Lateness gene Concerning Photosensitivity Increases Yield, by Applying Low to High Levels of Fertilization, in Rice, a Preliminary Report

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ABSTRACT

Various genes controlling heading time have been reported in rice. An isogenic-line pair of late and early lines “L” and “E” were developed from progenies of the F₁ of Suweon 258 × an isogenic line of IR36 carrying U rl gene. The lateness gene for photosensitivity that causes the difference between L and E was tentatively designated as “Ex(t)”, although it’s chromosomal location is unknown. The present study was conducted to examine the effects of Ex(t) on yield and related traits in a paddy field in two years. Chemical fertilizers containing N, P₂O₅, and K₂O were applied at the nitrogen levels of 4.00, 9.00 and 18.00 g/m² in total, being denoted by “N4”, “N9” and “N18”, respectively, in 2014. L was later in 80%–heading by 18 or 19 days than E. Regarding total brown rice yield (g/m²), L and E were 635 and 577, 606 and 548, and 590 and 501, respectively, at N18, N9 and N4, indicating that Ex(t) increased this trait by 10 to 18%. Ex(t) increased yield of brown rice with thickness above 1.5mm (g/m²), by 9 to 15%. Ex(t) increased spikelet number per panicle by 16 to 22% and spikelet number per m² by 11 to 18%. Thousand-grain weight (g) was 2 to 4% lower in L than in E. L was not significantly different from E in ripened-grain percentage. Hence, Ex(t) increased yield by increasing spikelet number per panicle. It is suggested that Ex(t) could be utilized to develop high yielding varieties for warmer districts of the temperate zone.

Keywords: Heading time, lateness gene, photosensitivity, yield, yield components. rice, breeding

सारांश

धानबाली बाला निस्कने समय नियन्त्रण गर्न विभिन्न जीवनहरू पता लगाउनको छ। सुवे० २५८ र आठ आर ३६ को उरल आइसोजेनिक लाइनको क्रोसिंग क्रमान्वयन एक जोडी दिनो बाला निकालने र छिडी बाला निर्माण आइसोजेनिक लाइन “एल” र “ई” विकसित गरिएको थियो। यीदृढ आइसोजेनिक लाइनको बीचको बाला निर्माण अवधि एउटा पक्षा संस्कृति विज्ञान जीवन Ex(t) भने नियन्त्रण गर्न अनुमान गरिएको, यद्यपि यो जीवनको क्षेत्रमा स्थायी अनुमति छ। यो अध्ययनमा विस्मय जीवन Ex(t) बालाबीको उपादान तथा समयको समन्निधित्व विशेषता पार्वतीय अनुपात असरको र स्नेहमा विशेषता गर्न बालाबालीको स्थायी अनुपात र समानांतर संचारण गरिएको थियो। उरल जात्रा, कोनौट, श्री चमकिला र साविक राजादेव महासहू ४००, ९०० र १८०० ग्राम प्रतिटिम नाइडीडिजन स्तरको धान पोषण गर्न खास बाला: “पन ५”, “पन ९” र “पन १८” ले जनाइएको थियो। एउटा बाली त्वन धानबाली उपादान (ग्राम प्रतिटिम) ६.३५ र ७.४०, ७.४२ र ६.४१, र ७.४० र ६.७५ त्यस बालाले भएको थियो। उरल जीवन Ex(t) बाली त्वन धानबाली उपादान १० देखि १८% बुद्धिभएको जनाइएको थियो। Ex(t) बाला १.५ हिमतिम भएको बहुत बालाको थिन धानबालीको उपादान (ग्राम प्रतिटिम) ९ देखि १५% बुद्धिभएको थियो। Ex(t) बाला प्रतिक्रिया स्थापना तथा प्रतिदिन नाइडीडिजन संख्या र प्रतिटिम धानबाली उपादान बाला बोधकाण्ड अनुपात र २% देखि २% कम गरिएको थियो। तर परिपक्व धानबाली बालाबादी प्रति दिन धानबाली उपादान Ex(t) बाला अस्त्र साहित्यकी रुपमा फरक पाइएको थिए।
INTRODUCTION

Growth duration is a major factor affecting regional adaptability and yield in rice (*Oryza sativa* L., 2n=2x=24). Longer growth duration allows rice plants to have sufficient vegetative growth to obtain higher biomass (Kawano and Tanaka 1968, Wada and Cruz 1989). In rice, growth duration is primarily determined by heading time. The duration from sowing to heading consists of the vegetative phase and panicle development phase. Vegetative phase is defined by the duration from sowing to the appearance of panicle primordium. Vegetative phase is further divided into basic vegetative phase and photoperiod sensitive phase (Chang et al 1969). Basic vegetative phase can be estimated by the minimum number of days from sowing to appearance of panicle primordium under the condition of short day-length and optimum temperature. Since the duration from appearance of panicle primordium to heading is about 30 days in rice varieties ordinarily (Akimoto and Togari 1939, Terao et al 1942, Saito et al 2007); length of vegetative phase can be approximated by number of days to heading.

In rice, more than 40 genes controlling heading time have been reported. Most of them control photosensitivity (Khun et al 2005, Ebana et al 2011). The major photosensitivity loci viz. *Sel* and *El*, located on chromosome 6 and chromosome 7, respectively, play important roles in determining heading time (Yokoo and Kikuchi 1977, Yokoo et al 1980, Yokoo and Kikuchi 1982; Okumoto et al 1991). The *Sel* locus involves at least three alleles, *Sel-u*, *Sel-n* and *Sel-e*: the first two are incompletely dominant alleles controlling photosensitivity, while *Sel-e* is a recessive allele with non-photosensitivity (Yokoo and Kikuchi 1977). *Sel-e* is distributed in rice varieties grown in Hokkaido prefecture and Tohoku district of Japan (Yokoo et al 1980, Ichitani et al 1997), while *Sel-n* is harbored in middle and late heading varieties grown in warmer regions of Japan (Yokoo et al 1980). *Sel-u* is harbored in an *indica* variety ‘Morak Sepilai’ (Yokoo and Fujimaki 1971). *Sel-u* delays heading by at least 20 days as compared with its photo-insensitive allele *Sel-e*.

Murai developed an isogenic-line pair of late and early lines, denoted by “L” and “E”, respectively, from descendants of the F1 from Suweon 258 × an isogenic line of IR36 carrying *Ur1* gene. L was later in heading by 15 to 21 days than E (Trieu et al 2010). The difference in heading time between the two lines is controlled by a lateness gene (allele) tentatively designated as “*Ex(t)*”. *Ex(t)* is an incompletely dominant allele controlling photosensitivity. Although the locus of *Ex(t)* is unknown, genetical analyses involving that by DNA markers suggest that it is different from either the *Sel* locus or the *El* locus (Murai unpublished).

*Ghd7* allele at the *El* locus delayed heading and had a pleiotropic effect of increasing spikelet number per panicle of main culm (Xue et al 2008, Saito et al 2011). However, any yield tests on field level regarding the effect of a lateness gene at *Sel*, *El* and other loci have not been performed, as far as we know. A field experiment was conducted for L and E in the present study. Besides yield, yield components and other traits such as culm length and sink size were measured. On the basis of the data obtained, the effects of *Ex(t)* on yielding ability was examined.

MATERIALS AND METHODS

Pair of isogenic lines

The isogenic-line pair of L and E was used in the present study. This isogenic-line pair was developed from descendents of the F1 from Suweon 258 × 36U. The maternal parent Suweon 258 is an *indica*-type semi-dwarf variety which had been developed by the cooperation between Korea and International Rice Research Institute (IRRI) 1980. The paternal line 36U was developed after 15 backcrosses by IR36 for the F1 of IR36 × N-55 (*Ur1*-carrying line) (Murai unpublished). IR 36 is a typical improved *indica* variety developed at International Rice Research Institute (Khush and Virk 2005). IR36 is a semi-dwarf variety carrying *sd1-d* which had been broadly grown in Southeast Asia during 1980s (Imbe 2002). The
heading-time characteristic of 36U is almost same as that of IR36. IR36 is an early-maturing variety in the tropics and subtropics (Khush and Virk 2005); nevertheless, it and Suweon 258 can be regarded as middle-heading in Kochi prefecture, Japan (Trieu et al 2010).

Figure 1 shows the developing process of a pair of early and late isogenic-lines, E and L. Suweon 258 was crossed with 36U. Comparatively early-heading, late-heading and intermediate-heading plants were segregated in the F2 population from the F1. Early-heading, late-heading and intermediate-heading plants appeared in the F3 population from an intermediate-heading plant of F2. Similarly, in F4 and later generations, plants with the three classes of heading appeared in a progeny population from an intermediate-heading plant of the previous generation. In the F8 population from an intermediate-heading F7 plant, a late-heading plant and an early-heading plant were selected for developing L and E, respectively (Trieu et al 2010). Any genic segregation regarding heading time, culm length and other traits within L as well as E were not observed from F9 generation and thereafter. From the results of genetic analyses by using L, E, Suweon 258 and 36U, and descendents of mutual crosses among them, it is suggested that Ex(t) is an incompletely dominant allele controlling photosensitivity over its recessive allele ex, and the former and latter alleles are harbored in L and E, respectively (Murai unpublished). 36U has ex/ex genotype (Trieu et al 2010). Suweon 258 harbors not only Ex(t) but also an inhibitor gene for it, resulting in the behavior of the middle heading in Kochi prefecture.

Cultivation and fertilization in the experimental field
Seeds were sterilized with a 1000-time solution of Benomyl\textsuperscript{\textregistered} (benzimidazol) in 2011. In 2014, seeds were soaked with hot water of 62 to 55°C for 15 minutes for sterilization, particularly countermeasure for bacterial panicle blight and/or grain rot. The seeds were sown on plastic trays filled with granulated soil containing N, P\textsubscript{2}O\textsubscript{5} and K\textsubscript{2}O and being adjusted at pH 4.5, on 2nd May in 2011 and 21st April in
2014. Seedlings were grown at 25°C for the first five days and 21°C for the subsequent seven days in a natural-light type growth chamber in both years. Twelve-day old seedlings were transplanted at a spacing of 30cm × 15cm (22.2 hills/m²) with two seedlings per hill to an experimental field of the Faculty of Agriculture (present name: Faculty of Agriculture and Marine Science), Kochi University (Nankoku 33°33′N). Yield test was performed with three replications for the two lines in each of the two years. Each plot comprised 29 hills × 5 rows (145 hills).

| Year | Fertilizer level | Basal or top-dressing | Chemical fertilizers applied | N (g/m²) | P₂O₅ (g/m²) | K₂O (g/m²) |
|------|------------------|-----------------------|-----------------------------|----------|-------------|-------------|
| 2011 | N17              | Basal                 | Ordinary chemical fertilizer | 5.00     | 5.00        | 5.00        |
|      |                   | Top-dressing          | ECOLONG® 413-140 type       | 12.00    | 12.00       | 12.00       |
|      |                   | Total                 |                             | 17.00    | 17.00       | 17.00       |
| 2014 | N18              | Basal                 | Ordinary chemical fertilizer | 1.33     | 1.33        | 1.33        |
|      |                   | Additional basal      | ECOLONG® 424-100 type       | 4.67     | 4.67        | 4.67        |
|      |                   | Top-dressing          | ECOLONG® 413-180 type       | 12.00    | 9.43        | 11.14       |
|      |                   | Total                 |                             | 18.00    | 15.43       | 17.14       |
|      | N9                | Basal                 | Ordinary chemical fertilizer | 1.33     | 1.33        | 1.33        |
|      |                   | Additional basal      | ECOLONG® 424-100 type       | 1.67     | 1.67        | 1.67        |
|      |                   | Top-dressing          | ECOLONG® 413-180 type       | 6.00     | 4.71        | 5.57        |
|      |                   | Total                 |                             | 9.00     | 7.71        | 8.57        |
|      | N4                | Basal                 | Ordinary chemical fertilizer | 1.33     | 1.33        | 1.33        |
|      |                   | Top-dressing          | ECOLONG® 413-180 type       | 2.67     | 2.10        | 2.48        |
|      |                   | Total                 |                             | 4.00     | 3.43        | 3.81        |

ECOLONG® 424-100 type, ECOLONG® 424-140 type and ECOLONG® 413-180 type: see text.

In 2011, just before ploughing, an ordinary chemical fertilizer, manufactured by Central Chemical Co., Ltd., Tokyo, Japan, containing N, P₂O₅ and K₂O was applied as basal dressing at the rate of 5.00 g/m² for each of N, P₂O₅ and K₂O (Table 1). Top-dressing was performed 54 days or 59 days before 80%-heading for L and E with a slow release and coated fertilizer ECOLONG® 424-140 type (about 5% of each nutrient element is readily available) containing N, P₂O₅ and K₂O at the rate of 14, 14 and 14%, respectively, manufactured by JCAM AGRI Co., Ltd. at the rate of 12.00 g/m² of each of the three nutrient elements. Chemical fertilizers containing N, P₂O₅ and K₂O were applied by both basal and top dressings at the nitrogen levels of 4.00, 9.00 and 18.00 g/m² in total in 2014, which were denoted by "N4", "N9" and "N18", respectively. Just before ploughing, the ordinary chemical fertilizer containing N, P₂O₅ and K₂O was applied as basal dressing at the rate of 1.33 g/m² for each of the three nutrient elements on April 10, 2014 (Table 1). Additional basal application was performed at the rate of 4.67 g/m².
g/m² and 1.67 g/m² of each of N, P₂O₅, and K₂O for N18 and N9, respectively, with a slow release and coated fertilizer ECOLONG® 424-100 type (about 7% of each nutrient element is readily available) manufactured by JCAM AGRI Co., Ltd. four days after transplanting. Hence, the totals of the basal application were 6.00 g/m², 3.00 g/m² and 1.33 g/m² for each of N, P₂O₅ and K₂O in N18, N9 and N4, respectively (Table 1). Moreover, top-dressing was performed 63 days or 64 days before 80%-heading (after 75 and 61 days after transplanting) for L and E with another slow release and coated fertilizer ECOLONG® 413-180 type (about 3% of each nutrient element is readily available) containing N, P₂O₅ and K₂O at the rate of 14, 11 and 13%, respectively, manufactured by the company mentioned above. Accordingly, 12.00, 6.00 and 2.67 g/m² of nitrogen were top-dressed for N18, N9 and N4, respectively. The total amounts of N, P₂O₅ and K₂O (basal application + top-dressing) for the three fertilizer levels were summarized in Table 1. Experiment for L and E in the three fertilizer levels was conducted by randomized block design with three replications in 2014.

Measurements of total dry matter and leaf area
At each of 80%-heading stage and maturity, panicle number per hill was counted for nine hills per plot at south, middle and north parts of the second row in the three plots of the first to third replications, respectively, in each of L and E in 2011. Five hills, which had intermediate panicle numbers, were sampled from the nine hills of each plot at each stage. Dry weight excluding roots of each hill was measured after oven drying at 75°C for two days. The total area of living leaves in each hill was measured using Automatic area meter AAM-8 manufactured by Hayashi Denko Co., Ltd., Tokyo, Japan at 80%-heading stage and maturity. Total dry matter weight per m² and leaf area index (LAI) was calculated from the measured values. However, yield and yield components were not measured, because the grain yield of L was seriously damaged by infection of bacterial panicle blight (grain rot) in 2011.

Measurements of yield, yield components and related traits
Yield, yield components, and other traits were measured in 2014 by the following procedure: all panicles of 25 hills were sampled from the 3rd to 27th hill of the 4th row of each plot at maturity and dried in hot air ovens with a temperature ranging from 30 to 45°C for 20 hours to 30 hours until the average moisture content of rough rice lowered to 11% or less. The panicle weight of each hill was measured after cutting at 1.5mm below panicle bases.

Out of nine hills randomly selected from 25 hills of each plot, five hills having intermediate panicle weights were selected. The panicles of the five hills were threshed, and all spikelets in each hill were counted. Each spikelet was dehulled and examined for endosperm development as described by Murai et al (2005): a spikelet containing any developed endosperm containing starch was regarded as fertilized. The number of fertilized spikelets was counted on a hill basis. Grains after hulling (hereafter “grains”) were sieved by both 1.7mm and 1.5mm sieves to separate grains into three ranks by thickness: above 1.7mm, from 1.5 to 1.7mm, and below 1.5mm. Grains in each rank were separately counted and weighed in each of the five hills. The number of grains below 1.5mm was estimated by subtracting the number of grains above 1.5mm from the fertilized spikelets in each hill. The percentage of grain weight of each of the three ranks to panicle weight was calculated in the five selected hills of each plot; then, the grain weight of each rank of 25 hills of each plot was estimated from these three percentages. The moisture content (%) of grains above 1.7mm in each plot was measured, and the grain weight at 15% moisture was estimated. For japonica rice in Japan, brown rice just after hulling is separated with 1.7mm-sieve or a wider one to select brown-rice grains with sufficiently high quality for milling from grains with lower quality. However, both in L and E, grains with thickness from 1.5 to 1.7mm can be regarded as possessing quality sufficient for milling (Figure 2). Accordingly, we included the grains from 1.5 to 1.7mm thickness into ripened grains.

RESULTS
Number of days to Heading
As shown in Table 2, 80%-heading dates of L and E were 27th and 8th of August, respectively, in 2011. In 2014, that of L was 24th August at all of the fertilizer levels, while that of E was 5th August at N9,
and 6th August at both N18 and N4. The numbers of days to 80%-heading from sowing in L were 117 and 125 days, respectively, in 2011 and 2014, and those of E was 98 days, and 106 or 107 days, respectively, in both years. Hence, L was 18 or 19 days later in 80%-heading than E in both years.

### Table 2. Dates of 80%-heading and heading durations in L and E

| Year | Traits | Fertilizer level | L | E | Difference |
|------|--------|-----------------|---|---|------------|
| 2011 | Date of 80%-heading | Aug. 27 | Aug. 8 |
|      | Number of days to 80%-heading from sowing | 117 | 98 | 19 |
| 2014 | Date of 80%-heading | Aug. 24 | Aug. 6 |
|      | N9 | Aug. 24 | Aug. 5 |
|      | N4 | Aug. 24 | Aug. 6 |
|      | Number of days to 80%-heading from sowing | N18 | 125 | 107 | 18 |
|      | N9 | 125 | 106 | 19 |
|      | N4 | 125 | 107 | 18 |

### Table 3. Total dry matter, LAI and panicles/m² in L and E in 2011

| Stage of growth | Traits | L | E |
|-----------------|--------|---|---|
| 80%-heading     | Total dry matter (g/m²) | 1402 | 1096 |
|                 | LAI    | 4.5 | 4.3 |
| Maturity        | Total dry matter (g/m²) | 1672 | 1562 |
|                 | LAI    | 0.8 | * 1.9 |
|                 | Panicles/m² | 276 | 296 |

* Significantly different between L and E at the 5% level.

### Total Dry matter and LAI at 80%-heading and maturity

In 2011, L was higher by 306 g/m² in total dry matter weight at 80%-heading than E, and the former was 110 g/m² higher in that at maturity than the latter, although being not statistically significant (Table 3). L was not significantly different from E in LAI at 80%-heading, while L was lower than E in that at maturity. Regarding the panicle number per m² at maturity, L was not significantly different from E.

### Table 4. Analysis of variance for yield and its components at the three fertilizer levels in L and E In 2014.

| Traits                                | Source of variation | Lateness gene (A) | Fertilizer level (B) | Interaction (AxB) |
|---------------------------------------|---------------------|-------------------|----------------------|-------------------|
| Total brown rice yield (g/m²)         |                     | 26.23 **           | 6.72 **              | <1                |
| Yield-1.5mm sieve (g/m²)              |                     | 20.00 **           | 8.50 **              | <1                |
| Spikelets / panicle                   |                     | 176.12 **          | <1                   | 1.17              |
| Panicles / m²                         |                     | 4.91               | 18.47 **             | <1                |
| 1000-grain weight 1.5mm-sieve (g)     |                     | 13.67               | 1.11                 | <1                |

Degrees of freedom for lateness gene, fertilizer level, and interaction are I, 2 and 2, respectively. Degrees of freedom for replication and error are 2 and 10, although these items are abridged in the table.

*, ** Significant at 5% and 1% levels, respectively.

### Yield and yield components

Table 4 shows the results of the analysis of variance for yield and yield components except for the percentage of ripened grains in the three fertilizer levels in L and E. Effects of the lateness gene and fertilizer level were statistically significant in total brown rice yield and yield-1.5mm sieve, but the interactive effect between them was not significant. Regarding spikelet number per panicle and 1000-grain weight, the effect of Lat(t) was significant, but the effects of fertilizer level and the interaction were not significant. In panicle number per m², the effect of the fertilizer level was significant but the effects of the lateness gene and the interaction were not significant.

The number of grains from 1.5 to 1.7mm thickness accounted for 10.3 to 11.6% and 9.0 to 10.0% of all fertilized grains in the three fertilizer levels in L and E, respectively (Table 5-(1)). Grain weight from 1.5 to 1.7mm thickness accounted for 9.5 to 11.1% and 8.0 to 9.1% of all fertilized-grain weight in the three fertilizer levels in L and E, respectively (Table 5-(2)). The total grain weight above 1.5mm thickness was denoted by “yield-1.5mm sieve”. Furthermore, “total brown rice yield” was the brown...
rice weight of all fertilized spikelets. Ripened-grain percentage was the ratio (%) of the number of ripened grains to the total number of spikelets. The average spikelet number per hill of the 25 hills in each plot was estimated from that of the five hills and the ratio of the average panicle weight of the 25 hills to that of the five hills. Sink size (single grain weight × spikelet number per m²) was estimated.

Regarding total brown rice yield including all grains with thicknesses above and below 1.5mm, L was higher by 58, 58 and 89 g/m², respectively, than E at N18, N9, and N4 (Table 6). Hence, $E_{x(t)}$ increased this trait by 10 to 18%. Regarding yield-1.5mm sieve, L was 597, 575 and 545 g/m² at N18, N9 and N4, respectively, which corresponded to 109, 110 and 115% to those of E. Regarding fertilizer response from N4 to N18, total brown rice yield was increased by 45 and 76 g/m², respectively, in L and E, and yield-1.5mm sieve was increased by 52 and 72 g/m² in the former and later, even though the lateness gene × fertilizer level interaction was not significantly effective. Spikelet number per panicle was 129.0 to 129.9 in L and 106.2 to 111.2 in E, showing that $E_{x(t)}$ increased this trait by 16 to 22% in the three fertilizer levels. $E_{x(t)}$ decreased 1000-grain weight by 2 to 4% in the three fertilizer levels.

Table 5. Classification of grains by thickness at each of the three fertilizer level in L and E

| Traits | Fertilizer level | L  | E  | LSD (0.05) |
|--------|------------------|----|----|------------|
| Yield > 1.7mm sieve | N18 | 66.6 b | 70.6 ab | 4.9 |
|         | N9  | 70.6 ab | 73.3 a  |       |
|         | N4  | 69.6 ab | 72.0 a  |       |
| Yield 1.5 - 1.7mm sieve | N18 | 11.6 a  | 9.4 a   | 3.4 |
|         | N9  | 11.0 a  | 9.0 a   |       |
|         | N4  | 10.3 a  | 10.0 a  |       |
| Yield < 1.5mm sieve | N18 | 21.7 a  | 20.0 a  | 4.4 |
|         | N9  | 18.4 a  | 17.7 a  |       |
|         | N4  | 20.1 a  | 18.0 a  |       |

(1) Percentage on the basis of grain number

Values followed by the same letter within each rank by grain thickness are not significantly different at the 5% level, determined by LSDs in the table. Analysis of variance was conducted for percentage data of all combinations of the fertilizer levels and lines in each rank by grain thickness (%).

Panicle number per m² was not significantly different between L and E at every fertilizer level. From N4 to N18, this trait was increased by 43 and 38, respectively, in L and E. In L and E, the positive fertilizer response in total brown rice yield as well as in yield-1.5mm sieve is considered to be caused by that in panicle number per m², because significant fertilizer response was not noticed in spikelet number per panicle.

Table 6. Yield and its components at the three fertilizer levels in L and E in 2014

| Traits | Fertilizer level | L  | E  | LSD (0.05) |
|--------|------------------|----|----|------------|
| Total brown rice yield (g/m²) | N18 | 635 a  | (110) 1  | 577 bc | 52 |
|        | N9  | 606 ab | (111) | 548 cd |       |
|        | N4  | 590 abc | (118) | 501 d |       |
| Yield -1.5mm sieve (g/m²) | N18 | 597 a  | (109) | 548 bc | 48 |
|        | N9  | 575 ab | (110) | 525 c |       |

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### Spikelets/panicle

|        | N4  | N18 | N9  | N4  |
|--------|-----|-----|-----|-----|
|        | 545 | 129.2 a | 129.0 a | 129.9 a |
|        | bc  | (115) | (118) | (122) |
|        | 111.2 b | 109.2 b | 106.2 b |
|        | 476 | 6.0 |

### Panicles/m²

|        | N4  | N18 | N9  | N4  |
|--------|-----|-----|-----|-----|
|        | 382 | 338 a | 308 bc | 295 c |
|        | (98) | (94) | (97) |
|        | 344 a | 327 ab |
|        | 21 |

### 1000-grain weight 1.5mm-sive (g)

|        | N4  | N18 | N9  | N4  |
|--------|-----|-----|-----|-----|
|        | 20.6 bc | 20.6 bc | 20.6 bc | 20.5 c |
|        | (96) | (97) | (98) |
|        | 21.5 a | 21.3 ab |
|        | 0.7 |

#### Ripened-grain percentage and other traits

As shown in Table 7, a statistically significant difference between L and E was not noticed for the ripened-grain percentage. The effects of fertilizer level and the interaction were not significant in this trait, as mentioned above.

Regarding spikelet number per m², sink size, culm length and panicle length; both lateness gene and fertilizer level were significantly effective but the interaction between them was not significant. Increases from E to L in spikelet numbers per m² were 5416, 3997 and 5859, respectively, at N18, N9 and N4 which corresponds to 18, 14 and 11% (Table 8). Regarding fertilizer response from N4 to N18, this trait was increased by 5309 and 5752, respectively, in L and E. Ext(t) significantly increased sink size (g/m²) by 7 to 15% in L in the three fertilizer levels. From N4 to N18, sink size was increased by 114 g/m², in L, and by 141 g/m² in E. Ext(t) increased culm length and panicle length by 6 or 7% and 4 or 8%, respectively, in the three fertilizer levels. Increases from N4 to N18 in culm length were 2.2 cm and 1.9 cm in L and E, and that in panicle length was 1.8 cm and 0.9 cm in L and E.

#### Table 7. Analysis of variance for ripened grain percentage and other traits at the three fertilizer levels in L and E in 2014

| Trait                  | Source of variation | Lateness gene (A) | Fertilizer level (B) | Interaction (A×B) |
|------------------------|---------------------|-------------------|---------------------|------------------|
| Ripened-grain percentage | <1                  | 3.76              | <1                  |
| Spikelets/m²           | 37.19               | **                | 14.81               | **               | <1               |
| Sink size (g/m²)       | 15.92               | **                | 13.85               | **               | <1               |
| Culm length (cm)       | 128.76              | **                | 9.93                | **               | <1               |
| Panicle length (cm)    | 20.57               | **                | 9.19                | **               | 1.36             |

Degrees of freedom for lateness gene, fertilizer level, and interaction are 1, 2 and 2, respectively. Degrees of freedom for replication and error are 2 and 10, although these items are abridged in the table.

*, ** Significant at the 5% and 1% levels, respectively.

† Single grain weight at 1.5mm sieve × spikelets/m².

#### Table 8. Ripened-grain percentage and other traits at the three fertilizer levels in L and E in 2014

| Trait                  | Fertilizer level | L | E | LSD (0.05) |
|------------------------|------------------|---|---|------------|
| Ripened-grain percentage | N18              | 66.3 a | (99) | 66.7 a |
|                        | N9               | 70.2 a | (101) | 69.1 a |
|                        | N4               | 69.2 a | (99) | 69.9 a |
| Spikelets/m²           | N18              | 43688 a | (114) | 38272 bc |
|                        | N9               | 39682 b | (111) | 35685 cd |
|                        | N4               | 38379 bc | (118) | 32520 d |
| Sink size (g/m²)       | N18              | 902 a | (109) | 825 ab |

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|          | N9  | 819  | b  | (107) | 762  | b  |
|----------|-----|------|----|-------|------|----|
|          | N4  | 788  | b  | (115) | 684  | c  |
| Culm length (cm) | N18 | 71.9 | a  | (107) | 67.2 | c  |
|          | N9  | 70.2 | b  | (106) | 66.4 | cd |
|          | N4  | 69.7 | b  | (107) | 65.3 | d  |
| Panicle length (cm) | N18 | 24.3 | a  | (108) | 22.6 | bc |
|          | N9  | 23.2 | b  | (104) | 22.3 | bc |
|          | N4  | 22.5 | bc | (104) | 21.7 | c  |

Values followed by the same letter within each trait are not significantly different at the 5% level, determined by LSDs in the table.

1 Percentage of L to E.
2 single grain weight with 1.5mm-sieve × spikelets/m².

DISCUSSION

Lateness allele Ex(t) in L delayed heading by 18 or 19 days compared with its photo insensitive allele ex in E. Statistically significant difference was not noticed between L and E for ripened-grain percentage and panicle number per m² in each fertilizer level. Ex(t) decreased 1000-grain weight by 2 to 4%. On the other hand, Ex(t) significantly increased total brown rice yield by 10 to 18%, spikelet number per m² by 11 to 18% and sink size by 7 to 15%, by increasing spikelet number per panicle by 16 to 22%. Hence, it is inferred that the effect of Ex(t) on yield was mainly due to its effect of increasing spikelet number per panicle. N18 is an outstandingly high fertilizer level in Japan. It is noteworthy that L (635 g/m²) was 9% higher than E in yield-1.5mm-sieve. IR36, a typical improved indica variety, occupied the widest cultivated area in Southeast Asia during 1980s (Imbe 2002). Suweon 258 achieved brown rice yield of about 1000 g/m² by heavy fertilizer-application in a field test in Kagawa Prefecture, a neighboring prefecture of Kochi (Komatsu et al 1984). L and E have typical plant type of improved indica variety with short culms and erect leaves, which are inherited from IR36 and Suweon 258. It is inferred that L and E are donated positive responsiveness to higher fertilizer application from Suweon 258. Late genotype types attain heading stages in late August or early September in the Kyushu region of Japan; and heading characteristic of L seems to be similar to those of them (Agriculture Production Bureau, Ministry of Agriculture, Forestry and Fisheries, Japan 1997). Sakai et al (2006) suggested that late varieties of the Kyushu region have the advantage to avoid high-temperature damage of grain filling because their maturing durations are mainly in September in which temperature is lower than in August ordinarily. Consequently, Ex(t) could be utilized to develop high yielding varieties for sufficiently warm districts in which late varieties can be cultivated.

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