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Surveying allelic diversity of Os *DEP1* regulating panicle architecture in rice

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Abstract
Rice (*Oryza sativa* L.) is the staple food for more than 60% of the world's population and the most widely grown cereal. In the context of rapidly increasing world's population there is a need to increase grain yield. Any further increase in rice yields has to overcome challenges due to yield plateau, declining resources and climate change. Increasing yield potential in rice requires discovery of novel genes and alleles from rice gene pool or genetic manipulation of novel traits like C4 photosynthesis. Therefore, identifying the superior alleles governing yield component traits is necessary to increase the rice grain yield. In this study, efforts were made to survey the allelic diversity of a major yield gene *DEP1* (Dense and Erect Panicle 1) in a diverse set of 217 rice germplasm by utilizing the sequence information available from IRRI 3K rice genome sequencing project. Allelic diversity analysis identified the presence of 18 INDELs and 44 SNPs (Single Nucleotide Polymorphisms) in *DEP1*. Haplotype analysis identified creation of three haplotypes *viz*., H1, H2 and H3 which can be utilized for further association mapping.

Keywords: rice, DEP1, haplotype analysis

1. Introduction
Rice (*Oryza sativa* L.) is the staple food for greater than 60% of the world's population and the most widely grown cereal in the world. In worldwide, rice is cultivated in 162.05 million hectare, produced 755.43 million tonnes and productivity of about 4.66 tonnes per hectare during 2019. Rice is cultivated in most of the states of India and contributes more than 40% of the area and production among food grains (Sekhara and Devarajulu 2019) [14]. In India, rice is cultivated in 43.78 million hectares, produced 177.64 million tonnes with the productivity of 4.05 tonnes per hectare (FAOSTAT 2019) [5]. To feed the rapidly increasing world's population, there is a need to double the food production by 2050. The rate of increase in rice yield is about 0.9 – 1.6% per year and it is not enough to feed the anticipated world's population in 2050 (Ray *et al*., 2013) [12]. During past decades, increasing yield was achieved by tedious and slow conventional breeding approaches such as plant introduction (Taichung Native-1 and IR-8), hybridization (CO 51), selection (M-351) and mutation breeding (Binasail) (Boopathi 2020) [4]. Through recent advancements in Marker Assisted Breeding (MAB), Transgenic and genome editing will accelerate yield improvement, but breeding efficiency in these approaches require the finding of superior alleles or creating superior alleles over the naturally available genotypes (Qaim 2020, Shamsudin *et al*., 2016) [11, 15]. For example, using CRISPR/Cas9 genome editing novel alleles of *DEP1* gene were created in rice, one of the novel allele results 51.1% yield increase. (Huang *et al*., 2018) [6]. Another approach to identify superior alleles is screening the germplasm having wide variation for the targeted trait (Abbai *et al*., 2019) [1]. *DEP1* (Os09g0441900) which is present in chromosome 9 (16411151 bp - 16415851 bp) coding for gamma subunit of G-protein and cysteine rich domain. Variation in this gene leads to difference in panicle architecture consequently increased yield (Li *et al*., 2016) [8]. The current study was undertaken to find out the allelic variations of *DEP1* in 3K RG panel subset.

2. Materials and Methods
2.1 Allelic diversity of *DEP1* in 3K RG panel subset
The SNP seek database was used to retrieve *DEP1* sequences of 217 lines (Mansueto *et al*., 2017) [9]. Multiple sequence alignment was performed using the BioEdit software (Version 7.2.5). *DEP1* sequence of *Oryza sativa indica* group from Ensembl Plants database (Bolser *et al*., 2016) [3] was used as the reference to find-out allelic variation (Repulles-Albelda *et al*., 1999) [13] among 217 lines.

2.2 Haplotype analysis of *DEP1* in 3K RG panel subset

Allelic variation of *DEP1* was accessed from the SNP seek database to perform haplotype analysis. The 3K filtered SNP dataset available in the SNP seek database was used for the haplotype analaysis. The 3K filtered SNP dataset was obtained by adopting criteria *viz*., alternative allele frequency at least 0.01 and missing calls per SNP 0.2 and this was readily available in the SNP seek database. Allele mining was carried out by selecting only the non-synonymous SNPs (SNPs in coding region which results amino acid change). This data was subsequently converted into haploview fileset using gPLINK (version 1.07) (Purcell *et al*., 2007) [10]. The generated haploview file set was used for performing haplotype analysis using Haploview (version 4.1) and the significant SNPs were chosen with the cut off value of 0.001 (Barrett *et al*., 2005) [2].

3. Results and Discussion

3.1 Allelic diversity of *DEP1*

DEP1 gene contains 62 variations (includes 18 INDELs and 44 SNPs) (Table 1) but only nine SNPs were non-synonymous and there were no changes in the exons as well as in splice donor/acceptor sites. Nine non-synonymous variations found in multiple sequence alignment leads to change of several amino acids (Lys→Arg, Cys→Tyr, Ser→Asn, Cys→Phe, His→Leu, Cys→Tyr, Ser→Trp, Ser→Cys) among them only four were significant (Fig. 1). Novel variation in *DEP1* allele's fifth exon, 637 bp is replaced by 12 bp substituition which leads to premature stop codon causing dense and erect panicle. (Huang *et al.*, 2009; Zhao *et al.*, 2016) [7, 16]. In our study, similar allelic variation as reported by Huang *et al.*, (2009) [7] was not found in all the genotypes of present study.

Table 1: Allelic variations in *DEP1* gene for the 3K RG panel subset

Region	Position	Alleles	Sequence polymorphisms	Type	MAF
5' UTR	16411393-16411396	4 bp	CGCT/----	INDEL	3.69
5' UTR	16411397	1 bp	C/T	SNP	0.46
5' UTR	16411529	1 bp	G/-	INDEL	20.28
5' UTR	16411562	1 bp	G/A	SNP	0.46
EXON 1 (NS)	16411598	1 bp	A/G	SNP	0.92
Intron 1-2	16411815	1 bp	A/T	SNP	6.91
Intron 1-2	16411954	1 bp	A/C	SNP	10.60
Intron 1-2	16411963	1 bp	A/C	SNP	30.41
Intron 1-2	16412067	1 bp	T/C	SNP	38.71
Intron 1-2	16412165	1 bp	A/G	SNP	38.25
Intron 1-2	16412257	1 bp	T/-	INDEL	40.55
Intron 1-2	16412686	1 bp	T/-	INDEL	0.92
Intron 1-2	16412867	1 bp	T/C	SNP	27.65
Intron 2-3	16413005	1 bp	T/A	SNP	1.38
Intron 2-3	16413204	1 bp	G/A	SNP	17.05
Intron 2-3	16413217	1 bp	G/A	SNP	16.13
Intron 2-3	16413221	1 bp	A/G/-	INDEL	1.38
Intron 2-3	16413222-16413224	3 bp	AAG/---	INDEL	45.16
Intron 2-3	16413225	1 bp	T/G/-	INDEL	1.38
Intron 2-3	16413241	1 bp	C/A	SNP	11.06
Intron 2-3	16413258	1 bp	G/A	SNP	17.51
Intron 2-3	16413274	1 bp	A/G	SNP	0.46
Intron 2-3	16414275-16413276	3 bp	CAA/---	INDEL	0.46
Intron 2-3	16413284	1 bp	A/G	SNP	27.65
Intron 2-3	16413299	1 bp	G/-	INDEL	18.89
Intron 2-3	16413300-16413304	5 bp	CATTT/-----	INDEL	18.89
Intron 2-3	16413305	1 bp	G/-	INDEL	18.89
Intron 2-3	16413344	1 bp	G/A	SNP	9.68
Intron 2-3	16413353	1 bp	C/T	SNP	26.73
Intron 2-3	16413391	1 bp	C/T	SNP	9.68
Intron 2-3	16413437	1 bp	C/-	INDEL	8.76
Intron 2-3	16413438-16413451	14 bp	TAAAACTTTTGCCA/CAAAACTTTTGCCA/----------	INDEL	14.75
Intron 2-3	16413452	1 bp	C/-	INDEL	29.49
Intron 2-3	16413464	1 bp	C/T	SNP	4.61
Intron 2-3	16413471	1 bp	C/T	SNP	1.84
Intron 2-3	16413488	1 bp	T/A	SNP	17.97
Intron 2-3	16413493	1 bp	A/C	SNP	26.73
Intron 2-3	16413750-16413763	14 bp	TAAATATATTGAAC/----------	INDEL	25.35
Intron 2-3	16413788	1 bp	A/G	SNP	26.73
Intron 2-3	16413808	1 bp	T/C	SNP	8.76
Intron 2-3	16413921	1 bp	C/T	SNP	17.97
Intron 2-3	16414037	1 bp	A/G	SNP	27.19
Intron 4-5	16414350	1 bp	A/C	SNP	10.14
Intron 4-5	16414408	1 bp	A/G	SNP	9.22
Intron 4-5	16414438	1 bp	A/G	SNP	17.97
Intron 4-5	16414484	1 bp	G/A	SNP	0.46
Intron 4-5	16414535	1 bp	C/T	SNP	24.42
Intron 4-5	16414576	1 bp	A/G	SNP	0.46

Intron 4-5	16414579	1 bp	A/-	INDEL	0.46
Intron 4-5	16414599-16414619	21 bp	TACATTAACATTGATTTTTTT/----------	INDEL	17.51
EXON 5 (NS)	16414745	1 bp	G/A	SNP	9.22
EXON 5 (S)	16414749	1 bp	T/C	SNP	17.97
EXON 5 (NS)	16415042	1 bp	A/G	SNP	33.64
EXON 5 (NS)	16415078	1 bp	G/T	SNP	5.99
EXON 5 (NS)	16415114	1 bp	A/T	SNP	8.76
EXON 5 (NS)	16415213	1 bp	G/A	SNP	6.91
EXON 5 (NS)	16415264	1 bp	G/C	SNP	33.64
EXON 5 (NS)	16415265	1 bp	C/G	SNP	20.74
EXON 5 (NS)	16415401	1 bp	A/T	SNP	9.22
3' UTR	16415734	1 bp	C/T	SNP	9.22
3' UTR	16415740	1 bp	T/-	INDEL	1.84
3' UTR	16415841	1 bp	G/C	SNP	7.37

MAF- Minor Allele Frequency; NS – Non-synonymous SNP; S - Synonymous SNP

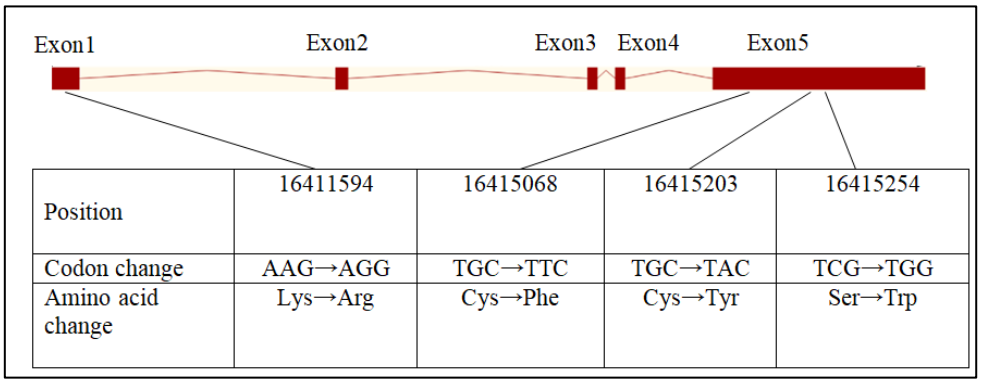

Fig 1: Four significant SNPs and their respective amino acid changes. (Solid blocks - exons, lines in between solid blocks – introns).

3.2 Haplotype analysis of 217 genotypes and diversity between haplotypes

Among the four significant non-synonymous SNP's found in *DEP1* gene, three haplotypes namely H1, H2, H3 were formed which comprises 116, 80 and 21 genotypes respectively based on only two SNP's (16415203 and 16415254) (Table 2). The LD plot (Fig. 2) showed the correlation of two SNPs involved in haplotype grouping and the correlation coefficient was low ($r^2 = 5$).

Table 2: List of significant SNPs in *DEP1* for the subset of 3K RG panel

Marker Number	SNP site	Position	HWpval	MAF	Alleles
1	10916411594	16411594	2.20E-05	0.008	A:G
2	10916415068	16415068	5.03E-24	0.053	G:T
3	10916415203	16415203	4.21E-31	0.091	G:A
4	10916415254	16415254	4.15E-67	0.34	C:G

HWpval- Hardy Weinberg p value, MAF- Minor Allele Frequency

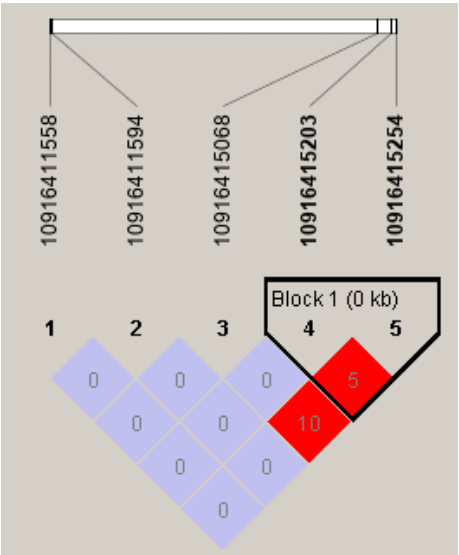

Fig 2: Linkage Disequilibrium (LD) plot of significant SNPs in *DEP1* using Haplo View (numbers in the block indicates LD r^2)

4. Conclusion

Out of the 62 genetic polymorphisms found in DEP1, only nine were non-synonymous and none of them were found to be non-sense mutations. Based on non-synonymous SNPs, three haplotype groups were identified. Extensive phenotyping of 217 lines for the yield related traits is needed to find out the elite haplotype for breeding applications. Identification of such superior haplotypes and haplotype based breeding can provide strategy to develop elite rice varieties with improved yield potential.

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