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Short Communication

Validation of hybrid rice seed vigour traits using SSR marker (*Oryza sativa* L.)

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Abstract

Seed vigour is an important characteristic of seed quality. Several quantitative trait locus (QTL) has been reported for seed vigour in many crops. In order to validate seed vigour traits, an experiment was conducted with three released rice hybrids and their respective female (A), and restorer/ male lines using SSR markers. A total of 18 quantitative traits loci (QTLs) were chosen for seed vigour characters for which 16 SSR markers that were dispersed throughout the 11 chromosomes of rice have been assessed. Eight SSR markers were polymorphic among the genotypes studied. These markers showed distinct bands in female (A), male (R) and hybrid (H). Further, genuineness of all the hybrids under study could be established using the primer RM 252.

Key words: Rice, hybrid, seed vigour traits, QTLs, SSR marker

Introduction

Rice is the principal food for more than half of the world's population (FAO, 2017). With the availability of improved varieties and hybrids, the production of rice has been steadily increasing. In addition to several factors, good quality seed is one of the most important requirements to achieve higher production and productivity in any crop. Seed vigour is an important characteristic of seed quality attributes that determines rate of early, rapid, uniform germination, emergence and growth of strong seedling in any environmental condition. Seed with high vigor may significantly improve the speed and uniformity of seed germination and the final percentage of seedling emergence, good crop performance and even high yield under different condition (Foolad et al. 2007). Seed vigor has been recognized as a complex trait that has been influenced by many factors, such as genetic background and environmental factors during seed development and storage (Sun et al. 2007). Genetic variation in seedling vigour as measured by shoot weight, shoot length and coleoptile length across environmental conditions was reported by Redona and Mackill (1996a, 1996b). Due to its complex background and quantitative nature, breeding for early seedling vigor by conventional approaches has long been unsuccessful (Li and Rutger, 1980; Redona and Mackill, 1996a). With the development of the techniques of DNA molecular marker and genome graphing, quantitative trait locus (QTL) analysis of seed vigor has been reported in rice (Redoña and Mackill, 1996b; Cui et al. 2002; Zhang et al. 2005a). Japonica and indica cultivars may have distinct sets of seedling vigor genes (Redona and Mackill, 1996c).

A large number of QTLs were reported for coleoptile length, germination index, germination rate, shoot length, fresh shoot weight, dry shoot weight, tiller number/plant, mesocotyl length, root fresh weight and root dry weight which directly or indirectly contributed to seedling vigour. However, there have been no reports on validation of seed vigour traits by using the already identified markers. Keeping these in view, present study was undertaken with the objective to validate seed vigour in hybrids and parental lines using known SSR markers and major QTLs detected.

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The seeds of 3 hybrids and their respective A, B, and R line of rice were used in this study (Table 1). The A, B and R line seeds for DRRH 2 and DRRH 3 were obtained from the Indian Institute of Rice Research, Hyderabad. The hybrid seeds of all the three hybrids were produced following the recommended package of practices in the field at ICAR-Indian Agricultural Research Institute, New Delhi during *kharif*, 2018.

Table 1. Hybrids and parental lines

Hybrids	A line	R line
DRRH-2	IR68897A	DR-714
PRH-10	PUSA6A	PRR-78
DRRH-3	APMS6A	RPHR1005

DNA was isolated from the fresh plant tissue by CTAB method (Doyle 1990). For polymerase chain reaction (PCR), the program comprises of initial denaturation at 94°C for 4 min. which was further followed by 35 cycles of 94°C for 1 min, 55°C for 30 sec. and 72°C for 1 min. Final extension was carried out at 72°C for 7 min. The amplified products were resolved on 3.5% metaphor agarose gel.

In the present study, a total of 18 quantitative traits loci (QTLs) were chosen for seed vigour characters for which 16 SSR markers that were dispersed throughout the 11 chromosome of rice have been assessed for association of these markers with the seed vigour characters. The results indicated that 8 SSR markers were polymorphic and the remaining was monomorphic.

For the QTLs, qGR-1 four SSR markers namely, RM3453, RM5389, RM9, RM234 was analyzed. All the four markers showed different marker alleles in at least one of three hybrid and parental lines (Table 2) (Fig. 1). All these four SSR markers could show hybridity of the rice hybrids in relation to that of the respective female (A) and male(R) line. RM3453 and RM234 identified DRRH2 and PRH10, while RM5389 to PRH10 and RM92 to DRRH2 only. For the QTLs, qSDW-4.2, RM3766 was used (Cheng et al. 2013). It indicated polymorphism with respect to the hybrid, DRRH3 and its parental lines. There was no difference in the marker allele in other two hybrids and its parental lines. For the QTL qGS-11/qGR-1-12, RM5609 was used which showed polymorphism with respect to the hybrids DRRH2 while in other two hybrids and parental lines only single band was expressed (Fig. 1B). The seed characters reported are shoot length, seed reserve utilization and seedling dry weight (Wang et al. 2010) (Table 2). The QTL,qDW-5 located in chromosome number 5 was analyzed with SSR marker RM315 that showed polymorphism in PRH-10 and its parental lines only. This QTL was attributed to control seedling length, leaf area and seedling dry weight (Cui et al. 2002b; Zhang et al. 2005a and Diwan et al. 2013). For the QTL, qGR-2/qGP-4 located in chromosome 2 and 4 analyzed with SSR marker, RM252. Polymorphism was shown in all three hybrid and the respective parental lines. This QTL was reported to attribute seed vigour traits namely seedling early vigour, germination percentage and rate (Diwan et al. 2013).

QTLs for seed early vigour traits in rice have been reported by several researchers (Cheng et al. 2013; Dang et al. 2014; Diwan et al. 2013). Using those reported information on QTLs and associated SSR markers, our study aimed to validate the presence of those markers in the specific genotypes including three hybrids. The assessment of the genotypes for various seed vigour related traits did show a variation among

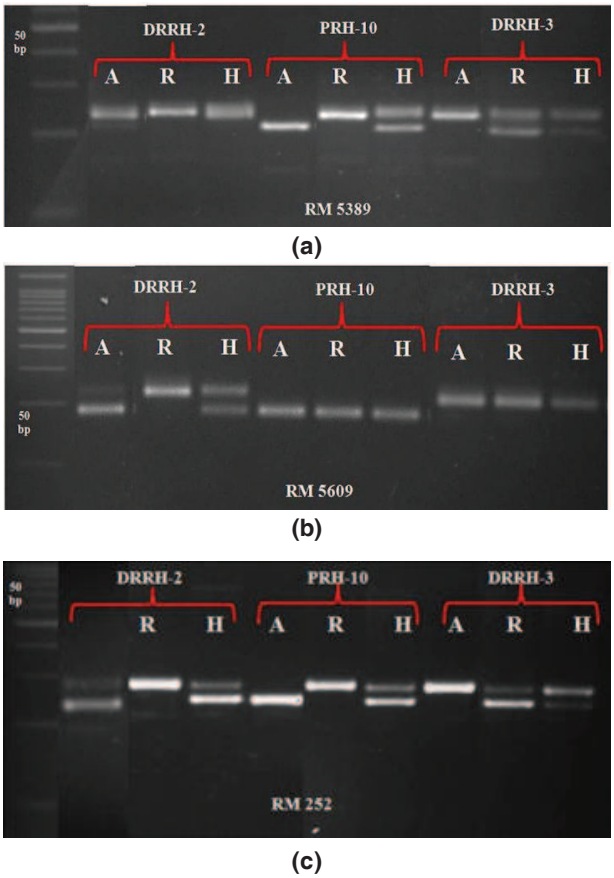

Fig. 1. Banding patterns of hybrid, female and male lines by polymorphic SSR markers

Table 2. Polymorphism in hybrids and its parental lines in SSR markers associated with QTLs for seed vigour traits

S.No.	SSR marker	Chromo-some no.	QTL	Traits*	Polymorphic/ monomorphic	References
1.	RM3453	1/11	qGR-1/qGI-11	RL,GR,GI	Polymorphic	Cui et al. (2002), Wang et al. (2010)
2.	RM5389	1	qGR-1	RL, SDW, TFW	Polymorphic	Cui et al. (2002), Wang et al. (2010)
3.	RM305	5	qDW-5	RL	Monomorphic	Cui et al. (2002b), Zhang et al. (2005a), Diwan et al. (2013)
4.	RM269	10	qFV-10	RL	Monomorphic	
5.	RM20	3	qSV-3-1	SL, SDW	Monomorphic	Zhang et al. (2005b)
6.	RM264	8/2/4/5	qSV-8-2/qSDW-2/qGP-4/qDW-5	SL, GR, RL, GP, SDW	Monomorphic	Wang et al. (2010), Cui et al. (2002b), Zhang et al. (2005a), Diwan et al. (2013)
7.	RM528	6	qDW-6	GP, SL, SDW	Monomorphic	
8.	RM573	2	qRL-2	SL	Monomorphic	
9.	RM201	9		SL	Monomorphic	
10.	RM480	5	qSDW-4.2	SL, RL, SDW	Monomorphic	Cheng et al. (2013)
11.	RM3766	3	qSDW-4.2	SDW	Polymorphic	Cheng et al. (2013)
12.	RM5609	11/12	qGI-11/qGR-1-12	SL, SR, SDW	Polymorphic	Wang et al. (2010)
13.	RM315	5	qDW-5	SL, LA, SDW	Polymorphic	Cui et al. (2002b), Zhang et al. (2005a), Diwan et al. (2013)
14.	RM252	2/4	qGR2/qGP-4	SEV, GP, GR	Polymorphic	Diwanet al.(2013)
15.	RM9	1	qGR -1	GR, SDW, SFW	Polymorphic	Wang et al. (2010)
16.	RM234	1	qGR-1	SL,GI	Polymorphic	Wang et al. (2010)

*: GI = germination index; GP = germination percentage; GR = germination rate; RL = root length; RA = root activity; SR = seed reserve utilization efficiency; SEV = seedling early vigor; TDW = Total dry weight; A, R, H = female, male and hybrid, respectively

Table 3. SSR markers distinguishing rice hybrids from its female (A) and Male(R) lines

SSR marker	Name of hybrid	Seed vigour traits*
RM 3453	DRRH -2	RL, GR, GI
RM3453	PRH-10	RL, GR, GI
RM5389	PRH-10	RL, SDW, TFW
RM3766	DRRH-3	SDW
RM5609	DRRH-2	SL, SR, SDW
RM315	PRH-10	SL, LA, SDW
RM252	DRRH-1	SEV, GP, GR
RM252	PRH-10	SEV, GP, GR
RM252	DRRH-3	SEV, GP, GR
RM9	DRRH-2	GR, SDW, SFW
RM234	DRRH-2	SL, GI
RM234	PRH-10	SL, GI

*: GI = germination index; GP = germination percentage; GR = germination rate; RL = root length; RA = root activity; SR = seed reserve utilization efficiency; SEV = seedling early vigor; TDW = Total dry weight

the genotypes. Out of 16 SSR markers associated with 18 QTLs dispersed in 11 chromosomes, 8 were monomorphic showing that those QTLs were not different among the genotypes studied. The other polymorphic SSR primers' presence validated the seed vigour related traits in the genotypes.

The QTLs for seed vigour traits in general validated in genotypes of the present study indicates conservation of genomic region across genotypes. Some of the QTLs or genomics region controlling seedling vigour detected in diverse rise population while the non-detection of other QTLs in the genotypes studied could depend largely on mapping population (Zhang et al. 2005b). Multiple QTLs governing seedling vigour on chromosome number 5 was reported by (Zhang et al. 2005).

Genotype-environment interaction in the expression of seed vigour traits has been common particularly in field level assessment. The QTLs mapping and its detection or validation using markers

become more complex. However, field phenotyping for seed vigour traits would be essential for a practical use in improvement of these traits. The same is also valid to finally validate or understand the inheritance of seed vigour trait factors in hybrids.

Seed vigour, particularly in hybrid rice being a very complex quantitative trait, it is of particular interest to know not only the position of QTLs in chromosomes but also proportion of phenotypic variance (PV) that can be contributed by each one. The large number of study made in the direction indicated variable phenotypic variance contributed by different seed vigour traits by one or many QTLs located in different region. The QTLs with high phenotypic variance would be useful to improve early seedling vigour using marker assisted selection.

Authors' contribution

Conceptualization of research (SKC); Designing of the experiments (SKC, SK); Contribution of experimental materials (PKB, ASH); Execution of lab experiments (SK,VJS); Analysis of data and interpretation (SK, SKC); Preparation of the manuscript (SK,SKC).

Declaration

The authors declare no conflict of interest.

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