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2 **Short communication**

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5 **Isolation of a major genetic interaction associated with an extreme**
6 **phenotype using assorted F2 populations in rice**

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1

2 **Abstract**

3 Detection of quantitative trait loci (QTLs) is dependent on materials used in the analysis, as
4 different combinations of parental materials may lead to different outcomes in QTLs for the
5 same trait. On the other hand, an extreme phenotype associated with a given trait implies the
6 potential involvement of a particular allele in various allelic interactions. A genetic factor
7 associated with such an extreme phenotype may frequently be identified from various genetic
8 populations consisting of different parental combinations. In this study, we attempted to
9 uncover the genetic factor associated with extremely early heading date in rice using various F2
10 populations. Heading date in rice has been characterized by at least 19 QTLs, from which 12
11 genes have been identified. A58, a rice strain with an extremely early heading date, is adapted to
12 Hokkaido, the northernmost limit of rice cultivation. Six F2 populations derived from crosses of
13 A58 with six other strains displayed a range of heading dates. Genotyping using 19 QTL
14 markers indicated that the A58 allele of the *Ghd7* locus was present in most F2 individuals
15 exhibiting extremely early heading dates. This analysis also demonstrated that when the
16 wild-type *Ehd1* allele was present, the *Ghd7* allele from A58 accelerated floral induction. The
17 results of this study demonstrate that assorted F2 populations are valuable materials for
18 comprehensive genotyping to explore major genetic factors for extreme phenotypes, and that
19 this methodology is broadly applicable to other unknown traits.

20

21 **Keywords**

22 Assorted F2 populations, Extreme phenotype, Heading date, *Ghd7*, *Ehd1*, Rice

23

1 **Text**

2 Analyses for quantitative trait loci (QTLs) have broadly and profoundly contributed to the
3 discovery of a complex of genes with minor effects for a particular phenotypic trait (Borevitz
4 and Chory 2004). An enormous number of QTLs have been identified using second filial
5 generation (F2) populations, recombinant inbred lines (RILs), backcross inbred lines (BILs),
6 and chromosomal substitution lines in various crops (Yamamoto et al. 2009; Wurschum 2012).
7 As technical development has boosted the ability to produce high-resolution genomic markers
8 throughout the genome, QTL detection accuracy and frequency have greatly advanced (Ganal et
9 al. 2009; Varshney et al. 2009). In rice, QTL analyses have generally employed RILs and BILs
10 (Yamamoto et al. 2009). Unlike F2 populations, RILs and BILs are genetically stable after
11 establishment, allowing recurrent use under different conditions. Such populations, however,
12 possess only the two parental alleles at each locus, limiting genetic variation to two entities.
13 Consequently, a trait of interest examined using RILs or BILs is defined by their parental
14 characteristics.

15

16 Ebana et al. (2011) successfully characterized known QTLs for rice heading date using multiple
17 F2 populations derived from different cross combinations. The different allele combinations
18 resulted in different phenotypic variations for the corresponding trait in the F2 populations.
19 Their study caused us to consider whether common genetic factors can be extracted from
20 individuals exhibiting phenotypes extremely different from the majority in various F2
21 populations. Different F2 populations are expected to show a wide range of phenotypic
22 variations. If particular F2 populations have distinct distributions of phenotypic variations, we
23 might be able to explore the loci associated with such distinct phenotype using F2 individuals
24 and isolate major genetic factors.

25

26 To attempt isolation of major genetic factors from various F2 populations, we focused on rice
27 heading date, a well-characterized, important agricultural trait. Approximately two dozen QTLs
28 (*Hd1-Hd17*, *Ehd1*, *Ehd2*, *Ehd3*, *Edh4*, and *Ghd7*) associated with heading date have been
29 reported over roughly the last decade (Yano et al. 1997; Yamamoto et al. 2000; Lin et al. 2003;
30 Takeuchi et al. 2003; Uga et al. 2007; Matsubara et al. 2008a; Matsubara et al. 2008b;
31 Matsubara et al. 2011; Shibaya et al. 2011; Gao et al. 2013), and genes have been identified and
32 characterized in 12 QTLs: *Hd1*, *Hd2*, *Hd3a*, *Hd5* (*Ghd8*), *Hd6*, *Hd16*, *Hd17* (*Hd3b*), *Ehd1*,
33 *Ehd2*, *Ehd3*, *Ehd4*, and *Ghd7* (Yano et al. 2000; Takahashi et al. 2001; Kojima et al. 2002; Doi

1 et al. 2004; Matsubara et al. 2008b; Xue et al. 2008; Matsubara et al. 2011; Yan et al. 2011;
2 Matsubara et al. 2012; Hori et al. 2013; Koo et al. 2013). Comprehensive gene identifications in
3 a quantitative trait facilitate direct mapping of genes having unknown genetic contributions to
4 their associated phenotypes.

5
6 An old rice strain, A58, is characterized by extremely early heading date, which thought to be
7 required for adaptation to northernmost limits of cultivation such as Hokkaido (Baruah et al.
8 2011). In studies of F₂ and BC₁F₁ populations between Hokkaido variety Hoshinoyume and
9 Nipponbare (Fujino and Sekiguchi 2005b), alleles of both *Hd2* and *Ghd7* (= *Hd4*) were found to
10 be prerequisite to conferral of extremely early heading date. Some other Hokkaido varieties
11 showing extremely early heading also possess these recessive alleles (Fujino and Sekiguchi
12 2005a; Nonoue et al. 2008). We did not know, however, whether Hokkaido strain A58 also
13 contained the same functional alleles possessed by the other Hokkaido varieties. In this study,
14 we produced F₁ seeds derived from six combinations, in which A58 as the seed parent was
15 crossed with three japonica, Nipponbare, Koshihikari, and Taichung 65 (T65), and three indica
16 strains, #108, IR36, and Kasalath. The F₁ plants were cultivated in a greenhouse equipped with
17 a short-day chamber at Hokkaido University (Sapporo, Japan) in 2012. To evaluate F₂
18 populations, about 200 seeds from each F₁ population were sown in nursery beds in the
19 greenhouse, with 4-week-old seedlings, then transplanted into a paddy field at the university.
20 Sowing and transplanting were performed on May 7 and June 4, 2012. Days to heading of the
21 earliest heading panicle among individual panicles were recorded for each plant to represent the
22 number of days required from sowing to heading.

23
24 Heading status was checked every 2 d, beginning in the middle of July, 2012. All six pollen
25 parents showed later heading dates (114–126 d in Sapporo, Hokkaido, Japan, 43°N latitude)
26 relative to A58 (81 d). The latest strain was IR36, which headed at 126 d. The six F₂
27 populations derived from the cross of A58 with the six pollen parents showed different
28 segregation patterns in terms of heading date (Supplementary Fig. S1). Based on earliest
29 observed heading date compared with that of A58, F₂ populations were classified into three
30 groups: 1) those contained individuals earlier than A58 (F₂ populations of A58/Kasalath
31 [F₂-A58/Kasalath] and F₂-A58/#108); 2) the earliest individuals in those were comparable to
32 A58 (F₂-A58/Nipponbare and F₂-A58/Koshihikari); and 3) those were later than A58
33 (F₂-A58/IR36 and F₂-A58/T65) (Supplementary Fig. S1).

1
2 We randomly selected 93 individuals from each F2 population (comprising about 200
3 individuals each), and performed genotyping using SSR markers (McCouch et al. 2002; Fujino
4 and Sekiguchi 2005a; Matsubara et al. 2008a; Matsubara et al. 2008b) for 19 loci related to
5 heading date (Table 1). Polymorphisms at each locus between A58 and the other strains were
6 assessed by PCR. No polymorphisms were detected between A58 and japonica strains at 11 or 8
7 loci because of genetic similarities to A58. Between A58 and indica strains, polymorphisms
8 were detected at all 19 loci (Table 1). Average heading dates were calculated for all individuals
9 that were homozygous or heterozygous at each locus. The significance of correlations between
10 heading date and alleles at each locus was assessed by ANOVA. The *Ghd7* locus was
11 significantly associated with heading date in four F2 populations (F2-A58/Nipponbare,
12 F2-A58/Kasalath, F2-A58/IR36, and F2-A58/#108) (Table 1), while a correlation with *Hd2* was
13 detected in three (F2-A58/Nipponbare, F2-A58/T65, and F2-A58/IR36) (Table 1). Other
14 significant correlations with heading date were found with *Hd1* (F2-A58/Nipponbare); *Hd6*,
15 *Hd16*, and *Ehd1* (F2-A58/Kasalath); *Hd17* (F2-A58/IR36); and *Hd3* and *Hd5* (F2-A58/#108)
16 (Table 1). Two-way ANOVA detected a strong interaction ($p < 0.01$) between *Ghd7* and *Ehd1*
17 in F2-A58/Kasalath (Supplementary Table S1). We subsequently focused on 20 individuals
18 from the four F2 populations (4 plants from F2-A58/Nipponbare, 2 plants from
19 F2-A58/Koshihikari, 12 plants from F2-A58/Kasalath, and 2 plants from F2-A58/#108) that had
20 earlier or identical heading dates to A58. These extreme individuals were genotyped at the 19
21 loci, and the resulting data were statistically evaluated for non-Mendelian distributions. As
22 shown in Table 2, the distribution of *Ghd7* genotypes was clearly divergent from the expected
23 Mendelian segregation ratio (1:2:1 for two alleles). Eighteen individuals were homozygous at
24 *Ghd7* for the allele from A58, implying its contribution to their extremely early heading dates.
25 In contrast, no contribution to early heading date was observed from the A58 allele at the *Ehd1*
26 locus (Table 2), implying that the homozygous *Ehd1* alleles from A58 tended to inhibit early
27 heading. In fact, a remarkable epistatic interaction ($P = 0.0044$) was detected between *Ghd7*
28 (A58 allele) and *Ehd1* (Kasalath allele) in F2-A58/Kasalath as suggested by two-way ANOVA
29 (Fig. 1). The trade-off interaction between both the wild-type alleles from *Ghd7* and *Ehd1* has
30 actually been observed with respect to their expressions under long-day conditions (Matsubara
31 et al. 2011). In the cited study, expression of *Ghd7* suppressed that of *Ehd1*, leading to delayed
32 floral induction under long-day conditions. Sequence analysis has determined that A58 contains
33 the same recessive *Ghd7* allele found in Hoshinoyume (Lu et al. 2012) (Supplementary Fig. S2),

1 and Doi et al. (2004) have reported that Kasalath contains a functional *Ehd1*. It has thus been
2 suggested that the combination of the A58 allele of *Ghd7* and the Kasalath allele of *Ehd1*
3 accelerates floral induction following basic vegetative growth (Tsuji et al. 2011). In our study,
4 this allele combination promoted a significantly shorter heading date than did A58 alone.

5
6 It is curious that when A58 was crossed with indica strains, some loci in the corresponding F2
7 populations did not segregate in a Mendelian manner irrespective to heading date, i.e., marked
8 genetic distortion occurred in *Hd8* and *Hd13* in F2-A58/IR36 and *Hd1* and *Ghd7* in
9 F2-A58/#108 (Table 1). Such genetic distortion has often been observed in hybrid progenies of
10 distantly related strains. Because of this genetic distortion, a large number of F2 individuals are
11 required to satisfy statistical testing.

12
13 The purpose of this pilot study was to demonstrate the use of assorted F2 populations, rather
14 than RILs or BILs, for rapid extraction of major genetic factors and/or interactions associated
15 with extreme phenotypes in complex QTLs. Heading date was a feasible trait with which to
16 search for functionally important genes: there are at least 19 heading date-related genes or loci
17 currently characterized, which allowed us to perform their genotyping directly. We successfully
18 detected one major genetic interaction associated with extremely early heading date from F2
19 populations derived from crosses between A58, a strain adapted to the northernmost rice
20 cultivation limit, and assorted other strains. The F2 populations examined here exhibited
21 different distributional patterns of heading date frequencies, implying that various combinations
22 of genetic factors were present. As shown in this study, individuals with extreme phenotypes in
23 assorted F2 populations are valuable materials for exploring the major genetic factor(s) involved
24 in such phenotypes. This approach has both strengths and weaknesses; the strengths are to select
25 the most effective one(s) among a broad range of the genetic interactions derived from multiple
26 alleles, and to accomplish isolation of such interaction(s) in a short period as F2 generation,
27 while the weakness is to required a number of F2 plants from the different cross combinations
28 to obtain the conclusive results. Combined with deciphered genomic information, this
29 methodology is broadly applicable to the investigation of various polygenic traits associated
30 with extreme phenotypes, such as biotic and abiotic stress tolerance and disease and insect
31 resistance.

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References

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- 11 Baruah AR, Onishi K, Oguma Y, Ishigo-Oka N, Uwatoko N, Sano Y (2011) Effects of
12 acclimation on chilling tolerance in Asian cultivated and wild rice. *Euphytica*
13 181:293-303.
- 14 Borevitz JO, Chory J (2004) Genomics tools for QTL analysis and gene discovery. *Curr Opin*
15 *Plant Biol* 7:132-136.
- 16 Doi K, Izawa T, Fuse T, Yamanouchi U, Kubo T, Shimatani Z, Yano M, Yoshimura A (2004)
17 *Ehd1*, a B-type response regulator in rice, confers short-day promotion of flowering and
18 controls FT-like gene expression independently of Hd11. *Gene Dev* 18:926-936.
- 19 Ebana K, Shibaya T, Wu JZ, Matsubara K, Kanamori H, Yamane H, Yamanouchi U,
20 Mizubayashi T, Kono I, Shomura A, Ito S, Ando T, Hori K, Matsumoto T, Yano M
21 (2011) Uncovering of major genetic factors generating naturally occurring variation in
22 heading date among Asian rice cultivars. *Theor Appl Genet* 122:1199-1210.
- 23 Fujino K, Sekiguchi H (2005a) Identification of QTLs conferring genetic variation for heading
24 date among rice varieties at the northern-limit of rice cultivation. *Breeding Sci*
25 55:141-146.
- 26 Fujino K, Sekiguchi H (2005b) Mapping of QTLs conferring extremely early heading in rice
27 (*Oryza sativa* L.). *Theor Appl Genet* 111:393-398.
- 28 Ganai MW, Altmann T, Roder MS (2009) SNP identification in crop plants. *Curr Opin Plant*
29 *Biol* 12:211-217.
- 30 Gao H, Zheng XM, Fei GL, Chen J, Jin MN, Ren YL, Wu WX, Zhou KN, Sheng PK, Zhou F,
31 Jiang L, Wang J, Zhang X, Guo XP, Wang JL, Cheng ZJ, Wu CY, Wang HY, Wan JM
32 (2013) *Ehd4* encodes a novel and *Oryza*-genus-specific regulator of photoperiodic
33 flowering in rice. *Plos Genet* 9.

1 Hori K, Ogiso-Tanaka E, Matsubara K, Yamanouchi U, Ebana K, Yano M (2013) Hd16, a gene
2 for casein kinase I, is involved in the control of rice flowering time by modulating the
3 day-length response. *Plant J*.

4 Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M (2002) Hd3a, a
5 rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream
6 of Hd1 under short-day conditions. *Plant Cell Physiol* 43:1096-1105.

7 Koo BH, Yoo SC, Park JW, Kwon CT, Lee BD, An G, Zhang Z, Li J, Li Z, Paek NC (2013)
8 Natural variation in OsPRR37 regulates heading date and contributes to rice cultivation
9 at a wide range of latitudes. *Mol Plant*.

10 Lin HX, Liang ZW, Sasaki T, Yano M (2003) Fine mapping and characterization of quantitative
11 trait loci Hd4 and Hd5 controlling heading date in rice. *Breeding Sci* 53:51-59.

12 Lu L, Yan WH, Xue WY, Shao D, Xing YZ (2012) Evolution and association analysis of Ghd7
13 in rice. *Plos One* 7.

14 Matsubara K, Kono I, Hori K, Nonoue Y, Ono N, Shomura A, Mizubayashi T, Yamamoto S,
15 Yamanouchi U, Shirasawa K, Nishio T, Yano M (2008a) Novel QTLs for photoperiodic
16 flowering revealed by using reciprocal backcross inbred lines from crosses between
17 japonica rice cultivars. *Theor Appl Genet* 117:935-945.

18 Matsubara K, Ogiso-Tanaka E, Hori K, Ebana K, Ando T, Yano M (2012) Natural variation in
19 Hd17, a homolog of Arabidopsis ELF3 that is involved in rice photoperiodic flowering.
20 *Plant Cell Physiol* 53:709-716.

21 Matsubara K, Yamanouchi U, Nonoue Y, Sugimoto K, Wang ZX, Minobe Y, Yano M (2011)
22 Ehd3, encoding a plant homeodomain finger-containing protein, is a critical promoter of
23 rice flowering. *Plant J* 66:603-612.

24 Matsubara K, Yamanouchi U, Wang ZX, Minobe Y, Izawa T, Yano M (2008b) Ehd2, a rice
25 ortholog of the maize INDETERMINATE1 gene, promotes flowering by up-regulating
26 Ehd1. *Plant Physiol* 148:1425-1435.

27 McCouch SR, Teytelman L, Xu YB, Lobos KB, Clare K, Walton M, Fu BY, Maghirang R, Li
28 ZK, Xing YZ, Zhang QF, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D,
29 Cartinhour S, Ware D, Stein L (2002) Development and mapping of 2240 new SSR
30 markers for rice (*Oryza sativa* L.). *DNA Res* 9:199-207.

31 Nonoue Y, Fujino K, Hirayama Y, Yamanouchi U, Lin SY, Yano M (2008) Detection of
32 quantitative trait loci controlling extremely early heading in rice. *Theor Appl Genet*
33 116:715-722.

- 1 Shibaya T, Nonoue Y, Ono N, Yamanouchi U, Hori K, Yano M (2011) Genetic interactions
2 involved in the inhibition of heading by heading date QTL, Hd2 in rice under long-day
3 conditions. *Theor Appl Genet* 123:1133-1143.
- 4 Takahashi Y, Shomura A, Sasaki T, Yano M (2001) Hd6, a rice quantitative trait locus involved
5 in photoperiod sensitivity, encodes the alpha subunit of protein kinase CK2. *Proc Natl*
6 *Acad Sci USA* 98:7922-7927.
- 7 Takeuchi Y, Lin SY, Sasaki T, Yano M (2003) Fine linkage mapping enables dissection of
8 closely linked quantitative trait loci for seed dormancy and heading in rice. *Theor Appl*
9 *Genet* 107:1174-1180.
- 10 Tsuji H, Taoka K, Shimamoto K (2011) Regulation of flowering in rice: two florigen genes, a
11 complex gene network, and natural variation. *Curr Opin Plant Biol* 14:45-52.
- 12 Uga Y, Nonoue Y, Liang ZW, Lin HX, Yamamoto S, Yamanouchi U, Yano M (2007)
13 Accumulation of additive effects generates a strong photoperiod sensitivity in the
14 extremely late-heading rice cultivar 'Nona Bokra'. *Theor Appl Genet* 114:1457-1466.
- 15 Varshney RK, Nayak SN, May GD, Jackson SA (2009) Next-generation sequencing
16 technologies and their implications for crop genetics and breeding. *Trends Biotechnol*
17 27:522-530.
- 18 Wurschum T (2012) Mapping QTL for agronomic traits in breeding populations. *Theor Appl*
19 *Genet* 125:201-210.
- 20 Xue WY, Xing YZ, Weng XY, Zhao Y, Tang WJ, Wang L, Zhou HJ, Yu SB, Xu CG, Li XH,
21 Zhang QF (2008) Natural variation in Ghd7 is an important regulator of heading date
22 and yield potential in rice. *Nat Genet* 40:761-767.
- 23 Yamamoto T, Lin HX, Sasaki T, Yano M (2000) Identification of heading date quantitative trait
24 locus Hd6 and characterization of its epistatic interactions with Hd2 in rice using
25 advanced backcross progeny. *Genetics* 154:885-891.
- 26 Yamamoto T, Yonemaru J, Yano M (2009) Towards the understanding of complex traits in rice:
27 substantially or superficially? *DNA Res* 16:141-154.
- 28 Yan WH, Wang P, Chen HX, Zhou HJ, Li QP, Wang CR, Ding ZH, Zhang YS, Yu SB, Xing
29 YZ, Zhang QF (2011) A major QTL, Ghd8, plays pleiotropic roles in regulating grain
30 productivity, plant height, and heading date in rice. *Mol Plant* 4:319-330.
- 31 Yano M, Harushima Y, Nagamura Y, Kurata N, Minobe Y, Sasaki T (1997) Identification of
32 quantitative trait loci controlling heading date in rice using a high-density linkage map.
33 *Theor Appl Genet* 95:1025-1032.

1 Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K,
2 Umehara Y, Nagamura Y, Sasaki T (2000) Hd1, a major photoperiod sensitivity
3 quantitative trait locus in rice, is closely related to the arabidopsis flowering time gene
4 CONSTANS. *Plant Cell* 12:2473-2483.

7 **Figure legends**

8 Fig. 1

9 Effect of genetic interactions between *Ghd7* and *Ehd1* on heading date in an F2-A58/Kasalath
10 population. Combinations of the two loci, *Ghd7* and *Ehd1*, from A58 and Kasalath were either
11 homozygous (A58 and Kasalath) or heterozygous (Hetero). Labels below bars refer to *Ghd7*
12 genotypes; bar colors refer to *Ehd1* genotypes (white: A58 homozygous; gray: heterozygous;
13 black: Kasalath homozygous). Numbers above bars correspond to number of individuals
14 possessing the genotype combination. Error bars indicate standard errors.

15
16 Supplementary Fig. S1

17 Frequency and distribution of days to heading in six F2 populations. (a) F2-A58/Nipponbare (n
18 = 209); (b) F2-A58/Koshihikari (n = 212); (c) F2-A58/T65 (n = 195); (d) F2-A58/Kasalath (n =
19 213); (e) F2-A58/IR36 (n = 194); (f) F2-A58/#108 (n = 238). Arrowheads indicate parental
20 mean heading dates.

21
22 Supplementary Fig. S2

23 Amino acid variation in the Ghd7 protein deduced from nucleotide sequences in the seven
24 studied rice strains. Positions correspond to those of the 257 amino acids of the Nipponbare
25 Ghd7 polypeptide. All variable positions characterized by Lu et al. (2012) are noted. A
26 nonsense mutation is present in the *Ghd7* allele of A58; mutations occurring in the other strains
27 most likely do not seriously impair proper function. The CCT (CONSTANS, CO-like, and
28 TOC1) domain is a highly conserved basic module of ~43 amino acids, and is characteristic of
29 the Ghd7 polypeptide.

30

Table 1 Summary of genotyping analysis for heading date using 19 markers in the six F2 populations

| F2 population (n=93) ^a | Target gene or QTL | Marker ^b | Mean of DTH ^c | | | Probability |
|--------------------------------------|-----------------------|---------------------|--------------------------|------------|------------|-------------|
| | | | A (n) | B (n) | H (n) | |
| A58/Nipponbare | <i>Hd1</i> | Hd1 | 97 (21) | 109 (25) | 101.3 (47) | 0.0002* |
| | <i>Hd2</i> | RM1306 | 96 (22) | 110 (23) | 102 (48) | 0.0000* |
| | <i>Ghd7</i> | RM21327 | 95 (29) | 106 (19) | 105 (45) | 0.0000* |
| | <i>Hd6</i> | RM15956 | 97 (17) | 103 (27) | 104 (49) | 0.0794 |
| | <i>Hd10</i> | RM17071 | 104 (25) | 100 (24) | 102 (44) | 0.3821 |
| | <i>Hd11</i> | RM16278 | 101 (28) | 104 (21) | 102 (44) | 0.5199 |
| | <i>Hd12</i> | RM23471 | 103 (30) | 102 (22) | 102 (41) | 0.9686 |
| | <i>Hd16</i> | 87C10-17 | 98 (22) | 104 (23) | 103 (48) | 0.0636 |
| A58/Koshihikari | <i>Hd1</i> | Hd1 | 105 (21) | 105 (31) | 103 (41) | 0.7492 |
| | <i>Hd2</i> | RM1306 | 103 (15) | 105 (21) | 104 (57) | 0.8553 |
| | <i>Ghd7</i> | RM21327 | 100 (24) | 104 (16) | 107 (53) | 0.0668 |
| | <i>Hd6</i> | RM15956 | 106 (22) | 106 (29) | 102 (42) | 0.3170 |
| | <i>Hd10</i> | RM17071 | 108 (22) | 104 (24) | 103 (47) | 0.2634 |
| | <i>Hd11</i> | RM16278 | 108 (27) | 105 (24) | 102 (42) | 0.0917 |
| | <i>Hd12</i> | RM23471 | 105 (24) | 105 (20) | 104 (49) | 0.8544 |
| | <i>Hd13</i> | RM27727 | 105 (21) | 105 (24) | 104 (48) | 0.9315 |
| | <i>Hd16</i> | RM1038 | 105 (23) | 107 (24) | 103 (46) | 0.3046 |
| | <i>Hd17</i> | P548D347 | 104 (26) | 103 (19) | 105 (48) | 0.8179 |
| A58/T65 | <i>Hd1</i> | Hd1 | 105 (22) | 100 (31) | 101 (40) | 0.0145 |
| | <i>Hd2</i> | RM1306 | 98 (25) | 107 (27) | 101 (41) | 0.0000* |
| | <i>Ghd7</i> | RM7110 | 104 (20) | 100 (20) | 102 (53) | 0.1167 |
| | <i>Hd7</i> | RM3857 | 103 (26) | 102 (25) | 102 (42) | 0.6281 |
| | <i>Hd11</i> | RM16278 | 101.4 (25) | 102.3 (21) | 102.1 (47) | 0.8582 |
| | <i>Hd12</i> | RM23471 | 101 (23) | 101 (24) | 103 (46) | 0.1357 |
| | <i>Hd16</i> | 87C10-17 | 102 (18) | 103 (22) | 102 (53) | 0.5486 |
| | <i>Ehd1</i> | RM25539 | 99 (18) | 104 (30) | 102 (45) | 0.0263 |
| A58/Kasalath | <i>Hd1</i> | Hd1 | 104 (14) | 98 (37) | 100 (42) | 0.1366 |
| | <i>Hd2</i> | RM1306 | 99 (25) | 104 (26) | 97 (42) | 0.0163 |
| | <i>Hd3</i> | RM204 | 106 (16) | 97 (37) | 100 (40) | 0.0099* |
| | <i>Ghd7</i> | RM5499 | 87 (23) | 108 (26) | 101 (44) | 0.0000* |
| | <i>Hd5</i> | GBR8001 | 95 (23) | 99 (23) | 102 (47) | 0.0253 |
| | <i>Hd6</i> | RM15956 | 94 (25) | 100 (17) | 102 (51) | 0.0061* |
| | <i>Hd7</i> | RM8030 | 99 (15) | 100 (29) | 100 (49) | 0.9222 |
| | <i>Hd8</i> | RM5442 | 94 (15) | 101 (31) | 100 (47) | 0.0602 |
| | <i>Hd9</i> | GBR3002 | 93 (18) | 102 (31) | 100 (44) | 0.0190 |
| | <i>Hd10</i> | RM17067 | 99 (31) | 104 (19) | 98 (43) | 0.1464 |
| | <i>Hd11</i> | RM16278 | 101 (17) | 101 (19) | 99 (57) | 0.7347 |
| | <i>Hd12</i> | RM23471 | 101 (30) | 100 (27) | 98 (36) | 0.5581 |
| | <i>Hd13</i> | RM27727 | 97 (28) | 102 (17) | 100 (48) | 0.3564 |
| | <i>Hd16</i> | RM1038 | 93 (25) | 101 (20) | 102 (48) | 0.0012* |
| | <i>Hd17</i> | P548D347 | 104 (18) | 96 (36) | 100 (39) | 0.0328 |
| | <i>Ehd1</i> | RM25539 | 106 (24) | 96 (29) | 98 (40) | 0.0006* |
| | <i>Ehd2</i> | SSR-1 | 104 (22) | 96 (31) | 99 (40) | 0.0164 |
| | <i>Ehd3</i> | RM1381 | 99 (17) | 99 (31) | 100 (45) | 0.9710 |
| | <i>Ehd4</i> | RM6349 | 94 (16) | 102 (34) | 99 (43) | 0.0466 |

| | | | | | | |
|-------------|-------------|------------|----------|----------|----------|----------|
| A58/IR36 | <i>Hd1</i> | Hd1 | 116 (19) | 114 (25) | 116 (47) | 0.6730 |
| | <i>Hd2</i> | RM1306 | 110 (23) | 120 (21) | 116 (47) | 0.0008* |
| | <i>Hd3</i> | RM204 | 113 (28) | 120 (21) | 115 (42) | 0.0115 |
| | <i>Ghd7</i> | RM5499 | 110 (14) | 119 (31) | 115 (46) | 0.0090* |
| | <i>Hd5</i> | GBR8001 | 117 (33) | 112 (17) | 116 (41) | 0.1248 |
| | <i>Hd6</i> | RM15956 | 117 (19) | 117 (27) | 114 (45) | 0.4337 |
| | <i>Hd7</i> | RM8030 | 114 (23) | 117 (28) | 116 (40) | 0.3218 |
| | <i>Hd8</i> | RM5442 | 107 (4) | 114 (40) | 118 (47) | 0.0176 |
| | <i>Hd9</i> | GBR3002 | 115 (18) | 116 (21) | 116 (52) | 0.8517 |
| | <i>Hd10</i> | RM17071 | 115 (19) | 115 (27) | 116 (45) | 0.8487 |
| | <i>Hd11</i> | RM16278 | 116 (17) | 117 (32) | 114 (42) | 0.3102 |
| | <i>Hd12</i> | RM23471 | 119 (25) | 116 (21) | 114 (45) | 0.0559 |
| | <i>Hd13</i> | RM27727 | 119 (7) | 114 (37) | 117 (47) | 0.2296 |
| | <i>Hd16</i> | RM1038 | 117 (17) | 119 (23) | 114 (51) | 0.1019 |
| | <i>Hd17</i> | 0007o20 | 114 (33) | 123 (19) | 114 (39) | 0.0006* |
| | <i>Ehd1</i> | RM25539 | 119 (16) | 116 (26) | 115 (49) | 0.2862 |
| | <i>Ehd2</i> | SSR-1 | 118 (18) | 116 (25) | 115 (48) | 0.3162 |
| | <i>Ehd3</i> | RM1381 | 115 (20) | 117 (23) | 115 (48) | 0.7285 |
| | <i>Ehd4</i> | RM6349 | 115 (24) | 116 (17) | 115 (50) | 0.8740 |
| | A58/#108 | <i>Hd1</i> | Hd1 | 97 (8) | 109 (43) | 104 (42) |
| <i>Hd2</i> | | RM1306 | 104 (28) | 105 (23) | 107 (42) | 0.5110 |
| <i>Hd3</i> | | RM204 | 101 (22) | 111 (29) | 104 (42) | 0.0044* |
| <i>Ghd7</i> | | RM5499 | 87 (9) | 112 (43) | 103 (41) | 0.0000* |
| <i>Hd5</i> | | GBR8001 | 110 (21) | 96 (25) | 109 (47) | 0.0000* |
| <i>Hd6</i> | | RM15956 | 103 (30) | 109 (26) | 105 (37) | 0.2099 |
| <i>Hd7</i> | | RM8030 | 106 (24) | 106 (20) | 105 (49) | 0.9150 |
| <i>Hd8</i> | | RM5442 | 104 (10) | 106 (51) | 105 (32) | 0.8096 |
| <i>Hd9</i> | | GBR3002 | 103 (17) | 109 (39) | 103 (37) | 0.0452 |
| <i>Hd10</i> | | RM17071 | 105 (31) | 105 (26) | 106 (36) | 0.8216 |
| <i>Hd11</i> | | RM16278 | 102 (19) | 108 (28) | 105 (46) | 0.2900 |
| <i>Hd12</i> | | RM23471 | 109 (18) | 107 (25) | 104 (50) | 0.1949 |
| <i>Hd13</i> | | RM27727 | 106 (19) | 103 (26) | 106 (48) | 0.5218 |
| <i>Hd16</i> | | RM1373 | 104 (27) | 108 (26) | 105 (40) | 0.3680 |
| <i>Hd17</i> | | P548D347 | 102 (24) | 110 (22) | 105 (47) | 0.0342 |
| <i>Ehd1</i> | | RM25539 | 110 (24) | 106 (30) | 102 (39) | 0.0206 |
| <i>Ehd2</i> | | SSR-1 | 108 (23) | 109 (29) | 107 (41) | 0.1131 |
| <i>Ehd3</i> | | RM1381 | 110 (22) | 106 (31) | 103 (40) | 0.0836 |
| <i>Ehd4</i> | | RM6349 | 104 (19) | 108 (32) | 105 (42) | 0.4207 |

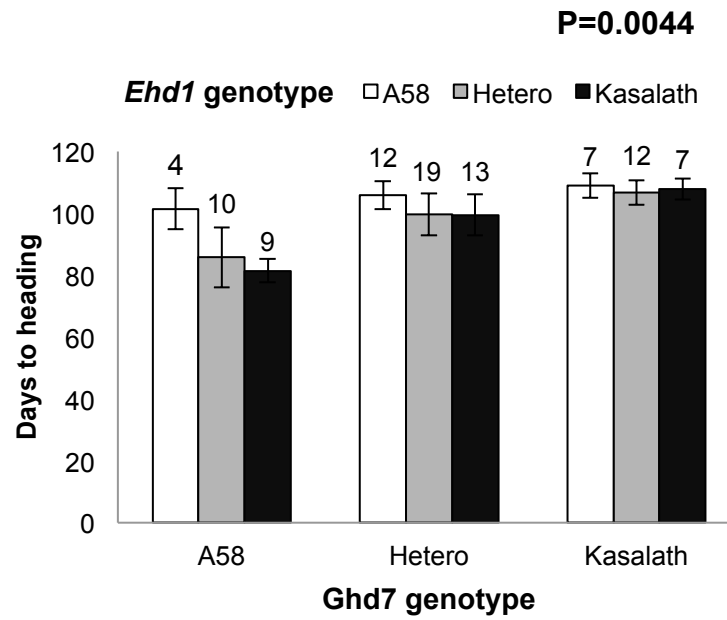
a: Paternal strains (A/B=femal/male), 93 individuals randomly selected from each of the F2 populations were examined; b: SSR markers were referred from following references; Fujino and Sekiguchi (2005a) for Hd1, GBR3002, GBR8001, McCouch et al. (2002) for all the markers with RM numbers, Matsubara et al. (2008a) for P548D347, 0007o20, Matsubara et al. (2008b) for SSR-1; c: DTH (days to heading), A represents homozygote of A58 allele, B is homozygote of the pollen parental allele, and H is heterozygote; * indicates a significance level of 1% as determined by ANOVA.

Table 2 Number of individuals expressing the extremely early heading trait categorized by genotype for 19 loci^a

| Genotype/locus | <i>Hd1</i> | <i>Hd2</i> | <i>Hd3</i> | <i>Ghd7</i> | <i>Hd5</i> | <i>Hd6</i> | <i>Hd7</i> | <i>Hd8</i> | <i>Hd9</i> | <i>Hd10</i> | <i>Hd11</i> | <i>Hd12</i> | <i>Hd13</i> | <i>Hd16</i> | <i>Hd17</i> | <i>Ehd1</i> | <i>Ehd2</i> | <i>Ehd3</i> | <i>Ehd4</i> |
|---|------------|------------|------------|-------------|------------|------------|------------|------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Homozygous for A58 | 2 | 8 | 2 | 18 | 7 | 7 | 3 | 4 | 6 | 5 | 4 | 2 | 6 | 7 | 3 | 0 | 2 | 3 | 6 |
| Heterozygous | 8 | 11 | 5 | 1 | 5 | 8 | 7 | 7 | 6 | 12 | 13 | 12 | 7 | 8 | 5 | 8 | 5 | 9 | 6 |
| Homozygous for pollen parent | 10 | 1 | 7 | 1 | 2 | 5 | 4 | 3 | 2 | 3 | 3 | 6 | 3 | 5 | 8 | 6 | 7 | 2 | 2 |
| No. of examined plants | 20 | 20 | 14 | 20 | 14 | 20 | 14 | 14 | 14 | 20 | 20 | 20 | 16 | 20 | 16 | 14 | 14 | 14 | 14 |
| Fit to Mendelian segregation ratio ^b | 0.027 | 0.078 | 0.095 | 2E-10 | 0.095 | 0.55 | 0.93 | 0.93 | 0.28 | 0.55 | 0.39 | 0.3 | 0.5 | 0.55 | 0.068 | 0.066 | 0.095 | 0.53 | 0.28 |

a: The 20 individuals showing extremely early heading comprised 4 in F2-A58/Nipponbare, 2 in F2-A58/Koshihikari, 12 in F2-A58/Kasalath and 2 in F2-A58/#108. b: *P*-values for the probability to Mendelian segregation of genotypes for each loci were obtained by a Chi-square test (1:2:1 ratio).

Figure 1



Supplementary Table S1 Two-way ANOVA based on Table 1 to detect additive interaction between different alleles shown by P-value.

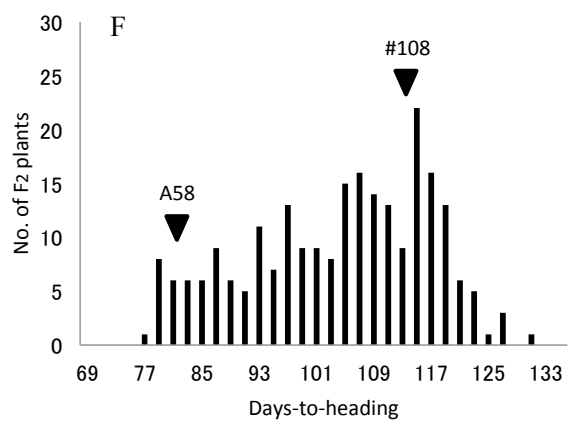
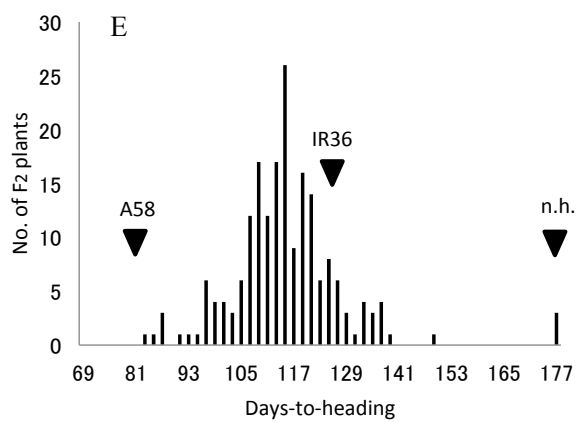
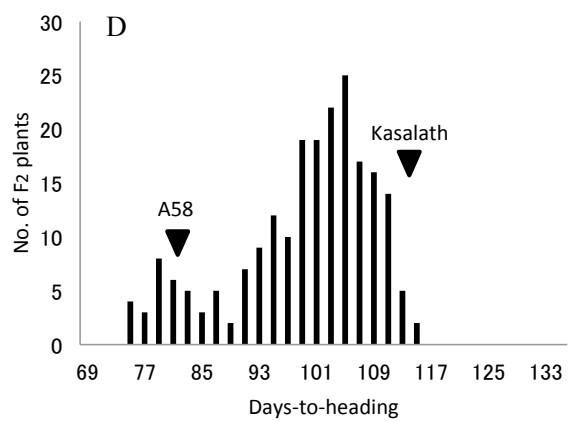
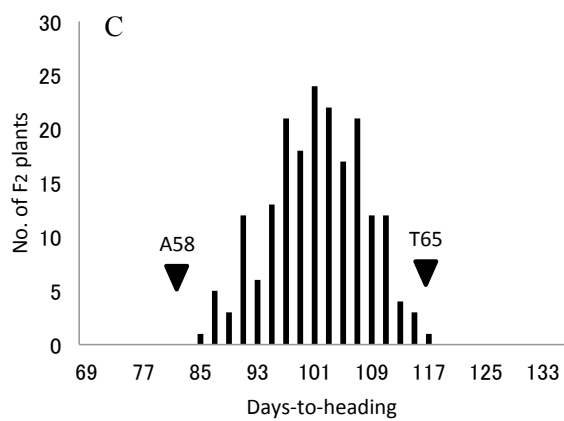
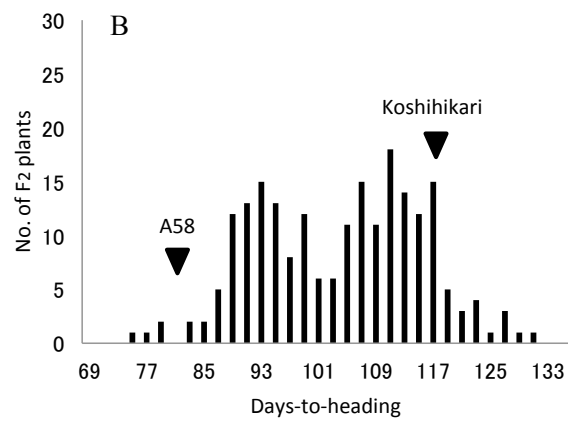
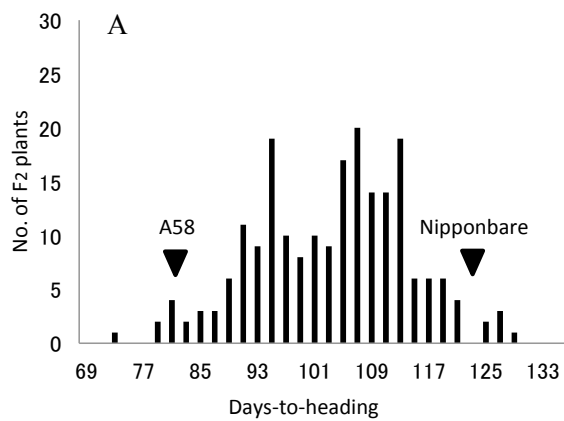
| A58/Nipponbare | Hd1 | Hd2 | Ghd7 |
|----------------|----------|---------|------|
| Hd1 | | | |
| Hd2 | 0.807855 | | |
| Ghd7 | 0.038454 | 0.64416 | |

| A58/Kasalath | Hd3 | Ghd7 | Hd6 | Hd16 | Ehd1 |
|--------------|----------|----------|-----------|---------|------|
| Hd3 | | | | | |
| Ghd7 | 0.411932 | | | | |
| Hd6 | 0.111289 | 0.329899 | | | |
| Hd16 | 0.054606 | 0.055915 | 0.789351a | | |
| Ehd1 | 0.721323 | 0.009632 | 0.592643 | 0.20737 | |

| A58/IR36 | Hd2 | Ghd7 | Hd17 |
|----------|-----------|-----------|------|
| Hd2 | | | |
| Ghd7 | 0.701702a | | |
| Hd17 | 0.727955 | 0.563473a | |

| A58/#108 | Hd3 | Ghd7 | Hd5 |
|----------|----------|----------|-----|
| Hd3 | | | |
| Ghd7 | 0.803396 | | |
| Hd5 | 0.031798 | 0.182031 | |

a: short of degree of freedom, yellow cell p < 5%, red cell p < 1%



Supplementary Figure S2

