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Identification of high grain number genes and assessment of genetic diversity in high and low grain number rice genotypes useful for marker-assisted selection breeding programs

GAYATRI GOUDA[1], MADHUCHHANDA PARIDA[1], RAVINDRA DONDE[1], MANOJ KUMAR GUPTA[2], JITENDRA KUMAR[1], SOUMYA MOHANTY[1], RUDRAKSH SHOVAN PANDA, SUSHANTA KUMAR DASH[1], SHARAT KUMAR PRADHAN[1], TRILOCHAN MOHAPATRA[3] AND LAMBODAR BEHERA*[1]

[1]Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack-753006, India

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ABSTRACT

The introgression of high grain number QTLs/ genes into popular rice varieties is one of the potential approaches for increasing grain yield. The primers specific for three high grain number genes, Gn1a, OsSPL14 and Dep1 were used to detect the presence of these genes in two high grain number rice genotypes, CR444-3-1-1-1 and CR3856-44-22-2-1-11 and one popular low grain number rice variety, MTU1010. These three high grain number genes were detected in CR444-3-1-1-1 while one gene, Gn1a was detected in CR3856-44-22-2-1-11. All the three genes were found to be absent in MTU1010. Further, genetic diversity study was carried out in these three genotypes using 58 microsatellite markers and 10 phenotypic traits. A total of 139 alleles were amplified with an average of 2.28 alleles per locus by 58 microsatellite markers. Major allelic frequency varied from 0.33 to 0.83 with an average of 0.61. The polymorphism information content (PIC) ranged from 0.24 to 0.67 with an average of 0.4. Gene diversity ranged from 0.28 to 0.72 with an average of 0.4. Genetic diversity was measured using 58 SSR and 3 gene specific markers. A similarity coefficient of 0.244 and 0.260 were found between MTU1010 and CR444-3-1-1-1, and MTU1010 and CR3856-44-22-2-1-11, respectively. Out of 58, 45 and 46 SSR markers were found to be polymorphic between MTU1010 and CR3856-44-22-2-1-11, and MTU1010 and CR444-3-1-1-1, respectively. The grain number per panicle ranged from 109.2 (MTU1010) to 294.50(CR444-3-1-1-1) with an average of 225.97.

Key words: Genetic diversity, grain number, microsatellite, multivariate analysis.

INTRODUCTION

Rice(*Oryza sativa* L.) is a major staple food crop in the world. About 92% of total rice is produced in Asian countries which supplies 60% energy in human population. Rapid industrialization, mechanization and increasing population took place in many parts of the worlds which led to the reduction of land, labor and water for agriculture (Varshney *et al.* 2011). The frequency of climate changes was also increasing day by day and it has adverse effects on crop productivity. Rice production is mainly reduced by climatic changes occurred in the environment (Godfray *et al.* 2010). In the past decade, due to the domestication process, there has been a significant slowdown in the production potential of modern cultivars. Therefore, the physiologists and breeder hypothesized that narrow bottleneck and loss of useful alleles might be responsible for having several unproductive tillers and limited sink size i.e., small panicles. Therefore, it is highly essential to produce new varieties with high and stable yield. Grain number is an important trait for grain yield. The rice yield is measured through grain numbers per panicle, tiller number per plant and spikelet number per panicle and thousand-grain weight. For increasing yield, it is important to detect the genes responsible for grain number as well as the panicle number. Increasing grain number is possible through introduction of the genes for high grain number into elite rice cultivars. This should maximize the food supply by farmers in a limited area that could reduce the yield gap. Pyramiding of favourable genes/QTLs for grain yield traits would make a significant role for the development of high-yielding rice varieties. It is now possible to pyramid several favourable genes/QTLs in popular rice varieties within shorter time period using marker-assisted selection approach.

Several QTLs/ genes for high grain number have been identified and few have been cloned. The genes responsible for high grain number such as *Gn1a, Dep1, APO1* and *OsSPL14* have been identified and cloned (Miura *et al.* 2010). A QTL *Gnp4.1* have been introgressed into 9 *indica*and 3 Basmati rice varieties through marker-assisted backcrossing approach for increasing grain number in rice (Singh *et al.* 2018). *Dep1* encodes phosphatydylethonoamine binding protein and is responsible for high grain number, erect and dense panicle. *OsSPL14*has positive effect on grain number by increasing the primary branches in corresponding panicles.Several high grain number genotypes have been obtained through *indica-indica*,*indica-japonica*, *japonica-japonica* crosses.Microsatellite markersare highly polymorphic, co-dominant, abundant and well distributed in the rice genome. These markers have been utilized for identification of genes/ QTLs, assessment of genetic diversity germplasm, introgression of desirable QTLs/ genes into rice cultivars, gene flow study, etc(Behera *et al.* 2012). In the present study, assessment genetic diversity was conducted in two high grain number genotypes(CR444-3-1-1-1 and CR3856-44-22-2-1-11) and one popular low grain number rice variety(MTU1010) at molecular and phenotipic levels. Further, primer specific for high grain number genes, *Gn1a*, *Dep1*, and *OsSPL14* were used for their detection in these genotypes.

MATERIALS AND METHODS

Plant materials

Two high grain number genotypes (CR444-3-1-1-1, CR3856-44-22-2-1-11) and one popular low grain number rice variety, MTU1010 were used in the present study. CR444-3-1-1 is a photosensitive and high yielding advance breeding line with maturity duration of 147 days and developed from*indica-tropical japonica* cross. CR3856-44-22-2-1-11 is a high yielding advance breeding line of *indica* type having duration of 130 days. MTU1010 is a popular rice variety developed by crossing of Krishnaveni and IR64. It is a semi dwarf variety with low grain number and long grain size having duration of 120 days and mainly cultivated in Odisha, Andhra Pradesh and Telangana.

Genomic DNA isolation and PCR amplification

The leaf samples were collected from one-month old transplanted seedlings. Genomic DNAs were isolated from 1-2 gleaf samples of each genotype by using CetylTrimethyl Ammonium Bromide (CTAB) method (Murray and Thompson 1980). The quality and quantity ofDNAs was checked by spectrometrically and 0.8% agarose gel electrophoresis using known concentration of Lambda DNA. The samples were diluted in $T_{10}E_1$ buffer to get final concentration of 15 ng/µl for PCR amplification. A set of 58 microsatellite markers distributed over 12 chromosomes of rice were used for PCR amplification (Table 2). The primer sequences for these markers are available in the Gramene website (http://www.gramene.org). The amplification was carried out in 20µl reaction mixture volume containing 30ng of genomic DNA, 1X PCR buffer {10 mMTris-HCl (pH 8.0), 5 mMKCl, 2.0 mM $MgCl_2$ }, 200 µM dNTP mix (MBI Fermantas, Lithuania, USA), 5 picomole of each of forward and reverse primers, and 1U of *Taq (Thermusaquaticus)* DNA polymerase (Kappa Biosystem, South Africa). The PCR was performed in a thermal cycler (Eppendorf, USA) as per following cycling parameters: initial denaturation at 94^0C for 4 min followed by 35 cycles of denaturation at 94^0C for 45sec, annealing at 55-60^0C (depending upon primer) for 1 min and extension at 72^0C for 1 min and final extension at 72^0C for 8 min. The amplified products were separated on 2.5 % agarose gels using 1X TBE buffer and stained with ethidium bromide (0.5µg/ml). The gels were visualized under UV radiation and photographed using a gel documentation system (Uvitech, Cambridge, UK) to detect the polymorphism.

Identification of genes for high grain number in rice genotypes

The gene specific primers for three high grain number genes, *Gn1a, OsSPL14* and *Dep1* were used to amplify alleles from three rice genotypes, CR444-3-1-1-1, CR3856-44-22-2-1-11 and MTU1010. Amplified PCR products were run in 3% agarose gel. Each amplified band was taken as an allele for each gene locus. The amplified bands of 275bp, 200bp and 110bp were taken as donor (positive) alleles for high

grain number genes, *Gn1a, OsSPL14* and *Dep1,* respectively. The amplified bands of 205bp, 220bp and 130bp were taken as alleles (negative) for low grain number (Jiao *et al.* 2010; Kim *et al.* 2016).

Evaluation of grain number and other phenotypic traits

Three rice genotypes, CR444-3-1-1-1, CR3856-44-22-2-1-11 and MTU1010 were grown in *Kharif* 2018 following Randomized Block Designed (RCBD) at 20cm x 15cm spacing. Twenty five days old seedlings of each genotype were transplanted in the experimental plots. Gap filling was done within a week in order to maintain uniform plant population. Fertilizer dose of 80 kg N, 40 kg P_2O_5, and 40 kg K_2O was applied. Entire dose of P_2O_5 and K_2O along with half dose of N was applied as basal dose at the time of final field preparation. Remaining amount of nitrogen was split in two equal doses and were applied at the time of tillering and grain filling stages. The standard agronomic practices were adopted for normal crop growth. Five plants were selected from middle of the row for data collection. Ten phenotypic traits, namely, 50% flowering (DFF), plant height (PH), panicle number per plant (PN), tiller number per plant (TN), panicle length(PL), fertile grain number per panicle(GN), chaff number per panicle(CH), total spikelet number (TSN), thousand-grain weight (TGW) and grain yield perplant were recorded.

Molecular Data Analysis

The size of each amplified band was determined based on its migration relative to molecular weight markers. The individual bands were assigned as alleles of the appropriate microsatellite locus. The amplified alleles were scored as present (1) or absent (0) for each genotype and marker locus combination. The data were entered into a binary matrix and subsequently analysed using POWERMARKER software. Total number of alleles, allele frequency, heterozygosity, gene diversity, PIC and P-value are calculated using POWER MARKER 3.0 (Liu and Muse 2005). Genetic diversity was analysed using NTSYS-PC 2.02(Rohlf 2004). A dendrogram was plotted by using UPGMA (Unweighted Pair Group Method with Arithmetic means) method.

Phenotypic Data Analysis

10 phenotypic traits were analysed using the software XL-STAT (https://www.xlstat.com/en/).The basic statistics like mean, standard deviation, correlation and p-value were calculated. The PCA and PCA Bi-plot analysis were done using the phenotypic traits (https://www.xlstat.com/en/). Heatmap cluster map was generated from the phenotypic data for trait identification and their dominance in respective genotypes (https://www. xlstat.com/en/).

RESULTS AND DISCUSSION

Identification of high grain number genes

Increasing grain number in rice genotypes is one of the potential approaches for increasing grain yield. Therefore, it is need of day to develop high grain yielding varieties. The present study based on identification three high grain yielding genes in three genotypes and assessment of geneticdiversity between them. The grain number genes were validated through gene linked markers between high grain number and low grain number rice varieties. The three high grain number genes, namely, *Gn1a, Dep1*and *OsSPL14,* which were used for detection of high grain number genes. The *Gn1a* marker was amplified with positive (high grain) allele of 275bp inCR3856-44-22-2-1-11and CR444-3-1-1-1 genotypes, while low grain number allele of 205bp was amplified in MTU1010 (Fig. 1). In case of *Dep1* gene, marker was amplified allele of 110bp (positive) in CR444-3-1-1-1 while 130bp (negative) in CR3856-44-22-2-1-11 and MTU1010. In case of *OsSPL14*, gene linked marker amplified alleles of 200bpand 220bp for high grain and low grain number, respectively. The CR444-3-1-1-1 genotype having three genes combinations i.e., *Gn1a, Dep1*, and *OsSPL14*was identified. Therefore, this genotype could have produce high grain numbersvarying from 280 to 350. The present study reported that CR3856-44-22-2-1-11 genotypehas high grain number gene i.e., *Gn1a*. The MTU1010 is a low yielding popular farmer rice variety having grain number 110 to 130 (Table 1).

Table 1:List of gene specific primers used for identification of high grain number genes in high and low grain rice genotypes

Gene	Primer sequence	Size of amplified alleles		
		CR444-3-1-1-1	CR3856-44-22-2-1-11	MTU1010
Gn1a	F-GATCTAGATGCTCCAAAGTCC R-CTGTACGTACGTGCACGTAG	275*	275*	205**
Dep1	F-AGTTTCTTGGTTTCCGATCA R-CATATTGGAATGCTCCCTCCT	110*	130**	130**
OsSpl14	F- TTCCAGCAGAAAGAAGACGC R- GGAGGAAGCAAATCATTAGTG	200*	220**	220**

**Positive allele for high grain number, **Negative allele for high grain number*

The assessment of genetic diversity is important for varietal identification and development. Joshi *et al.*(2006) used molecular markers to assess genetic diversity in basmati rice. The molecular markers help breeders in the selection of diverse and unique genotypes for using in breeding programs. We identified two genotypes CR444-3-1-1-1 and CR3856-44-22-2-1-11 having high grain number genes with high grain yield. The gene specific primer of three high grain number genes, namely, *Gn1a, Dep1*, and *OsSPL14* were used for detection of their presence in these high grain number genotypes. The positive alleles of 275bp, 110bp and 200bp were detected at three high grain number gene loci, *Gn1a*, *Dep1*, and *OsSPL14*, respectively in CR444-3-1-1-1 while positive allele of 275bp was detected in CR3856-44-22-2-1-11 at *Gn1*a locus, indicating that three genes are present in CR444-3-1-1-1 while only one gene, *Gn1a* is present in CR3856-44-22-2-1-11. There were several reports that varieties having *Gn1a* gene for higher grain number have grain yield (Feng *et al.* 2013; Kim *et al.* 2016; Huang *et al.* 2018).

Identification of polymorphic markers between genotypes

Out of 58, 45 (77.58%) microsatellite markers were found to be polymorphic between MTU1010 and CR3856-44-22-2-1-11 while 46 (79.31 %) microsatellite markers were found to be polymorphic between MTU1010 and CR444-3-1-1-1(Table 2).Mohanty *et al.* (2017) identified the maximum polymorphism of 34.02 % between Reeta and WAB56-50, and 31.76% between AC38562 and Pimpudibasa, whereas 27.22% polymorphism was found between PDKV Shriram and Heera.

Table 2: Identification of polymorphic markers between high and low grain genotypes

Genotype combination	No of SSR markers used	No. of polymorphic markers	Percentage of polymorphic markers
CR444-3-1-1-1 and MTU1010	58	46	79.31
CR3856-44-22-2-1-11 and MTU1010	58	45	77.58

Allelic diversity of microsatellite markers

A total of 58 SSR markers were used for assessing genetic diversity between two high grain genotypes and one popular rice variety. All SSRs were found to be polymorphic. A total of 139 polymorphic alleles were amplified with an average of 2.28 alleles per locus. The number of alleles varied from 2 to 4 with an average of 2.28. Four alleles were amplified by RM26898. Fifteen markers, namely, RM11258, RM1282, HvSSR02-60, HvSSR02-44, RM12353, RM175, RM15981, HvSSR04-27, HvSSR04-19, RM164, RM21858, HvSSR07-52, RGNMS2758, RM23698 and HvSSR12-23 amplified 3 alleles per marker locus. Major allelic frequency varied from 0.33 to 0.83 (RM495, RM18107, RM25735, RM25935 and RM26213) with an average of 0.61(Table 3). Similarly, several researchershave been reported variation in allelic diversity (Behera *et al.* 2012; Behera*et al.* 2013; Choudhary*et al.* 2013; Singh *et al.* 2016). Singh *et al.* (2014) detected 79 alleles for 28 markers and the number of alleles varied from 2

to 4 with an average of 2.82 per locus.Sajib *et al.*(2012) assessed the genetic diversity in 12 aromatic landraces using 9 SSR markers and reported two to nine alleles with an average of 3.33 alleles per locus. Microsatellite markers have been widely used for assessment of genetic diversity among genotypes, gene mapping and improvement of genetic traits for development of new rice varieties (Singh *et al.* 2016). Donde *et al.*(2019) studied the genetic diversity in 16 land races using 63 SSR markers.Nadia and teamreported 0.23 of major alleles in 30 rice accessions with allele frequency ranging from 0.10 to 0.43 (Nadia *et al.* 2 014).

Table 3: Data on total number of alleles, major allele frequency, gene diversity, PIC, heterozygosity and P-values

Parameter	Total	Minimum	No of loci/ Name of marker	Maximum	No of loci/ Name of marker	Mean	%
Total SSR marker used	58	-	-	-	-	-	
No of alleles	139						
No. of polymorphic allele	139	-	-	-	-		100
No of alleles per locus	-	2	42	4	RM26898	2.28	-
Major allele frequency	36.24	0.33	13	0.83	RM495, RM18107, RM25735, RM25935, RM26213	0.61	-
Gene diversity	29.04	0.28	4	0.72	RM26898	0.49	-
Heterozygosity	3.65	0.00	49	0.67	HVSSR04-27, RM26898	0.06	-
PIC	24.13	0.24	5	0.67	RM26898,	0.40	-
P-Value	15.42	0.06	3	1	RM495, HVSSR04-27, RM18107, RM25735, RM25935, RM26213	0.26	-

Gene diversity and Heterozygosity

Gene diversity varied from 0.28 (RM495, RM18107, RM25735, RM25935, and RM26213) to 0.72 (RM26898) with an average of 0.49 (Table 3). Heterozygosity determines the number of heterozygous alleles present at the marker locus. The heterozygosity varied from 0.0 to 0.67 (HVSSR04-27, RM26898). HVSSR04-27 and RM26898 markers were able to distinguish three rice genotypes. Moderate level (0.33) of heterozygosity was observed at seven markers, namely, RM495, RM12353, RM18107, RM23698, RM25735, RM25935, RM26213 (Table 3).

Figure

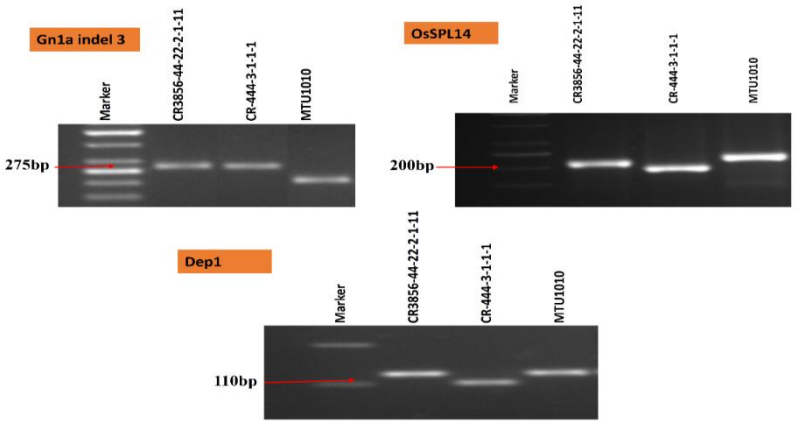

Fig. 1: Amplification of genomes of high and low grain genotypes with primers specific to high grain number genes, a) *Gn1a,* b) *OsSPL14*, and c) *Dep1,* Marker= 50bp DNA ladder.

Polymorphism information content (PIC) and p-value for population differentiation

The PIC value ranged from 0.24 to 0.67 (RM26898) with an average of 0.40 (Table2). Population differentiation test (P-Value) was calculated among three rice genotypes and was used to differentiate genotypes based on allelic frequency. The P-value varied from 0.06 to 1.00(RM495, HVSSR04-27, RM18107 and RM25735) with an average of 0.26 (Table 3). Polymorphism Information Content (PIC) varied from 0.24 to 0.67 with an average of 0.40. Patel *et al.* (2014) reported the PIC value ranged from 0.36 to 0.78. Jasim *et al.* (2018) reported the PIC value 0.25 to 0.98 with an average of 0.61. Similarly, several researchers have been reported the different PIC values, 0.26 to 0.65 (Singh *et al.* 2014).

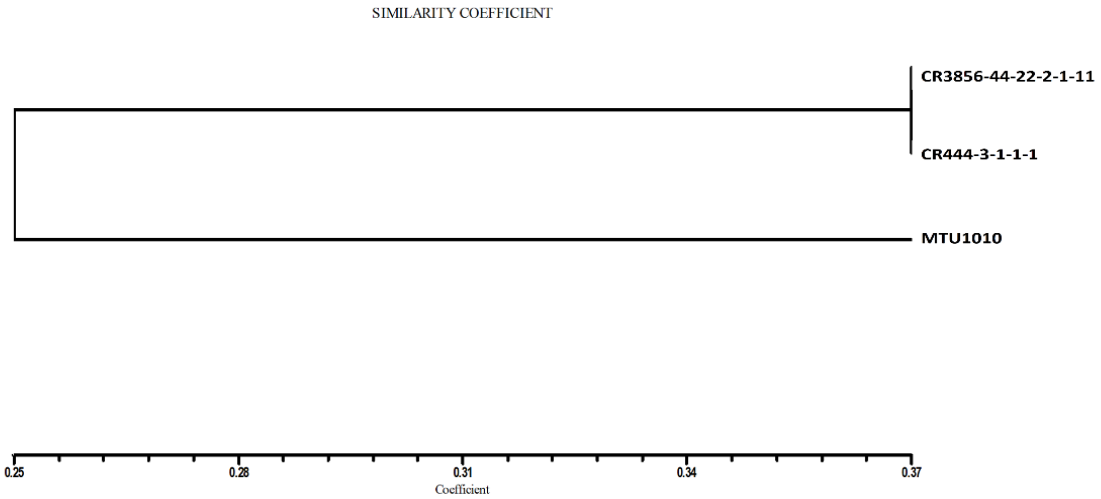

Fig.2: Dendrogram showing genetic relationship between high and low grain rice genotypes

Genetic diversity and Cluster Analysis

Alleles of 58 SSR markers and three gene specific markers (*Gn1a, Dep1* and *OsSPL14*) were used for assessing genetic diversity and plotting a dendrogram to differentiate three genotypes. The genetic similarity between MTU1010 and CR444-3-1-1-1 was found to be 0.244 while 0.260 was found between MTU1010 and CR3856-44-22-2-1-11. A highest similarity, 0.371 was found amongCR3856-44-22-2-1-11 and CR444-3-1-1-1 (Table 4).

Table 4: Similarity coefficient between high and low grain number genotypes

	MTU1010	CR3856-44-22-2-1-11	CR444-3-1-1-1
MTU1010	1.000		
CR3856-44-22-2-1-11	0.260	1.000	
CR444-3-1-1-1	0.244	0.371	1.000

Two major clusters were obtained amongst three genotypes with a similarity coefficient of 0.37. Cluster-I composed of 2 high grain genotypes, CR3856-44-22-2-1-11 and CR444-3-1-1-1 while cluster-II composed of one low grain genotype, MTU1010 (Fig. 2). The genetic diversity ranged from 0.04 to 0.66 with an average of 0.33 (Singh *et al.* 2016).Shah *et al.* (2016) identified the genetic diversity of 0.42 in 105 rice accessions. Three major clusters are obtained from the genotypes having the genetic distance ranged from 0.31 to 0.39. This data revealed the genetic similarity of rice genotypes with respect to the morphological characters responsible for increasing yield. Genetic similarity was calculated by using 28 SSR markers where two major clusters were found having a dissimilarity coefficient of 0.26 (Singh *et*

al. 2014).Similarly, Choudhary *et al.*(2013) reported 0.02 heterozygosity at RM27840 locus.

Phenotypic diversity

Phenotype diversity assessment was carried out to differentiate three rice genotypes on the basis of 10 agro-morphological traits. Plant height (PH), tiller number (TN), fertile grain number per panicle (GN), chaff number (CH), panicle number (PN), panicle length (PL), total spikelet number (TSN), single panicle weight (SPW), thousand-grain weight (TGW) and yield per plant (YPP) are important agro-morphological traits which are directly or indirectly influencing grain yield. Wide phenotypic variations were observed in all the 10 phenotypic traits. The fertile grain number per panicle was found to be 110.30, 274.20 and 294.50 in MTU1010, CR3856-44-22-2-1-11 and CR444-3-1-1-1, respectively. The plant height (PH) was found to be 101.2cm, 130.67cm and 169.2cm in MTU1010, CR3856-44-22-2-1-11 and CR444-3-1-1-1,respectively. Similarly, tiller number was found to be 6.67, 8.67 and 10.4 in CR444-3-1-1-1, CR3856-44-22-2-1-11 and MTU1010, respectively (Fig.3).

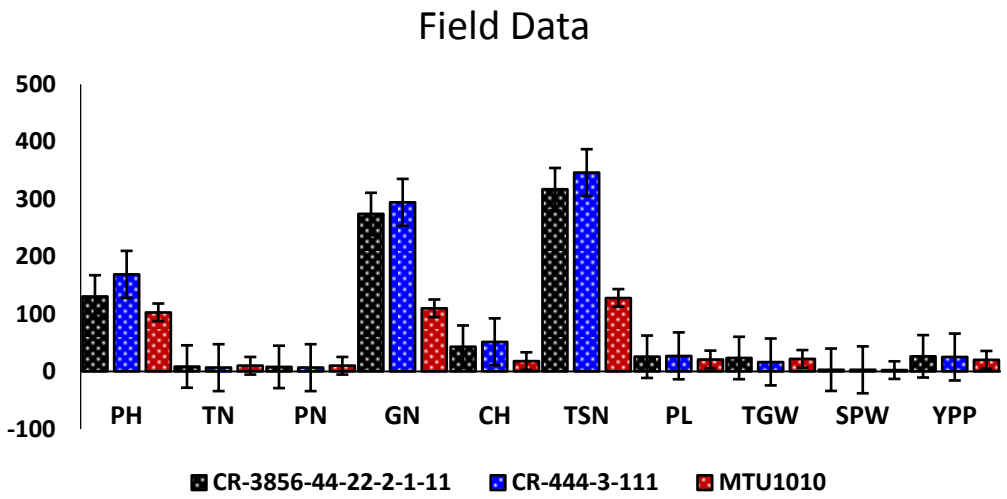

Fig.3:Bar diagram showing values of ten phenotypic traits of high and low grain genotypes

The thousand-grain weight varied from 16.6 to 23.5 with an average of 20.83. Panicle length varied from 22 to 27.3 (Table 5). Phenotypic variations were observed in grain number, plant height and tiller number in our study. Plant height showed positive correlation with panicle number, grain number and panicle length. Highest variationswere observed in grain number, tiller number, panicle length and plant height (Singh *et al.* 2013). In *O. rufipogon* populations, variations in morphological traits were observed. Singh *et al.*(2014) have reported the variations in panicle length, plant height and grain number per panicle.

Table 5: Mean phenotypic traits of high and low grain rice genotypes

Traits	CR3856-44-22-2-1-11	CR444-3-1-1-1	MTU1010	Mean	SD
TN	8.67	6.67	9.4	8.25	1.41
PN	8.3	6.67	9.2	8.06	1.28
GN	274.2	294.5	109.2	225.97	101.63
CH	43.2	51.7	18.1	37.67	17.47
TSN	317.4	346.2	127.3	263.63	118.94
PL	25.67	27.23	22.2	25.03	2.58
TGW	23.5	16.6	22.4	20.83	3.71
SPW	3.05	2.92	2.4	2.79	0.34
YPP	26.44	25.2	20.4	24.01	3.19
PH	130.67	169.2	101.2	133.69	34.10

Principal Component and PCA biplotAnalysis

Principal component analysis (PCA) was conducted to understand the genetic relationships among three genotypes.The PCA percentage varied from F1 (15.69%) to F2 (84.31%). PCA-Biplot was used to calculate the association between morphological traits with respective rice genotypes. CR3856-44-22-2-1-11 was included under 1st quadrant having dominant traits like grain number (GN), total spikelet number (TSN), total grain yield, single panicle weight (SPW). CR-444-3-1-1-1 was included under 4th-quadrant having higher plant height (PH) and grain number (GN). MTU1010 belongs to 3rd quadrant and close to 2nd quadrant. So, it is having higher thousand-grain weight (TGW), panicle number (PN), tiller number (TN) (Fig. 4).

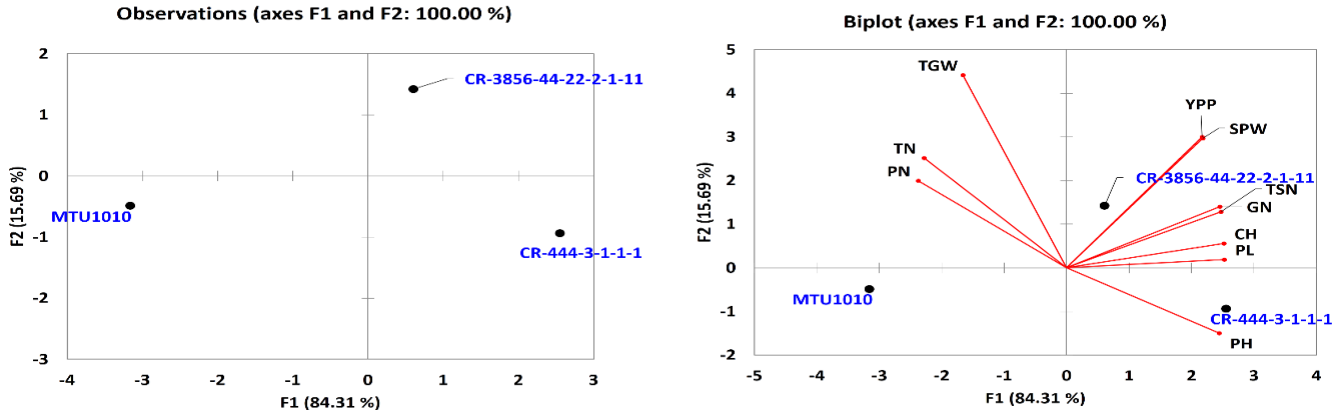

Fig. 4: **(**a) PCA and (b) PCA Bi-plot analysis of three rice genotypes showing variation among them

Table 6: Correlation among phenotypic traits

Variables	TN	PN	GN	CH	TSN	PL	TGW	SPW	YPP	PH
TN	1	0.995	-0.774	-0.858	-0.787	-0.888	0.917	-0.560	-0.556	-0.983
PN	0.995	1	-0.832	-0.903	-0.843	-0.929	0.874	-0.638	-0.634	-0.996
GN	-0.774	-0.832	1	0.989	1.000	0.978	-0.457	0.958	0.957	0.877
CH	-0.858	-0.903	0.989	1	0.992	0.998	-0.581	0.907	0.904	0.938
TSN	-0.787	-0.843	1.000	0.992	1	0.982	-0.476	0.952	0.950	0.887
PL	-0.888	-0.929	0.978	0.998	0.982	1	-0.632	0.878	0.875	0.958
TGW	0.917	0.874	-0.457	-0.581	-0.476	-0.632	1	-0.184	-0.178	-0.828
SPW	-0.560	-0.638	0.958	0.907	0.952	0.878	-0.184	1	1.000	0.703
YPP	-0.556	-0.634	0.957	0.904	0.950	0.875	-0.178	1.000	1	0.700
PH	-0.983	-0.996	0.877	0.938	0.887	0.958	-0.828	0.703	0.700	1

Values at significance level alpha=0.95

Phenotypic Correlation and p-value analysis of phenotypic traits

The phenotypic traits were measured through correlation analysis. The positive correlations were found between plant height with panicle number, grain number, panicle length and single panicle weight while negative correlations were found with tiller number, panicle number and thousand-grain weight. Grain number showed positive correlation with chaff number (0.989), panicle length (0.978), plant height (0.877) and single panicle weight (0.958) (Table 6). The P-values were measured for all the phenotypic traits. The grain number (GN) was found to be highly significant (i.e. 0.014) with total spikelet number (TSN) while chaff number (CH) was found to be significant(i.e. 0.04) with panicle length (PL). Similarly, total grain yield per plant (YPP) was found to be significant (i.e. 0.003) with single panicle weight (SPW) (Table 7).

Table 7: The analysis of p-values among three rice genotypes and screened for high grain number per panicles

Variables	TN	PN	GN	CH	TSN	PL	TGW	SPW	YPP	PH
TN	0	0.062	0.437	0.344	0.423	0.304	0.261	0.621	0.625	0.118
PN	0.062	0	0.375	0.282	0.361	0.242	0.323	0.559	0.563	0.056
GN	0.437	0.375	0	0.093	0.014	0.133	0.698	0.185	0.188	0.319
CH	0.344	0.282	0.093	0	0.079	0.040	0.605	0.277	0.281	0.226
TSN	0.423	0.361	0.014	0.079	0	0.119	0.684	0.198	0.202	0.305
PL	0.304	0.242	0.133	0.040	0.119	0	0.565	0.318	0.321	0.185
TGW	0.261	0.323	0.698	0.605	0.684	0.565	0	0.883	0.886	0.379
SPW	0.621	0.559	0.185	0.277	0.198	0.318	0.883	0	0.003	0.503
YPP	0.625	0.563	0.188	0.281	0.202	0.321	0.886	0.003	0	0.507
PH	0.118	0.056	0.319	0.226	0.305	0.185	0.379	0.503	0.507	0

Values in bold are different from 0 with a significance level at alpha =0.05

Heat Map

Heatmap of three rice genotypes was compared with 10 agronomic traits. The heat map clearly showed the dominance of specific agronomic traits with respect to genotypes. Heatmap cluster showed the association morphological traits between the genotypes.The traits showing darker or dense in colour are higher association with respective genotypes (Fig.5).

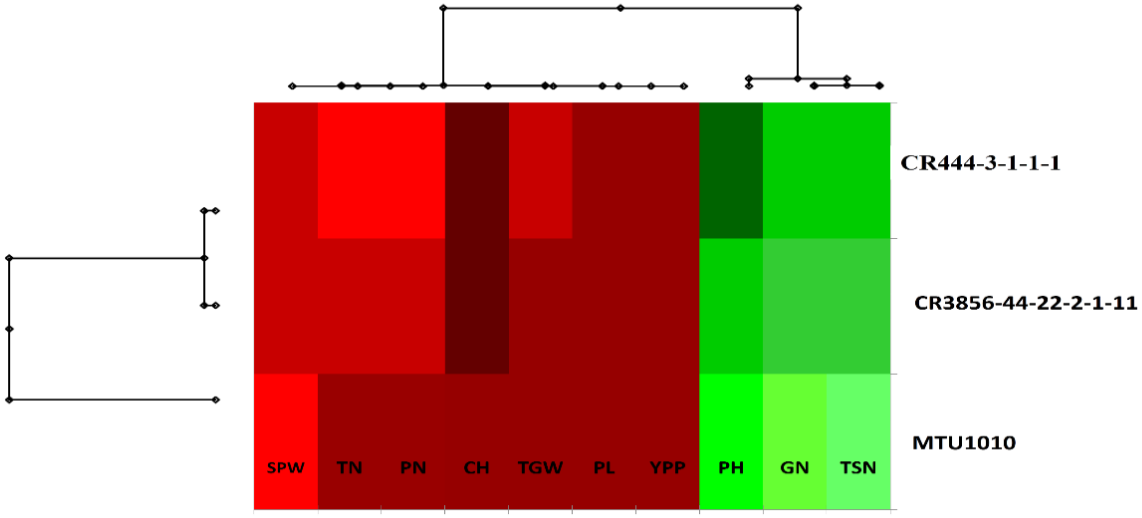

Fig.5: Heat map showing variation in phenotypic traits among of rice genotypes.*Note: Deeper colour indicates higher value of particular trait*

In the case of MTU1010, a heat map was obtained dominancy in TN and PN traits. CR444-3-1-1-1 showed dominancy in grain number per panicle, chaff number, total spikelet number, panicle length and plant height. CR3856-44-22-2-1-11 showed dominancy in single panicle weight and single plant yield. The grain number variation was found between genotypes. Two major clusters were plotted against three genotypes. A strong association of plant height with grain number and total spikelet number which were grouped into one cluster. SPW, TN, PN, CH, TGW, PL and YPP were grouped into another cluster. The dark colour specifies the trait association among genotypes. CR3856-44-22-2-1-11 had equal association with PH and GN whereas CR444-3-1-1 had negative association with plant height. As the plant height increases, the decrease in TN was observed. Similarly, chaff number was strongly associated with panicle length (PL). Therefore, the genetic and phenotypic diversity information of genotypes would be useful in donor and variety identification, marker-assisted selection and design effective breeding platform to introgress high grain number genes into popular rice

varieties to produce higher yielding varieties. The finding of the present study has used in marker-assisted breeding programme, to introgress high grain responsive genes i.e. *Gn1a, Dep1*and*OsSpl14*into popular MTU1010 variety.

Present study has identified two genotypes, CR444-3-1-1 and CR3856-44-22-2-1-11 having three genes (*Gn1a, Dep1* and *OsSPL14*) and one gene (*Gn1a*) for high grain number, respectively. MTU1010, a popular *indica*rice variety lacks these genes. This study revealed the genetic and phenotypic variations among three rice genotypes. Marker-based breeding approach is highly useful tool and is used to develop variety in a short period of time with high grain number. 58 SSR polymorphic markers were identified, which could be used in MAS programs. PIC value ranged from 0.24 to 0.67 (RM26898) with an average of 0.40. The P-value varied from 0.06 to 1.00 (RM495, HVSSR04-27, RM18107 and RM25735) with an average of 0.26. The 10 agronomic traits were used for assessing genetic diversity among three genotypes. The highly potential morpho-physiological traits TN, TSN, YPP, GN and PL showed positive association with grain yield. The assessment of genetic diversity at molecular and phenotypic levels could be helpful for the identification highly diverse genotypes having multiple genes for grain yield and related traits, which might be useful for bi-parental/multi-parental crosses combinations to develop high yielding rice varieties.

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FINDING

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REFERENCES

Behera, L., Mohanty, S., Pradhan, S.K., Singh, S., Singh, O.N., Sahu,R.K., Sahu, S.C., Dash, S.K. and Mohapatra,T.(2013). Assessment of genetic diversity of rainfed lowland rice genotypes using microsatellite markers.*Indian Journal of Genetics and Plant Breeding***73**(2):142-152.

Behera, L., Patra, B.C., Sahu, R.K., Nanda, A., Sahu, S.C., Patnaik, A., Rao, G.J.N. and Singh, O.N.(2012)Assessment of genetic diversity in medicinal rices using microsatellite markers. *Australian Journal of Crop Science***6**(9):1369-1376.

Choudhary,G., Ranjitkumar, N., Surapaneni, M., Deborah, D.A., Vipparla, A., Anuradha, G., Siddiq, E.A. and Vemireddy, L.R. (2013)Molecular genetic diversity of major Indian rice cultivars over decadal periods. PLoS One8: p.e66197.

Donde, R., Kumar, J., Gouda, G., Gupta, M.K., Mukherjee, M., Baksh, Y.S.K., Mahadani, P., Sahoo, K.K., Behera, L. and Dash, S.K. (2019) Assessment of genetic diversity of drought tolerant and susceptible rice genotypes using microsatellite markers. *Rice Science***26**(4):239-247.

Godfray, H.C., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty,J., Robinson, S., Thomas, S.M. and Toulmin, C.(2010) Food Security: The challenge of feeding 9 billion people. *Science***327**:812–818.

Huang, L., Zhang, R., Huang, G., Li, Y., Melaku, G., Zhang, S., Chen, H., Zhao, Y., Zhang, J., Zhang, Y. and Hu, F. (2018) Developing superior alleles of yield genes in rice by artificial mutagenesis using the CRISPR/Cas9 system.*The Crop Journal***6**:475–481.

Jasim Aljumaili, S., Rafii, M.Y., Latif, M.A., Sakimin,S.Z., Arolu, I.W. and Miah, G.(2018) Genetic diversity of aromatic rice germplasm revealed by SSR markers. BioMed Research International: 1–11.

Jiao, Y., Wang, Y., Xue, D., Wang, J., Yan, M., Liu, G., Dong, G., Zeng, D., Lu, Z., Zhu, X., Qian, Q. and Li, J. (2010) Regulation of *OsSPL14* by OsmiR156 defines ideal plant architecture in rice. Nature Genetics **42**:541–544.

Joshi, R.K. and Behera.L. (2006) Identification and differentiation of indigenous non-basmati aromatic rice genotypes of India using microsatellite markers.*African Journal of Biotechnology***6**(4): 348-354.

Kim, S.R., Ramos, J., Ashikari, M., Virk, P.S., Torres, E.A., Nissila, E., Hechanova, S.L., Mauleon, R. and Jena, K.K.(2016) Development and validation of allele-specific SNP/indel markers for eight yield-enhancing genes using whole-genome sequencing strategy to increase yield potential of rice, *Oryza sativa* L. *Rice***9**(1): 12.

Liu, K. and Muse, S.V. (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics***21**:2128–2129.

Miura. K., Ikeda, M., Matsubara, A., Song, X.J., Ito, M., Asano, K., Matsuoka, M., Kitano, H. and Ashikari, M. (2010) *OsSPL14* promotes panicle branching and higher grain productivity in rice. Nature Genetics **42**:545–549.

Mohanty, S., Mohanty,N., Sulakshana, S., Pradhan, S.K., Dash, S.K., and Behera, L. (2017) Assessment of polymorphism at molecular level, association studies, multivariate analysis and genetic diversity among recombinant inbred lines of rice (*Oryza sativa* L.). Oryza **54**:176–187.

Murray, M.G. and Thompson, W.F. (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research 8:4321–4325.

Nadia,I., Mohiuddin, A.K.M., Sultana, S. and Ferdous, J. (2014) Diversity analysis of indica rice accessions (*Oryza sativa* L.) using morphological and SSR markers.*Annals of Biological Research***5**(11): 20-31.

Rohlf, FJ. (2004) NTSY Spc, ver. 2.2 use rguide. https://www.exetersoftware.com/cat/ntsyspc/ntsyspc.html. Accessed 24 May 2019

Sajib, A.M. (2012) SSR marker-based molecular characterization and genetic diversity analysis of aromatic landraces of rice (*Oryza sativa* L.*). Journal of Bioscience and Biotechnology***1**(2): 107-116.

Shah, S.M., Arif, M., Aslam, K., Shabir, G. and Thomson, M.J.(2016) Genetic diversity analysis of Pakistan rice (*Oryza sativa*) germplasm using multiplexed single nucleotide polymorphism markers. *Genetic Resources and Crop Evolution***63**:1113–1126.

Singh, A., Saini, R., Singh, J., Arya, M., Ram, M., Pallavi., Mukul. and Singh, P.K. (2014) Genetic diversity studies in rice (*Oryza sativa* L.) using microsatellite markers. International *Journal of Agriculture, Environment and Biotechnology***8**:143-152.

Singh, A., Singh, B., Panda, K., Rai,V.P., Singh, A.K., Singh, S.P., Chouhan, S.K., Rai, V., Singh, P.K. and Singh, N.K. (2013) Wild rices of eastern Indo-Gangetic plains of India constitute two sub-populations harbouring rich genetic diversity.*Plant Omics Journal***6**(2): 121-127.

Singh, N., Choudhury, D.R., Tiwari, G., Singh, A.K., Kumar, S., Srinivasan, K., Tyagi, R.K., Sharma, A.D., Singh, N.K. and Singh, R. (2016) Genetic diversity trend in Indian rice varieties: an analysis using SSR markers. BMC Genetics **17** (1):127.

Singh, V.K., Ellur, R.K., Singh, A.K., Nagarajan, M., Singh, B.D. and Singh, N.K. (2018).Effect of *qGN4.1* QTL for grain number per panicle in genetic backgrounds of twelve different mega varieties of rice. Rice 11 (1):8.

Varshney, R.K., Bansal, K.C., Aggarwal, P.K., Datta, S.K. and Craufurd, P.Q. (2011). Agricultural biotechnology for crop improvement in a variable climate: hope or hype? Trends in Plant Science **16**:363–371.