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Genome Wide Association Studies for Flowering Time and Plant Height in *Indica* MAGIC Lines of Rice (*Oryza sativa L.*)

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ABSTARCT

Genome wide association studies in a subset of *indica* MAGIC lines using MLM and GLM analysis through TASSEL identified 15 significant SNPs for 15 quantitative trait loci out of 27,041 SNP markers. Two previously identified QTLs viz., *qFDN-1* and *RFT-1* located on chromosomes 3 and 6 respectively were identified for flowering time and three QTLs were detected on chromosome 1 (*qPHT-1* and *ph1.1*) and chromosome 6 (*ph6*) for plant height. Six novel QTLs (*qDFF2*, *qDFF3*, *qDFF6*, *qDFF6-1*, *qDFF8* and *qDFF10*) for flowering time and four QTLs (*qPH3*, *qPH4*, *qPH8* and *qPH12*) for plant height were detected in the present investigation. Further, eleven candidate genes with unknown function were also identified by GWAS using MLM and GLM analysis.

Key words: *Flowering, GWAS, MAGIC lines, Plant height, Rice, SNP, TASSEL*

Rice (*Oryza sativa.* L) is one of the most important cereals and feeds more than half of the world's population. Exploiting new quantitative trait loci (QTLs) for complex traits is essential for effective crop improvement. Generally, bi-parental populations are used to identify the location and effects of QTLs controlling trait of interest by conventional QTL mapping. Recently, a multi-parent advanced generation intercross (MAGIC) strategy has been proposed to introgress multiple alleles and to provide increased recombination and mapping resolution (Cavanagh *et al*., 2008). Genome wide association studies (GWAS) have been widely used to identify QTL underlying quantitative traits in humans and animals, and has recently become a popular method of mapping QTL in plants. Genome-wide association study (GWAS), also known as whole genome association study (WGAS), is evaluation of many common genetic variants in different individuals to see whether any variant is associated with a specific trait of interest. GWAS typically focuses on associations between single-nucleotide polymorphisms (SNPs) and traits. By using this method, several QTLs for agronomic, yield and quality traits have been identified.

MATERIAL AND METHODS

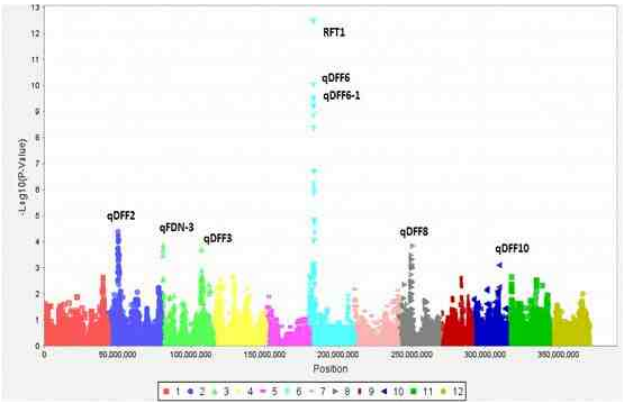
The present investigation "Genome wide association studies on flowering time and plant height in *indica* MAGIC lines in rice" was carried out during *kharif*, 2017 at Regional Agricultural Research Station, Maruteru, West Godavari District of Andhra Pradesh state located at 81.44^0E longitude, 26.30 latitude and 5m above mean sea level. The soils are characterized by black alluvial clay soils, neutral to slightly alkaline in reaction with medium levels of phosphorus and potassium. A subset of 395 *indica* MAGIC lines and its genotypic data received from International Rice Research Institute (IRRI), Philippines was used as experimental material for genome wide association studies (GWAS) to identify QTLs. The seeds of 395 *indica* MAGIC lines were sown in seedling nursery on 21st of June, 2017 and transplanted in 2 rows of 3.3 m length in a plot with 44 plants in each plot at 25 days after sowing. The spacing was 20 cm between rows and 15 cm between plants. Augmented randomized complete block design was adopted for the trial. Six popular varieties *viz*., Sahabhagi Dhan, Rasi, IR64, MTU 1010, CSR36 and MTU 1075 were used as check varieties and were replicated six times. Phenotypic data was collected on the phenological traits *viz*., days to 50% flowering and plant height at maturity (cm). Statistical analysis of data was done for GWAS using a software called as TASSEL (Trait Analysis by Association, Evolution and Linkage). TASSEL implements general linear model (GLM) and mixed linear model (MLM) approaches for controlling population and family structure. The genotypic and phenotypic data was run through TASSEL for GWAS to obtain marker trait associations (MTA). The genotypic data containing 27,041 filtered SNP marker sites for all the 12 chromosomes were used for marker trait associations. The QTLs were identified from QTARO database (qtaro.abr.affrc.go.jp).

RESULTS AND DISUSSION

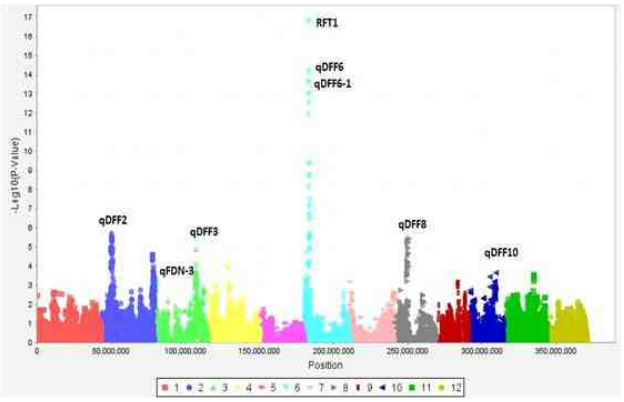
In the present investigation, two known QTLs *viz*., *qFDN-3* on chromosomes 3 and *RFT1* on

Table. 1. QTLs identified by GWAS through TASSEL in a subset of *indica* MAGIC lines of rice

Characters	Markers	Chromo-some	Marker Region	Allele	P-value	QTL	Physical position of QTL	Length of QTL	References
Days to 50% flowering	S3_1817619	3	1817619	A/T	1.41E-04	qFDN-3	1429107-3509693	2.081 Mbp	Hittalmani *et al*. (2002)
	S6_2939487	6	2939487	A/C	3.75E-13	RFT1	2925824-2927475	1.652 kbp	Ogiso-Tanaka *et al*. (2013)
	S2_6921840	2	6921840	G/T	4.24E-05	qDFF2			
	S3_28098531	3	2.80E+07	C/T	2.03E-04	qDFF3			
	S6_2962502	6	2962502	C/T	3.75E-13	qDFF6			
	S6_2950009	6	2950009	A/G	1.02E-10	qDFF6-1			
	S8_10098424	8	1.00E+07	C/T	1.44E-04	qDFF8			
	S10_18074213	10	1.80E+07	A/G	8.03E-04	qDFF10			
Plant height at maturity	S1_37368297	1	3.70E+07	C/G	5.85E-08	qPHT-1	36697294-42364623	5.6 Mbp	Hittalmani *et al*. (2002)
	S1_38286772	1	3.80E+07	A/G	9.98E-08	ph1.1	38490966-38491404	0.43 kbp	Hittalmani *et al*. (2003); Huang *et al*. (1994); Cho *et al*. (1998).
	S6_27043225	6	2.70E+07	A/G	2.37E-04	ph6	6927624-29906021	22.9 Mbp	Xiao *et al*. (1996)
	S3_29584573	3	3.00E+07	G/T	5.13E-04	qPH3			
	S4_31800256	4	3.20E+07	A/G	6.97E-04	qPH4			
	S8_25611811	8	2.60E+07	A/G	9.22E-05	qPH8			
	S12_3148965	12	3148965	A/G	5.14E-05	qPH12			

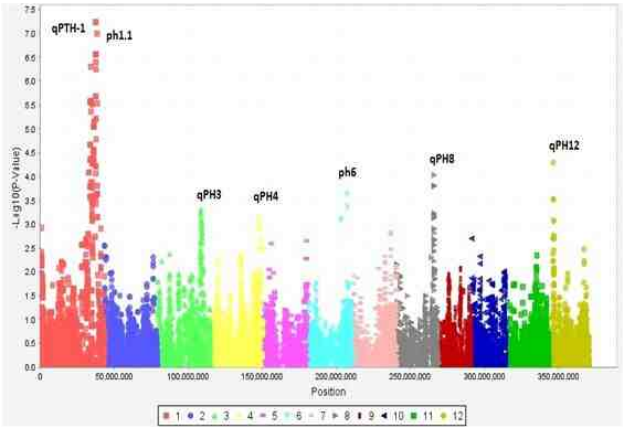


1 (a) Flowering time by MLM analysis

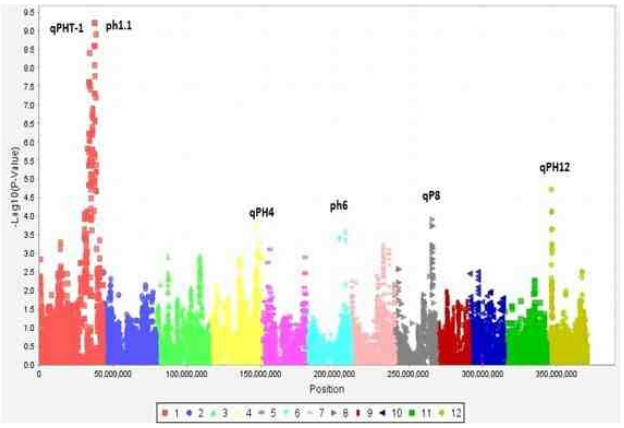


1 (b) Flowering time by GLM analysis

Fig 1. Manhattan plots of genome wide association mapping using MLM and GLM analysis for *indica* MAGIC lines of rice for flowering time. Black horizontal lines represent the threshold at P<0.001



2 (a) Plant height by MLM analysis



2 (b) Plant height by GLM analysis

Fig 2. Manhattan plots of genome wide association mapping using MLM and GLM analysis for *indica* MAGIC lines of rice for plant height. Black horizontal lines represent the threshold at P<0.001

chromosome 6 known to be associated with days to 50% flowering. The two QTLs, were detected by both GLM and MLM (p<0.001) (Fig.1) procedures. Further, *qFDN-3*, the QTL controlling flowering duration was found to be associated with a significant marker (S3_1817619) around 1.81 Mb on chromosome 3 which falls within the physical position of QTL region from 1429107- 3509693 bp and it has a length of 2.081 Mbp. This was also previously identified by Hittalmani *et al*. (2002) while studying on double haploid population (DH) of 125 lines derived from IR64 × Azucena, an *indica – japonica* cross. They observed that the QTL was located between the marker interval RZ329- RG348 with a LOD score of 4.19 and phenotypic variance of 18.3%.

GWAS analysis for days to 50% flowering using MLM analysis (p<0.001) detected a significant SNP site *i.e*., S6_2939487 on chromosome 6 (2.9 Mbp) where *RFT1* locus was located. The phenotypic variance of the marker is 14.51% and hence, it is identified as major QTL for flowering time. *RFT1* gene has a physical position at 2925824 bp to 2927475 bp and length of 1.652 kbp. This gene known to control flowering time divergence in rice was earlier reported by Ogiso-Tanaka *et. al.* (2013) while studying F_3 progeny of the cross Koshihikari x SL520.

GWAS by both MLM and GLM studies (p<0.001) in the present investigation identified a total of a total of six novel QTLs *viz*., *qDFF2*, *qDFF3*, *qDFF6*, *qDFF6-1*, *qDFF8* and *qDFF10* on chromosomes 2, 3, 6, 8 and 10, respectively (Table 1 and Fig. 1). *qDFF2* is associated with S2_6921840 SNP marker (physical position: 6921840 bp) on chromosome 2. S3_28098531 is located at the physical position 28098531 bp on chromosome 3 and is the SNP marker associated with *qDFF3*. *qDFF6* and *qDFF6-1* QTLs are associated with S6_2962502 (physical position: 2962502 bp) and S6_2950009 (physical position: 2950009 bp) SNP markers on chromosome 6. *qDFF8* and *qDFF10* are the QTLs associated with S8_10098424 and S10_18074213 SNP markers which are having a physical position at

10098424 bp and 18074213 bp on chromosomes 8 and 10, respectively. The phenotypic variances of the markers are given in Table 1 in which *qDFF6* and *qDFF6-1* having phenotypic variances of 14.51% and 11.33 %, respectively. Nine candidate genes were identified by GWAS using MLM and GLM analysis whose function was not known.

The present study also resulted in the identification of three known QTLs for plant height (Fig.2). A total of 136 significant SNP markers were detected on chromosomes 1, 3, 4, 6, 8 and 10 by both GLM and MLM analysis ($p<0.001$). On chromosome 1, in the region 37- 38 Mbp, two significant SNP markers (S1_37368297 and S1_38286772) were detected co-localized with 2 plant height QTLs *viz*., *qPHT-1* and *ph1.1,* respectively. A significant SNP marker *i.e*., S6_27043225 was detected on chromosome 6 in the region of 27.04 Mbp pointing to ph6, a plant height QTL. Further, *qPHT-1* on chromosome 1 has a length of 5.6 Mbp and physical position at 36697294 bp to 42364623 bp was found encompassing *sd*-1, a major semi-dwarf gene affecting plant height. This QTL was found to be associated with S1_37368297 marker which is having phenotypic variance of 7.85%. Similar results were also reported by Hittalmani *et al*. (2002). *ph1.1* on chromosome 1 at the physical position starting from 38490966 bp to 38491404 bp has a length of 0.43 kbp, which is also the map position of gene *sd*-1. This QTL is also having phenotypic variance of 7.56%. These results are in accordance with the reports of Hittalmani *et al*. (2003), Huang *et al*. (1994), Cho *et al*. (1998). QTL, *ph6* with a length of 22.9 Mbp occupies physical position of 6927624- 29906021 bp on chromosome 6. This QTL is having phenotypic variance of 3.53%.

Three novel QTLs associated with significant SNP markers were identified by GWAS using MLM and GLM analysis ($p<0.001$) on chromosomes 4, 8 and 12, respectively in the present study and one QTL was identified by MLM analysis (Table 1 and Fig.2). On chromosome 3, *qPH3* was found associated with S3_29584573 SNP marker which is located at 29.6 Mbp. *qPH4* is the QTL on chromosome 4 and was found to be associated with S4_31800256 SNP marker which is located at 31.8 Mbp. *qPH8* and *qPH12* are the QTLs on chromosomes 8 and 12, respectively. These QTLs were found to be associated with two significant SNP markers *viz*., S8_25611811 at 25.6 Mbp and S12_3148965 at 3.1 Mbp, respectively. The phenotypic variances of the markers are given in Table 1. Two candidate genes were also identified in the present study by GWAS for plant height. However their function could not be ascertained.

CONCLUSION

The QTLs *viz., RFT1, qDFF6* and *qDFF6-1* identified by GWAS for flowering time and are having phenotypic variance greater than 10% and hence, are considered as major QTLs. The introgression of these QTLs may help the rice breeder for effecting further improvement in rice. The QTLs for plant height *viz., qPHT-1* and *ph1.1* are having phenotypic variance between 5-10%. All the remaining fall below 5% of phenotypic variance and hence, are considered as minor QTLs.

LITERATURE CITED

Cavanagh C, Morell M, Mackay I and Powell W 2008 From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants. *Current opinion in plant biology*, 11: 215-221.

Cho Y G, McCouch S R, Kuiper M, Kang M, Pot J, Groenen J T M and Eun M Y 1998 Integrated map of AFLP, SSLP and RFLP markers using a recombinant inbred population of rice (*Oryza sativa* L.). *Theoretical Application of Genetics*, 97: 370-380.

Hittalmani S, Shashidhar H E, Bagali P G, Huang N, Sindhu J S, Singh V P and Khush G S 2002 Molecular mapping of quantitative trait loci for plant growth, yield and yield related traits across tree diverse locations in a doubled haploid rice population. *Euphytica*, 125: 207-214.

Hittalmani S, Huang N, Courtois B, Venuprasad R, Shashidhar H E, Zhuang J Y, Zheng K L, Liu G F, Wang G C, Sindhu J S, Srivantaneeyakul S, Singh V P Bagali P G, Prasanna H C, McLaren G and Khush G S 2003 Identification of QTL for growth and grain related traits in rice across nine locations of Asia. *Theoretical and Applied Genetics*, 107: 679-690.

Huang N, McCouch S R, Mew T, Parco A and Guiderdoni E 1994 Development of an RFLP map from a doubled haploid population in rice. *Rice Genetics Newsletter*, 11: 134-137.

Ogiso-Tanaka E, Matsubara K, Yamamoto S-i, Nonoue Y, Wu J, Fujisawa H, Ishikubo H, Tanaka T, Ando T, Matsumoto T and Yano M 2013 Natural variation in RICE FLOWERING LOCUS T 1 contributes to flowering time divergence in rice. *PLoS ONE*, 8(10): e75959.

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