and were grown in a natural-light growth chamber being set at 25ºC and 21ºC, respectively, for the first 5 days and thereafter. Seedlings raised were transplanted on May 12, with one seedling per hill, in an experimental field of Faculty of Agriculture, Kochi University (Nankoku (33º35'N), Kochi) at a spacing of 45 × 15cm. An ordinary chemical fertilizer was applied as basal application at the rate 2.0 g/m^2 for each of N, P_2O_5 and K_2O. ECOLONG® 180 type was applied at a rate of 6.0 g/m^2 for each of N, P_2O_5 and K_2O by top-dressing on 7th July. First heading date of each plant was recorded: the panicle of main stem emerged from its flag leaf sheath earliest within a plant in most of the cases.

E. Allelism Test of Ex to $Se1^{pat}$

An isogenic line of *japonica* variety Taichung 65 carrying both $Se1^{pat}$ and *wx*, viz. T65LF3 was used in the present study, which was developed using the wx isogenic line of Taichung 65 ("T65wx") as recurrent parent and an *indica* variety 'Patpaku' as donor parent, after seven backcrosses [6], [17]. We presume that $Se1^{pat}$ is identical with *Se1-u*, because their effects on photoperiod sensitivity are similar to each other. L was crossed with T65LF3. The F_2 population of the cross was grown together with the parents and F_1. First heading date of each plant was recorded as mentioned above.

Seventeen plants out of a total of 161 plants in F_2 did not head before 18th October. The 17 plants were shoveled up with roots and soil into 1/1000 pots and were brought to a natural-light type growth chamber set at 24ºC through day and night, and the observation and record were continued until their heading.

III. Results and Discussion

A. Days to Heading from Sowing under Short-Photoperiod Condition

Table I shows days to heading of parental varieties and isogenic-line pair under the 10-hour photoperiod condition and under natural field condition in 2012. Under the field condition, L is 20.8 days later than E. Under the short photoperiod condition, on the contrary, L is 2.0 days earlier than E. These results indicate that *Ex* in L has responsibility for photoperiod sensitivity.

TABLE I
NUMBER OF DAYS TO HEADING FROM SOWING IN THE PARENTAL AND ISOGENIC LINES UNDER THE FIELD CONDITION AND GROWTH CHAMBER CONDITION OF 10-HOUR PHOTOPERIOD

Variety/Line	Field	Growth Chamber
S	93.8	105.1
36U	96.0	88.4
E	96.2	89.9
L	117.0	87.9
T65wx	99.0	94.6
T65LF3	133.7	65.2

B. Segregation of Days to Heading from Sowing in Five F_2 Populations among S, 36U, L and E

In the F_2 of 36U × L (Fig. 1), rather late to late plants (106 to 121 days from sowing), and middle heading plants (93 to 97 days) were separately segregated as 117: 39. This ratio fits to the ratio of 3: 1 ($\chi^2 = 0.00$, P = 1.00). The former and the latter groups of segregants should carry *Ex-* and *ex/ex* genotypes, respectively. In the F_2 of 36U × E (Fig. 2), only middle heading plants (92 to 99 days from sowing) appeared almost within the range of the parents.

Therefore, 36U harbors *ex/ex* genotype like E. In the F_2 of S × L (Fig. 3), middle heading plants (89 to 107 days from sowing), and rather late to late plants (110 to 131 days) were segregated as 114: 42. This ratio fits to the ratio of 3: 1 ($\chi^2 = 0.31$, P = 0.58). The former and the latter groups of segregants

should carry *Ex/Ex I-Ex-* and *Ex/Ex i-Ex/i-Ex* genotypes, respectively, by supposing that "*I-Ex*" is a dominant inhibitor gene to *Ex*. This segregation implies that S carries both *Ex* and *I-Ex*, and L carries *Ex* only. Besides, their F_1 can be regarded as middle heading, supporting that *I-Ex* is dominant to *i-Ex* (see Table II). In the F_2 of S × 36U (Fig. 4), rather late to late plants (108 to 131 days from sowing) and early to middle heading plants (83 to 107 days) were segregated as 51: 261. This ratio fits to the ratio of 3: 13 (χ^2 = 1.18, P = 0.28). However, segregants earlier than S and those later than L suggest genetic difference of minor gene(s) concerning heading time between S and 36U.

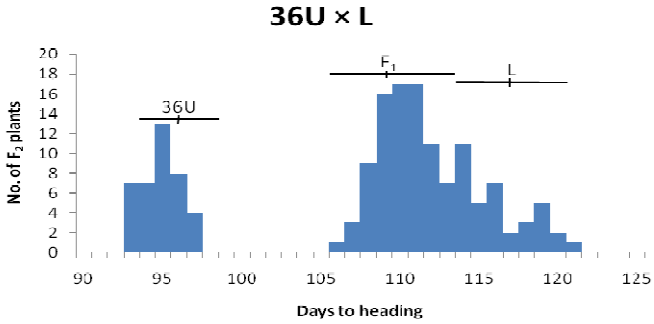

Fig. 1 Frequency distribution of days to heading in F_2 population of 36U × L

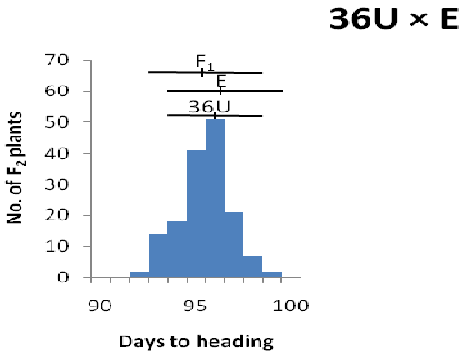

Fig. 2 Frequency distribution of days to heading in F_2 population of 36U × E

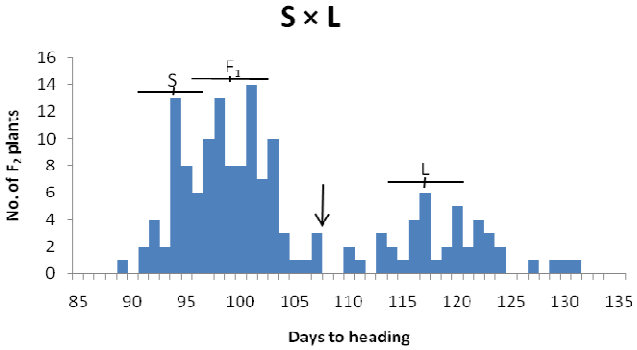

Fig. 3 Frequency distribution of days to heading in F_2 population of S × L

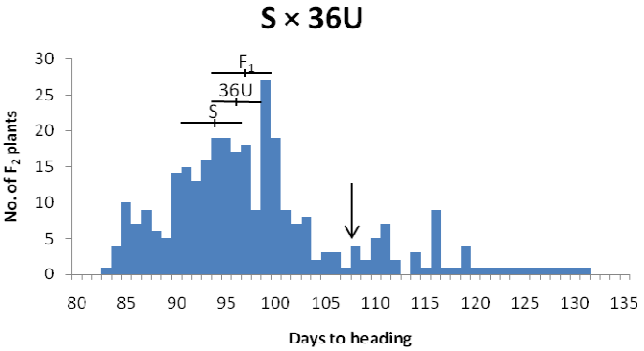

Fig. 4 Frequency distribution of days to heading in F_2 population of S × 36U

TABLE II
NUMBER OF DAYS TO HEADING FROM SOWING IN SEVEN F_1S

Cross	Days
36U X L	109.2
36U X E	95.4
S X L	99.0
S X 36U	96.9
S X E	98.5
E X L	111.8
L X T65LF3	150.2

Similarly, in the F_2 of S × E (Fig. 5), rather late to late plants (109 to 133 days from sowing) and early to middle heading plants (84 to 107 days) were segregated as 62: 248. This ratio also fits to the ratio of 3:13 (χ^2 = 0.32, P = 0.57). The 3:13 ratio of segregation in S × 36U and S × E may be caused by that S harbors both *Ex* and *I-Ex* while either of 36U and E harbors *ex* and *i-Ex* (see Table III); so that the ratio of 3 and that of 13 may involve the *Ex- i-Ex/i-Ex* genotype (3), and the *Ex- I-Ex-*, *ex/ex I-Ex-* and *ex/ex i-Ex/i-Ex* genotypes (9 + 3 +1), respectively.

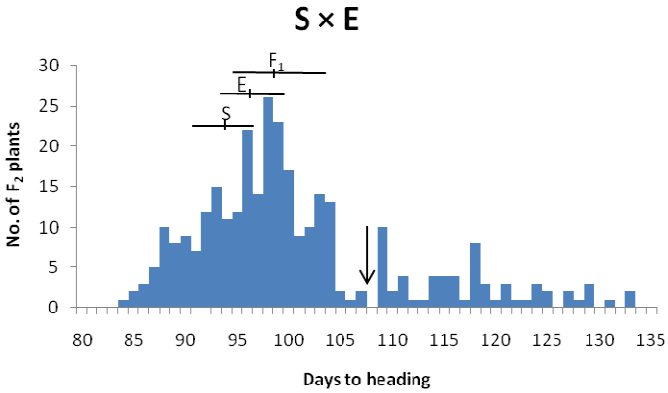

Fig. 5 Frequency distribution of days to heading in F_2 population of S×E

TABLE III
ESTIMATED GENOTYPE OF S, 36U, E AND L

Line/Variety	Phenotype	Genotype
S	middle-heading	*Ex/Ex I-Ex/I-Ex*
36U	middle-heading	*ex/ex i-Ex/i-Ex*
E	middle-heading	*ex/ex i-Ex/i-Ex*
L	late-heading	*Ex/Ex i-Ex/i-Ex*

C. Segregation of Days to Heading from Sowing in the F_2 of L × T65LF3

In the F_2 of L × T65LF3 (Fig. 6), a wide range from 90 to 204 days from sowing, comprising middle and extremely late heading times, was observed. Many plants were distributed around the mode of 157 days from sowing. Introgressive distribution over the mean heading date of T65LF3 (Fig. 6) occupied 108 out of the whole 161 F_2 plants. On the other hand, plants earlier than 100 days from sowing segregated in this F_2. Accordingly, the variation in the F_2 may be caused by two or more genes originating from different parents. T65LF3 harbors Se^{pat}. Taichung 65 carries *E1* [2]; hence, T65wx and T65LF3 should carry *E1* also. According to Ichitani et al. [5], an *indica* variety 'Kasalath' harbors *E1-k* which is an allele with higher effect of delaying heading than *E1*. Supposing that *Ex* is *E1-k* or another allele with similar effect and L harbors a recessive allele *se1* at the *Se1* locus, most of plants with the *Ex-*$Se1^{pat-}$ genotype may have headed later than plants with the *Ex- se1/se1* and *E1/E1* $Se1^{pat-}$ genotypes. Number of plants earlier than 100 days from sowing inclusive were 16, which is more than the expected number of plants with the *E1/E1 se1/se1* genotype, viz. 10.1 (= 161 × 1/16). These 16 segregants were included into the continuous distribution from 90 to 116 days from sowing. Plants distributing around L (the *Ex/Ex se1/se1 genotype*) seems to be too few. According to Tsai and Oka [4] and Sato et al. [13], Taichung 65 harbors *efl*, i. e. a lateness allele at the locus responsible for basic vegetative phase on chromosome 10. Assuming that L carries an allele for earliness *Efx* (not so high effect as that of *Efl*), segregation of this dominant allele may have affected to produce early plants more frequent; e. g., plants with the *Efx/Efx Ex/E1 se1/se1* genotype may have been earlier than 100 days from sowing. This assumption is supported by the segregation of earlier plants more than expected in the F_2; however, it could be substantiated by a dominant earliness allele at another locus by the place of *Efx*. To sum up, it is inferred that *Ex* is an allele with a higher effect of delaying heading than *E1* allele, and accumulation of effects of *Ex-* and $Se1^{pat-}$ was one of causes of the introgressive distribution.

Sano [20] demonstrated that an incompletely dominant gene *En-Se1* enhances the effect of $Se1^{pat}$ resulting in extremely late heading. Assuming that *En-Se1* or a gene with similar enhancing effect is carried by L (tentatively designated as "*Enhancer-Se1*"), the *Enhancer-Se1/Enhancer-Se1* $Se1^{pat}/Se1^{pat}$ *Ex/Ex* genotype should segregate in the rate of 1/64 (= $1/4^3$). As shown in Fig. 6, the latest four plants (201 to 204 days from sowing, viz. 17th to 20th of November) and the other plants (90 to 187 days from sowing) were segregated as 4:157. This ratio fits to the ratio of 1:63 (χ^2 = 0.89, P = 0.35). The F_1 headed 150.2 days from sowing in average (see Table II) and its genotype could be estimated as *Enhancer-Se1/enhancer-Se1* $Se1^{pat}$*/se1 Ex/E1*. It is inferred that most of the plants, which headed from 150 to 187 days from sowing, were heterozygous or homozygous-dominant at all of the *Enhancer-Se1*, *Se1*and *Ex* loci. Consequently, the enhancer gene to $Se1^{pat}$ may be a principal cause of the introgressive distribution and it may bring about extremely late segregants with the *Enhancer-Se1/Enhancer-Se1* $Se1^{pat}$/ $Se1^{pat}$ *Ex/Ex* genotype.

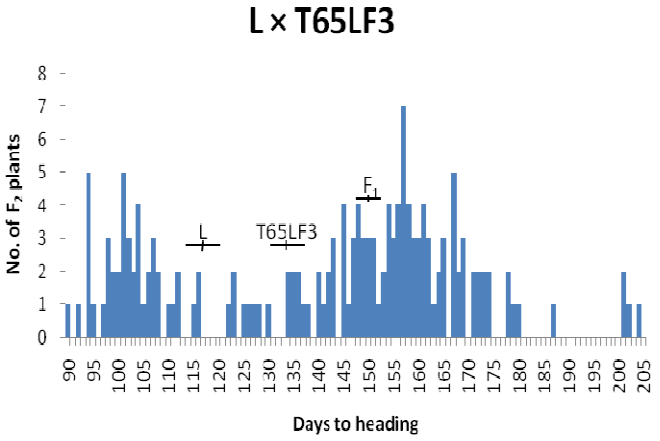

Fig. 6 Frequency distribution of days to heading in F_2 population of L × T65LF3

D. Inhibitor to E1 or Its Allele

An inhibitor gene to *Se1-u* was reported by Ohshima *et al.* [3]. However, there has been no previous report regarding an inhibitor to *E1* or its allele, as far as we know. The inhibitor to a dominant allele at the *E1* locus i.e. *I-Ex* is a novel finding of the present study.

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