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Genetic analysis of dormancy and shattering traits in the backcross inbred lines derived from *Oryza sativa* cv. Swarna / *O. nivara* Ac. CR100008

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ABSTRACT

Seed dormancy and shattering are important traits influencing the economics of rice farming. The genetic basis of dormancy and shattering traits were investigated in 174 Backcross Inbred Lines (BILs) derived from Oryza sativa cv. Swarna and O. nivara ac. CR100008. Significant variation was observed among the BILs for dormancy and shattering traits. Dormancy of 4-40 days was observed among BILs harvested at 35 days after heading and all the BILs attained > 80% germination by 6th week. Among all the BILs, least dormancy period (4 days) was found in SN-1, 13, 23, 25 and SN-28. Highest dormancy period (40 days) was found in 4 BILs i.e., SN-108, SN-116, SN-117 and SN-122 (40 days). None of the BILs were found to have non-shattering trait, while 2 BILs (SN-38 and SN-163) showed low shattering and 18 BILs were found with very high grain shattering percent. Of the 312 SSRs screened, 94 were polymorphic between the parents. A strategy of combining the DNA pooling from phenotypic extremes and genotyping was employed to detect the putative markers associated with dormancy and shattering traits. Single marker analysis revealed co-segregation of two putative markers RM488 on chromosome 1 and RM247 on chromosome 12 were with dormancy and shattering traits respectively. The putative marker RM488 identified is suitable for the marker-assisted transfer of the dormancy shown by O. nivara accession CR100008 for addressing pre harvest sprouting in modern cultivars. Interestingly, O. nivara type allele at RM247 was observed in BILs with low shattering phenotype.

Key words: *BILs, dormancy, O. nivara, rice, shattering, Swarna*

INTRODUCTION

The rice (*Oryza sativa* L.) is a staple food crop to millions across the globe. Due to its wider adaptability under different environmental conditions, rice has been regarded as a strategic crop for food security worldwide by the Food and Agriculture Organization (FAO) (Montano et al., 2014). It has been estimated that 40% more rice is needed to be produced by 2050 to meet the food demands of the ever increasing population (Milovanovic and Smutka, 2017). In addition to the biotic and abiotic factors limiting the rice production levels, there are two major factors grain shattering and pre-harvest sprouting causing severe economic losses to the farmers.

Seed dormancy (SD) is the temporary failure of an intact viable seed to germinate under favourable conditions (Bewley, 1997). Rice seed dormancy is a tricky trait. On one hand, weak or no dormancy leads to a higher PHS rate in rainy weather and results in production losses and poor quality. On the other hand, dormancy for a relatively longer period interferes with the timely sowing of crops. It is a complex trait influenced by both genetic and environmental factors. In crop production, SD has advantages as well as

disadvantages. For succeeding crops and accelerating breeding processes, the crop cultivars or breeding materials need to be sown for a second/next crop quickly (Seshu and Sorrells, 1986). For grain quality, however, the cultivars with moderate dormancy are needed to protect the grains from germinating before harvesting. In the recent past, India, witnessed untimely rains and floods in the form of Phailin in 2013, Hudhud in 2014, Vardah in 2016, Ockhi in 2017, Titli, Gaza and Phethai in 2018 and Fani in 2019 that resulted in pre harvest sprouting of fully matured grain in the standing crop.

A study on postharvest loss in India estimated a 10.3% increase (1.74% to 1.92%) in paddy harvesting losses due to delayed harvesting (Kannan et al., 2013). Uncontrolled grain shattering results in severe yield losses on one hand and non-shattering poses difficulty in threshing on the other hand. 'Seed shattering' refers to the release of mature seeds from their mother plants, which allows offspring dispersal in the natural environment. In domesticated crops, however, the reduction of shattering is a desirable trait, as keeping the seeds on their mother plants facilitates efficient harvesting and prevents yield loss. A critical evolutionary step during rice domestication was the elimination of seed shattering. Wild rice disperses seeds freely at maturity to guarantee the propagation, while cultivated rice retains seeds on the straws to make easy harvest and decrease the loss of production. Wild species are important donors in rice breeding programme as they have many beneficial alleles for rice improvement (Tanksley and McCouch, 1997; Swamy and Sarla, 2008; Swamy et al., 2011 and Eizenga et al., 2013) which were eliminated during domestication. The wild progenitor species *O. nivara* is easily crossable with cultivated rice and can be readily exploited in breeding programmes without embryo rescue procedures (Niroula and Bimb, 2009) and has been used in inter-specific crosses as a source of new alleles (Wickneswari et al., 2012 and Haritha et al., 2018). *O. nivara* accessions have abundant genetic diversity (Sarla et al., 2003 and Juneja et al., 2006) however, this species has not been used extensively in breeding programs. Therefore, wild species derived backcross inbred lines are deployed in the study for seed dormancy and shattering traits.

It is necessary to identify the molecular markers closely linked with important traits like rice shattering and dormancy genes in order to advance the generations for early selection. The utility of molecular markers relies on finding tight linkages between markers and genes of interest which permits indirect selection for the presence of a desirable gene and thus shorten the breeding procedure (Tanksley et al., 1989). Tightly linked DNA markers to the important genes may be used as molecular tools for MAS in plant breeding, which helps in more efficient, reliable and commercial phenotype selection compared to the classical plant breeding methods (Collard et al., 2005). A bulked segregant analysis (BSA) is a simple and rapid approach to identify molecular markers tightly linked to the target genes or QTLs (Michelmore et al., 1991). The present study focuses on estimating the variation present in backcross inbred lines derived from *O. sativa* cv. Swarna and *O. nivara* CR100008 and marker-trait linkages for shattering and dormancy traits in rice.

MATERIALS AND METHODS

Development of mapping population

Mapping population consisting of 174 Backcross Inbred Lines (BILs) were derived from the cross of Swarna, an elite cultivar and *O. nivara*, a wild species accession (CR100008). F_1 was generated by crossing Swarna and *O. nivara* in *rabi* 2014, F_1 was back crossed twice to Swarna in *rabi* 2015 and **kharif** 2015 to develop BC_2F_1 and selfed to produce BC_2F_2 under ICAR National Professor Project, IIRR, Hyderabad (Rao et al., 2018). Further, this material was advanced by single seed descent for 5 seasons to get 174 BC_2F_6 lines at ICAR-IIRR farm, Ramachandrapuram, ICRISAT campus during 2016-2018. The development of BILs is schematically presented in Fig. 1.

Assessment of dormancy traits

Seeds from each BIL after harvest were sown in 9cm petri dish lined with Whatman no.1 filter paper, wetted with 10-ml deionized water, and then incubated at 30°C and 100% relative humidity in the dark. Sowings were taken up at regular weekly intervals till germination was observed in all the BILs. Germination percentage and time taken for germination after sowing was noted to estimate dormancy period in each BIL.

Germination percentage

It is calculated based on the number of seeds

germinated to the total number of seeds sown and expressed as percentage in whole number. Based on germination percentage, 174 BILs were classified as per International Rice Research Newsletter (IRRN), 1988 into > 80%, 40-80% and <40% germination. Percentage of germination less than 80% was considered to estimate dormancy.

Germination percentage (%) =

$$\frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Dormancy period

Dormancy period is calculated as the time taken to observe sprouting. Dormancy period of 174 BILs were taken at weekly intervals after harvesting (Hanumanthappa et al., 2015). Loss of dormancy and germination was confirmed by radical emergence till 2 mm longer.

Phenotyping for shattering

174 BILs (BC_2F_6) were sown during *kharif* 2018 at Ramachandrapuram farm of ICAR-IIRR located in ICRISAT campus. To ascertain immediate and 100% germination in the BILs as the seed was freshly harvested from previous crop, seed was kept in hot air oven at 60ºC for 48 hours to break dormancy. Hot air oven treated seeds were sown in raised nursery beds and transplanted at 30 days after sowing (DAS). The field management included timely irrigation, recommended fertilizer application and hand weeding. The fertilizer was applied at the rate of 30 kg N ha^{-1}, 15 kg P ha^{-1} and 15 kg K ha^{-1} as a basal dose and two additional nitrogen applications of 30 kg ha^{-1} at 44th and 66th day after sowing.

The degree of shattering was measured following grasping of each panicle by hand to dislodge spikelets (Voleti et al., 2013). First emerged panicle was tagged and used for noting shattering data at physiological maturity. The proportion of seeds shed by hand gripping (shattered) and non-shed by hand gripping (non-shattered) were recorded and shattering percentage is calculated by the following formula. Based on shattering percentage, 174 BILs were scored on a scale of 1-9 as given in Table 1.

Shattered grains (%) =

$$\frac{\text{Number of shattered grains}}{\text{Total number of grains}} \times 100$$

Genotyping

Young leaves were collected 20 d after transplantation and genomic DNA was isolated using CTAB method (Doyle and Doyle, 1987). Purity and concentration of DNA were checked with 0.8% agarose gel using uncut λ DNA as standard. Polymerase chain reactions (PCR) were carried out in thermal cycler (Veriti PCR, eppendorff, USA) with the total reaction volume of 10 µl containing 25 ng of genomic DNA, 1× assay buffer, 200 µM of dNTPs, 1.5 mM $MgCl_2$, 5 pmol of forward and reverse primer and 1 unit of Taq DNA polymerase (KAPA Taq). PCR cycles were programmed as initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 45 s, 55°C for 30 s, 72°C for 45 s and a final extension of 10 min at 72°C. Amplified products were resolved on 3% agarose gels prepared in 1x TAE buffer and electrophoresis was conducted at 120 V for

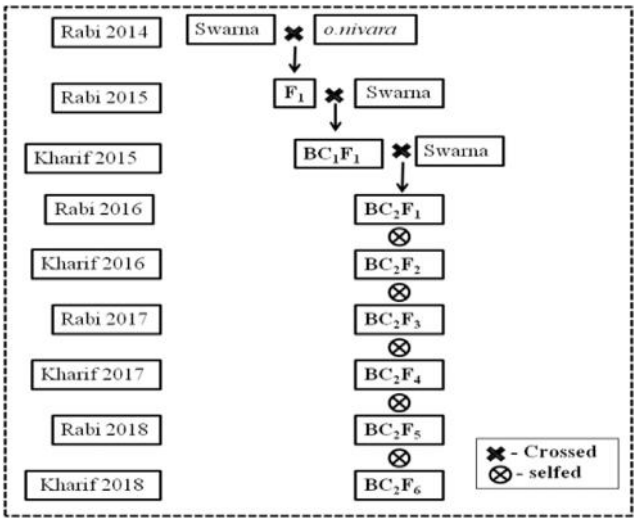

Fig. 1. Schematic representation of development of BILs from Swarna/*O. nivara*

Table 1. Scale for shattering trait in rice.

Shattering %	Scale	Type
0	1	Non-shattering
1-5	3	Low shattering
5-25	5	Moderately shattering
25-50	7	High shattering
>50	9	Very high shattering

2 h. Gels were stained with ethidium bromide and documented using gel documentation system (BIORAD, USA).

Marker trait segregation in phenotypic extremes

Two DNA bulks were made from the genomic DNA of 16 in each of the phenotypic extremes in terms of non-dormant BILs and BILs with maximum dormancy period. Similarly, two other bulks were prepared from the genomic DNA of 20 extreme phenotypes for the trait 'shattering'. Polymorphic markers were screened in bulks along with parental DNA. Segregation pattern of the polymorphic SSRs was assessed in the bulks. The SSR markers that could clearly distinguish the phenotypic extremes were used for single marker analysis.

RESULTS AND DISCUSSION

Germination percentage

In the first week of sowing immediately after harvesting (35 days after heading), of the 174, 13 BILs showed >80% of germination, 41 BILs showed 40-80% of germination, 29 BILs showed <40% of germination and 91 BILs were found with no germination. Number of lines with >80% of germination increased with time, and after six weeks all lines were observed to show > 80% germination.

Dormancy period

The duration of dormancy ranged from one week to six weeks (Table 2). Dormancy period for 13 BILs is one week, 12 BILs had two weeks, 34 BILs had three weeks, 48 BILs had four weeks, 51 BILs had five weeks and 16 BILs had six weeks of dormancy. The general mean of dormancy period was 22 days and the mean values ranged from 4-40 days. Among all the BILs, least dormancy period (4 days) was found in SN-1, 13, 23, 25 and SN-28. Highest dormancy period (40 days) was found in 4 BILs *i.e.*, SN-108, 116, 117 and SN-122 (40 days). The frequency distribution of 174 BILs for dormancy period showed continuous distribution indicating quantitative naure of the trait (Fig. 2).

Table 3. Evaluation for seed shattering percentage by using hand gripping.

Shattering degree	Shattering scale	Shattering percent (%)	No. of BILs
Non-shattering	1	<1	0
Low	3	1-5	2
Moderate	5	5-25	47
	7	25-50	112
High	9	>50	18

Shattering percentage

174 BILs showed a variable seed shattering pattern. None of the BILs were found to have non-shattering trait, 2 BILs with low shattering, 47 BILs showed moderate resistance to shattering 112 BILs were found with high grain shattering and 18 BILs with very high grain shattering percentage as per the shattering scale of 1-9 (Table 3). The frequency distribution of 174 BILs for shattering trait showed a typical bell shaped curve with continuous distribution indicating quantitative nature of the shattering trait (Fig. 3).

Shattering percentage ranged from 5 to 68.08 % with a general mean of 33. 67 %. Lowest shattering percentage was observed in two BILs SN-38 and SN-163 (5%) and highest in SN- 96 (68.08%) and SN161 (65.5%).

Parental polymorphic survey

Of the 296 SSR primer pairs used for polymorphism survey between Swarna and *O. nivara* accession (CR 100008), 94 were polymorphic and 168 were monomophic and 34 SSRs didn't amplify. Number of polymorphic markers ranged from a minimum of 4 on

Table 2. Percent of germination of BILs at weekly intervals after harvesting.

Germination percentage (%)	No. of BILs germinated at weekly intervals after harvesting					
	1st week	2nd week	3rd week	4th week	5th week	6th week
> 80 %	13	25	59	107	158	174
40-80 %	41	84	86	47	12	0
< 40 %	29	22	5	0	0	0
0	91	43	24	20	4	0

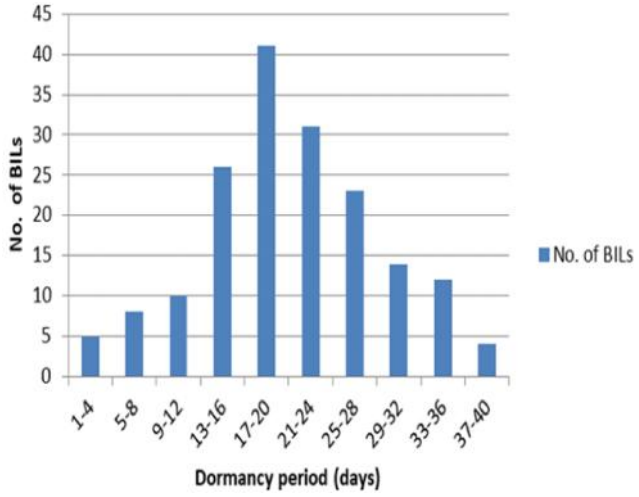

Fig. 2. Phenotypic distribution of germination pattern in BILs at regular intervals after harvesting.

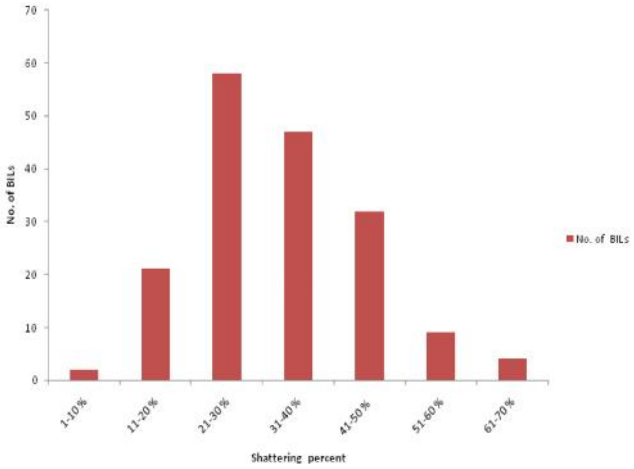

Fig. 3. Phenotypic distribution of shattering percentage of 174BILs.

chromosomes 10 and 11 to a maximum of 15 on chromosome 5, while 5 each on chromosomes 7 and 8 and 6 on chromosome 6, 7 each on chromosomes 3 and 9, 8 on chromosome 1 while 9 on chromosome 12 and 14 on chromosome 2. Maximum polymorphism between the parents was observed on chromosomes 2 and 5.

Marker-trait segregation for dormancy and shattering traits

Two bulks of the phenotypic extremes were screened with 94 polymorphic SSRs. Two markers RM488 on chromosome 1 and RM219 on chromosome 9 were polymorphic with clear and distinctive bands in two bulks for dormancy trait (Fig. 4a) and while another two markers RM314 on chromosome 6 and RM247 on chromosome 12 were found to be polymorphic between the bulks for shattering trait (Fig. 5a). To assess the co-segregation of RM488 and RM219 with dormancy trait, single marker analysis was performed with these two markers in each of the 32 individual BILs that made up the bulks. RM 488 co-segregated with the dormancy trait with 10 out of 16 BILs showing Swarna type allele in > 5 weeks dormant BILs and 10 out of 16 BILs showing *O. nivara* type allele in < 1 week dormant BILs (Fig. 4b, c). RM219 didn't show co-segregation with the dormancy trait. Hence, RM488 on chromosome 1 can be considered as tagged with dormancy trait in rice.

Likewise, for shattering, the individual BILs of the bulks were screened with RM314 and RM247 to identify marker-trait linkage. RM 314 didn't show co-segregation in the individuals BILs with 10 out of 10 BILs of low shattering bulk and 8 out of 10 heavily shattering bulk showing similar banding pattern of Swarna type. RM247 showed co-segregation with shattering trait with clear and similar banding pattern of Swarna type in 8 out of 10 BILs of low shattering bulk and similar banding pattern of *O. nivara* type in 7 out of 10 BILs in heavily shattering bulk (Fig. 5b, c). Thus RM247 can be considered as tagged with shattering trait in rice. Single marker analysis revealed co-segregation of RM488 on chromosome 1 with dormancy trait and RM247 on chromosome 12 with shattering trait.

O. nivara, considered to be the direct progenitor of *O. sativa* (Vaughan et al., 2003) can be exploited in breeding programmes having high cross compatibility without any need for embryo rescue procedures (Niroula and Bimb, 2009). *O. nivara* is considered to be resourceful for novel alleles (Wickneswari et al., 2012 and Haritha et al., 2018) and has been used as donor to produce BILs (Addanki et al., 2018). Earlier, *O. nivara* accessions were used in the development of BILs in crosses with Swarna and many traits related to yield and quality were mapped (Sarla et al., 2003; Swamy et al., 2009; Swamy et al., 2014). These studies indicated that transgressive segregants showed improved yield traits implying that

alleles from *O. nivara* were favourable in the genetic background of Swarna.

Beneficial alleles can be introgressed into elite cultivars from wild rice through BIL approach (Brar and Khush, 1997; Monforte and Tanksley, 2000; Li et al., 2004). Thus developed BILs contain wild segments in the chosen cultivars genetic background (Tian et al. 2006; Tan et al., 2007; Barone et al., 2009). BILs can be considered valuable resources which help to accelerate molecular breeding carrying important agronomic traits with mere linkage drag from the wild parent.

In this study, 174 BILs derived from the cross of Swarna and *O. nivara* were used in evaluating dormancy and shattering related traits. Seed germination is influenced by the precise induction and release of seed dormancy. However, weak or deep seed dormancy can have adverse effects on seed germination causing non uniformity and delay in the sowings. Seed dormancy with both advantages and disadvantages can be considered to explain domestication and utilization. Thus, it becomes indispensable for the breeder to establish balance. Weak dormancy can lead to pre harvest sprouting reducing the quality of harvested seeds. In other terms, strong dormancy prevents germination during short spells of favorable conditions and allowing the plant to grow in an unsuitable environment.

Based on germination percentages 174 BILs were grouped into four categories on the number of germinated introgression lines as >80%, 40-80%, <40% and lines with no germination in the first seven days. Number of lines germinated increased with increase in days till 6th week. BILs showed good variation in germination indicating significant differences between Swarna and *O. nivara*. The results were in conformity with (Kumar et al., 2009; Ye et al., 2010; Hori et al., 2010; Angrish and Panwar, 1996; Mutinda et al., 2017; Magwa et al., 2016; Cai and Morishima, 2000; Lee et al., 2016; Sanghamitra et al., 2018) who had reported variation in germination percentage in rice with the increase in duration after harvesting.

Seed dormancy has evolved to make the plant grow in its favourable conditions and continue its growth cycle. However, untreated seeds showed less germination representing initial poor germination. When 174 BILs were tested for germination, seeds showed initial dormancy but, within 7-14 days of time, more than 50% of the lines showed germination (Fig. 4). Maximum mean germination (82.86%) was recorded at 45°C for 72 hrs (Hanumanthappa et al., 2016). In the present study, to assure uniform germination during *kharif* 2018, the freshly harvested BILs of previous season (*rabi*, 2018) were incubated at 60ºC for 48h that helped in breaking dormancy in some of the BILs which otherwise had 2-6 weeks dormancy post harvesting. The increase in germinability may be due to increase in cracks which allows in imbibition and the results agree with the study conducted by Waheed et al. (2012), who observed an increase in germination in wild rice and cultivated rice after hulling at 50°C.

These results agree largely with previous reports that rice in general has dormancy from weak to strong (Hanumanthappa et al., 2015; Padma and Reddy, 2000). Varying degrees of dormancy have also been reported for the seeds of weedy rice (Franco et al., 1997) wild species of the *Oryza* genus (Oka and Morishima, 1967; Takahashi, 1984; Shimamoto et al., 1994) and cultivated species of *O. sativa* and *O. glaberrima* (Roberts, 1961; Misra and Misro, 1970; Kalita et al., 1994). The present study indicates that variation in dormancy period among BILs is due to the involvement of wild species accession *O. nivara* (CR 100008) as one of the parents.

In the marker-trait analysis for dormancy trait, RM488 on chromosome 1 and RM219 on chromosome 9 showed polymorphism between bulks of phenotypic

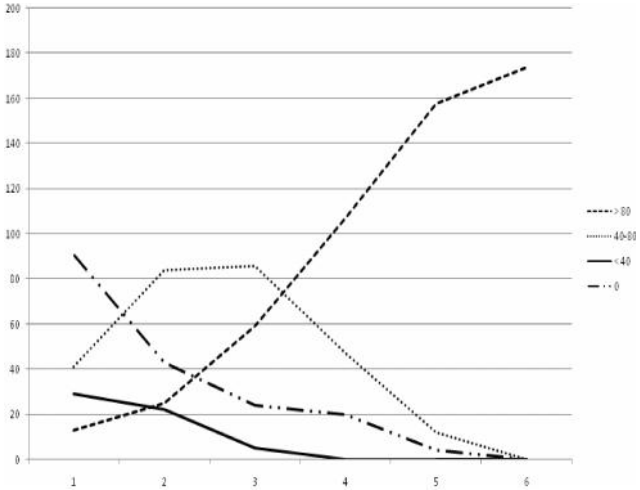

Fig. 4. Germination at weekly intervals after harvesting in 174 BILs.

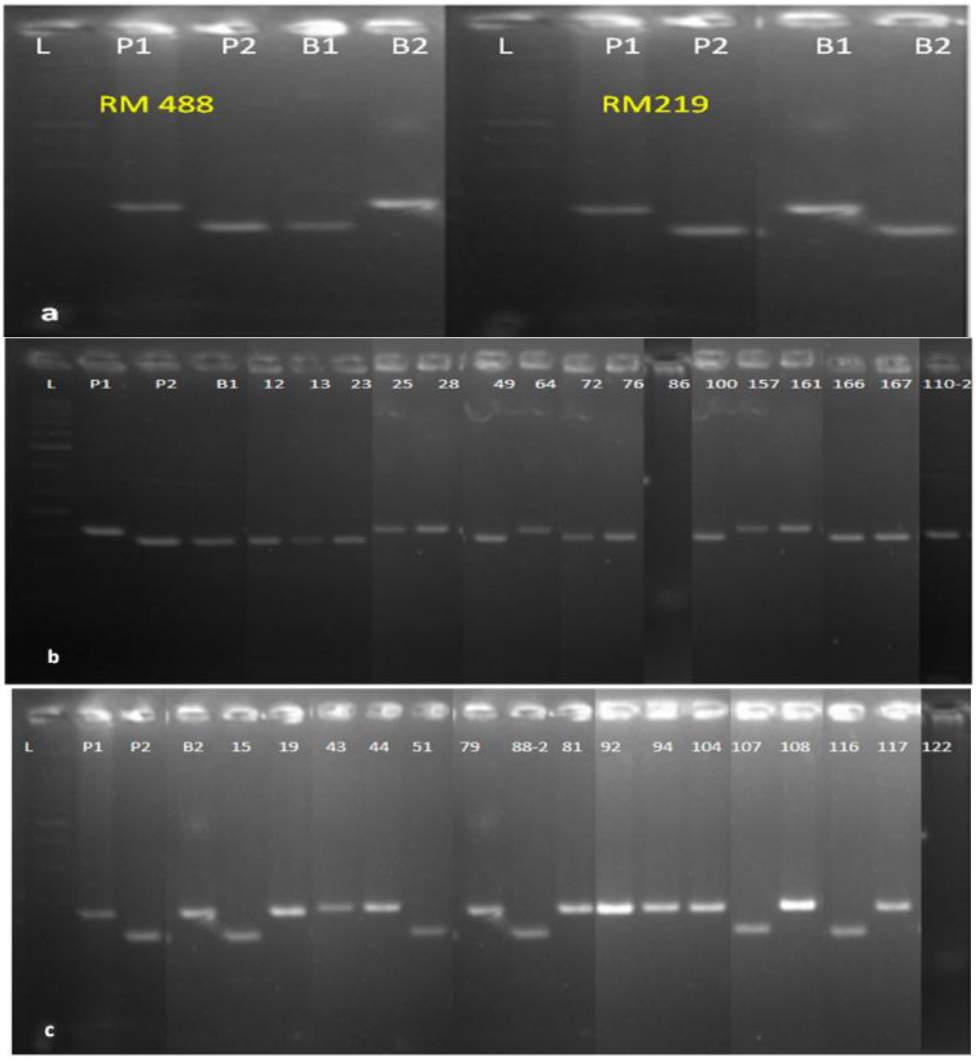

Fig. 4. (a) Bulked segregant analysis for dormancy trait, L- ladder -200bp, P1- *O. nivara*, P2-Swarna, B1- >5 week bulk, B2- <1 week dormant bulk. (b) Single marker analysis with RM488 in the individual BILs of the B1, 12 to 110-2 are the BILs (SN lines) that made up B1 (c) Single marker analysis with RM488 in the individuals of the B2, and 15 to 122 are the BILs (SN) lines that made up B2.

extremes for the trait dormancy, while RM488 allele alone co-segregated with the dormancy trait in the individual BILs of the bulk and is from *O. nivara*. RM 488 was found associated with eight other traits related to yield and quality (Swamy et al., 2018). Nearly 165 dormancy related QTLs on almost all the chromosomes have been reported in Gramene QTL database. However, review of literature indicates significance of dormancy QTL on chromosome 1. Wan et al., 2006 identified five QTLs for seed dormancy, *i.e.*, *qSdn-1*, *qSdnj-3*, *qSdn-5*, *qSdn-7* and *qSdn-11*, on chromosomes 1, 3, 5, 7 and 11, respectively and of all QTLs, *qSdn-1* provided the most obvious target for marker-assisted selection (MAS) due to its stability for improving rice pre-harvest sprouting tolerance. In support of these results, Lu et al. (2010) showed that *qSdn-1* displayed major effects on seed dormancy in N22. In another study by Xie et al. (2010), three seed dormancy QTLs, *qSd-1*, *qSd-2* and *qSd-3*, were detected. Furthermore, *qSd-1* was detected to be a major dormancy QTL in the advanced backcross population of Nanjing35/N22/N22 (BC_6F_2), and resolved into *qSd-1-1* and *qSd-1-2* (Xie et al., 2010). In addition, Lu et al. (2011) suggested that *qSdn-1* and

qSdn-5 are major effect seed dormancy QTL in N22 and qSdn-1 is flanked by RM488-RM11669. The association of RM488 with dormancy trait in the previous reports as well as in the present study and its association with several important agronomic traits in rice indicate the significance of RM488. Hence, genomic region at RM488 on chromosome 1 can be further explored for practical breeding purposes.

Shattering percentage

Similar to dormancy trait, the trait 'shattering' is also a tricky trait. Heavily shattering types lead to grain loss and economic losses to the farmers while non-shattering types pose difficulty in threshing. Thus, it is important to focus on developing rice varieties with some amount of shattering that prevents grain fall in the field or at the time of harvest and enables ease in threshing. Rice varieties having moderate shattering are preferred for both hand and combined threshing whereas non shattering varieties may not be threshing friendly which again poses a problem in its end use (Kobayashi, 1990). The precise determination of the shattering trait will guide the breeder, to develop an acceptable shattering ability in rice. Shattering habit of rice has been evaluated by the estimation of the number of shattering grains by grasping panicles (Kikuchi et al., 1985; Oba et al., 1990), the estimation of the rate of shattering by grasping panicles (Okubo et al., 2012; Okubo, 2013). Similar

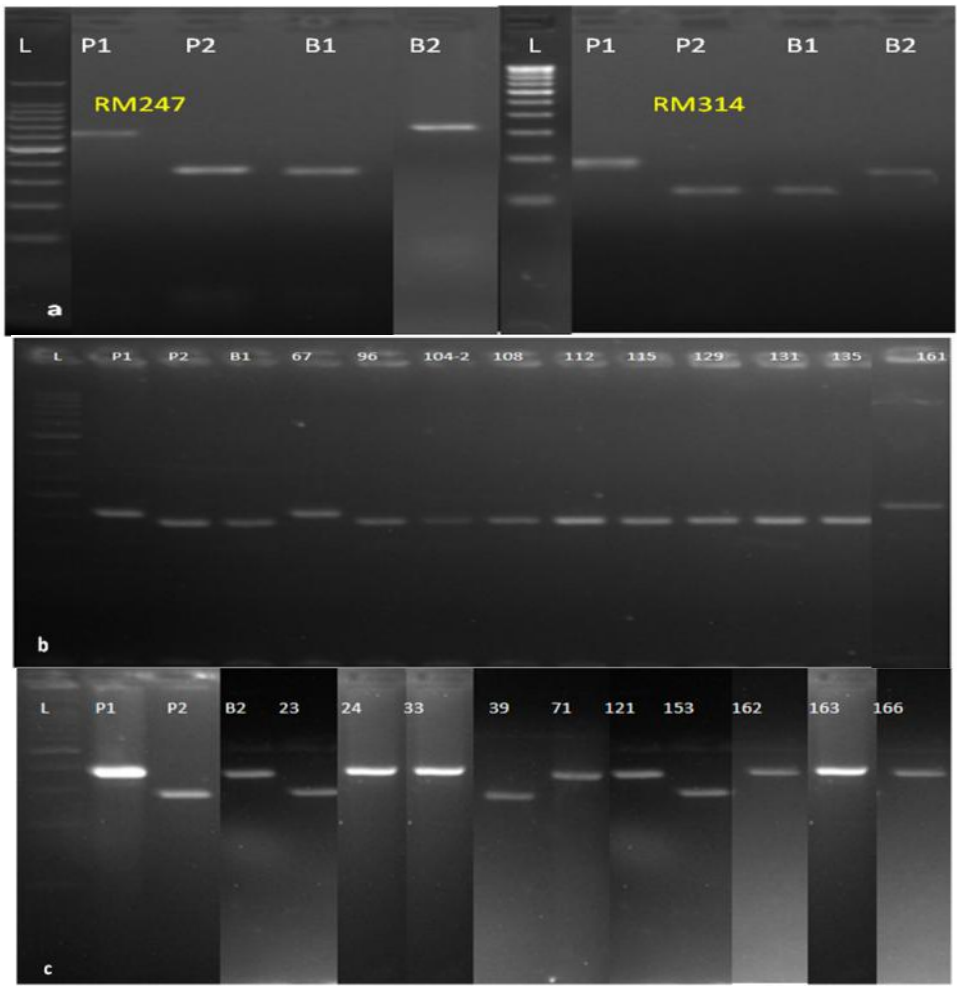

Fig. 5. (a) Bulked segregant analysis for shattering trait, L- ladder -200bp, P1-*O. nivara*, P2-Swarna, B1- low shattering bulk, B2- heavily shattering dormant bulk. (b) Single marker analysis with RM247 in the individual BILs of the B1, 16-161 are the BILs (SN lines) that made up B1 (c) Single marker analysis with RM247 in the individuals of the B2, and 23 to 166 are the BILs (SN) lines that made up B2.

approach was followed in our study in phenotyping for shattering trait by grasping of panicles in fist.

A breeding objective that targets grain shattering and associated traits at the initial stages of the breeding process could lead to optimum grain shattering ability. The frequency distribution of 174 BILs for shattering trait showed a typical bell shaped curve with continuous distribution indicating quantitative nature of the shattering trait. Similar findings were reported by (Cai and Morishima, 2000; Lin et al., 2007; Chin et al., 1999; Niruntrayakul et al., 2009) reported in wild rice, Hurber et al., 2010 reported in weedy rice. In contrast, Kwon et al., 2015, reported more low shattering RILs. Complex polygenic control for shattering was observed in *O. nivara* (Li et al., 2006b) in weedy rice (Bres-Patry et al., 2001; Gu et al., 2005; Thurber et al., 2013) and cultivated rice (Qin et al., 2010).

From 174 BILs, two bulks each containing distinct phenotypic extremes for seed shattering namely shattering bulk 1 (high shattering) and 2 (low shattering) respectively were screened with 94 SSRs which were found polymorphic with Swarna and *O. nivara*. Two markers RM314 on chromosome 6 and RM247 on chromosome 12 were found to be polymorphic between the bulks, however only RM247 showed co-segregation with shattering trait when individual BILs of the bulks were screened with these two markers to identify marker-trait linkage. Molecular marker studies demonstrate that many QTLs widely distributed over the genome control seed shattering in rice. There are several reports on QTLs for dormancy related traits on other chromosomes. Cai and Morishima, 2000 reported on chromosomes 1, 4, 8 and 11, Onishi et al., 2007 on chromosomes 1, 3 and 4, Qin et al., 2010 on chromosomes 1, 3, 4 and 5, Yao et al., 2015 on chromosomes1, 3 and 5 and Ishikawa et al., 2010 on chromosomes 1 and 4. Though Thurber et al., 2013 reported *qSS12s* on chromosome 12 at 21.8 cM and and Qi et al., 2015 reported *qSH12B* at 30.63 cM, the present study identified a novel putative marker RM247 for shattering trait at 3.18 cM on chromosome 12. Interestingly, allele at RM 247 among the low shattering BILs is from *O. nivara*.

Both shattering and dormancy cause economical loss to the farmer. These traits have been under selection during domestication which can be easily differentiated between wild and the cultivated rice. The present study elucidates the importance of dormancy and shattering traits in rice, amount of variation observed for these traits employing BILs derived from the cross of cultivated and wild species. The results contribute to the understanding of the genetic basis for dormancy and shattering traits in rice and identified putative markers will pave way for employing sequence based approach in these regions to identify the candidate genes responsible for the traits.

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