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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 F_1 of Suweon 258 × an isogenic line of IR36 carrying *Ur1* 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, P_2O_5 and K_2O were applied at the nitrogen levels of 4.00, 9.00 and 18.00 g/m^2 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/m^2), 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/m^2), by 9 to 15%. *Ex*(t) increased spikelet number per panicle by 16 to 22% and spikelet number per m^2 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

धानमा बाला निस्कने समय नियन्त्रय गर्ने विभिन्न जीनहरु पत्ता लगाइएका छन । सुवेवन २५८ र आइ आर ३६ को *Ur1* आइसोजेनिक लाइनको क्रसिङ्गबाट एक जोडि ढिलो बाला निस्कने र छिटो बाला निस्कने आइसोजेनिक लाइन “एल” र “इ” विकसति गरिएको थियो । ती दुइ आइसोजेनिक लाइनको बीचको बाला निस्कने अवधि एउटा प्रकाश संवेदनशील विलम्ब जीन *Ex(t)* ले नियन्त्रण गर्ने अनुमान गरिएको छ, यद्यपी यो जीनको क्रोमोजोमको स्थान अज्ञात छ । यस अध्ययनमा विलम्ब जीन *Ex(t)* ले धानबालीको उत्पादन तथा यससंग सम्बन्धित विशेषताहरुमा पार्ने असरको बारेमा विश्लेषण गर्नको लागि धानखेतमा परिक्षणहरु संचालन गरिएको थियो । नाइट्रोजन, फोस्फोरस र पोटासियम समाविष्ट रासायनिक मलहरु ४.००, ९.०० र १८.०० ग्राम प्रतिमिटर नाइट्रोजन स्तरको रुपमा प्रयोग गरि क्रमशः “एन ४” , “एन ९” र “एन १८” ले जनाइएको थियो । एल र इ को कुल खैरो चामल उत्पादन (ग्राम प्रतिमिटर) ६३५ र ५७७, ६०६ र ५४८, र ५९० र ५०१ क्रमशः एन ४, एन ९ र एन १८ मा भएको थियो, जसले विलम्ब जीन *Ex(t)* ले कुल खैरो चामल उत्पादन १० देखि १८% बृद्धि भएको जनाउदछ । *Ex(t)* ले १.५ मिलिमिटर भन्दा बढी मोटाइ भएको खैरो चामलको उत्पादन (ग्राम प्रतिमिटर) ९ देखि १५% बृद्धि गरेको थियो । *Ex(t)* ले प्रतिबाला स्पाइकलेट संख्या र प्रतिमिटर स्पाइकलेट संख्या क्रमशः १६ देखि २२ र ११ देखि १८% बृद्धि गरेको थियो । *Ex(t)* ले एक हजार खैरो चामलको तौल (ग्राम) २ देखि ४% कम गरेको थियो । तर परिपक्क खैरो दानाको प्रतिशतमा *Ex(t)* को असर सांख्यिकीय रुपमा फरक पाइएको थिएन

। अतः *Ex(t)* ले प्रतिबाला स्पाइकलेटको संख्या बृद्धि गरि धानको उत्पादन बृद्धि गरेको थियो । *Ex(t)* जीन समशितोष्ण क्षेत्रका गर्मी हावापानी भएका जिल्लाहरुमा धानको उत्पादन बृद्धि गर्नको लागि प्रयोग गर्न सकिन्छ ।

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. *Se1* and *E1*, 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 *Se1* locus involves at least three alleles, *Se1-u*, *Se1-n* and *Sel-e*: the first two are incompletely dominant alleles controlling photosensitivity, while *Se1-e* is a recessive allele with non-photosensitivity (Yokoo and Kikuchi 1977). *Se1-e* is distributed in rice varieties grown in Hokkaido prefecture and Tohoku district of Japan (Yokoo et al 1980, Ichitani et al 1997), while *Se1-n* is harbored in middle and late heading varieties grown in warmer regions of Japan (Yokoo et al 1980). *Se1-u* is harbored in an *indica* variety ‘Morak Sepilai’ (Yokoo and Fujimaki 1971). *Se1-u* delays heading by at least 20 days as compared with its photo-insensitive allele *Se1-e*. *E1-k* allele at the *E1* locus delays heading by about two weeks, as compared with its non-photosensitive allele *e1* (Ichitani et al 1998).

Murai developed an isogenic-line pair of late and early lines, denoted by “L” and “E”, respectively, from descendants of the F_1 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 *Se1* locus or the *E1* locus (Murai unpublished).

Ghd7 allele at the *E1* 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 *Se1*, *E1* 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 F_1 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 F_1 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).

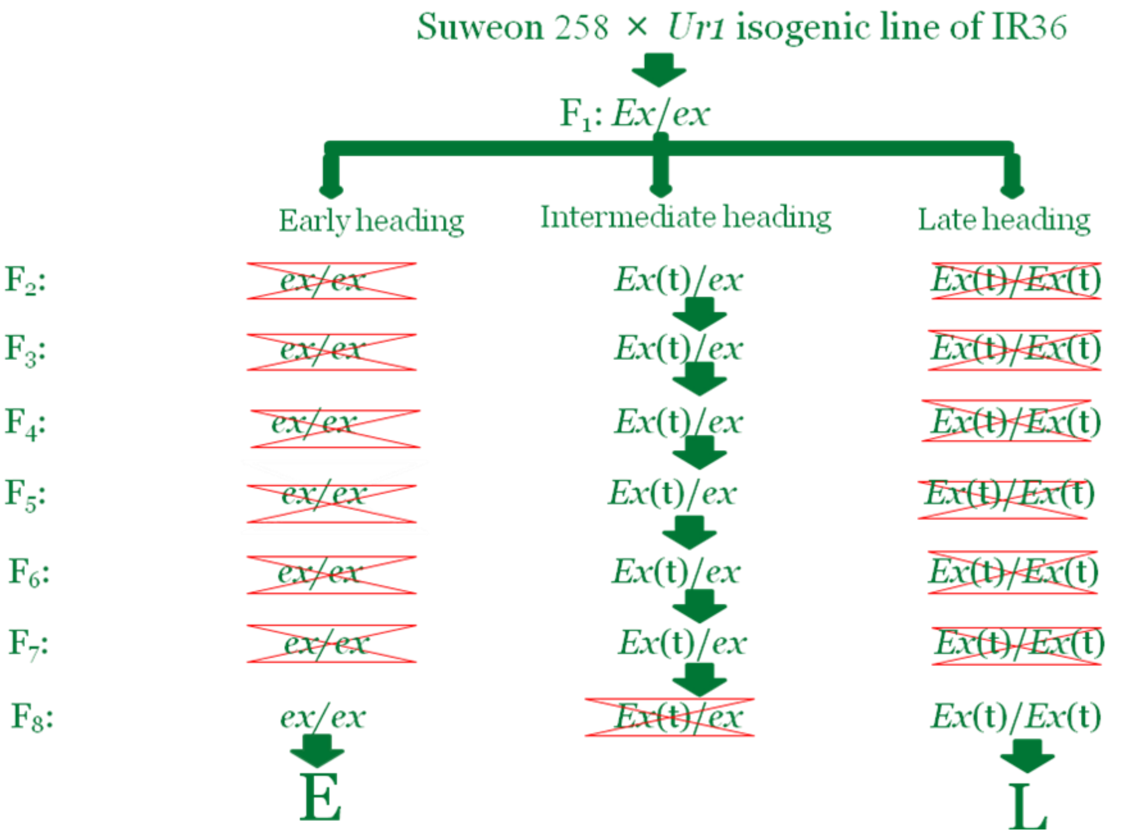

Figure 1. Procedure of developing early and late isogenic-line pair of E and L, from the initial cross to establishment.

☒ : not selected for later generations.

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 F_2 population from the F_1. Early-heading, late-heading and intermediate-heading plants appeared in the F_3 population from an intermediate-heading plant of F_2. Similarly, in F_4 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 F_8 population from an intermediate-heading F_7 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 F_9 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$^{®}$ (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_2O_5 and K_2O 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/m^2) 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).

Table 1. Chemical fertilizers applied in the experimental field in 2011 and 2014

Year	**Fertilizer level**	**Basal or top-dressing**	**Chemical fertilizers applied**	**N (g/m^2)**	**P_2O_5 (g/m^2)**	**K_2O (g/m^2)**
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.

Figure 2. Classification of brown rice by thickness in L.

In 2011, just before ploughing, an ordinary chemical fertilizer, manufactured by Central Chemical Co., Ltd., Tokyo, Japan, containing N, P_2O_5 and K_2O was applied as basal dressing at the rate of 5.00 g/m^2 for each of N, P_2O_5 and K_2O (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, P_2O_5 and K_2O at the rate of 14, 14 and 14%, respectively, manufactured by JCAM AGRI Co., Ltd. at the rate of 12.00 g/m^2 of each of the three nutrient elements. Chemical fertilizers containing N, P_2O_5 and K_2O were applied by both basal and top dressings at the nitrogen levels of 4.00, 9.00 and 18.00 g/m^2 in total in 2014, which were denoted by "N4", "N9" and "N18", respectively. Just before ploughing, the ordinary chemical fertilizer containing N, P_2O_5 and K_2O was applied as basal dressing at the rate of 1.33 g/m^2 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/m^2 and 1.67 g/m^2 of each of N, P_2O_5, and K_2O 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/m^2, 3.00 g/m^{2,} and 1.33 g/m^2 for each of N, P_2O_5 and K_2O 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, P_2O_5 and K_2O at the rate of 14, 11 and 13%, respectively, manufactured by the company mentioned above. Accordingly, 12.00, 6.00 and 2.67 g/m^2 of nitrogen were top-dressed for N18, N9 and N4, respectively. The total amounts of N, P_2O_5 and K_2O (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℃ 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 m^2 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	N18	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/m^2 in L and E in 2011

Stage of growth	Traits	L	E
80%-heading	Total dry matter (g/m^2)	1402	1096
	LAI	4.5	4.3
Maturity	Total dry matter (g/m^2)	1672	1562
	LAI	0.8 *	1.9
	Panicles/m^2	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/m^2 in total dry matter weight at 80%-heading than E, and the former was 110 g/m^2 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 m^2 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. Numerals in the table show F-values.

Traits	Source of variation		
	Latenessgene (A)	Fertilizer level (B)	Interaction(A×B)
Total brown rice yield (g/m^2)	26.23 **	6.72 *	<1
Yield-1.5mm sieve (g/m^2)	20.00 **	8.50 **	<1
Spikelets / panicle	176.12 **	<1	1.17
Panicles / m^2	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 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 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 *Ex*(t) was significant, but the effects of fertilizer level and the interaction were not significant. In panicle number per m^2, 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 m^2) 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/m^2, respectively, than E at N18, N9, and N4 (**Table** 6). Hence, *Ex*(t) increased this trait by 10 to 18%. Regarding yield-1.5mm sieve, L was 597, 575 and 545 g/m^2 at N18, N9 and N4, respectively, which correspond 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/m^2, respectively, in L and E, and yield-1.5mm sieve was increased by 52 and 72 g/m^2 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 *Ex*(t) increased this trait by 16 to 22% in the three fertilizer levels. *Ex*(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

(1) Percentage on the basis of grain number

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}	

(2) Percentages on the basis of grain weight

Traits	Fertilizer level	L	E	LSD (0.05)
Yield > 1.7mm sieve	N18	83.0 ^{a}	86.5 ^{a}	4.9
	N9	84.6 ^{a}	87.7 ^{a}	
	N4	83.0 ^{a}	85.8 ^{a}	
Yield 1.5 - 1.7mm sieve	N18	11.1 ^{a}	8.6 ^{a}	3.5
	N9	10.2 ^{a}	8.0 ^{a}	
	N4	9.5 ^{a}	9.1 ^{a}	
Yield < 1.5mm sieve	N18	6.0 ^{a}	4.9 ^{a}	3.3
	N9	5.2 ^{a}	4.2 ^{a}	
	N4	7.5 ^{a}	5.1 ^{a}	

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 m^2 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 m^2, 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/m^2)	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/m^2)	N18	597	a	(109)	548	bc	48
	N9	575	ab	(110)	525	c	

	N4	545	bc	(115)	476	d	
Spikelets/panicle	N18	129.2	a	(116)	111.2	b	6.0
	N9	129.0	a	(118)	109.2	b	
	N4	129.9	a	(122)	106.2	b	
Panicles/m^2	N18	338	a	(98)	344	a	21
	N9	308	bc	(94)	327	ab	
	N4	295	c	(97)	306	bc	
1000-grain weight 1.5mm-sive (g)	N18	20.6	bc	(96)	21.5	a	0.7
	N9	20.6	bc	(97)	21.3	ab	
	N4	20.5	c	(98)	21.0	abc	

[1] Percentage of L to E.

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

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 m^2, 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 m^2 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. *Ex*(t) significantly increased sink size (g/m^2) by 7 to 15% in L in the three fertilizer levels. From N4 to N18, sink size was increased by 114 g/m^2, in L, and by 141 g/m^2 in E. *Ex*(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. Numerals in the table show F-values.

Trait	Source of variation				
	Lateness gene (A)		Fertilizer level (B)		Interaction（A×B）
Ripened-grain percentage	<1		3.76		<1
Spikelets/m^2	37.19	**	14.81	**	<1
Sink size [1] (g/m^2)	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.*

[1] single grain weight at 1.5mm sieve × spikelets/m^2.

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)[1]	66.7	a	4.1
	N9	70.2	a	(101)	69.1	a	
	N4	69.2	a	(99)	69.9	a	
Spikelets/m^2	N18	43688	a	(114)	38272	bc	3221
	N9	39682	b	(111)	35685	cd	
	N4	38379	bc	(118)	32520	d	
Sink size (g/m^2) [2]	N18	902	a	(109)	825	ab	77

	N9	819	b	(107)	762	b	
	N4	788	b	(115)	684	c	
Culm length (cm)	N18	71.9	a	(107)	67.2	c	1.5
	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	1.0
	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/m^2.*

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 m^2 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 m^2 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/m^2) 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/m^2 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 genotypes 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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