An active transposon *mPing* facilitates the discovery of useful flowering time mutant genes in rice

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2014

Chapter Ⅰ Introduction

Rice is a major cereal crop that provides a dietary staple for more than half of the world population and is commercially planted in a wide range of latitude from 55N to 35S. This wide regional adaptability of rice was attained by the complex regulation system of flowering time. Flowering time of rice is controlled by the basic vegetative growth period (BVP) and photoperiod sensitivity (PS). BVP is a period of the photoperiod insensitive juvenile phase securing the sufficient vegetative growth under flowering inductive short day-length condition. PS is a degree of flowering retardation under long day-length condition. Recent molecular biological studies have successfully revealed that *Hd1* and *Ghd7* were the key controlling genes of PS. Furthermore, *Ghd7* also control number of grains per panicle and plant height and the combined effects of *Hd1* and *Ehd1* reduce the number of primary branches in panicles. Thus, functional analysis of flowering time genes is very important to improve the adaptability and productivity in rice breeding.

In Arabidopsis (*Arabidopsis thaliana*), many studies on flowering time have been conducted by utilizing a rich resources of flowering time mutants induced in various ecotypes. Thus, induced mutants are useful materials for the investigation of novel functions of flowering time genes in rice. Tanisaka et al. (1992) investigated the flowering time of the mutant lines (HS lines) induced by gamma-ray irradiation to the seeds of japonica rice variety 'Gimbozu' (GB).

mPing was reported as the first active miniature inverted-repeat transposable element (MITE) as well as the first active DNA transposon in rice. A subsequent study revealed that *mPing* copy number was increased about 40 copy per plant per generation in GB. In addition, *mPing* copy number exceeds over 1000 in GB, while *mPing* copy number is less than 50 in most of the Japanese cultivars. *mPing SCAR* (Sequence characterized amplified region) marker that is based on the polymorphic insertion of *mPing* between GB and other Japanese cultivars was reported as an usable DNA marker for QTL analysis.

In this study, I demonstrated the successful mapping and characterization of a novel flowering time gene *Se15* with the use of *mPing SCAR* marker system (Chapter II). Furthermore, *mPing SCAR* markers could be used to map the mutant genes using the cross combinations between mutant lines and their original variety GB (Chapter III). Lastly, I evaluated productivity of three photoperiod insensitivity mutant alleles induced in GB under the deficient-*Ehd1* genetic background (Chapter IV). This shows the advantage of using mutants induced in the same genetic background in the investigation of the pleiotropic effects of PS genes on the productivity.

Chapter Ⅱ Mapping and characterization of a novel flowering time gene *Se15*

Results

***Se15* conferring a weak photoperiod sensitivity and a high yield potential**

HS254 that is an early mutant line induced in GB flowered earlier than GB under field condition and long day-length condition (LD) but later than GB under short day-length condition (SD). This indicates that HS254 has a weak photoperiod sensitivity (Fig.1a). In comparison with GB, the number of grains per panicle of HS254 are increased due to the increase of secondary branches (Fig.1b) . The single recessive mutant gene in HS254 was named as *se15*.

Mapping of *Se15*

Using the F_2 population of the cross HS254 × Nipponbare (NB), I narrowed down the candidate region of *Se15* into 1.2 Mb region on the long arm of chromosome 10 (Fig.1c). Among the annotated genes at this region, *Os10g0577600* showed significant similarity with *ELF6* that is one of flowering-time related genes in Arabidopsis. The sequence analysis of *Os10g0577600* revealed the presence of a 7bp-deletion at the 6th exon only in HS254. This caused a frame shift mutation and a premature stop codon in *Os10g0577600* of HS254 (Fig.1c).

Flowering time of DMG lines

A double recessive mutant line (DMG line) of DMG26 (*se15hd1*) flowered earlier than HS110 (*Se15hd1*) and HS254 (*se15Hd1*). DMG28 (*se15ehd1*) flowered earlier than HS169 (*Se15ehd1*) but later than HS254 (*se15Ehd1*). DMG29 (*se15ghd7*) flowered earlier than EG2 (*Se15ghd7*) and HS254 (*se15Ghd7*). These suggested that functions of *Se15* on PS was independent of *Hd1* and *Ghd7* pathway. DMG28 flowered earlier than HS169, which showed that *Se15* did not repress flowering through *Ehd1* pathway. Thus, under LD, *Se15* represses flowering independently of *Hd1*, *Ehd1* and *Ghd7*.

Discussion

With the efficient usage of *mPing* SCAR marker, *Se15* is identified as *Os10g0577600. Se15* is classified as a JmjC domain-containing histone lysine demethylases family gene which regulates gene expression and plant development through the chromatin remodeling.

   Rice is a typical short day plant well adapted to warm intermediate latitudes, and its commercial production had been impractical in high latitudes before the introduction of the extremely early variety insensitive to photoperiod. Therefore, the photoperiod insensitive varieties are required for the rice cultivation in the high latitudes. Besides the weak photoperiod sensitivity, *se15* mutant showed several advantages in agronomic traits, such as the bigger panicle and the larger number of grains per panicle. Thus, detail investigation of *Se15* will provide us a beneficial knowledge to improve the productivity of photoperiod insensitive varieties.

Chapter Ⅲ *mPing* **SCAR marker is applicable to a cross combination between Gimbozu and its mutants**

Results

Photoperiodic responses of the mutant lines

Both HS57 and HS113 flowered earlier than GB under natural day length condition and LD conditions but later than GB under SD condition. Thus, HS57 and HS113 harbor mutations in the genes regulating PS.

Variations of copy number and insertion sites of *mPing* in GB HS57 and HS113

Results of quantitative PCR exhibited that the copy number of *mPing* was not significantly different among GB, HS57 and HS113 (Fig.2a). However, the *mPing* insertion sites of three lines are quite different from each other (Fig. 2b). This indicates that *mPing SCAR* markers based on the unique insertion sites of GB are suitable DNA markers for the mapping of mutant gene in the cross combination between HS line and GB.

Mapping and Identification of mutant genes

Sixty and Sixty-two *mPing* SCAR markers were found to be polymorphic between GB and HS57, and between GB and HS113, respectively. In HS113, the candidate region of mutant gene was narrowed down into the 6 Mb region between MK5_16 and the end of long arm of chromosome 5. Among the annotated genes in this region, five genes were considered as PS related genes. The sequence analysis revealed no sequence difference contributing to the mutation. In HS57, the chromosomal location of the mutant gene was narrowed down to 6.2 Mb region between MK6_47 and MK6_64 on the short arm of chromosome 6. *Hd1* gene was also located at this candidate region. The sequence analysis revealed the insertion of a transposable elements *Ping* at the 239bp upstream of the *Hd1* transcriptional start site. The expression level of *Hd1* in HS57 was decreased during ZT12 ~ ZT21 under LD. This might be caused by the *Ping* insertion at the promoter region of *Hd1* in HS57. Consequently, the low expression level of *Hd1* in HS57 causes the low PS of HS57.

Discussion

mPing SCAR marker system was applicable not only in the closely related cross combination, but also in the cross combination of a HS line and its original variety GB. Therefore, the combined use of GB mutant lines and *mPing* SCAR marker system is a highly efficient way to identify a novel flowering time mutant gene induced in GB. The understanding of the flowering regulation mechanism and the efficient evaluation of the pleiotropic effects of flowering time genes on the productivity will provide us crucial knowledge to breed high yielding varieties adapted to the wide range of latitudes.

Chapter Ⅳ The effects of the photoperiod insensitive alleles, se13, hd1 and ghd7, on yield components

Results

I investigated the effect of three recessive photoperiod insensitive alleles, *se13*, *hd1* and *ghd7*. Their effects on the yield components could be deserved under *Ehd1*-deficient genetic background by ensuring vegetative growth of these lines. Compared to HS169, all DMG lines showed a decrease in their total biomass at the mature stage. The grain yields of DMG2 (*se13ehd1*) and DMG3 (*hd1ehd1*) were significantly decreased in comparison to HS169 (*Se13Hd1ehd1*). The reduction in grain yield was observed in all DMG lines. However, the effects of three photoperiod insensitive alleles on yield components were not the same.

The grain yield of DMG2 was 19% lower than that of HS169. Main cause of this reduction is the lower number of panicles. The grain filling percentage and 1000-grain weight also tended to be decreased. The number of tillers of DMG2 was fewer than that of HS169 throughout the vegetative growth period. The grain yield of DMG3 was 28% lower than that of HS169. This was mainly attributed to the reduction in the filled grain percentage (Table 1). Although the number of tillers in DMG3 was significantly less than that of HS169 during the 5th to 10th week after transplanting, the degradation of tillers in DMG3 during the late vegetative growth period was not as obvious as that of HS169. The grain yield of DMG10 was decreased by 9% in comparison to HS169 (Table 1). The main cause of this reduction is a reduced number of the grains per panicle. The number of grains per panicle and the number of secondary branches also tended to be decreased in DMG10. Among the three photoperiod-insensitive alleles, only *ghd7* reduced the PS without decreasing the number of tillers (Table. 1). This is the unique advantage of *ghd7* in the breeding of a photoperiod insensitive variety.

Discussion

In tropical and subtropical countries, most of the improved varieties have been introduced the photoperiod-insensitivity gene and they can be cultivated. The present study demonstrated the effect of three photoperiod insensitive alleles on grain yield and yield component traits. This will provide us with beneficial information that can be used to breed an elite photoperiod insensitive variety and to develop novel ecotypes to realize massive increases in rice yield.

Chapter Ⅴ Conclusions

The objectives of this study are: (1) Isolate novel flowering time gene to bring new insight into the flowering time pathway (Chapter II). (2) Create an efficient mapping system (Chapter III). (3) Evaluate the pleiotropic effects of flowering time gene on yield components (Chapter IV). *mPing* plays an irreplaceable role in this study. *mPing SCAR* marker system provided enough polymorphic DNA markers applicable for the mapping. The *mPing SCAR* markers also provide polymorphic insertion sites between GB and its mutant lines (HS lines). As the results of previous study, there are more than 200 HS lines induced from GB. These materials are great resources not only for the clarification of the genetic factors controlling flowering time, but also for the investigation of productivity affected by the flowering time genes. As those mutant lines were induced in the same genetic background, the effects of the mutant genes on productivity can be directly compared. Thus, the combined use of mutant lines induced in GB and *mPing* SCAR marker will provide critical information to create photoperiod insensitive varieties which can be adapted to a wide range of latitudes.
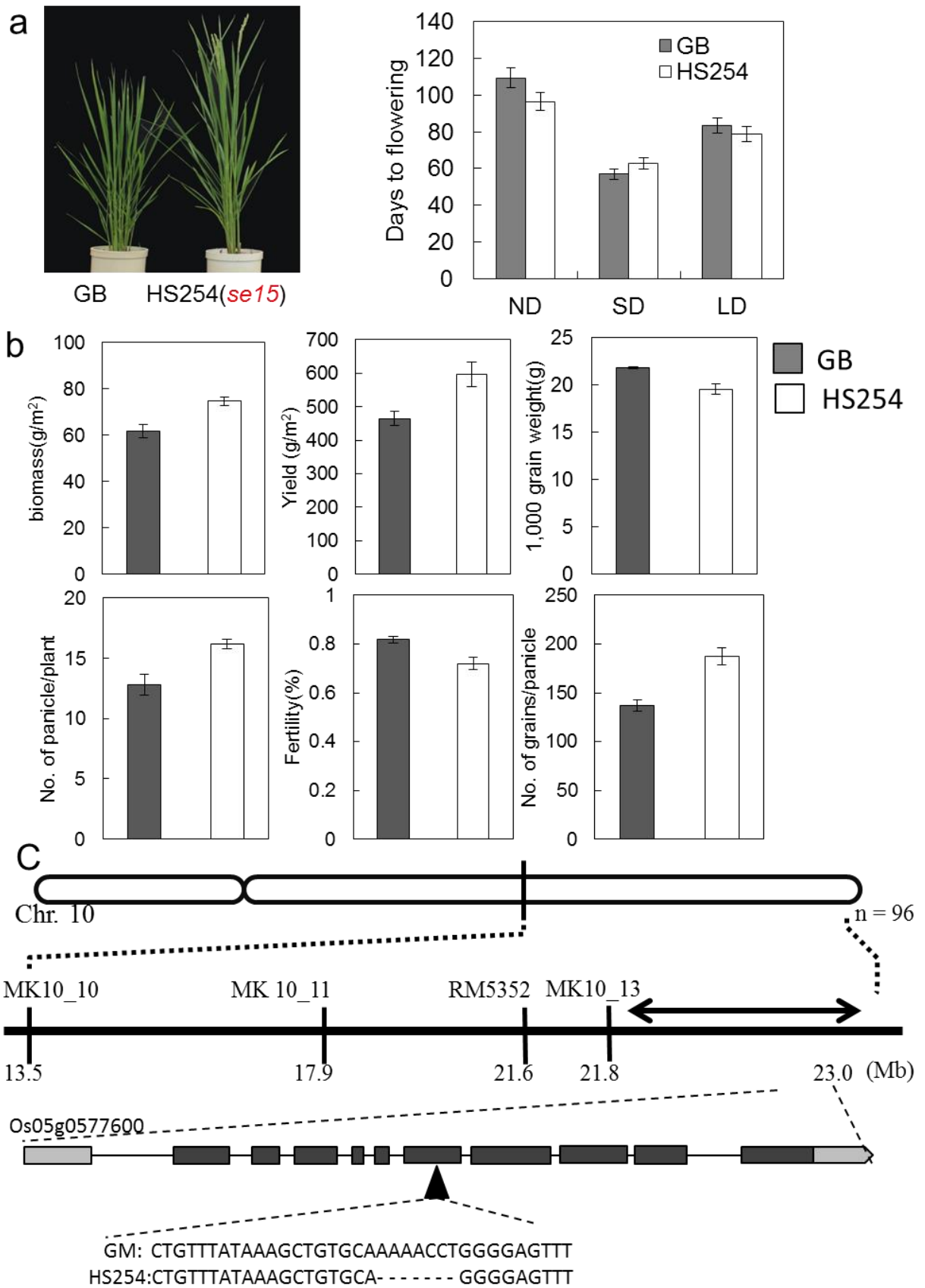
Figure 1 Phenotype of the HS254 and the GB (a). Comparisons of yield components between GB and HS254 (b).
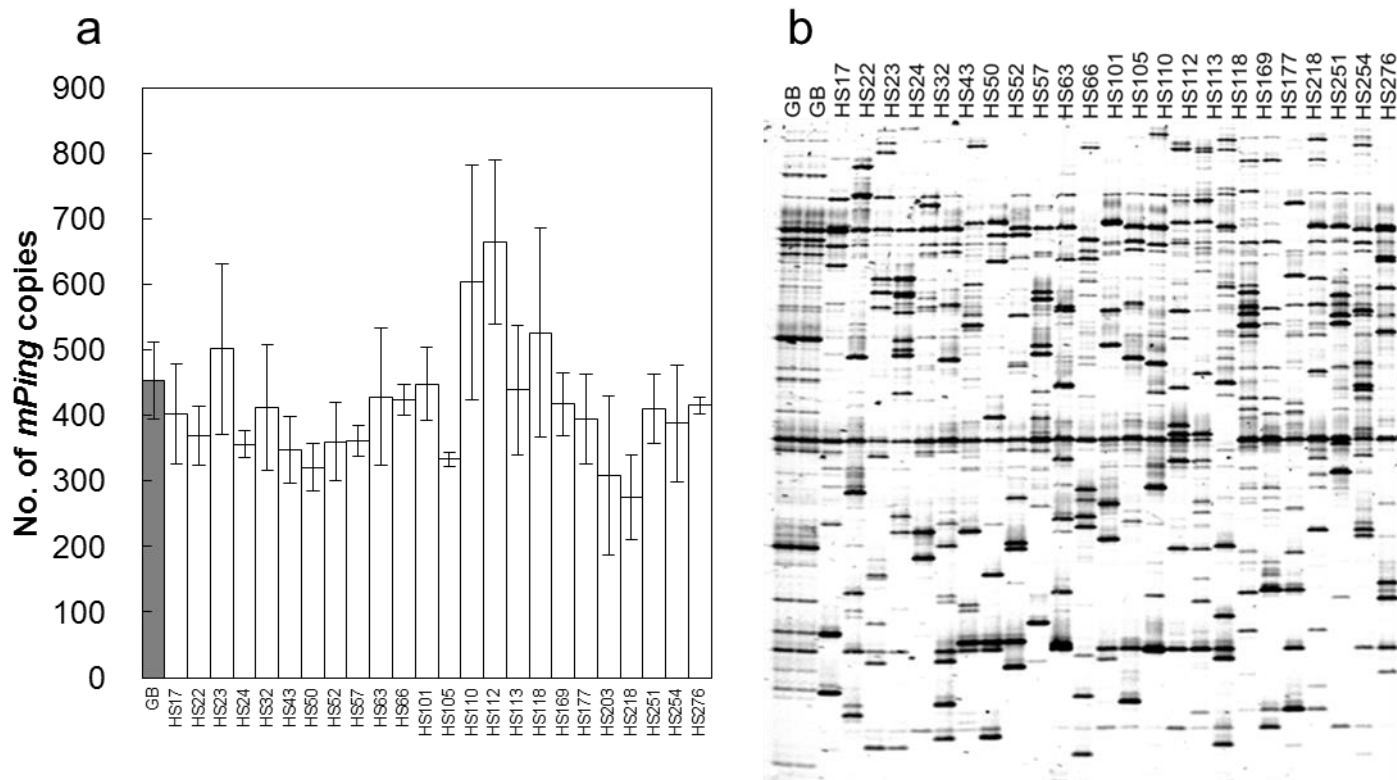
Mapping of the *Se15* gene (c).

Figure 2 Copy numbers of *mPing* in the GB and HS lines estimated by quantitative PCR (a). Result of a transposon display by using TTG selective base in GB and HS lines (b).

Table 1 Effects of the photoperiod insensitive alleles *se13*, *hd1* and *ghd7* on yield components

Line	HS169	DMG2	DMG3	DMG10
Genotype	*ef1-h*	*ef1-h/se13*	*ef1-h/hd1*	*ef1-h/ghd7*
Dry weight at mature stage (g/ m^2)	1297.1[aA]	1000.3[bB]	1097.9[bB]	1151.2[bAB]
Grain yield (g/ m^2)	528.2[aA]	409.9[bB]	360.0[cB]	490.3[aA]
Number of panicles (/m^2)	314.5[aA]	280.6[bA]	317.6[aA]	316.4[aA]
Number of grain (/m^2)	33789[aA]	29498[bA]	33852[aA]	30578[abA]
Number of grains per panicle	107.5[aA]	105.5[aA]	106.8[aA]	97.0[aA]
Filled grains (%)	72.0[aA]	66.9[aAB]	49.6[bB]	74.3[aA]
1000-grain weight (g)	21.7[aA]	20.7[aA]	21.4[aA]	21.5[aA]
Number of primary branches per panicle	15.2[bA]	15.9[abA]	16.1[aA]	15.5[abA]
Number of secondary branches per panicle	19.7[abA]	21.1[aA]	16.0[bcA]	14.7[cA]

Lower case letter difference indicates significance at 5% probability level, capital letter difference means significance at 1% probability level by the Duncan's new multiple range method.