（様式５）(Style5)

学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

氏名：　Rana Birendra Bahadur
Name

学位論文題目：
Title of Dissertation

Genetic analyses of genes controlling heading time and their effects on yield and related traits in rice
(イネの出穂性に関する遺伝的解析、および、それに係わる遺伝子が収量および関連形質に及ぼす作用)

学位論文要約：
Dissertation Summary

Rice is the dominant dietary energy source in Asia and some countries in Africa. Rice supplies about 19% of human calorie in the world. In middle and low income countries of the world, rice provides 27% of calories. Rice was a staple food for about 80% of total malnourished human beings in the world in 2016. Therefore, increase in production and productivity of rice is the key to tackle with hunger and malnutrition in the world. Short day ("SD") length is the critical environmental factor for transition from vegetative to reproductive phase in rice. SD length promotes heading (flowering) in rice. However, Japanese *japonica* rice varieties introduced to Taiwan from 1885 did not adapt well in Taiwan's subtropical environmental conditions, viz. short day lengths in summer and high temperature, because headings of those *japonica* varieties were too early to secure sufficient vegetative growth, resulting low yields. Taichung Agricultural Research Station successfully developed "Ponlai" (*japonica*) varieties such as Taichung 65 which was derived from the F_1 between two *japonica* varieties 'Kameji' and 'Shinriki'. Taichung 65 has wider adaptability for the first and second cropping seasons in there, due to its sufficiently long basic vegetative phase and low photosensitivity.

The growth period in rice from sowing to heading consists of three phases: basic vegetative growth phase, photoperiod sensitive phase and panicle development phase. Since the latter phase is rather constant of about 30 days among *japonica* and *indica* rice varieties, heading time is principally governed by the former two phases. Chang *et al.* (1969) indicated that photoperiod sensitivity can be eliminated under optimum photoperiod for panicle initiation. The duration of basic vegetative phase can be estimated by subtracting 30 days from days to heading under SD conditions and higher temperature, and the duration of photoperiod sensitivity can be calculated by subtracting days to heading under SD condition from days to heading under long-day ("LD") condition. Times of heading are important for obtaining sufficient and stable yields in wider latitudes and altitudes. In cool-weather rice areas such as Hokkaido of Japan and high altitude areas in Nepal, short basic vegetative phase and low photoperiod sensitivity is essential to complete harvest before the beginning of frost. In Southeast Asia and other tropical and subtropical areas, rather early elite varieties with low photosensitivity can adapt well for various cropping seasons (Khush *et al.* 2001).

The conserved photoperiodic model for flowering consists of *GI-CO-FT* (GIGANTEA – CONSTANS - FLOWERING LOCUS T) signaling pathway, where *GI* (a circadian clock controlling gene) up-regulates expression of *CO*, and *CO* activates expression of *FT* (Tsuji *et al.* 2010). The *GI-CO-FT* pathway is active only during LD conditions in Arabidopsis. The rice homologue of the *GI-CO-FT* pathway corresponds to *OsGI – Hd1* (*Se1*) – *Hd3a*, which is active only in SD conditions (Kojima *et al.* 2002). *Se1* accelerates heading by enhancing the activity of *Hd3a* under SD conditions. During LD conditions, the function of *Se1* is changed into repressor to diminish the activity of *Hd3a*, resulting delay of heading (Lin *et al.* 2000). Although, *Hd3a RNAi* (loss of function of *Hd3a*) plants were strongly delayed in heading during SD conditions, *Hd3a RNAi* plants flowered quite normally during LD conditions, indicating presence of another key factor promoting flowering during LD conditions. Komiya *et al.* (2009) revealed that this factor is *RFT1* (RICE FLOWERING LOCUS T1), which is active during LD conditions. Hence, *Hd3a* and *RFT1* are major floral activators under SD and LD conditions, respectively, in rice (Komiya *et al.* 2008). The activity of *Hd3a* is also up-regulated by *Ef1* (=*Ehd1*), but it is suppressed by *E1* (*Ghd7* = *Hd4*) under LD conditions (Itoh *et al.* 2010). Koo *et al.* (2013) indicated that *Hd2*

(OsPRR37) controls the activity of *Hd3a* independently of expression of *Se1* and *Ef1*.

A photoperiod sensitive gene *Se1* (Photosensitivity 1) located on chromosome 6, plays an important role in controlling heading in rice. *E1* and *Hd2* are other major photoperiod sensitive genes, which are located at different loci on the same chromosome 7 (Okumoto *et al.* 1996, Lin *et al.* 2000). These three genes are deeply related with diversity in heading and regional adaptabilities (Yokoo *et al.* 1980, Yamagata *et al.* 1986, Okumoto *et al.* 1996, Ichitani *et al.* 1998, Ebana *et al.* 2011). *Se1* locus involves at least three alleles: the magnitudes of delaying heading are in the order of *Se1-u* > *Se1-n* > *Se1-e*, where the latter allele is non-functional for photosensitivity. *Se1-u* is harbored in middle and late-heading varieties grown in warmer regions of Japan. *Se1-u* is harbored in an *indica* variety 'Morak Sepilai'. *E1* is harbored in early, middle and late heading varieties grown in south-western region of Japan. *Ef1* (Earliness 1) is a dominant allele for earliness at the *Ef1* locus on chromosome 10 controlling basic vegetative phase (Tsai and Oka 1976, Tsai 1993). *Ef1* accelerates heading by 7 to 13 days. *Ef1* is widely distributed in early-heading varieties of northern areas in both Japan and China. On the other hand, its recessive allele *ef1* for longer basic vegetative phase is found only in some Taiwanese *japonica* varieties (Saito *et al.* 2009). *Efx* is a dominant allele for earliness at a locus on chromosome 3, which controls basic vegetative phase (Sato *et al.* 1992, Sumi *et al.* 1998). *Efx* is thermo sensitive: its effect of reducing basic vegetative phase is accelerated by higher temperature; this accelerating effect is masked by *Ef1*. A recessive allele, *m-Ef1*, is the enhancer for *Ef1* (Tsai 1976), which is identical with *e1* allele at the *E1* locus. The *se-pat* is a recessive allele for lateness, which is located between *C* (Chromogen) and *wx* (Glutinous) locus on chromosome 6 (Dung and Sano 1996).

Table 1. Genotypes of five early NILs andT65-R, T65-T, and three late NILs and T65wx

Line/ Variety	Genes/alleles					
	Ac-ef1	*Ef1*	*Efx*	*m-Ef1*^{b1,2}	*Se1-pat*(t)	*se-pat*^{3}
ER50	*Ac-ef1*	*Ef1*^{A58}	*Efx*	*m-Ef1*^{b}	*se1*	*se-pat*$^{+}$
ER40	*Ac-ef1*	*Ef1*^{A58}	*efx*	*m-Ef1*^{b}	*se1*	*se-pat*$^{+}$
ER20	*Ac-ef1*	*Ef1*^{A58}	*Efx*	*M-Ef1*	*se1*	*se-pat*$^{+}$
ER1	*Ac-ef1*	*Ef1*^{A58}	*efx*	*M-Ef1*	*se1*	*se-pat*$^{+}$
ER21	*Ac-ef1*	*ef1*	*Efx*	*M-Ef1*	*se1*	*se-pat*$^{+}$
T65-R	*Ac-ef1*	*ef1*	*efx*	*M-Ef1*	*se1*	*se-pat*$^{+}$
T65-T	*ac-ef1*	*ef1*	*efx*	*M-Ef1*	*se1*	*se-pat*$^{+}$
T65wx	*ac-ef1*	*ef1*	*efx*	*M-Ef1*	*se1*	*se-pat*$^{+}$
LF3	*ac-ef1*	*ef1*	*efx*	*M-Ef1*	*Se1-pat*(t)	*se-pat*$^{+}$
LF1	*ac-ef1*	*ef1*	*efx*	*M-Ef1*	*se1*	*se-pat*
LF2	*ac-ef1*	*ef1*	*efx*	*M-Ef1*	*Se1-pat*(t)	*se-pat*

[1] *m-Ef1*^{b} was donated from Bozu 5.

[2] *m-Ef1*^{b} *and M-Ef1* are identical with *E1* and *e1*, respectively (Okumoto *et al.* 1992)

[3] *se-pat* is identical with RFT1 (Hagiwara *et al.* 2009).

Professor Shigetoshi Sato (University of the Ryukus) developed five early near-isogenic lines (NILs) of an accession of Taichung 65 maintained in the university ('T65-R'), which harbor either of both of *Ef1* and *Efx* in all of the five NILs, and *m-Ef1* in two of them additionally (Sato *et al.* 1988, Sato *et al.* 1992, Itoh *et al.* 2010) (Table 1). In addition, Professor Yoshio Sano (Hokkaido University) developed three late NILs of T65wx carrying either or both of *Se1-pat*(t) at the *Se1* locus and *se-pat* at the *se-pat* locus derived from the same *indica* variety 'Patpaku'. T65wx is an isogenic line of T65-T carrying *wx* from a *japonica* variety 'Kinoshita mochi'. T65-T is another accession of Taichung 65 maintained at National Chung Hsing University of Taiwan. T65-R carries *Ac-ef1*, which accelerates heading by 4.2 and 7.7 days as compared to T65-T under first (January to June) and second crop (July to October) seasons, respectively, in Taichung, Taiwan (Tsai 1993). T65-T carries its recessive allele *ac-ef1*. Table 1 shows the detail of the materials lines with their genotypes used in the experiments.

The five early NILs, T65-R, T65-T, three late NILs, and T65wx were grown under six different environmental

conditions: 10h photoperiod (SD) and 13.5h photoperiod conditions in an artificial light-type ("Art-L") growth chamber and natural light-type ("Nat-L") growth chamber from autumn to winter (SD condition), and two spring-sowing paddy field ("PF") conditions and a summer sowing PF condition.

Under the spring-sowing PF condition in 2011, the days to heading ("DTH") of the five early lines and T65-R were in the order of ER50 < ER20 = ER40 < ER1 < ER21 < T65-R (< indicates significant difference). The DTH of T65-R was 88.8 days. The DTH of ER50 (*m-Ef1*, *Ef1*, *Efx*) was 23.8 days shorter than that of T65-R. Both ER40 (*m-Ef1*, *Ef1*) and ER20 (*Ef1*, *Efx*) were 19.9 days earlier in heading than T65-R, and ER1 (*Ef1*) and ER21 (*Efx*) were 10.3 and 7.5 days earlier, respectively, than T65-R. The order of DTH in the early lines and T65-R in 2018 was identical with that in 2011, with one exception that ER40 = ER20 in 2011 but ER40 ≤ ER20 in 2018 (≤ indicates non-significant difference). Under the PF conditions in 2011 and 2018, day lengths at the critical stage (35 days before heading) in the five early NILs, T65-R, T65-T and T65wx were from 14:13 (hours : minutes) to 14:23 and 14:10 to 14:23 in 2011 and 2018, respectively, indicating LD conditions. The order of DTH in the lines and T65-R under the summer-sowing PF condition in 2014 was identical with that in 2018, in which day lengths at the critical stage was from 13:04 to 13:44. On the other hand, the lines and T65-R were in the order of ER20 ≤ ER50 < ER40 ≤ ER1 < ER21 < T65-R under the Nat-L growth chamber condition in which the day lengths at the critical stage were short; this order is the same with that in the 10-h photoperiod condition. Consequently, *Ef1*, *Efx*, their combination, and the additional enhancing effect of *m-Ef1*^{b} decreased DTH more or less under the two LD conditions, the day length condition of 13:04 to 13:44, and the two SD conditions. However, under each of the two SD conditions, ER40 (*e1 = m-Ef1*, *Ef1*) was not significantly different from ER1 (*E1 = M-Ef1*, *Ef1*), and similarly, ER50 (*e1= m-Ef1*, *Ef1*, *Efx*) was not significantly different from ER20 (*E1 = M-Ef1*, *Ef1*, *Efx*), probably because *E1* did not increased DTH under the SD conditions.

T65-R (*Ac-ef1*) was 5.1 and 2.8 days earlier in heading, respectively, than T65-T (*ac-ef1*) under the spring-sowing PF conditions in 2011 and 2018. Day lengths at the critical stage for T65-R and T65-T were 14:22 or 14:23 in both years indicating LD conditions. T65-R was identical with T65-T in DTH under the 13.5h photoperiod condition. T65-R was 0.9 day later (not significant statistically) in heading than T65-T under the summer-sowing PF condition, in which day lengths at the critical stage were 13:04 and 13:07, respectively. Under the Nat-L growth chamber condition, T65-R was 9.9 days later than T65-T, in which day lengths at their critical stage were 9:59 and 10:07, respectively, for the former and the latter accessions. Moreover, DTH of T65-R was 19.1 days shorter than T65-T under 10h-photoperiod condition. Notably, the response of *Ac-ef1* to day length was found to delay heading under the SD conditions, because other photoperiod sensitive alleles such as *Se1*, *E1*, *Hd2*, etc. reduce DTH under SD conditions.

Under the PF condition in 2011, LF3 (*Se1-pat*(t)), LF1 (*se-pat*) and LF2 (*Se1-pat*(t), *se-pat*) were 33.2, 38.9 and 51.9 days later in heading, respectively, than T65wx. The four lines were in the same order in 2018 as in 2011. Day lengths at the critical stage in 2011 and 2018, respectively, were 14:21 and 14:23, 13:45 and 13:34, 13:35 and 13:18, and 13:10 and 12:57, respectively, in T65wx, LF3, LF1 and LF2. Under the 13.5h-photoperiod condition, LF3, LF2 and T65wx were in the same order as those in the two spring-sowing PF conditions, whereas LF1 was 6.9 days earlier than LF3. Under the summer-sowing PF condition, LF2, LF3 and T65wx were similar to one another in heading although the difference between the latter two lines was significant; however, LF1 was 17.7 days later than T65wx. Day lengths at the critical stage were 13:04, 12:59, 12:28 and 13:02, respectively, in T65wx, LF3, LF1 and LF2. Under the 10h-photoperiod condition, LF3 was 5.3 days earlier in heading than T65wx. LF2 was 1.4 days earlier than T65wx although being not statistically significant. LF1 was 33.8 days later than T65wx. Under the Nat-L growth chamber condition, in which day lengths at the critical stage were from 9:56 to 10:24 in the four lines, LF3 and LF2 were 16.3 and 15.2 days earlier in heading, respectively, than T65wx, and the difference between the former two lines was not significant. On the other hand, LF1 was 24.7 days later than T65wx. Hence, *Se1-pat*(t) increased DTH under LD conditions but decreased it under SD conditions, while *se-pat* elongated DTH under both SD and LD conditions indicating that *se-pat* is responsible for basic vegetative phase. The *se-pat* increased DTH by adding its effect over that of *Se1-pat*(t) under LD conditions. However, this increasing effect of DTH was almost completely masked when *se-pat* co-existed with *Se1-pat*(t) under the SD conditions.

In rice, grain yield is composed of four components: number of panicles per plant (hill), number of spikelets per panicle, ripened-grain percentage and single grain weight. Number of panicles is dependent on the ability of producing tillers and rate of effective tillers having panicles. Number of spikelets per panicle is mainly

determined by numbers of primary and secondary rachis branches. Single-grain weight is mainly determined by grain size, which is specified by its three dimensions (length, width and thickness), and the degree of filling.

In general, longer growth duration (late heading or late maturity) allows greater biomass accompanied by larger leaf area in cereal crops. Kawano and Tanaka (1968) showed positive relationship between biomass yield at flowering and heading period among early and late varieties under sub-tropical cultivated conditions. Nevertheless, harvest index has negative relationship with growth period. The highest grain yield was obtained at the range of optimum growth duration in each of dry and wet seasons at a subtropical area (Wada and Cruz 1989).

Professor Masayuki Murai at the Faculty of Agriculture and Marine Science, Kochi University developed an isogenic line pair of late and early lines, denoted by "L" and "E", respectively, from descendents of the F_1 from Suweon 258 × an isogenic line of IR36 carrying *Ur1* gene. Suweon 258, an *indica*-type variety developed by the cooperation between Korea and International Rice Research Institute (IRRI), was crossed with 36U: an *Ur1* (Undulate rachis-1) isogenic line of IR36. In the F_2 population, comparatively early-heading, late-heading and intermediate-heading plants were segregated. Similar segregations were observed in the F_3 and later generations. A late-heading plant and an early-heading plant were selected in the F_8 population from an intermediate-heading F_7 plant, for developing L and E of the isogenic-line pair, respectively. 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. However, the locus of *Ex*(t) is unknown. L and E were grown under PF with three replications. Twelve day old seedlings were transplanted on May 3 at a planting distance of 30 cm × 15 cm with two seedlings per hill to an experimental field of the Faculty of Agriculture, Kochi University, (Nankoku 33°35′N) (Current Name: Faculty of Agriculture and Marine Science, Kochi University). 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. 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 grain 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%. *Ex*(t) decreased 1000-grain weight by 2%. 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 regions of the temperate zone.

Lodging could be a factor of reducing yield in rice by diminishing percentage of ripened grains and 1000-grain weight. Higher single panicle weight owing to more number of spikelets upon longer culm makes rice plant lodge easier. Effects of *Ex*(t) on lodging and its related traits were examined under the same PF condition mentioned above using L and E. The same three fertilizer levels viz. N4, N9 and N18 were set in the PF, as mentioned above. *Ex*(t) significantly increased the length from the base of 4th internode to panicle top (a) and the total fresh weight above the 4th internode inclusive (b), by 6 to 8% and 10 to 18%, respectively, at the three fertilizer levels. On the other hand, *Ex*(t) increased breaking strength at the 4th internode with leaf sheaths (c) by 10 to 18%, at the three fertilizer levels. As the result, index of lodging (ab/c × 100) was increased by 6 to 13% in L in comparison with E, indicating that *Ex*(t) diminishes lodging resistance. When we pursue higher yield by introducing *Ex*(t) in rice breeding, decrease in lodging resistance might be expected even though its extent would not be serious.