

Breeding strategies for optimum heading date using genotypic information in rice

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Abstract Heading date (HD) is a key trait for the adaptation of rice cultivar to a specific growing region. Here, we report conventional and marker-assisted breeding strategies using genetic information related to the determination of HD, where the breeding objectives were to avoid the delayed heading common in *indica* × *japonica* hybrids, to increase the efficiency in selecting hybrid rice combinations having a suitable growth duration, and to develop cultivars with target growth duration by quantitative trait locus (QTL) pyramiding. The allelic constitution at the major HD loci was determined for a set of 109 leading Chinese rice cultivars by crossing them with HD tester lines. It was shown that the late heading in *indica* × *japonica* hybrids can be overcome by replacing the strong photoperiod-sensitivity

allele *Se-1ⁿ* with *Se-1^e*. A breeding strategy to enable the selection of hybrid combinations with suitable growth duration was proposed, based on HD genotypic information in rice. Meanwhile, a QTL analysis for HD was conducted over five years based on a recombinant inbred line population, derived from two parents Asominori (*japonica*) and IR24 (*indica*). Four QTLs, located on chromosomes 2, 3, 6, and 8, respectively, could be detected in all five years, indicating they were stably expressed QTL. According to this QTL information, and taking Asominori as an example, the HD genotypes for improving the growth duration were designed, and the best breeding selection schemes were determined by use of a genetic breeding simulation tool. Results obtained in this study demonstrate that genetic information related to HD can make a significant contribution to rice breeding.

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Introduction

Heading date (HD) is one of the most important traits for rice to adapt to different areas and cropping seasons. Time taken for a cultivar to head is mainly determined by a combination of, or interaction between, basic vegetative growth period (BVG) and

its photoperiod-sensitivity (PS) and temperature-sensitivity (TS). Among numerous HD genes that have been identified, *Ef-1* plays a major role in controlling the BVG by accelerating the switch to reproductive growth independently of photoperiod, and partially counteracting the effects of the PS gene under long-day conditions (Tsai 1986; Kinoshita 1995; Sato et al. 1988; Nishida et al. 2002; Xu et al. 2006). *Se-1* and *E₁* are known as strong PS genes, proven to be the most ubiquitous genes controlling HD (Ichitani et al. 1997, 1998; Okumoto et al. 1996). Many other genes also affect HD through interaction with *Se-1* or *E₁*. For example, *i-Se-1* can inhibit the effect of *Se-1*, producing an early heading type even under long-day conditions (Ohshima et al. 1993; Ohshima and Kikuchi 1994; Luo et al. 2002). Co-presence of *E₁* and *Se-1* produces a very strong level of PS, manifested as a very late heading type under long-day conditions (Cai et al. 1987; Luo et al. 2002).

To date, a growing number of QTL for HD has emerged from analysis of various mapping populations (Li et al. 1995; Yano et al. 1997; Lin et al. 1998, 2002; Maheswaran et al. 2000; Yamamoto et al. 2000; Yu et al. 2002), and some (*Hd1*, *Hd3a*, *Hd6*, *Ehd1* and *Ghd7*) have been isolated by map-based cloning (Yano et al. 2000; Takahashi et al. 2001; Kojima et al. 2002; Doi et al. 2004; Xue et al. 2008). The coincidence of both the phenotypic effect and chromosomal location suggests that *Se-1* is either identical or allelic to *Hd1*, *Ef-1* to *Ehd1*, and *i-Se-1* to *hd2* (Yano et al. 2000; Doi et al. 2004; Ohshima et al. 1993; Ohshima and Kikuchi 1994; Luo et al. 2002; Xu et al. 2006).

HD is genetically diversified in rice, which provides a wealth of variation for rice to adapt in a vast range of growing areas worldwide. However, it also increases the breeding complexity for a specific area with optimum growth duration (Luo et al. 2001). Late heading in *indica* × *japonica* hybrids limits the exploitation of substantial inter-species heterosis. Conflict between early heading and high yielding always appears in hybrid rice breeding (Deng et al. 2001). Along with a growing number of sterile and restorer lines with different HDs, breeding hybrids for certain areas was even harder than before. To overcome these difficulties, breeders have to blindly make many crosses for repeated testing in the field, which results in tremendous work and low breeding efficiency. If an optimum HD for a certain area can

be designed using known genetic information, breeding will become more directed and efficient than the conventional way (Peleman and van der Voort 2003; Wang et al. 2005; Wan 2006; Wang and Pfeiffer 2007). In order to avoid the delayed heading common in *indica* × *japonica* hybrids, to increase selection efficiency in hybrid rice, and to obtain lines with an HD suited to a particular rice-growing region, in this paper we first describe the HD genetic make-up of a sample of 109 leading rice cultivars from across China, and then report use of a QTL approach to analyze the genetic determination of HD in a recombinant inbred population. On the basis of this genotypic information, more efficient strategies were devised for breeding rice cultivars with optimum HD.

Materials and methods

Materials

The following genetic stocks, cultivars, hybrids and populations were employed:

- HD near-isogenic lines (NILs) EG0, EG1, ER, LR, T65m, and T65E^bm (Yamagata et al. 1986; Ichitani et al. 1997, 1998; Inoue et al. 1998), and two HD QTL near-isogenic lines NIL(*Hd1*) and NIL(*Hd2*) (Yamamoto et al. 1998; Lin et al. 2000) (Table 1);
- 109 leading cultivars in production that are representative of different ecological regions in China (Supplementary Table 1) and cv. Nipponbare; F₁ hybrids between the 109 leading cultivars and the genetic stock lines;
- 71 recombinant inbred lines (RILs) derived from Asominori (*japonica*) × IR24(*indica*) (Tsunematsu et al. 1996); and
- 66 chromosome segment substitution lines (CSSLs) including segments of IR24 in Asominori background (Kubo et al. 1999).

Determination of HD genotype for each cultivar

Stock lines EG0 and EG1 are a pair NILs differing only at the *E₁* locus (EG0 is *E₁*, and EG1 is *e₁*) (Ichitani et al. 1997, 1998) (Table 1). The PS allele *E₁* is dominant over the *e₁* (Okumoto et al. 1992,

Table 1 The genotypes and heading dates of near isogenic lines (NIL)

NIL	Genotype	Days to heading	Gene effect (days)
EG0	$e_1e_1 e_2e_2 e_3e_3 Se-1^n Se-1^n Ef-1Ef-1$	71.2 ± 0.6	–
EG1	$E_1E_1 e_2e_2 e_3e_3 Se-1^n Se-1^n Ef-1Ef-1$	90.4 ± 0.7	19.2
ER	$E_1E_1 e_2e_2 E_3E_3 Se-1^e Se-1^e Ef-1Ef-1$	80.7 ± 1.3	–
LR	$E_1E_1 e_2e_2 E_3E_3 Se-1^u Se-1^u Ef-1Ef-1$	102.7 ± 1.0	22.0
T65m	$e_1e_1 E_2E_2 E_3E_3 Se-1^e Se-1^e ef-1ef-1$	96.9 ± 1.7	–
T65E ^b m	$e_1e_1 E_2E_2 E_3E_3 Se-1^e Se-1^e Ef-1Ef-1$	75.6 ± 1.6	21.3
Nipponbare	$E_1E_1 e_2e_2 e_3e_3 Se-1^n Se-1^n Hd2Hd2$	86.9 ± 1.0	–
NIL(<i>Hd1</i>)	$E_1E_1 e_2e_2 e_3e_3 Se-1^e Se-1^e Hd2Hd2$	77.4 ± 1.0	9.5
NIL(<i>Hd2</i>)	$E_1E_1 e_2e_2 e_3e_3 Se-1^n Se-1^n hd2hd2$	79.9 ± 1.5	7.0

Values are expressed as mean ± SD

1996). The HD of EG1 was 19.2 days later than that of EG0 under natural long-day conditions at Nanjing (latitude 32°N), when photoperiods in May, June, July, and August were 13.7 hours light (hL)/10.3 hours dark (hD), 14.2 hL/9.8 hD, 14.0 hL/10.0 hD, and 13.2 hL/10.8 hD, respectively. If the HD of EG1 × “cultivar A” F₁ hybrid is about 19.2 days later than that of EG0 × cultivar A hybrid, cultivar A was considered to carry the no-PS allele e_1 . If HDs are similar in two F₁ hybrids, cultivar A should be deemed to carry the PS allele E_1 . If the HD of EG1 × cultivar A F₁ hybrid is later but not 19.2 days later than that of EG0 × cultivar A F₁ hybrid, cultivar A should be assumed to carry a PS allele at E_1 locus, but the PS of this allele should be weaker than E_1 allele. The weak PS allele at E_1 is named E_1^e . NILs ER and LR differ only at $Se-1$ (LR is $Se-1^u$, and ER is $Se-1^e$). Nipponbare carries the PS allele $Se-1^n$, whereas NIL(*Hd1*) has *hd1* (synonymous with $Se-1^e$) (Yano et al. 1997, 2000; Lin et al. 2000; Yamamoto et al. 1998) (Table 1). $Se-1^u$ and $Se-1^n$ are both dominant over $Se-1^e$, and the PS imposed by $Se-1^u$ is greater than that by $Se-1^n$ (Yokoo and Kikuchi 1977). In Nanjing, the HD of ER was 22.0 days earlier than that of LR, and NIL(*Hd1*) was 9.5 days earlier than that of Nipponbare. The genotype of each of the 109 leading cultivars at $Se-1$ was determined from the HD of their F₁ hybrids with ER, LR, Nipponbare, and NIL(*Hd1*), respectively. Similarly, the allelic state at $Ef-1$ was identified by comparing the F₁ HD of each cultivar with NILs T65m ($ef-1$) and T65E^bm ($Ef-1$), respectively. The *Hd2* genotypes was determined by comparison of the

F₁ HD of each cultivar with Nipponbare (*Hd2*) and NIL(*Hd2*) (*hd2*), respectively. For each of these heading comparisons, ten parental and ten F₁ plants were grown in the field from 2000 to 2006 at Nanjing. Sowing was around May 15 and seedlings were transplanted about June 15 at a density of 13.3 cm × 26.7 cm. Crop management followed commercial rice-production practices.

HD QTL analysis for designing rice cultivars with target growth duration

The linkage map was built from the Asominori/IR24 RIL population genotyped using 375 RFLP loci that cover 1275.3 cM, with an average inter-marker interval of 3.4 cM (Tsunematsu et al. 1996). Additive HD QTLs were detected by inclusive composite interval mapping (ICIM) using QTL IciMapping v2.1 software (Li et al. 2007). In the first step of stepwise regression of ICIM, the *P* value for entering variables (PIN) was set at 0.01 and for removing variables (POUT) was set at 0.02 to select the significant markers; while in the second step, a threshold LOD of 2.5 was used to declare the significant QTL. The 66 CSSLs were selected from 268 BC₃F₁ plants by a whole-genome survey at 116 RFLP loci and nominated as CSSL1–CSSL66, which represented the whole IR24 genome (Kubo et al. 1999). The CSSLs carrying the QTL of HD were used to design rice cultivars with target growth duration. The target genotypes were designed based on breeding goals and the QTL information. To obtain the target genotypes by QTL pyramiding, various breeding schemes were

compared using the software QuLine (Wang et al. 2003, 2004, 2007), and the best breeding scheme was selected.

RILs and CSSLs were planted in Nanjing from 2003 to 2007. The time of sowing and transplant and the plant-density were same as those of the leading cultivars. Also, crop management followed commercial rice-production practices.

Measurement of HD and HD heterosis

HD of each line was measured by the number of days from seeding to heading when the leading panicle emerged about 1 cm beyond the leaf sheath of its flag leaf. The HD heterosis of each hybrid was calculated as:

$$\text{Mid-parent heterosis (\%)} = \frac{F_1 - (P_1 + P_2)/2}{(P_1 + P_2)/2} \times 100\%$$

where F_1 , P_1 , and P_2 represented the HD of F_1 hybrid, its male parent, and female parent, respectively.

Results

HD genotypes of leading cultivars and their distribution in China

Genotypes of the 109 leading Chinese rice cultivars on the major HD gene loci E_1 , $Se-1$, $Ef-1$, and $Hd2$ were determined, and are presented in Supplementary Table 1. Distribution of the HD genotypes over geographic regions and cropping seasons is summarized in Table 2. This showed that the dominant allele at $Ef-1$ determining early heading widely distributed among cultivars adapted to a wide range of ecological regions. *Japonica* cultivars grown in northeast and northwest China carried either one or no PS-determining dominant allele at E_1 and $Se-1$, and some carried the recessive allele $hd2$. Most *japonica* cultivars from north China and central China had the PS allele at either E_1 or $Se-1$, while those from southwest China included either both or one of the PS alleles at these loci. Cultivars adapted to higher altitudes in southwest China carried the dominant allele $Ef-1$ for early heading, while those from low altitudes had the recessive allele $ef-1$ for late heading. In the middle-lower regions of the Yangtze River and

south China, early-season *indica* cultivars had either one PS allele or none at E_1 and $Se-1$; middle-season ones had either one or two; and most late-season ones had both of the PS alleles. Some middle-season cultivars also carried the late-maturing allele $ef-1$ (Table 2; Fig. 1). Distribution pattern of the PS alleles demonstrated that the PS was gradually strengthened with decrease of latitude and elevation of rice growing areas (Fig. 1). These results on HD genotypes distribution in different ecological regions and cropping systems provided important information for subsequently genotype-based breeding.

Manipulation of PS genes to make *indica* × *japonica* hybrids head properly

Late heading among *indica* × *japonica* hybrids is mainly because both parents carry PS alleles at one of the two independent major PS genes (such as E_1 and $Se-1$) (Cai et al. 1987; Luo et al. 2002). Thus, any of three requirements has to be satisfied in selecting parents for *indica* × *japonica* hybrid rice in order to achieve proper heading. Both parents have none of the PS alleles, or the same PS-determining allelic constitution at one of the PS alleles, or independent but non-complementary PS alleles. However, the F_1 hybrids of *japonica* Nipponbare and LR with *indica* rice cultivars identically and strongly exhibited late heading, indicated by their mid-parent heterosis between 4.1 and 26.2% (Table 3). This could be explained by the non-allelic complementary effects between strong PS genes in these hybrids. *Japonica* Nipponbare and LR carry the strong PS gene $Se-1^n$ or $Se-1^u$ whereas the *indica* cultivars have the PS-determining allele at E_1 (Table 3; Supplementary Table 1).

Therefore, in order to make *indica* × *japonica* hybrids head properly, the non-allelic complementary effects between E_1 and $Se-1^n$ or $Se-1^u$ need to be avoided. Either the E_1 allele in *indica* cultivars (such as Minghui63) should be replaced by the non-PS allele e_1 , or $Se-1^n/Se-1^u$ in *japonica* parent should be replaced by the non-PS allele $Se-1^e$. The replacement of PS genes has proven to be effective in breeding practice using successive backcrossing with molecular marker-assisted selection based on mapping information of the target genes. According to the fine mapping of $Se-1(Hd1)$, the $Se-1^n$ allele in Nipponbare has been substituted by $Se-1^e$ allele in the BC_4F_2 , and the photoperiod sensitivity becomes

Table 2 The heading date genotype of rice cultivars adapted to various rice-production regions in China

Ecology type	Genotype for heading date	The typical cultivar	Characteristic of growth duration ^a
Middle-lower regions of the Yangtze river and South China regions			
Early-season <i>indica</i>	<i>e₁e₁ Se-1^eSe-1^e Ef-1Ef-1 hd2hd2</i>	XieqingzaoB	Weak or no PS, short BVP, short GD
	<i>e₁e₁ Se-1ⁿSe-1ⁿEf-1Ef-1 hd2hd2</i>	Zaoxian14	
	<i>E₁E₁ Se-1^eSe-1^e Ef-1Ef-1 hd2hd2</i>	Changlixian	
Middle-season <i>indica</i>	<i>E₁E₁ Se-1^eSe-1^e Ef-1Ef-1 hd2hd2</i>	Peiai64S	Moderate PS, long BVP, long GD
	<i>E₁E₁ Se-1ⁿSe-1ⁿ ef-1ef-1 hd2hd2</i>	II-32B	
	<i>e₁e₁ Se-1ⁿSe-1ⁿ ef-1ef-1 hd2hd2</i>	Bo B	
Late-season <i>indica</i>	<i>E₁E₁ Se-1^uSe-1^u Ef-1Ef-1 hd2hd2</i>	9194	Strong PS, short BVP and long GD
	<i>E₁E₁ Se-1^eSe-1^e Ef-1Ef-1 hd2hd2</i>	Qinghui756	
	<i>E₁E₁ Se-1ⁿSe-1ⁿ Ef-1Ef-1 hd2hd2</i>	Xiangwanxian10	
<i>Japanica</i> rice in Northeast China	<i>e₁e₁ Se-1^eSe-1^e Ef-1Ef-1 Hd2Hd2</i>	Kongyu131	Weak or no PS, short BVP, short GD
	<i>e₁e₁ Se-1ⁿSe-1ⁿ Ef-1Ef-1 Hd2Hd2</i>	Heijiao951	
	<i>e₁e₁ Se-1ⁿSe-1ⁿ Ef-1Ef-1 hd2hd2</i>	Jijing80	
	<i>e₁e₁ Se-1^eSe-1^e Ef-1Ef-1 hd2hd2</i>	Hejiang19	
<i>Japanica</i> rice in Northwest China	<i>E₁E₁ Se-1^eSe-1^e Ef-1Ef-1 Hd2Hd2</i>	Fulei419	Weak or no PS, short GD
	<i>e₁e₁ Se-1^eSe-1^e ef-1ef-1 hd2hd2</i>	Ningjing18	
<i>Japanica</i> rice in North and central China	<i>E₁E₁ Se-1^eSe-1^e Ef-1Ef-1 hd2hd2</i>	Xiangjing9402	Weak PS, short BVP, short GD
	<i>E₁E₁ Se-1^eSe-1^e ef-1ef-1 hd2hd2</i>	Jijing14	
	<i>E₁E₁ Se-1ⁿSe-1ⁿ Ef-1Ef-1 Hd2Hd2</i>	Zhonghua11	
<i>Japanica</i> rice in Southwest China	<i>E₁E₁ Se-1^eSe-1^e Ef-1Ef-1 hd2hd2</i>	Honggu	Moderate PS, short BVP in high altitude, long in low altitude
	<i>E₁E₁ Se-1^eSe-1^e ef-1ef-1 hd2hd2</i>	Yundao1	
<i>Japanica</i> rice in Southeast China	<i>E₁E₁ Se-1^eSe-1^e Ef-1Ef-1 hd2hd2</i>	Wuyunjing21	Moderate or strong PS, short BVP, long GD
	<i>E₁E₁ Se-1^eSe-1^e Ef-1Ef-1 hd2hd2</i>	Nanjing35	
	<i>E₁E₁ Se-1^eSe-1^e Ef-1Ef-1 Hd2Hd2</i>	Xudao3	
	<i>E₁E₁ Se-1ⁿSe-1ⁿ Ef-1Ef-1 Hd2Hd2</i>	Suxiangjing2	
	<i>E₁E₁ Se-1ⁿSe-1ⁿ Ef-1Ef-1 hd2hd2</i>	Yangjing9538	

^a PS, photoperiod sensitivity; BVP, basic vegetative phase; GD, growth duration

weak in the resulting near isogenic line NIL(*Hd1*) (Lin et al. 2000). In the same way, replacement of the strong *Se-1^u* in LR with the weak *Se-1^e* has resulted in another near isogenic line ER (Yamagata et al. 1986). We crossed both the near isogenic lines with *indica* cultivars and the HD of these F₁ hybrids was similar to that of the mid-parent value, demonstrated by their mid-parent heterosis ranging from -13.2 to 6.9% (Table 3). Thus, breeding based on prior knowledge of HD genotype should be effective for avoiding late heading in *indica* × *japonica* hybrids.

Genotype design and HD prediction in hybrid rice breeding

Early heading and high yielding are often paradoxical and the HD of hybrids is not known until they are

grown out, which takes an entire season in the breeding program (Deng et al. 2001). Based on the genetic effects of major HD genes and the HD genotypes widely utilized in hybrid rice parents, we have designed genotypes of hybrids suitable for photo-temperature conditions in different cropping regions of China (Table 4). These genotypes provide direction in parental selection for hybrid breeding in a target region. Obviously, the HD of derivative hybrids is predictable, so that breeders could pay more attention to yield than heading objective. The shift of attention should help better balance early heading and high yielding and improve efficiency in hybrid rice breeding.

Here, we want to obtain a middle-season *indica* hybrid suitable for the Yangtze River region, and south and southwestern China. According to local climatic

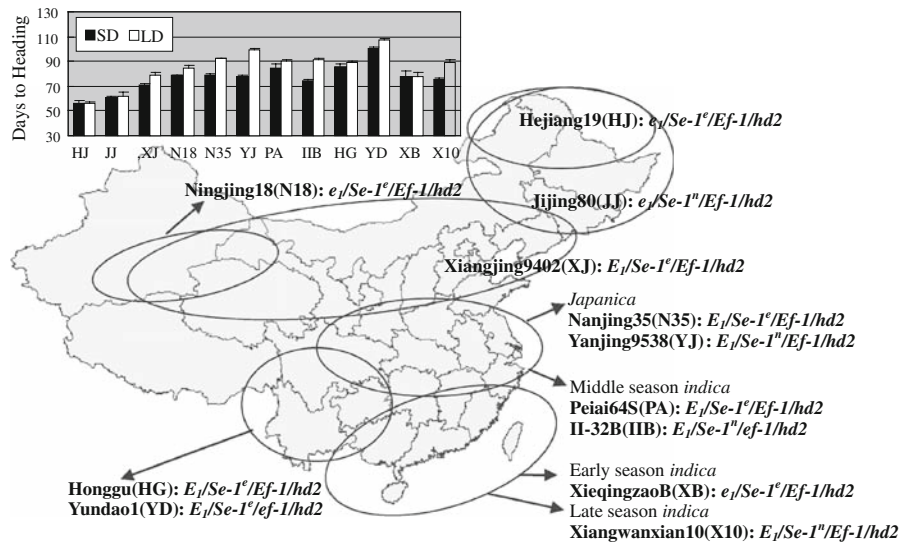


Fig. 1 The typical heading date genotypes of rice cultivars adapted to specific rice-production areas in China. The chart on the top right indicates the heading date of the cultivars grown

under both short-day (SD, 9 h light/15 h dark) and long-day (LD, 14 h light/10 h dark) conditions

Table 3 Strategy to avoid late heading in the *indica* × *japonica* F₁ hybrid

<i>Indica</i> cultivars	Genotype	HD of <i>indica</i> cultivars	HD (and mid-parents heterosis, %) of the <i>indica-japonica</i> hybrid F ₁ ^a			
			×Nipponbare	×NIL(<i>Hd1</i>)	×LR	×ER
Minghui63	<i>E₁E₁Se-1^eSe-1^e</i>	90.7 ± 2.1	103.9 ± 2.2 (17.0)	82.7 ± 2.5 (-1.6)	118.7 ± 1.6 (22.8)	84.5 ± 4.2 (-1.4)
Peiaif64S	<i>E₁E₁Se-1^eSe-1^e</i>	92.0 ± 1.8	108.3 ± 2.3 (21.1)	84.8 ± 3.8 (0.11)	116.0 ± 2.2 (19.15)	85.0 ± 0.8 (-1.6)
Teqing	<i>E₁E₁Se-1^eSe-1^e</i>	96.0 ± 1.1	105.0 ± 5.4 (14.8)	90.0 ± 2.7 (3.8)	115.0 ± 3.3 (15.8)	86.0 ± 2.5 (-2.7)
Jiahezaozhan	<i>E₁E₁Se-1^eSe-1^e</i>	81.0 ± 0.7	92.0 ± 1.8 (9.6)	79.8 ± 0.3 (0.8)	101.0 ± 3.2 (10.0)	77.2 ± 1.4 (-4.5)
Ce64	<i>E₁E₁Se-1^eSe-1^e</i>	77.0 ± 0.5	103.4 ± 0.3 (26.2)	82.6 ± 4.4 (6.9)	110.8 ± 0.3 (23.3)	79.4 ± 2.9 (0.7)
Qinghui756	<i>E₁E₁Se-1^eSe-1^e</i>	91.0 ± 3.0	93.0 ± 1.3 (4.6)	79.6 ± 2.0 (-5.5)	104.4 ± 0.9 (7.8)	79.0 ± 5.1 (-8.0)
752	<i>E₁E₁Se-1^eSe-1^e</i>	99.0 ± 1.8	100.0 ± 3.9 (7.6)	88.8 ± 3.3 (0.7)	105.0 ± 1.5 (4.1)	78.0 ± 2.6 (-13.2)

Value is expressed as mean ± SD

^a The value in brackets represents the mid-parents heterosis (%) of the F₁ hybrid

conditions (temperature and photoperiod), this hybrid rice should bear weak PS, a short basic vegetative phase, and moderate growth duration. These requirements could be met by type IV in Table 4, and the recommended genotypes were *e₁e₁Se-1^eSe-1^eEf-1_{Hd2}*, *e₁e₁Se-1ⁿEf-1_{hd2hd2}*, *E₁Se-1^eSe-1^eEf-1_{hd2hd2}*, and *E₁Se-1ⁿEf-1_{hd2hd2}*. If *E₁Se-1ⁿEf-1_{hd2hd2}* is taken as the target genotype, HD genotypes of their derivative male sterile/restorer lines should be either *E₁E₁Se-1^eSe-1^eEf-1_{hd2hd2}/e₁e₁Se-1ⁿSe-1ⁿef-1_{hd2hd2}* or *E₁E₁Se-1ⁿSe-1ⁿEf-1_{hd2hd2}/e₁e₁Se-1^eSe-1^eef-1_{hd2hd2}*. For example, two elite middle-season hybrid cultivars Shanyou63

and Liangyoupeijiu are widely grown in these regions. Male sterile/restorer lines are Zhenshan 97A/Minghui 63 for Shanyou63 and PeiAi 64S/93-11 for Liangyoupeijiu. HD genotypes of the parental lines are known (Supplementary Table 1). Genetic combination of both parents resulted in an HD genotype *E₁Se-1ⁿEf-1_{hd2hd2}* for Shanyou63 and *E₁Se-1^eSe-1^eEf-1_{hd2hd2}* for Liangyoupeijiu (Table 5). The resultant genotypes fit perfectly into type IV for middle-season rice in vast areas of China. These results validated the feasibility and effectiveness of genotype design and HD prediction in breeding hybrid rice.

Table 4 Growth duration and suitability of hybrid rice with particular heading date genotypes

Types	Major heading date genotype of hybrid rice	Character of growth duration and suitable planting scope ^a
I	<i>E₁Se-1ⁿef-1ef-1 Hd2_</i> <i>E₁Se-1^eSe-1^eef-1ef-1 Hd2_</i> <i>e₁e₁Se-1ⁿef-1ef-1 Hd2_</i>	Strong PS, long BVP, and GD; suitable for growing as middle-season rice in the Yangtze regions, south China, and southwest China
II	<i>E₁Se-1ⁿEf-1 Hd2_</i> <i>E₁Se-1^eSe-1^eEf-1 Hd2_</i> <i>e₁e₁Se-1ⁿEf-1 Hd2_</i>	Strong PS, short BVP, and moderate GD; suitable for growing as middle-season or late-season rice in the Yangtze regions, south, southwest, and central China
III	<i>e₁e₁Se-1^eSe-1^eef-1ef-1 Hd2_</i> <i>e₁e₁Se-1ⁿef-1ef-1hd2hd2</i> <i>E₁Se-1^eSe-1^eef-1ef-1hd2hd2</i> <i>E₁Se-1ⁿef-1ef-1 hd2hd2</i>	Weak PS, long BVP, and moderate GD; suitable for growing as early or middle-season rice in the Yangtze regions, south, southwest, and mid China
IV	<i>e₁e₁Se-1^eSe-1^eEf-1 Hd2_</i> <i>e₁e₁Se-1ⁿEf-1 hd2hd2</i> <i>E₁Se-1^eSe-1^eEf-1 hd2hd2</i> <i>E₁Se-1ⁿEf-1 hd2hd2</i>	Weak PS, short BVP, and moderate GD; suitable for growing as early or middle-season rice in the Yangtze regions, south, and southwest China regions, and as middle-season rice in northeast, northwest, and north China

^a BVP, basic vegetative phase; GD, growth duration

Table 5 The genotype and heading date of the hybrid cultivars Shanyou63 and Liangyoupeijiu, and of their parents

Genotype and hybrid rice	Genotype and sterile lines	Genotype and restorer lines
Shanyou 63 (91.0 ± 2.5) ^a <i>E₁e₁Se-1ⁿSe-1^eEf-1Ef-1hd2hd2</i>	Zhenshan 97A (67.0 ± 4.3) <i>e₁e₁Se-1ⁿSe-1ⁿEf-1Ef-1hd2hd2</i>	Minghui 63 (90.7 ± 2.1) <i>E₁E₁Se-1^eSe-1^eEf-1Ef-1hd2hd2</i>
Liangyou peijiu (103.0 ± 1.7) <i>E₁E₁Se-1ⁿSe-1^eEf-1ef-1hd2hd2</i>	PeiAi 64S (92.0 ± 1.8) <i>E₁E₁Se-1^eSe-1^eEf-1Ef-1hd2hd2</i>	93-11 (103.0 ± 2.6) <i>E₁E₁Se-1^eSe-1^eef-1ef-1hd2hd2</i>

^a The number in the brackets is the heading date of the cultivars. Values are expressed as mean ± SD

Breeding strategies based on HD QTL information

Breeding will become more powerful and effective if HD genotype can be engineered for a cultivar to fit in a specific environment. *Japonica* cultivar Asominori heads at about 88 days in Nanjing, and it would be desirable for this cultivar either to have an earlier HD in order to be grown as a late double-season crop, or to have a later HD in order to be grown as a single-season intermediate maturity crop in Nanjing. We conducted a QTL analysis for HD over five years using a RIL population derived from Asominori (*japonica*) and IR24 (*indica*) (Table 6). A total of seven QTLs (located on chromosomes 2, 3, 6, 8, and 12) were identified, for which accountable phenotypic variances ranged from 5.09 to 70.43%. Four of these, *qDTH-2*, *qDTH-3b*, *qDTH-6*, and *qDTH-8* were detected in all five years, indicating they were stably

expressed for HD in multiple environments and considered to be suitable for gene design. The HDs of CSSLs containing the IR24 allele at *qDTH-6* and *qDTH-8* were, respectively, ~6 days and ~8 days earlier than for Asominori in Nanjing. In contrast, those CSSLs including the IR24 allele at *qDTH-2* and *qDTH-3b* headed ~7 days and ~8 days later than Asominori. The CSSLs with the IR24 alleles at both *qDTH-2* and *qDTH-3b* flowered ~12 days later than Asominori (Fig. 2). Thus, to achieve later heading, the IR24 alleles at *qDTH-2* and *qDTH-3b* should be introduced into Asominori; for earlier heading, the IR24 alleles at *qDTH-6* and *qDTH-8* need to be transferred. Our approach was to use recurrent marker-assisted backcrossing to obtain the desired genotypes.

Two target genotypes were designed in the Asominori background: the first (TG1) was to introduce the IR24 alleles at both *qDTH-6* and *qDTH-8* for earlier heading, and the second (TG2) was to

Table 6 Putative heading date QTL detected in recombinant inbred lines derived from Asominori and IR24 from 2003 to 2007 under Nanjing natural long-day conditions

QTL locus	Year	Chr.	Near markers	LOD scores	PVE (%)	Additive effect
<i>qDTH-2</i>	2003	2	C621	2.53	5.15	−2.54
	2004			4.27	8.59	−3.18
	2005			2.74	5.92	−2.78
	2006			3.91	8.31	−3.15
	2007			3.03	5.09	−2.15
<i>qDTH-3a1</i>	2004	3	XNpb212-2	2.55	5.11	−2.45
<i>qDTH-3a2</i>	2005		G1015	2.59	5.36	−2.64
<i>qDTH-3b</i>	2006	3	XNpb279	2.88	6.27	−2.73
	2003			5.52	12.68	−3.98
	2004			4.47	8.66	−3.19
	2005			3.57	7.45	−3.11
	2006			3.38	7.26	−2.94
<i>qDTH-6</i>	2007	6	R2171	4.84	8.40	−2.76
	2003			3.87	9.00	3.86
	2004			3.92	8.10	3.58
	2005			3.43	7.72	3.68
	2006			2.50	5.74	3.01
<i>qDTH-8</i>	2007	8	R2976	6.28	12.26	3.81
	2003			16.55	55.03	8.32
	2004			17.91	56.04	8.12
	2005			17.23	59.88	8.83
	2006			17.81	62.20	8.62
<i>qDTH-12</i>	2007	12	R3375	21.62	70.43	8.02
	2006			2.75	5.61	−2.59
	2007			3.96	6.73	−2.50

LOD, likelihood of odds; PVE, phenotypic variance explained

Genotype	M2-5	M2-6	M2-7	M2-8	M3-1	M3-2	M3-3	M3-4	M3-5	M3-6	M6-1	M6-2	M6-3	M6-4	M6-5	M6-6	M8-1	M8-2	M8-3	M8-4	M8-5	Days to Heading (Day)	
Asominori	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	88.3	
CSSL31	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	B	B	B	A	A	79.0
CSSL57	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	82.5
CSSL61	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	78.1
CSSL43	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	82.6
CSSL45	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	A	A	A	A	A	A	84.5
CSSL47	A	A	A	A	A	A	A	A	A	A	A	A	B	B	A	A	A	A	A	A	A	A	81.4
CSSL16	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	95.2
CSSL23	B	A	B	B	A	A	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	100.0
CSSL20	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	96.0
TG1	A	A	B	A	A	A	B	A	A	A	A	A	B	A	A	A	A	B	A	A	A	A	77.5
TG2	A	A	B	A	A	A	B	A	A	A	A	A	B	A	A	A	A	B	A	A	A	A	101.2

Fig. 2 A designed genotype from heading date QTL pyramiding for HD. The shaded area represents genome segments from the donor parent IR24, and the unshaded area represents the genetic background of Asominori. Markers from M2-5 to

M8-5 are RFLP loci were used to track each IR24 segments. M2-7, M3-3, M6-3 and M8-2 were the nearest markers of *qDTH-2*, *qDTH-3b*, *qDTH-6*, and *qDTH-8*, respectively

introduce the IR24 alleles at *qDTH-2* and *qDTH-3b* for later heading (Fig. 2).

According to the graphical genotypes of the CSSLs, the best strategy to achieve TG1 could be

marker-assisted selection among the progenies of CSSL47 × CSSL57. TG2 could be achieved by progeny selection assisted by molecular markers from either crossing CSSL16 with CSSL20 or

Table 7 Efficiency of each cross or backcross and selection strategy

Scheme for marker-assisted selection	F ₂ size before selection	F ₂ size after selection	F ₄ families before selection	F ₄ families after selection (standard error)	Total DNA samples to be tested	DNA samples per selected F ₄ family
Cross:CSSL47 × CSSL57						
Scheme 1	200	200	4000	11.7 (6.13)	4000	341
Scheme 2	200	50.4	1008	11.3 (5.52)	1208	108
Cross:CSSL16 × CSSL20						
Scheme 1	200	200	4000	7.9 (4.89)	4000	507
Scheme 2	200	40.7	813	7.8 (4.53)	1013	131
Backcross:CSSL23 × Asominori						
Scheme 1	200	4000	4000	4.1 (2.97)	4000	986
Scheme 2	200	30.5	609	4.0 (2.91)	809	202

backcrossing CSSL23 with Asominori (Fig. 2). If the two target genotypes are expected in F₄, there are many schemes for marker-assisted selection. Here, we take two as examples. In scheme 1, 200 F₂ individuals are obtained from a cross or backcross, and each F₂ individual is allowed to produce 20 F₃ progenies. 4,000 F₄ individuals are produced by single-seed descent, and then target genotypes are selected by marker analysis. In scheme 2, marker analysis is applied to 200 F₂ individuals, and only those carrying the target alleles are advanced to F₄ where marker analysis is applied again. A simulation experiment was carried out using QuLine software (the RFLP markers used in the simulation experiment are given in supplementary Table 2). From the DNA samples per selected F₄ family needed, we can draw a conclusion that the optimum approach to achieving TG1 was selection from the progenies of CSSL47 × CSSL57, coupled with marker-assisted selection of scheme 2. Similarly, for TG2, the optimum approach was scheme 2 from the progenies of CSSL16 × CSSL20 (Table 7). The time taken for CSSL31, 57, 61, 43, 45, 47, and TG1 to flower was shorter than that for Asominori whereas that for CSSL16, 20, 23, and TG2 was longer. The former lines were suitable for double-season late rice cropping and the latter for single-season middle rice in Nanjing (Fig. 2).

Discussion

Growth duration or HD is an important agronomic trait in rice, which is critical for rice to adapt to

specific cultivation conditions and cropping seasons. Breeding for high yield cultivars with suitable growth duration has been a major focus. Now, with the genetic mapping and isolation of many genes and QTL controlling rice HD (Li et al. 1995; Yano et al. 1997, 2000; Lin et al. 1998, 2002; Yamamoto et al. 2000; Maheswaran et al. 2000; Yu et al. 2002; Takahashi et al. 2001; Kojima et al. 2002; Doi et al. 2004; Xue et al. 2008), the genetic basis of HD has been under elucidation (Hayama et al. 2003; Izawa et al. 2003). At the same time, the rapid development of marker technology in recent years has also encouraged the elaboration of the concept of breeding based on genotype, rather than on phenotype (Pelerman and van der Voort 2003; Wang et al. 2005; Wan 2006; Wang and Pfeiffer 2007). Thus, the time is right for addressing the challenge of engineering HD in rice by design breeding.

Both major genes and QTL are involved in the determination of HD, and the HD genotypes for different ecotypes in rice have been investigated (Okumoto et al. 1992, 1996; Ichitani et al. 1997, 1998; Tsai 1985; Xu et al. 2007; Wei et al. 2008). However, the best means of using this genotypic information is so far lacking. In the work discussed in this paper, the leading rice cultivars from different ecological regions of China were genotyped by analysis of the phenotype of their hybrids with a set of HD tester lines. On the basis of this information we showed that the late heading in the *indica* × *japonica* F₁ hybrid is because of interactions between alleles at *E₁* and *Se-1*. This result was consistent with previous research, which indicated that late heading of the *indica* × *japonica* F₁ hybrid is highly dependent on

the identity of the two parents (Cai et al. 1987; Luo et al. 2002).

In recent years, a growing number of HD QTL have been identified (Li et al. 1995; Yano et al. 1997; Lin et al. 1998, 2002; Yamamoto et al. 2000; Maheswaran et al. 2000; Yu et al. 2002), but there is little evidence that any of these is being used as an aid to rice improvement. In fact, we can design the target genotype for the rice cultivar with required HD in different ecological regions using the QTL information and then achieve the target phenotype by pyramiding of the QTLs by molecular marker-assisted selection. Here, a total of seven QTLs were identified using recombinant inbred lines in five years under the natural long-day growing conditions of Nanjing. But the genetic location of *qDTH-2*, *qDTH-3a1*, *qDTH-3a2*, *qDTH-3b*, *qDTH-6*, *qDTH-8*, and *qDTH-12* was the same as that of the previously reported QTLs *Hd7*, *Hd16*, *Hd6*, *Hd9*, *Hd1* (*Se1*), *Hd5*, and *Hd13*, respectively (Yano et al. 1997, 2000; Yamamoto et al. 2000; Lin et al. 2002; Matsubara et al. 2008). Among the seven QTLs, *qDTH-2*, *qDTH-3b*, *qDTH-6*, and *qDTH-8* could be detected in all five years, indicating they were stably expressed for HD in multiple environments. We believe the main effect and stably expressed QTLs could be preferentially applied for gene design. The best breeding or selection schemes could be finally determined by use of a genetic breeding simulation tool, for example QuLine (Wang et al. 2003, 2004, 2007).

The results in this report show that breeding for suitable HD in rice using genotypic information is very efficient, and represents an applied example of “design breeding” (Peleman and van der Voort 2003; Wang et al. 2005; Wan 2006; Wang and Pfeiffer 2007). As yet we have only considered the major HD genes, although it is clear that HD is also affected by other minor genes. Allelic variation in these minor or unknown HD genes probably underlies differences in HD between cultivars that share the same major HD gene allelic constitution. Using crosses with the test NILs to determine the HD alleles is simple and useful, but the results need further validation by study of DNA sequences of the HD genes. The HD genes *Se-1* (*Hd1*) and *Ef-1* (*Ehd1*) have been cloned (Yano et al. 2000; Doi et al. 2004), providing the opportunity to generate DNA-based assays to determine the identity of the allele(s) carried by any given rice plant. Although several major QTLs can be identified

using recombinant inbred lines in five years under the growing conditions at Nanjing, whether they will also be expressed in other environments is not clear. In addition, the outcomes from the simulation study will, in the end, also need to be validated by field experiments. Even so, the results here showed that genetic information related to HD can make a significant contribution to rice breeding, and it has important significance in guiding design breeding for important agronomic traits of rice.

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