

# Electronic Journal of Plant Breeding



## Research Article

### Genetic diversity and QTL-marker association analysis of rice germplasm for grain number per panicle and its contributing traits

M. Arunkumar<sup>1</sup>, S. Geetha<sup>2\*</sup>, K. Amudha<sup>3</sup>, R. Suresh<sup>4</sup>, V. Ravichandran<sup>5</sup> and K. Geetha<sup>6</sup>

<sup>1</sup>Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore 641003, India

<sup>2</sup>Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641003, India

<sup>3</sup>Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641003, India

<sup>4</sup>Tamil Nadu Rice Research Institute, Tamil Nadu Agricultural University, Aduthurai – 612 101, India

<sup>5</sup>Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore 641003, India

<sup>6</sup>Regional Research Station, Tamil Nadu Agricultural University, Paiyur- 635 112, India

\*E-Mail : geethagovind1@gmail.com

#### Abstract

Rice is one of the leading cereal crops that consumed over half of the world's population. The number of grains per panicle is the main trait to decide grain yield potential of rice. Rice germplasm have rich diversity for panicle related trait. Hence, a study was undertaken to screen 77 diverse rice germplasm for grain number per panicle and its contributing traits. Mahalanobis' D<sup>2</sup> analysis was performed to study genetic diversity among the germplasm. Eight clusters were formed and cluster IV was found to be the largest cluster with 37 genotypes. Cluster V had 20 genotypes with the highest cluster mean value of 298 for the number of grains per panicle. Grain yield per plant and the number of spikelets per panicle were found to be the biggest contributor for total divergence. QTL linked markers were screened in diverse rice germplasm. Among the six QTLs screened, five QTLs were significantly associated with grain number per panicle and its relevant traits. For grain number per panicle the QTLs, *Gn1a* (0.1058), *qGN 4.1* (0.117) and *NGP 4* (0.062) recorded high R<sup>2</sup> values. Among the markers, *Gn1a* indel 3 (*GN1a*), NKSSR 04-19 (*q GN 4.1*), RM 6314 (*NGP 4*) and RM1183 (*NOG 1*) were identified as the most informative markers for utilization in marker assisted breeding programme. From this study, genotypes viz., *Kallukar*, CO52, IET 29504, IET 29506, RP-5594-97-5-1, MTU1360, ADT 54 possess high mean value for grain number per panicle and grain yield, linked with functional markers could be used as a donor in marker assisted breeding programme.

**Keywords:** Rice, grain number, QTLs, diversity analysis, grain yield

#### INTRODUCTION

Rice (*Oryza sativa* L.) is the major staple food crop which is consumed by more than 300 crore people globally and supports in the fight against hunger and poverty. In India, it is grown in an area of 43.7 million ha with production and productivity of 118.9 million tonnes and 2.72 tonnes/ha, respectively (Indiastat, 2021). It accounts for 40–43

per cent of total food grain production and also plays a key role in the national food and livelihood security in India. Due to the rapid increase in the global population, it is expected to feed 9 billion people by 2050. Hence, further yield improvement is essential to meet the future food demand. The yield potential of rice depends on the number

of panicles, grain number per panicle and grain weight (Xie *et al.*, 2019). Among the three traits, grain number per panicle is very crucial for yield determination and it can be improved *via* modifying the panicle related characteristics *viz.* panicle length, the number of primary rachis branches and the number of secondary rachis branches (Ikeda *et al.*, 2010). So, assessing the diversity for panicle related traits present among the available genetic resources is essential for identifying the potential donors for grain yield improvement in rice. Mahalanobis'  $D^2$  analysis is the powerful multivariate analysis method for genetic diversity analysis.

The number of grains per panicle formation come across series of physiological and molecular functions *viz.*, conversion of shoot apical meristem into inflorescence meristem, formation of the first order and second order lateral meristem and conversion of lateral meristem into spikelet meristem (Huang *et al.*, 2018). These physiological activities are governed and regulated by the number of genes *viz.*, *GNP 1*, *GNP4*, *DEP 1* and *NOG 1* which increases grain number per panicle *via* positive regulation of rachis branch development (Wang *et al.*, 2020) (Huo *et al.*, 2017), while *LP/EP3* and *GN1a* improves by negative regulation of rachis branch related genes (Li *et al.*, 2011; Wang *et al.*, 2015). Molecular markers such as Simple sequence repeats (SSR), Single nucleotide polymorphism (SNP), Sequence tagged site (STS) and indel markers are codominant markers abundantly present in the genome, positioned within and nearby genic regions and could be effectively utilized in marker assisted breeding (MAB) programme. The gene/QTL linked markers have the high chances of co-segregation with the specific loci associated with a phenotypic trait (Chen *et al.*, 2021). Anand *et al.*, (2013) validated QTLs for grain size (*GS3*), grain weight (*GW2*) and seed width (*qSW5*) in rice germplasm and found that *SF28* linked with *GS3* was more robust and explained 32.5 % of phenotypic variation.

Hence, the present investigation was attempted to assess the genetic diversity of tremendous genetic variation present in *indica* subspecies for grain number per panicle and characterize them using grain number per panicle related genic markers to identifying the informative markers or allele which can be used in marker assisted breeding programme for improvement of grain yield

## MATERIALS AND METHODS

A set of 77 rice genotypes belongs to different regions of India were selected for this study and also described in **Table 1**. The genetically pure seeds were obtained from the Department of Rice, Tamil Nadu Agricultural University, Coimbatore, India. The field experiment was laid out in Randomized Block Design (RBD) with two replications at Regional Research Station (RRS), Paiyur, Tamil Nadu, during *Rabi*, 2021 following standard agronomical practices.

At anthesis, the first formed primary panicle was tagged in three plants of each genotype and the tagged panicles were collected at physiological maturity. The data recorded on each accession were: plant height (PH), total productive tillers (TPT), flag leaf length (FL), flag leaf width (FW), panicle length (PL), total spikelets per panicle (TSP), total filled grains per panicle (TFGP), total primary rachis branch (TPRB), total secondary rachis branch (TSRB), spikelets in primary rachis branches (SPRB), spikelets in secondary rachis branches (SSRB), spikelets density (SD), length of primary branches (LOPB), length of secondary branches (LOSB) and grain yield per plant (GYP). A total of thirteen markers were linked to six grain number related QTLs namely *Gn1a*, *LP/EP3*, *NOG1*, *GNP1*, *qGN 4.1*, *NGP 4* (Ashikari *et al.*, 2005; Wang *et al.*, 2015; Li *et al.*, 2011; Wang *et al.*, 2020) (Ashikari *et al.*, 2005; Wang *et al.*, 2015; Li *et al.*, 2011; Wang *et al.*, 2020; Singh *et al.*, 2020) were selected for this study. The linked molecular markers namely *Gn1a indel3*, *RM1195*, *Gn1 a indel1*, *F35*, *F57*, *RM1183*, *RM 85*, *RM227*, *NKSSR04-11*, *NKSSR04-19*, *RM 3604*, *RM 5424* and *RM6314* and the details of markers are given in **Table 2**.

The genomic DNA was isolated from young leaves by following the cetyl-trimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980) with some minor modifications and stored at  $-40^{\circ}$  C. The DNA quality and quantity were checked using a spectrophotometer (Tecan's NanoQuant Plate, Mannedorf, Switzerland). The PCR amplified product was separated on 3 % agarose gel and then visualized under UV light of a gel documentation system (Bio-Rad Gel Doc XR + UV Gel Doc. Imaging System). The banding size of amplified fragments was determined by 100 bp ladder (Thermo Fischer scientific, USA) and the gel images were documented in Lab system for further scoring.

ANOVA for the randomized block design (RBD) was calculated at the 5% significance level. Phenotypic data of rice germplasm were subjected to Mahalanobis'  $D^2$  analysis using the INDOSTAT services, Hyderabad. Each gene specific markers scored for the presence or absence of band on respective alleles among 77 germplasm. The binary data '0' and '1' were used to score the absence and presence of the specific band. The binary data matrix developed by scored data was subjected to analysis. The marker and phenotype association explained by *P* value (significance) and  $R^2$  value (fraction of variation) were analysed using SPSS version 16. The correlation coefficient was calculated by Pearson's method using R software version 4.2.0.

## RESULTS AND DISCUSSION

Analysis of variance for randomized block design showed that all the studied traits were significant at a 5 % level of significance. The genetic diversity of 77 genotypes was measured using Mahalanobis (1936)  $D^2$  statistics by numerical method. At the Tocher's cut off value of

**Table 1. List of 77 rice genotypes used in this study**

Code	Genotypes	Code	Genotypes	Code	Genotypes
GN1	Sembil priyan	GN27	IET29519	GN53	AD 16148
GN2	Karuka	GN28	CRAC 3998-43-1	GN54	KPS 6097
GN3	Kallukar	GN29	IET29494	GN55	MTU 1360
GN4	Kalarkar	GN30	IET29530	GN56	NVSR-566
GN5	T396 peria samba	GN31	NWGR-15026	GN57	GNV 1974
GN6	Nootripattu	GN32	IET29535	GN58	RNR 29094
GN7	Muttakar	GN33	IET29523	GN59	IET29506
GN8	Kothamalli samba	GN34	IET29499	GN60	IET29536
GN9	CO52	GN35	IET29529	GN61	IET29497
GN10	RG192	GN36	IET29504	GN62	BPT 3059
GN11	Mikuruvai	GN37	CB 16142	GN63	IET29522
GN12	IW ponni	GN38	CSR 27SM59	GN64	JGL 35071
GN13	IARI3	GN39	IET 29539	GN65	RNR 29176
GN14	IET 28835	GN40	IET29516	GN66	Anna(R)4
GN15	IET 28834	GN41	RP 5594-97-5-1	GN67	CO43
GN16	ADT(R)48	GN42	AD 16105	GN68	CB15138
GN17	BPT3004	GN43	IET29501	GN69	CR1009
GN18	CO51	GN44	WGL-1283	GN70	CO(R)48
GN19	CO(R)50	GN45	KNM 7714	GN71	ADT 54
GN20	RRG1	GN46	NP 9253-13	GN72	TKM 13
GN21	ADT37	GN47	IET29491	GN73	VDG1
GN22	MDU 5	GN48	ADT 49	GN74	GEB 24
GN23	ADT45	GN49	KNM 7715	GN75	ADT50
GN24	ADT43	GN50	IET29509	GN76	CO 53
GN25	TRY2	GN51	IET29538	GN77	CO 54
GN26	IET29537	GN52	IET29503		

80.00 the genotypes were grouped into eight clusters (**Table 3**). Among the eight clusters, cluster IV was the biggest which included 37 genotypes followed by cluster V which had 20 genotypes. Fifteen genotypes were grouped into cluster II and the rest of the clusters viz., cluster I, cluster III, cluster VI, cluster VII and cluster VIII were solitary clusters containing only one genotype.

The average intra and inter cluster distance were calculated and presented in **Table 4**. The maximum intra cluster value was found in cluster II (57.55) followed by cluster V (55.08) which shows that maximum divergence was present among the genotypes within these clusters. Similarly, a minimum intra cluster value of 52.02 was observed in cluster IV and it indicated that the least variation was present among the genotypes of the cluster. Solitary clusters had zero intra cluster distance. A similar kind of results was reported by Srinivas (2018).

The highest inter-cluster distance was found between clusters I and III (660.58) followed by clusters I and V (508.80). The genotypes presented among these two

clusters exhibited maximum genetic distance and similar results were observed by Sanju Kumari *et al.* (2018) and Umesh *et al.* (2015). Similarly, minimum inter cluster distance was observed between clusters II and VIII (80.86) followed by clusters VII and VIII (82.44). The lower values of genetic distances indicate closeness and similarities of genotypes in these clusters.

The inter cluster values were ranged between 80.86 and 660.58 which indicates moderate genetic divergence among the clusters. The significant exploitation of heterosis was noticed in the crosses made between the genotypes that have moderate genetic distance (Parhe *et al.*, 2014 and Sreewongchai *et al.*, 2021 ).

The cluster mean values for 15 traits are given in **Table 5**. Among the clusters, cluster V recorded the highest mean value for five panicle related traits viz., total spikelets per panicle (323.10), total filled grains per panicle (298.53), total secondary rachis branches (55.83), spikelets in secondary rachis branches (270.5), spikelets density (3.32) followed by cluster III which recorded the highest

**Table 2. List of QTLs and its linked markers used in this study**

S. No.	QTLs name	Primer Name	Marker type	Forward Primer	Reverse Primer	Annealing temperature (°C)
1	<i>qGN 4-1</i> (LOC_Os04g52540)	NKSSR04-11	SSR	CCATCAGTTGAAGGGCTCTC	CTGGAATCACAAACCACGAC	60
		NKSSR04-19		CTGGAATCACAAACCACGAC	GCTACCTCAAGCTCCACGAC	60
2	<i>NOG 1</i> (LOC_Os01g54860.1)	RM1183	SSR	GGGCACGAATAAAACCAGAG	GGGATGGTCCAATGACAAAAG	60
3	<i>NGP 4</i>	RM6314	SSR	GATTCGTGTCGGTTGTCAAG	GGTTCAGGGACGAATTTTCAG	60
		RM5424	SSR	CACCAGACAGACGCCACAG	CGTATATATCGCATGCACCG	
4	<i>GNP1</i> (LOC_Os03g63970.1)	RM85	SSR	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC	60
		RM 227		ACCTTTCGTCATAAAGACGAG	GATTGGAGAGAAAAGAAGCC	
5	<i>Gn1a</i> (LOC_Os 01g10110)	RM3604	SSR	ATGTCAGACTCCGATCTGGG	TCTTGACCTTACCACCAGGC	60
		Gn1a-indel3	INDEL	GATCTAGATGCTCCAAAGTCC	CTGTACGTACGTGCACGTAG	60
		Gn1a-indel1		GCCACCTTGTCCTTCTACA	TGCCATCCTGACCTGCTCT	60
6	<i>EP7</i> (LOC_Os02g15950)	RM 1195		ATGGACCACAAACGACCTTC	CGACTCCCTTGTCTTCTGG	60
		F57	STS	ATACCCAAATGGAGCTAG	TTTGGATCTAGAGTTGGG	55
		F35	STS	ATACCCAAATGGAGCTAG	TTTGGATCTAGAGTTGGG	55

**Table 3. Distribution of rice genotypes into eight clusters**

Cluster number	Number of genotypes	Name of the genotypes
I	1	GN1
II	15	GN2, GN5, GN14, GN10, GN37, GN11, GN51, GN54, GN74, GN44, GN76, GN8, GN12, GN4, GN31
III	1	GN3
IV	37	GN6, GN13, GN7, GN39, GN15, GN69, GN20, GN16, GN58, GN27, GN24, GN62, GN22, GN66, GN50, GN52, GN43, GN75, GN67, GN33, GN18, GN17, GN65, GN73, GN19, GN77, GN23, GN29, GN72, GN57, GN28, GN25, GN47, GN68, GN63, GN64, GN30
V	20	GN9, GN36, GN59, GN41, GN55, GN46, GN60, GN53, GN35, GN21, GN45, GN71, GN34, GN40, GN26, GN32, GN48, GN49, GN56, GN61
VI	1	GN38
VII	1	GN42
VIII	1	GN70

mean value for flag leaf length (43 cm), flag leaf width (1.70 cm), total primary rachis branch (14.50) and grain yield per plant (47.96 g). Cluster I exhibited the highest mean values for plant height (154.50 cm) and panicle length (27.95 cm), whereas cluster VI recorded the highest mean value for total productive tillers (17.50) and spikelets in primary rachis branch (84.50). Devi *et al.* (2019) observed maximum cluster mean value for panicle length and total grains per panicle and Sanju

Kumari *et al.* (2018) noticed maximum cluster mean value for grains per panicle. Based on cluster mean values, genotypes belonging to clusters V and III exhibited desirable grain number per panicle and its contributing traits along with high grain yield. These genotypes are suitable for use as parents in the grain yield improvement of rice. The per cent contribution of different traits to genetic divergence is presented in **Table 6**. Among different traits, the maximum contribution was recorded in

Table 4. Intra (bold) and inter cluster distance of 77 rice genotypes for 15 traits

Cluster number	I	II	III	IV	V	VI	VII	VIII
I	<b>0</b>	269.00	660.58	387.63	508.80	300.35	444.04	314.89
II		<b>57.55</b>	215.61	100.72	120.82	98.85	97.76	80.86
III			<b>0</b>	342.53	232.81	275.97	108.55	133.46
IV				<b>52.02</b>	90.33	91.55	135.88	180.68
V					<b>55.08</b>	134.22	109.73	133.54
VI						<b>0</b>	113.18	179.33
VII							<b>0</b>	82.44
VIII								<b>0</b>

Table 5. Cluster mean for different quantitative traits

CLUSTER	PH	TPT	FLL	FLW	PL	TSP	TFGP	TPRB	TSRB	SPRB	SSRB	LOPB	LOSB	SD	GYP
I	154.50	11.50	36.00	1.40	27.95	209.00	186.00	11.50	43.50	68.00	141.00	11.55	3.20	2.06	13.82
II	103.57	13.27	30.30	1.26	22.66	151.87	135.10	9.67	27.10	50.23	101.63	10.15	3.17	1.93	25.25
III	106.00	15.00	43.00	1.70	23.90	221.00	198.00	14.50	37.50	80.50	140.50	9.45	3.05	1.82	47.96
IV	83.80	11.92	25.22	1.33	20.76	180.18	164.42	9.41	34.46	44.05	136.12	10.11	3.46	2.41	16.32
V	88.05	12.55	28.19	1.50	22.78	323.10	298.53	11.20	55.83	52.60	270.50	10.83	3.84	3.32	25.16
VI	101.00	17.50	20.00	1.50	22.55	234.50	215.50	14.50	48.00	84.50	150.00	9.80	3.45	2.55	22.46
VII	93.00	12.50	30.00	1.50	23.00	180.00	163.50	12.50	55.00	62.00	118.00	9.95	3.75	3.13	33.53
VIII	120.50	12.50	26.00	1.50	26.15	261.50	237.00	11.00	52.00	45.50	216.00	12.70	4.25	2.33	34.67

PH-Plant height (cm), TPT-Total productive tiller, FLL- Flag leaf length (cm), FLW-Flag leaf width (cm), PL- Panicle length (cm), TSP- Total spikelets per panicle, TFGP- Total filled grains per panicle, TPRB- Total primary rachis branches, TSRB- Total secondary rachis branches, SPRB- Spikelets in primary rachis branches, SSRB- Spikelets in secondary rachis branches, SD- Spikelets density, LOPB- Length of primary branches (cm), LOSB- Length of secondary branches (cm), GYP- Grain yield per plant (g)

Table 6. Contribution of different quantitative characters towards genetic divergence among 77 genotypes of rice

S.No.	Character	Contribution (%)
1	Plant height	17.635
2	Total productive tiller	1.401
3	Flag leaf length	0.239
4	Flag leaf width	3.725
5	Panicle length	1.982
6	Total spikelets per panicle	22.453
7	Total filled grains per panicle	0.375
8	Total primary rachis branch	2.597
9	Total secondary rachis branch	2.323
10	Spikelets in primary rachis branch	3.451
11	Spikelets in secondary rachis branch	0
12	Length of primary branch	0.512
13	Length of secondary branch	0.956
14	Spikelets density	2.187
15	Grain yield per plant	40.157



grain yield per plant (40.15%), followed by total spikelets per panicle (22.45%), plant height (17.63%), flag leaf width (3.72%) and spikelets in primary rachis branches (3.45%). The characters viz., total primary rachis branches, total secondary rachis branches, spikelets density and panicle length contributed 2.59, 2.52, 2.18 and 1.98%, respectively. The least contribution for divergence was recorded by flag leaf length (0.23) and total filled grains per panicle (0.37). On contrary, grain per secondary branch had no contribution towards genetic divergence. The relative contribution of each trait proved that grain yield per plant and number of spikelets per panicle were the top two contributors to total divergence, so that the selected genotypes had enough variation to meet the objective of this study. Similar results in rice were also reported by Dey *et al.* (2020) for grain number per panicle and Singh *et al.* (2020) for plant height and single plant yield.

The correlation coefficient among yield attributing traits is presented in Fig. 1. The values of correlation coefficient revealed that single plant yield had a significant and positive correlation with the number of productive tillers (0.53), flag leaf length (0.40), panicle length (0.38), the number of primary branches (0.38), plant height (0.35), grains per primary branches (0.33),

flag leaf width (0.26), the number of secondary branches (0.22), the number of spikelets (0.21), the number of filled grains (0.20) and grains per secondary branches (0.17) and total filled grains per panicle had significant and positive correlation with total spikelets per panicle (1.00), spikelets in secondary rachis branches (0.99), total secondary rachis branch(0.91), total primary rachis branch(0.91), spikelets density(0.65), flag leaf width(0.48) and panicle length (0.45). These traits could be considered as selection criteria for yield improvement in rice. These observations support the earlier findings of Prasannakumari *et al.* (2020) for the number of productive tiller and plant height. Bhargava *et al.* (2021) reported a similar positive association between panicle length, the number of productive tillers and fertility of spikelets.

The seventy-seven germplasm characterized using thirteen functional markers for six QTLs were analysed for single marker analysis and it showed that the QTLs, *Gn1a*, *qGN 4-1* and *NGP 4* (Fig. 2) had a significant association with a number of grains per panicle with the phenotypic association percentage ( $R^2$ ) of 0.977, 0.114 and 0.619 (Table 7). The QTL *qGN 4.1* had a significant association with nine important traits viz., flag leaf width (0.13), total spikelets per panicle (0.114), total filled

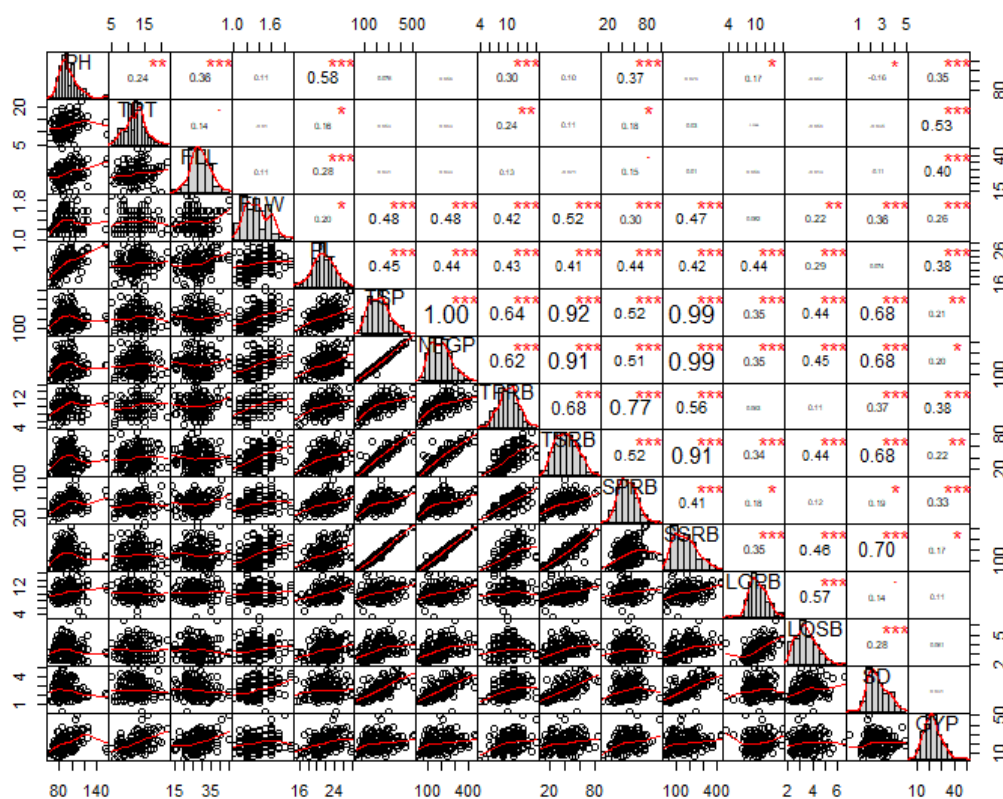


Fig. 1. The Pearson's Correlation co efficient among grain number per panicle and its contributing traits (\*, \*\* and \*\*\* indicates significance at 5 %, 1 % and 0.1 % level, respectively)

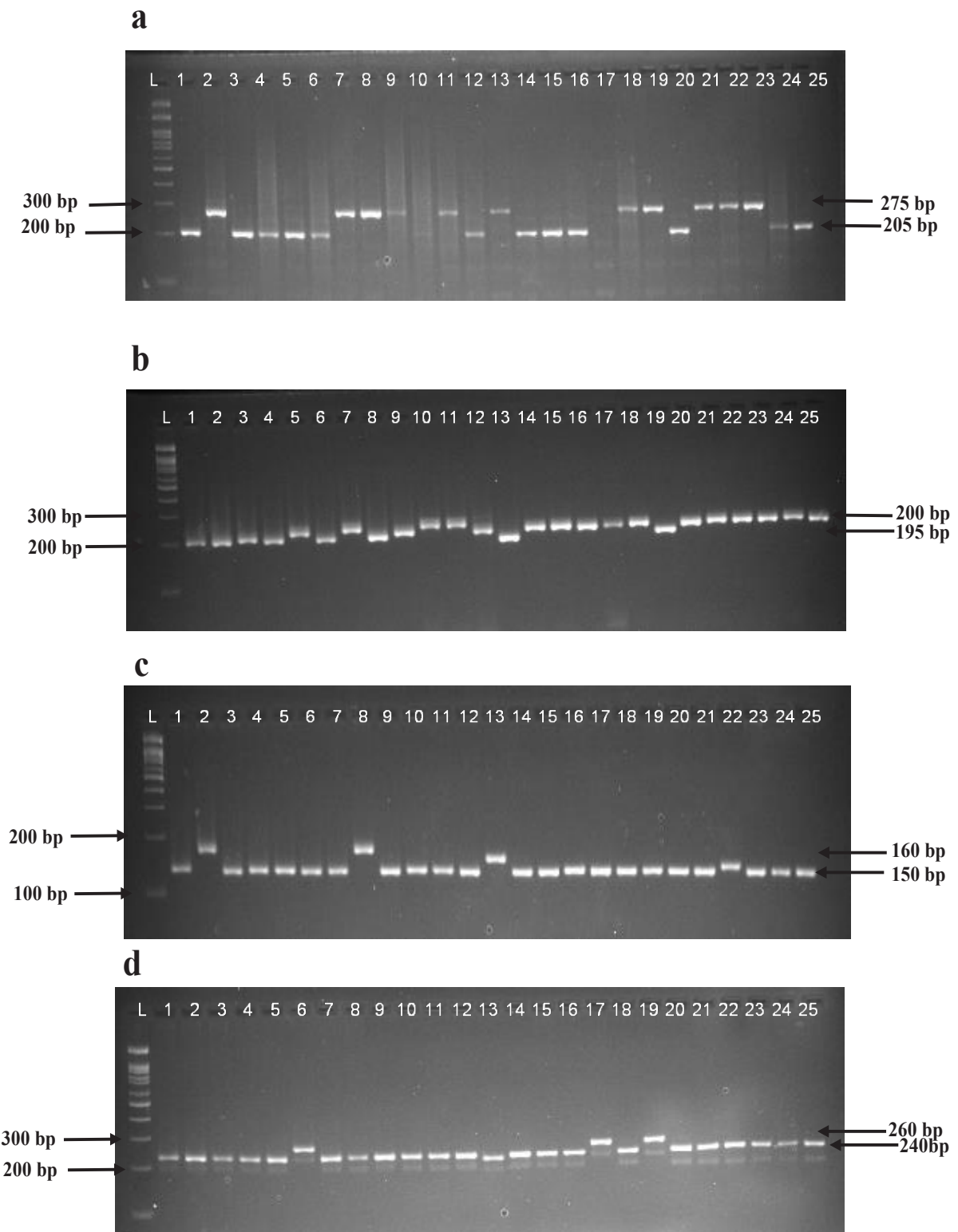


Fig. 2. QTL linked marker screening in rice germplasm, a- GN1a indel3, b- NKSSR 04-19, c- RM1183, d- F 57, 1 to 25 – GN 1 to GN 25 germplasm, L = ladder (100 bp)

Table 7. Gene specific markers and its R<sup>2</sup> value for fifteen quantitative traits

Markers	PH	TPT	FLL	FLW	PL	TSP	TFGP	TPRB	TSRB	SPRB	SSRB	LOPB	LOSB	SD	GYP
F 57	0.0232	0.0010	0.0348	0.0057	0.0222	0.0179	0.0202	0.0002	0.0243	0.0066	0.0240	0.0137	0.0106	0.0684*	0.0431
Gn1a	0.0226	0.0012	0.0176	0.0297	0.0117	0.0977**	0.1058**	0.0084	0.0552	0.0306	0.0981**	0.0000	0.0077	0.1816**	0.0152
NK-SSR-025811	0.0045	0.0064	0.0391	0.0335	0.0912*	0.0870*	0.0222	0.0781*	0.0155	0.0957**	0.0038	0.0168	0.0669	0.0006	
NK-SSR-000619	0.0004	0.0011	0.1370**	0.0054	0.1141**	0.1172**	0.1069**	0.1612**	0.0906*	0.1005**	0.0008	0.0788*	0.1231**	0.0026	
RM85	0.0000	0.0067	0.0000	0.0057	0.0228	0.0084	0.0054	0.0000	0.0037	0.0120	0.0067	0.0103	0.0047	0.0005	0.0004
RM1183	0.0042	0.0402	0.0215	0.0275	0.0409	0.0556*	0.0520	0.0822*	0.0750*	0.0317	0.0510	0.0419	0.0526	0.0207	0.0275
RM6314	0.0012	0.0088	0.0944**	0.0981	0.0015	0.0670*	0.0619*	0.0854	0.1184**	0.0272	0.0637*	0.0364	0.0780*	0.0809*	0.0092

\* significant at 5 % (P<0.05), \*\* significant at 1 % (P<0.01)

grains (0.117), spikelets in primary rachis branch (0.090), spikelets in secondary rachis branch (0.10), length of secondary branch (0.07), spikelets density (0.12), total primary rachis branches (0.10) and total secondary rachis branches (0.16). The QTL *Gn1a* is associated with four important grain number related traits viz., total spikelets per panicle (0.09), total filled grains per panicle (0.10), spikelets in secondary rachis branches (0.098) and spikelets density (0.1816). The QTL, *NGP 4* had a significant association with seven characters viz., flag leaf length (0.09), total spikelets per panicle (0.067), total filled grains per panicle (0.0619), total secondary rachis branches (0.1184), spikelets in primary rachis branches (0.063), length of secondary branch (0.078) and spikelets density (0.08). Similarly, *NOG1* had a significant association with total spikelets per panicle (0.056), total primary rachis branches (0.082) and total secondary rachis branches (0.075). The markers GN1a indel 3 in *GN1a*, NKSSR 04-19 in *q GN 4.1*, RM 6314 in *NGP 4* and RM1183 in *NOG 1* were found as the most informative markers and also had a higher association with phenotype of the respective traits. These markers could be used to characterize the germplasm or any other population for validation of respective QTLs and will be much useful in marker assisted breeding programmes. Bagudam *et al.* (2021) used seven gene specific markers including GN1a to screen new plant type-based rice accessions for yield and culm strength traits in rice. Anand *et al.* (2013) screened three QTLs i.e., grain size 3 (*GS3*), grain weight 2 (*GW2*) and seed width 5 (*qSW5*) in 242 rice accessions using QTL linked markers and found that one marker SF28 had significantly associated with grain length at 37 % of phenotypic variance. Hyun *et al.* (2015) screened 180 japonica rice accessions with low germinability linked markers and found eight SNP markers had a close association with the trait.

From this study, 77 diverse rice germplasm were grouped into eight clusters and cluster V and cluster III comprised of 21 genotypes had high cluster mean

value for grain number per panicle and grain yield per plant. These genotypes can be used for grain yield improvement breeding programme of rice. Among the six QTLs screened in the rice germplasm, five QTLs had a significant association with grain number per panicle and its contributing traits viz., *Gn1a*, *q GN 4.1*, *EP 7*, *NOG 1* and *NGP 4*. These QTL linked markers can be used in marker assisted backcross breeding for transferring the grain number per panicle related genes into a rice variety to enhance the grain yield.

## REFERENCES

- Anand, D., Baunthiyal, M., Singh, A., Krishnan, S. G., Singh, N., Prabhu, K. and Singh, A. 2013. Validation of gene based marker-QTL association for grain dimension traits in rice. *Journal of plant biochemistry and biotechnology*, **22**(4): 467-473. [Cross Ref]
- Ashikari, M., Sakakibara, H., Lin, S., Yamamoto, T., Takashi, T., Nishimura, A., Angeles, E.R., Qian, Q., Kitano, H. and Matsuoka, M. 2005. Cytokinin oxidase regulates rice grain production. *Science*, **309** (5735):741-745. [Cross Ref]
- Bagudam, R., Eswari, K., Badri, J., Devi, G.L., JaiVidhya, L., Bhavani, P., Swathi, G., Bharath, M., Rao, P.R. and Kumar, J.A. 2021. Morphological and molecular characterization of new plant type core set for yield and culm strength traits in rice (*Oryza sativa* L.). *Journal of Plant Biochemistry and Biotechnology*, **30** (2):233-242. [Cross Ref]
- Bhargava, K., Shivani, D., Pushpavalli, S., Sundaram, R., Beulah, P. and Senguttavel, P. 2021. Genetic variability, correlation and path coefficient analysis in segregating population of rice. *Electronic Journal of Plant Breeding*, **12** (2):549-555. [Cross Ref]
- Chen, X., Dang, X., Wang, Y., Yang, Y., Yang, G., Sun, J., Yin, H., Liu, E. and Hong, D. 2021. Association



- mapping of thousand grain weight using SSR and SNP markers in rice (*Oryza sativa* L.) across six environments. *Tropical Plant Biology*, **14** (2):143-155. [Cross Ref]
- Devi, M., Jyothula, D., Krishnaveni, B. and Rao, V. 2019. Genetic divergence studies in rice (*Oryza sativa* L.) hybrids for yield, yield component traits and quality parameters. *Int. Journal of Current Microbiology Applied Science*, **8** (6):1577-1583. [Cross Ref]
- Dey, S., Badri, J., Eswari, K. and Prakasam, V. 2020. Diversity analysis for yield traits and sheath blight resistance in rice genotypes. *Electronic Journal of Plant Breeding*, **11** (01):60-64. [Cross Ref]
- Huang, Y., Zhao, S., Fu, Y., Sun, H., Ma, X., Tan, L., Liu, F., Sun, X., Sun, H. and Gu, P. 2018. Variation in the regulatory region of FZP causes increases in secondary inflorescence branching and grain yield in rice domestication. *Plant Journal*, **96** (4):716-733. [Cross Ref]
- Huo, X., Wu, S., Zhu, Z., Liu, F., Fu, Y., Cai, H., Sun, X., Gu, P., Xie, D. and Tan, L. 2017. *NOG1* increases grain production in rice. *Nature Communication*, **8** (1):1-11. [Cross Ref]
- Hyun, D. Y., Lee, G. A., Kang, M. J., Burkart-Waco, D., Kim, S. I., Kim, J. Y., Lee, M. C., Gwag, J. G., Kim, Y. G. and Tai, T. H. 2015. Development of low-temperature germinability markers for evaluation of rice (*Oryza sativa* L.) germplasm. *Molecular Breeding*, **35**(4): 1-16. [Cross Ref]
- Ikeda, M., Hirose, Y., Takashi, T., Shibata, Y., Yamamura, T., Komura, T., Doi, K., Ashikari, M., Matsuoka, M. and Kitano, H. 2010. Analysis of rice panicle traits and detection of QTLs using an image analyzing method. *Breeding Science*, **60** (1):55-64. [Cross Ref]
- Indiastat, 2021 <http://www.indiastat.com>. (Accessed 09 June 2021).
- Li, M., Tang, D., Wang, K., Wu, X., Lu, L., Yu, H., Gu, M., Yan, C. and Cheng, Z. 2011. Mutations in the F-box gene *larger panicle* improve the panicle architecture and enhance the grain yield in rice. *Plant Biotechnology Journal*, **9** (9):1002-1013. [Cross Ref]
- Murray, M. and Thompson, W. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic acids research*, **8** (19):4321-4326. [Cross Ref]
- Parhe, S.D., Harer, P. and Nagawade, D. 2014. Investigation of genetic divergence in chickpea (*Cicer arietinum* L.) genotypes. *The Bioscan*, **9** (2):879-882.
- Prasannakumari, M., Akilan, M., Kalaiselvan, S., Subramanian, A., Janaki, P. and Jeyaprakash, P. 2020. Studies on genetic parameters, correlation and path analysis for yield attributes and Iron content in a backcross population of rice (*Oryza sativa* L.). *Electronic Journal of Plant Breeding*, **11** (03):881-886. [Cross Ref]
- Sanju Kumari, P., Singh, P., Bisen Bapsila, L., Rai, V. and Sinha B. 2018. Genetic diversity analysis of rice (*Oryza sativa* L.) germplasm through morphological markers. *International journal of agriculture environment and biotechnology*, 953-957.
- Singh, S., Pandey, V., Mounika, K., Singh, D., Khaire, A., Habde, S. and Majhi, P.K. 2020. Study of genetic divergence in rice (*Oryza sativa* L.) genotypes with high grain zinc using Mahalanobis' D2 analysis. *Electronic Journal of Plant Breeding*, **11** (02):367-372. [Cross Ref]
- Sreewongchai, T., Sripichitt, P. and Matthayathaworn, W. 2021. Parental genetic distance and combining ability analyses in relation to heterosis in various rice origins. *Journal of Crop Science and Biotechnology*, **24**(3): 327-336. [Cross Ref]
- Srinivas, T. 2018. Genetic diversity studies in rice (*Oryza sativa* L.). *Electronic Journal of Plant Breeding*, **9** (4):1335-1341. [Cross Ref]
- Umesh, U., Jaiswal, H., Sravan, T., Waza, S.A. and bhardwaj, R. 2015. Estimation of genetic variability, heritability and genetic advance for yield and quality traits in some indigenous Basmati rice (*Oryza sativa* L.) genotypes. *International Journal of Farm Science*, **5** (4):32-40.
- Wang, J., Xu, H., Li, N., Fan, F., Wang, L., Zhu, Y. and Li, S. 2015. Artificial selection of *Gn1a* plays an important role in improving rice yields across different ecological regions. *Rice*, **8** (1):1-10. [Cross Ref]
- Wang, Y., Zhai, L., Chen, K., Shen, C., Liang, Y., Wang, C., Zhao, X., Wang, S. and Xu, J. 2020. Natural sequence variations and combinations of *GNP1* and *NAL1* determine the grain number per panicle in rice. *Rice*, **13** (1):1-15. [Cross Ref]
- Xie, J., Li, F., Khan, N.U., Zhu, X., Wang, X., Zhang, Z., Ma, X., Zhao, Y., Zhang, Q. and Zhang, S. 2019. Identifying natural genotypes of grain number per panicle in rice (*Oryza sativa* L.) by association mapping. *Genes and genomics*, **41** (3):283-295.