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Biswajit Sahoo
Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India

Sunil Kumar Nair
Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India

Debarchana Jena
ICAR- National Rice Research Institute, Cuttack, Odisha, India

Diptibala Rout
ICAR- National Rice Research Institute, Cuttack, Odisha, India

Vineeta Singh
ICAR- National Rice Research Institute, Cuttack, Odisha, India

P Arsode
ICAR- National Rice Research Institute, Cuttack, Odisha, India

Amrita Giri
Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India

Sadhna Saha
Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India

Anushree Pramanik
Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India

DK Rana
ICAR- National Rice Research Institute, Cuttack, Odisha, India

JL Katara
ICAR- National Rice Research Institute, Cuttack, Odisha, India

SD Mohapatra
ICAR- National Rice Research Institute, Cuttack, Odisha, India

Sanghamitra Samantaray
ICAR- National Rice Research Institute, Cuttack, Odisha, India

Ramlakhan Verma
ICAR- National Rice Research Institute, Cuttack, Odisha, India

Corresponding Author:
Ramlakhan Verma
ICAR- National Rice Research Institute, Cuttack, Odisha, India

Genetic diversity analysis among parents and derivative NILs of rice hybrid Rajalaxmi improved for BPH tolerance

Biswajit Sahoo, Sunil Kumar Nair, Debarchana Jena, Diptibala Rout, Vineeta Singh, P Arsode, Amrita Giri, Sadhna Saha, Anushree Pramanik, DK Rana, JL Katara, SD Mohapatra, Sanghamitra Samantaray and Ramlakhan Verma

Abstract
Extent of genetic diversity among parents is very crucial for planning research strategies in crop plants. This study is pertaining to the trait development of brown plant hopper (BPH) resistance in popular rice hybrid Rajalaxmi. Parents of Rajalaxmi along with donors (CRMS32B, IR42266-29-3R, CR 2711-76, Dhobanumberi) and 15 NIL derivatives were taken for diversity analysis. Total 48 hyper-variable STMS markers were used to assess the genetic diversity among recurrent parents (CRMS32B, IR 42266-29-3R) and donors (CR 2711-76 and Dhobanumberi). Jaccard's similarity coefficient value was found to be ranged from 0.56 to 0.82 indicates substantial genetic distance among the genotypes. Clustering patterns showed a clear-cut distinction among 4 parental genotypes which categorized into major and minor groups. Besides, results of genetic similarity among parents and NILs which was estimated utilizing 70 SSR markers was ranged 0.71 to 0.91. Results of this study reflects the utility of donor parents for enhancing BPH resistance and yield consistency in rice hybrid Rajalaxmi.

Keywords: Similarity coefficient, genetic diversity, recurrent parents NILs, STMS

Introduction
Rice (*Oryza sativa* L.) is a self-pollinated crop, family Gramineae (Poaceae), order Cyperales and one of major staple food crop of Asia and majority of world's population. It is a major crop of Asia, Latin America and Africa (Rao *et al.* 2016) [13]. A thorough understanding of genetic diversity among existing cultivars of any crop is required for long term success of breeding program and higher exploitation of the genetic resources (Belaj *et al.*, 2002) [1]. Hybridization technique is used for the purpose of the analysis of genetic diversity for the selection of parents (Singh, 1983) [17]. Indigenous crop varieties and traditionally cultivated varieties contain high level of genetic diversity which is the source of high potential genetic potential for improving yield, resistance to pests and pathogens, and agronomic performance (Choudhury *et al.*, 2013) [2]. Indigenous crop varieties and traditionally cultivated varieties are rich in genetic variability to fulfil the food demand in the present scenario of climate change (Pusadee *et al.*, 2009) [12]. Genetic diversity is also helpful in the evaluating the important source genes of particular traits within the existing germplasm (Roy and Panwar, 1993) [16]. To feed the growing population we need to produce more rice from limited area through varieties or hybrids which should perform well in the different adverse conditions to produce higher yield (Khush, 2005) [6]. Rice accessions are rich source of genetic variability that can be harnessed for rice improvement programme (Rasmi *et al.* 2017). Genetic diversity is the foremost thing to carry out any crop breeding programme which out come in to a superior recombinants (Manonmani and Fazlullah Khan 2003) [7] and proper identification and selection of donor and recipient parents having wider adaptability and variability for different traits (Nayak *et al.*, 2004) [11].
Genetic diversity assessment between and within groups or clusters is very important to trace valuable parents having desirable traits and also to search the genotypes responsible for higher heterosis to use in Hybrid rice development programmes (Murty and Arunachalam, 1966) [9]. In the recent pasts, rice has faced a tremendous loss in Bio-diversity due to advancement and modernization of civilization, constructions roads and building etc. which needs replacement of native varieties with high yielding varieties (Choudhary *et al.*, 2013 and Heal *et al.*, 2004)

[5]. Genetic divergence analysis recognizes the genetical distance and genetic potential among the selected genotypes and also the relative contribution of specific traits towards the genetic pool (Iftekharuddaulae *et al.*, 2002). Heterosis has paramount importance that could be achieved through crosses between genetically distant parents (Falconer 1960) [3]. This study was undertaken to determine the genetic diversity in rice for the maximum utilization of the genetic resources and proper selection of donor parents.

Material and Methods

Genetic diversity analysis among recurrent and donor parents were assessed to avoid undesirable linkage drag as well as quick succession in pyramiding of target gene (s). Materials used for the study includes CRMS32B, IR42266-29-3R, CR 2711-76, Dhobanumberi and their 15 NILs derivatives. Total 48 hyper-variable STMS markers were used to assess the genetic diversity among recurrent parents (CRMS32B, IR 42266-29-3R) and donors (CR 2711-76 and Dhobanumberi). Molecular data generated were subjected to construct dendrogram by using UPGMA method of pooled SSR (STMS) data. This method is well suited for diversity analysis since it groups the individuals into several clusters which help to illustrate the arrangement of the clusters and discriminate the clustered parental lines.

Result and Discussion

The genetic coefficients measured using molecular data revealed varying degrees of genetic relatedness among the recurrent parent CRMS32B and donors CR 2711-76, and Dhobanumberi; and IR 42266-29- 3R and CR 2711-76 and Dhobanumberi. Jaccard's similarity coefficient ranged from 0.56 to 0.82 due to diversification in morphology and pedigree among the genotypes. Clustering results showed a clear-cut distinction among 4 parental genotypes, categorizing those into major and minor groups in (Fig 1 and Table 1). All 4 genotypes (2 RP and 2 donor lines) were grouped into 2 major clusters. The cluster I includes 1 genotype, Dhobanumberi which had least similarity coefficient value with RP, CRMS32B (0.62) and IR 42266-29-3R (0.56), whereas cluster II which includes 3 genotypes is further subdivided into 2 sub clusters includes 2 RP, CRMS32B, IR 42266-29-3R and donor, CR 2711-76. Donor CR 2711-76 (Bph31) was found to have the most close genetic relation with recurrent parent CRMS32B (0.71) and with IR 42266-29-3R (0.68).

The matrix value ranged from 0.06 to 0.77 (Table 2, Fig. 2). Hence, to avoid undesirable linkage drag and quick fixation of segregating loci with substantial RP genome recovery, CR 2711-76 having maximum genetic relatedness with recurrent parent CRMS32B and IR 42266-29- 3R was selected as donor for *Bph31* gene introgression. Cluster study in Pusa Basmati-1, Tarori Basmati, Basmati-385 and Vallabh Basmati-21, varieties Type 3, Ranbir Basmati specified a greater affinity in newly developed variety Vallabh Basmati-21 with the traditional varieties in company with the popular released varieties CSR-30 and PB-1 (Nagaraju *et al.*, 2002 and Singh, 2008) [10, 18].

Table 1: Genetic relationship among 04 different parental lines based on SSR marker.

Genotypes	IR 42266-29-3R	CRMS32B	CR 2711-76	Dhobanumberi
IR 42266-29-3R	1.0			
CRMS32B	0.82	1.0		
CR 2711-76	0.68	0.71	1.0	
Dhobanumberi	0.62	0.56	0.68	1.0

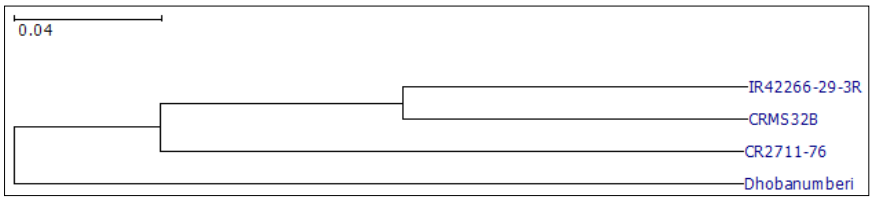

Fig 1: Genetic relationship among 04 different parental lines based on SSR marker.

Trait improvement/development strategy is worked upon stacking desirable trait (s) in a popular variety with intact product profile of recurrent parent. In view of selecting improved lines with >95% genome recovery of recurrent parent and least linkage drag from donor, genetic relatedness analysis amongst parents and NILs was done based on similarity metrics data of 70 informative SSR markers which ranged 0.71 to 0.91 (Table-3, Figure 3). Altogether, 17 genotypes (2 parents and 15 NILs) of CRMS32B/CR 2711-76 crosses were could be distinguished into 2 major clusters, where cluster I-A consists of 1 genotype, CR 2711-76 (donor) with least similarity coefficient value of 0.71. Whereas, cluster-II were found to be distributed with RP and 15 NIL derivatives which were 117 further subdivided into sub-sub clusters with maximum similarity coefficient value of 0.91 between CRMS32B (RP) and NIL-4 (0.91) followed by NIL-7 (0.91) and NIL-3 (0.90).

Whereas, NIL lines of IR42266-29-3R/CR 2711-76, all 17 genotypes (2 parents and 15 NILs) were grouped into two clusters, where cluster -1 pertained to the donor parent with 0.68 genetic similarity with RP and derivative NILs. Cluster-II pertained 16 genotypes including RP had maximum 0.90 similarity coefficient between NIL-7 (0.90), NIL-3 (0.89) and NIL-4 (0.89) found to have substantial recurrent parent genome recovery in the improved lines. Intra-cluster distance between NILs revealed close genetic similarity between improved lines with varying phenomic provides it to be more sustainable in biotic/abiotic adversity. Similarity varies from 0.77 to .98 in the 16 accessions of Iranian rice traditional varieties (Moumeni *et al.*, 2003) [8]. 45 accessions of the AA-genome of rice possessed a similarity value from 0.36 to 0.96 from wide genetic variable germplasms (Ren *et al.*, 2003). In an experiment similarity value varies from 0.22 to 0.68 in 193 accessions at IRRI collected from 26 different countries (Yu *et al.*, 2003) [19].

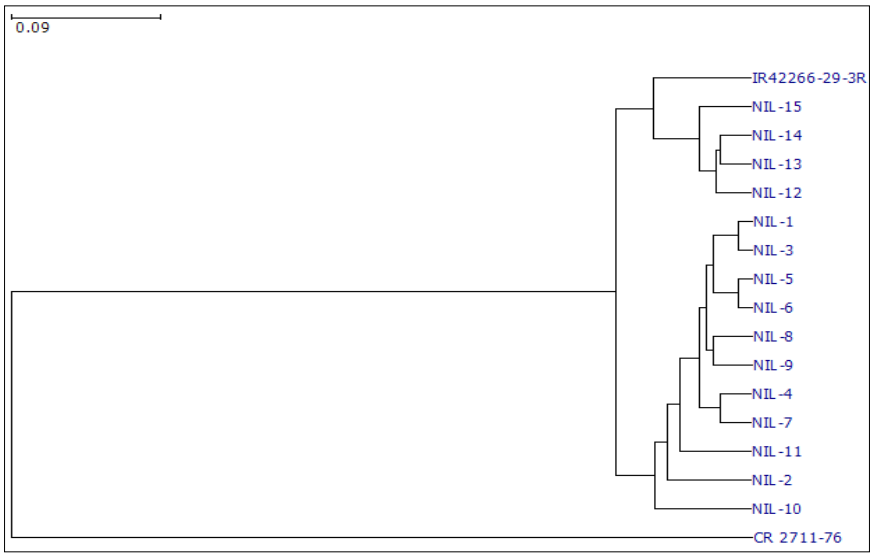

Fig 2: Genetic diversity between parentage (IR 42266-29-3R, CR 2711-76) and improved NILs derivative.

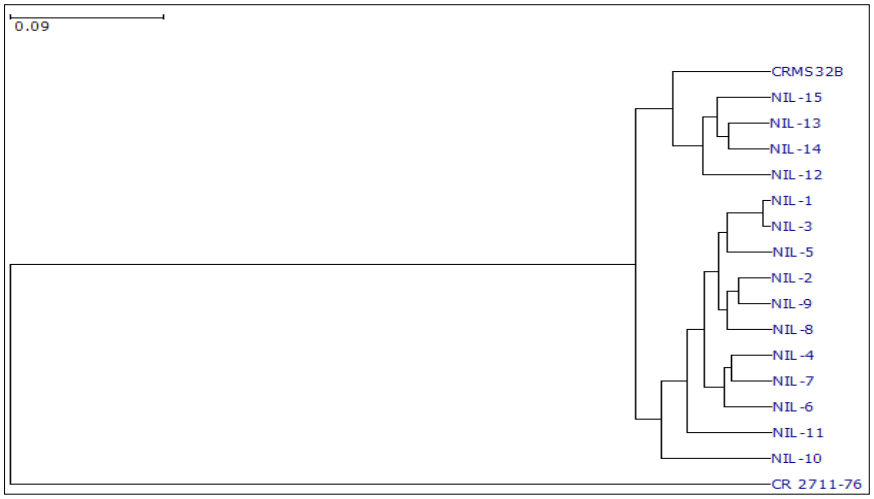

Fig 3: Genetic diversity between parentage (CRMS32B, CR 2711-76) and improved NILs derivative.

Table 2: Similarity coefficient values revealing relatedness among parent (CRMS32B) and NIL derivatives.

	CRMS32B	CR 2711-76	NIL-1	NIL-2	NIL-3	NIL-4	NIL-5	NIL-6	NIL-7	NIL-8	NIL-9	NIL-10	NIL-11	NIL-12	NIL-13	NIL-14	NIL-15
CRMS32B	1.0																
CR 2711-76	0.71	1.0															
NIL-1	0.89	0.09	1.0														
NIL-2	0.84	0.11	0.93	1.0													
NIL-3	0.90	0.09	0.99	0.91	1.0												
NIL-4	0.91	0.05	0.93	0.88	0.94	1.0											
NIL-5	0.85	0.07	0.95	0.90	0.94	0.91	1.0										
NIL-6	0.88	0.07	0.91	0.91	0.90	0.94	0.94	1.0									
NIL-7	0.91	0.07	0.93	0.94	0.96	0.93	0.95	0.94	1.0								
NIL-8	0.85	0.10	0.94	0.95	0.93	0.94	0.94	0.93	0.94	1.0							
NIL-9	0.88	0.09	0.94	0.93	0.88	0.94	0.94	0.93	0.91	0.95	1.0						
NIL-10	0.87	0.09	0.88	0.90	0.88	0.89	0.89	0.16	0.85	0.87	0.89	1.0					
NIL-11	0.85	0.06	0.90	0.89	0.89	0.93	0.93	0.10	0.85	0.89	0.94	0.88	1.0				
NIL-12	0.89	0.09	0.88	0.89	0.89	0.89	0.93	0.93	0.88	0.91	0.94	0.93	0.90	1.0			
NIL-13	0.89	0.07	0.83	0.78	0.88	0.83	0.84	0.84	0.87	0.83	0.83	0.93	0.93	0.93	1.0		
NIL-14	0.87	0.07	0.84	0.77	0.87	0.82	0.83	0.83	0.85	0.82	0.82	0.84	0.84	0.91	0.95	1.0	
NIL-15	0.87	0.09	0.84	0.76	0.88	0.83	0.84	0.82	0.84	0.87	0.80	0.87	0.82	0.91	0.94	0.93	1.0

Table 3: Similarity coefficient values revealing relatedness among parent (IR42266-29-3R) and NIL derivatives.

Genotype	IR42266-29-3R	CR 2711-76	NIL-1	NIL-2	NIL-3	NIL-4	NIL-5	NIL-6	NIL-7	NIL-8	NIL-9	NIL-10	NIL-11	NIL-12	NIL-13	NIL-14	NIL-15
IR42266-29-3R	1.0																
CR 2711-76	0.68	1.0															
NIL-1	0.89	0.09	1.0														

NIL-2	0.84	0.11	0.88	1.0													
NIL-3	0.89	0.10	0.98	0.88	1.0												
NIL-4	0.89	0.07	0.93	0.90	0.95	1.0											
NIL-5	0.85	0.10	0.96	0.89	0.96	0.94	1.0										
NIL-6	0.85	0.10	0.94	0.91	0.94	0.94	0.98	1.0									
NIL-7	0.90	0.09	0.94	0.91	0.94	0.96	0.93	0.95	1.0								
NIL-8	0.87	0.09	0.95	0.90	0.95	0.93	0.94	0.94	0.94	1.0							
NIL-9	0.87	0.10	0.95	0.90	0.93	0.88	0.94	0.94	0.91	0.95	1.0						
NIL-10	0.84	0.11	0.88	0.82	0.90	0.88	0.89	0.89	0.89	0.88	0.88	1.0					
NIL-11	0.85	0.07	0.91	0.89	0.89	0.89	0.93	0.93	0.88	0.91	0.94	0.87	1.0				
NIL-12	0.89	0.09	0.85	0.78	0.88	0.83	0.84	0.84	0.87	0.83	0.83	0.85	0.79	1.0			
NIL-13	0.88	0.09	0.84	0.77	0.87	0.82	0.83	0.83	0.85	0.82	0.82	0.87	0.78	0.96	1.0		
NIL-14	0.87	0.08	0.85	0.76	0.88	0.83	0.84	0.82	0.84	0.87	0.80	0.85	0.77	0.95	0.96	1.0	
NIL-15	0.87	0.11	0.82	0.76	0.84	0.80	0.80	0.80	0.84	0.79	0.79	0.84	0.76	0.91	0.95	0.94	1.0

Conclusion

Although the traditional and indigenous varieties are low yielding having valuable genes which can combat against the different biotic and abiotic stresses to increase the yield potential of the existing high yield varieties. Use of molecular marker (SSR, SNPs and STS marker) speed up the study of genetic diversity at the DNA level which maximizes the manipulation of genetic potential of various landraces for future use. The knowledge about the genetic diversity and genetic potential are very crucial for proper identification and selection of appropriate parents for breeding programs which involves gene mapping and also useful for the introgression of various traits to correct the susceptibility of high yielding varieties to increase the yield potential.

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