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Title	Isolation of a major genetic interaction associated with an extreme phenotype using assorted F2 populations in rice
Author(s)	Ota, Yuya; Ishiguro, Seiya; Aoyama, Eiko et al.
Citation	Molecular Breeding, 33(4), 997-1003 https://doi.org/10.1007/s11032-013-9998-6
Issue Date	2014-04
Doc URL	https://hdl.handle.net/2115/60043
Rights	The final publication is available at Springer via http://dx.doi.org/10.1007/s11032-013-9998-6
Type	journal article
File Information	Ota et al Molucular Breeding MS.pdf



Short communication

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

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Abstract

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

Keywords

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

Text

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

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

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

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

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

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

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

Acknowledgments

We are especially grateful to Ms. Takako Takeuchi (Research Faculty of Agriculture, Hokkaido University) for technical assistance and Drs. Kaworu Ebana and Masahiro Yano (QTL Genomic Research Center, National Institute of Agrobiological Sciences) for technical advice regarding the field data recording system. This research was supported by funding from the Scientific Technique Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry.

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Figure legends

Fig. 1

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

Supplementary Fig. S1

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

Supplementary Fig. S2

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

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)	0.0130
	<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

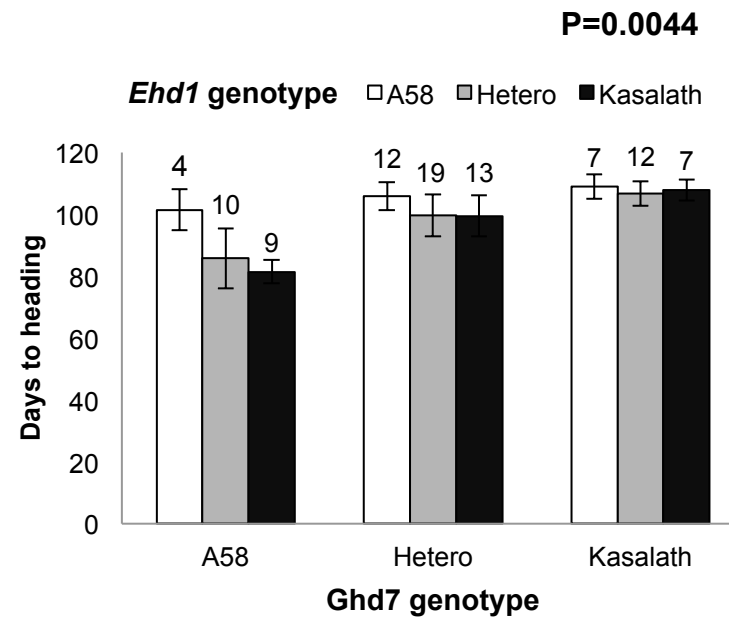
a: Paternal strains (A/B=femal/male), 93 individuals randomly selected from each of the F2 populations were examined; b: SSR markers were referred from following references; Fujino and Sekiguchi (2005a) for Hd1, GBR3002, GBR8001, McCouch et al. (2002) for all the markers with RM numbers, Matsubara et 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.

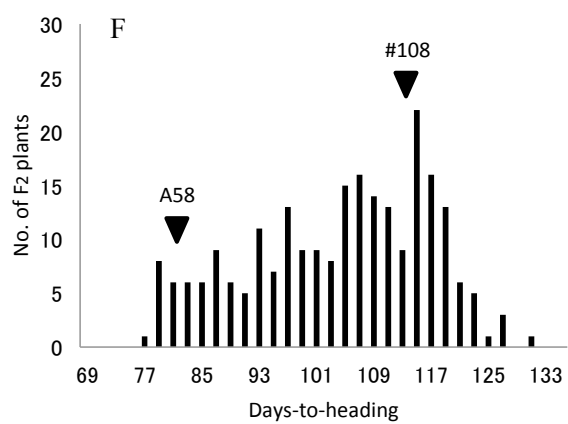
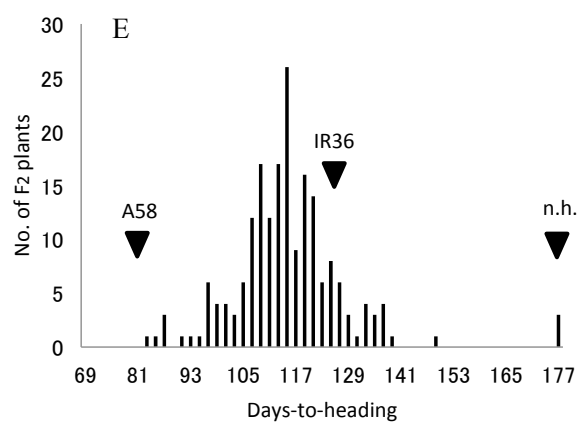
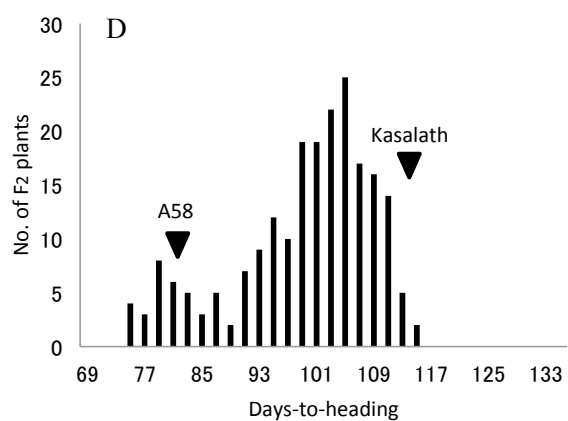
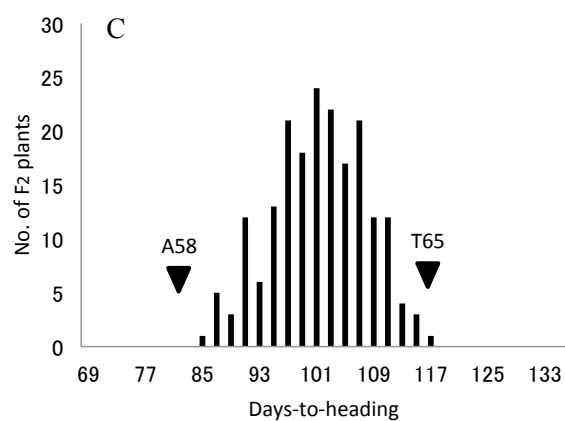
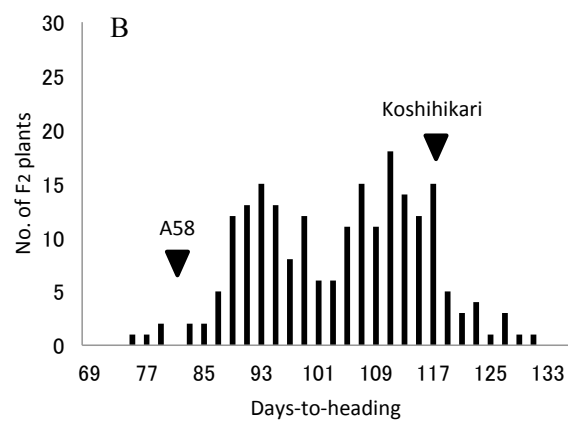
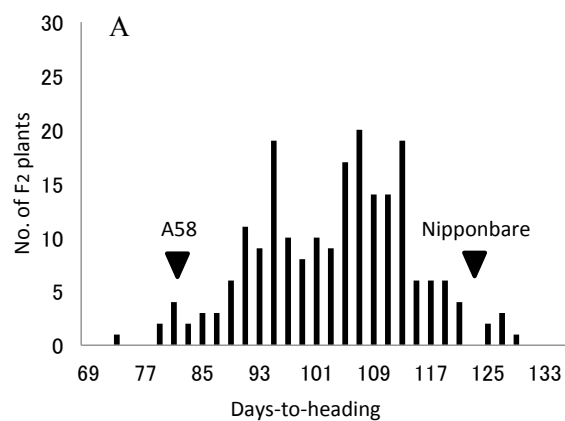
A58/Nipponbare	Hd1	Hd2	Ghd7
Hd1			
Hd2	0.807855		
Ghd7	0.038454	0.64416	

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

