Research Journal of Biology
Volume 1: 24 – 30 (Published: 16 June, 2013)
ISSN: 2322-0066

RESEARCH ARTICLE

Molecular mapping of early vigour related QTLs in rice

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ABSTRACT

Upland rice plays a major role in the sustainable food security of the country. Early vigour trait is important in the drill-sown rice for weed suppression as well as initial establishment. The IR64/Azucena was used as a reference population to identify Quantitative Trait Loci (QTLs) for early vigour related traits. The Simple Sequence Repeat (SSR) markers flanking QTLs in IR64/Azucena and other populations were used for validation in the new populations (BPT5204/A67 and BPT5204/Dodiga). Twenty-two QTLs distributed on chromosomes 1 to 6 were identified for different vigour related traits. The QTLs controlling germination were located on chromosome 1 in the region RM5-RM306 near to alpha-amylase genes which controls germination. The congruence of QTLs was observed on Chromosome 5 in the region RM87-RM334 for rate of germination, seedling dry weight and vigour index. RM253 (Chromosome 6) was associated with the shoot length at 10th and 14th day after sowing (DAS) with 9.6% and 10.1% contribution in BPT5204/A67. RM178 (Chromosome 5) was associated with shoot length (18%) and root length (11.2%) at 10th DAS in BPT5204/Dodiga.

Key Words: Early vigour, IR64/Azucena, QTLs, rice, shoot length.
(Received: 29/05/2013; Accepted: 11/06/2013; Published: 16/06/2013)

INTRODUCTION

Early vigour is an important trait which governs initial crop establishment and favours weed suppression in upland rice system. It is a complex trait and found to be associated with germination and seedling growth (Perry, 1972). Genetic improvement of genotypes for early vigour is a better option to control weeds than hand weeding or use of herbicide due to economic and ecological considerations. Breeding for early seedling vigour via conventional strategies has not been very successful for a long time because of its complex inheritance and quantitative nature (Li and Rutger, 1980; Redona and Mackill, 1996a)

DNA marker and genome mapping techniques are powerful tools for genetic analysis of quantitative traits loci (QTLs) controlling complex traits (Lander and Botstein 1989; Tanksley, 1993) and have been extensively used for genetic dissection of agriculturally important traits in rice (Xio *et al.*,1996; Li *et al.*, 1997; Yano *et al.*, 1997; Yu *et al.*, 1997). The QTL mapping using abundant and codominant marker like Simple Sequence Repeats (SSR) will be useful in determining number, location and effects of loci controlling early vigour related traits. A few efforts have mapped QTLs governing seedling vigour in rice (Redoña and Mackill 1996b; Cui *et al.*, 2002; Zhang *et al.*, 2005a). These studies used populations derived from parents such as Labelle/Black Gora, Zhenshan 97/Minghui 63 and Lemont/Teqing. However, there have been no reports on validation of the already identified markers. Results of QTL mapping are largely dependent on mapping populations, i.e., only those genes segregating in the mapping populations could be identified. Hence there is a need to identify many other QTLs for early vigour using diverse mapping populations. This would not only advance our understanding of the genetic diversity for seedling vigour in rice, but also favour pyramiding of more favourable alleles by marker-assisted selection (MAS) in molecular breeding programs.

Constructing a molecular map for an entirely new population is time-consuming and resource intensive. Therefore, detecting seedling vigour associated markers in a reference population and validating them in a new population would be a better approach. This exercise would also reflect the conserved functional genomic regions across genotypes. In the present study, IR64/Azucena double haploid population (DH), which is nearly saturated with molecular markers, was used to detect the chromosomal regions associated with early seedling vigour related traits. Markers thus identified, apart from other reported ones were validated in F_2:F_3 populations generated between a low vigour and good quality variety, BPT5204, and high vigour, medium quality and upland landraces, Dodiga and A67 *viz*., BPT5204/Dodiga and BPT5204/A67 populations.

MATERIALS AND METHODS

The experiment was carried out in the Department of Genetics and Plant Breeding, College of Agriculture, Dharwad, University of Agricultural Sciences, Dharwad (India) during 2002-2005.

Plant material and phenotyping experiments for QTL detection:

One hundred and twenty one lines of IR64/Azucena double haploid (DH) population originally developed by Guiderdoni *et al.* (1992) and later maintained at Barwale Foundation (formerly known as Mahyco Research Foundation), Hyderabad were used. The matrix of the genotypic data of the 269 SSR markers spanning all 12 chromosomes of rice was downloaded from www.gramene.org.

Seeds were treated with dry heat at 50°C for four days to eliminate possible dormancy. For germination, seeds were kept in rolled paper towels at ambient temperature (25° ± 3°C) in dark. Seeds germinated by 48 and 72h in the standard

germination test were counted and expressed as percentage in first and second count, respectively. Rate of germination was calculated as the ratio of the first count to the final count of germination and expressed as percentage as follows:

Rate of germination (%) = [(First count of germination / Final count of germination)] x 100

Five normal seedlings were randomly selected from each replication of the standard germination test on the 14^{th} day and shoot length (SL) was measured from collar region to the tip of the top most leaf and expressed (cm). Root length (RL) was measured on 14th day from collar region down to the tip of longest root. The seedlings used for measuring root and shoot lengths on 14th day were dried in hot air oven to constant weight. The total weight (mg) of seedlings divided by number of seedlings gave average weight of each seedling.

Vigour Index (VI) = Rate of germination (%) x Seedling dry weight (mg/seedling)

Plant material, genotyping and phenotyping for marker validation:

The populations were generated by effecting crosses between BPT5204 as female parent and Dodiga and A67 as male parents. The F_1s were selfed to obtain F_2 seeds, which were space planted to obtain F_3 seeds. DNA was extracted from parents, F_1 and F_2 plants for SSR analysis. Poly Acrylamide Gel Electrophoresis (PAGE) for chosen markers was carried out to assess polymorphism in parents, heterozygosity in F_1 and segregation in F_2. The molecular analysis was carried out as described in the manual (Mahyco Research Foundation, 2003). Forty two chosen markers from different studies as well as present study on IR64/Azucena were analyzed for parental polymorphism in BPT5204, Dodiga and A67 genotypes. The polymorphic markers were screened in ten high vigour and low vigour F_2 individuals to know initial association. Only those markers showing initial association were used to screen more number of F_2 segregants. The markers were assessed for segregation and goodness of fit was tested for 1:2:1 ratio using chi-square test in both the populations. F_3 families were analyzed for early vigour related traits i.e., root (RL) and shoot lengths (SL) in two replications on 10th (RL10 and SL10) and 14^{th} (RL14 and SL14) day after sowing (DAS). The measurements on ten seedlings were taken and expressed as family means.

Simple interval marker analysis:

This was carried out by MAPMAKER program. The linkage analysis and map construction was done using Mapmaker/Exp, while chromosomal location of putative QTLs was determined by using Mapmaker/QTL program (Lander *et al.*, 1987; Lincoln *et al.*, 1992). The phenotypes of DH lines were superimposed on already available genotypes to identify QTLs. The proportion of trait variation accounted for by each QTL was also estimated.

Single Marker Analysis:

The genotype of F_2 plants and phenotype of corresponding F_3 family were used to know association between markers and the trait. The analysis was done using IRRISTAT program. The percent phenotypic variance explained by association was calculated using linear regression approach.

RESULTS

Identification of QTLs:

QTLs with flanking SSR markers were identified through interval mapping using MAPMAKER program at a threshold LOD score of 2.5 for all the traits. The QTLs associated with six vigour-related traits along with percent phenotypic variance; LOD score and additive effect are presented in table 1. The chromosomal position of QTLs is depicted in figure 1. As many as 22 QTLs were identified for different vigour related traits and they were distributed on chromosomes 1 to 6.

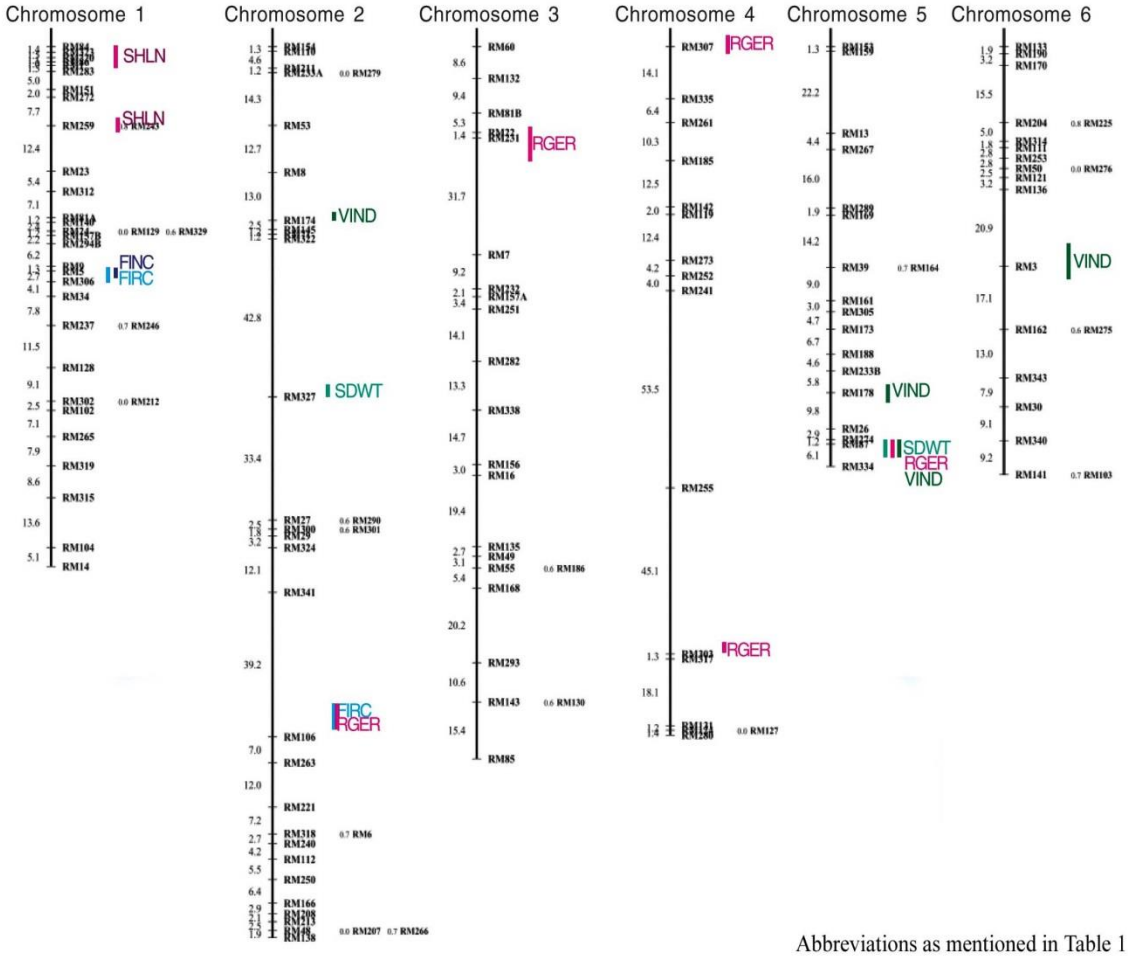
Table 1. QTLs associated with early vigour related traits in IR64/Azucena- DH population identified by interval mapping (MAPMAKER/QTL v. 1. 1)

Trait	Marker Interval	Chr. No.	Peak LOD	Phenotypic Variance (%)	QTL position	Distance between the markers (cM)	Additive effect	No. of QTLs
SHLN	RM 84- RM323	1	2.8	13.1	0.0	01.5	-1.08	6
	RM323- RM220	1	2.8	13.3	0.0	01.6	-1.12	
	RM220-RM86	1	2.6	12.2	0.0	01.1	-1.11	
	RM272-RM259	1	2.7	13.7	8.0	08.3	-1.29	
	RM259-RM243	1	2.7	13.7	0.0	00.8	-1.30	
	RM243-RM23	1	3.1	15.1	0.0	01.3	-1.35	
SDWT	RM327-RM27	2	2.5	40.2 (MQ)	0.0	43.9	2.97	3
	RM153-RM159	5	2.6	13.8	0.0	01.3	-1.13	
	RM87-RM334	5	2.9	17.1	0.0	06.5	-1.79	
FIRC	RM5-RM306	1	2.6	12.3	0.0	02.8	0.02	2
	RM341-RM106	2	2.8	49.4 (MQ)	36.0	53.3	-0.05	
FINC	RM5-RM306	1	2.5	10.5	0.0	02.8	0.01	1

RGER	RM341-RM106	2	4.5	56.2 (MG)	28.0	53.3	-8.74	5
	RM231-RM7	3	2.6	47.8 (MQ)	16.0	41.1	-9.20	
	RM307-RM335	4	3.1	32.4 (MQ)	0.0	16.1	-10.35	
	RM303-RM317	4	2.5	25.5 (MQ)	0.0	01.3	-7.99	
	RM87-RM334	5	2.5	28.0 (MQ)	6.0	06.5	-8.98	
VI	RM174-RM145	2	2.5	16.3	0.0	02.6	-191.40	5
	RM178-RM26	5	2.6	18.2	4.0	10.7	-196.60	
	RM87-RM334	5	2.5	16.0	0.0	06.5	-196.20	
	RM3-RM162	6	4.4	39.2 (MQ)	8.0	20.0	356.03	
	RM136-RM3	6	3.6	28.5 (MQ)	24.0	25.1	335.30	

SHLN, Shoot length; SDWT, Seedling dry weight; FIRC, First count of germination; FINC, Final count of germination; RGER, Rate of germination; VI, Vigour Index. Bold letters indicate common genomic regions controlling different traits; MQ, Major QTL (25-50% phenotypic variance); MG, Major gene (> 50% phenotypic variance).

Fig 1. QTL mapping for early vigour related traits in IR64/Azucena population



Germination:

Totally eight QTLs were identified for germination parameters *viz*., first count (2), final count (1) and rate (5) of germination. The QTLs controlling first and final counts of germination were located on chromosome 1 in the region (RM5-RM306) near to marker RM5 with phenotypic variance explained is 12.3% and 10.5% respectively. The RM341-RM106 region on chromosome 2 is common for the first count and rate of germination with 49.4% (first count of germination) and 56.2% (rate of germination) contribution towards phenotypic variation. This QTL is found to be a major determinant of seed germination. Other QTLs for the rate of germination were present on chromosome 3 (RM231-RM7), chromosome 4 (RM307-RM335, RM303-RM317) and chromosome 5 (RM87-RM334).

Morphological traits:

Six QTLs contributing for variation in shoot length were located in the region RM84-RM23 on the long arm of chromosome 1 and all were found to be minor QTLs as they contributed only 10-15% of the phenotypic variance. Seedling dry weight was found to be controlled by three QTLs. A single QTL (RM327-RM27) on chromosome 2 contributed highest phenotypic variance of 40.2%. Two QTLs on chromosome 5 (RM153-RM159, RM87-RM334) contributed 13.8% and 17.1% to phenotypic variance, respectively. Vigour index was conditioned by five QTLs distributed on chromosome 2

(RM174-RM145), 5 (RM178-RM26, RM87-RM334) and 6 (RM3-RM162, RM136-RM3). The major QTLs (39.2%, 28.5%) on chromosome 6 and minor QTLs (16.0-18.2%) on chromosome 2 and 5 were identified.

The congruence of QTLs was observed on chromosome 5 in the region RM87-RM334 for three traits, *viz*., rate of germination, seedling dry weight and vigour index.

Validation of markers:

The two new F_2:F_3 populations *viz*., BPT5204/A67 and BPT5204/Dodiga were used for validation of markers. Segregation of two markers RM253 (chr.5) and RM220 (chr.1) in the cross between BPT5204/A67 showed 1:2:1 ratio as expected (table 2). Similarly, when segregation of two markers RM178 (chr. 5) and RM163 (chr. 5) in BPT5204/ Dodiga was tested, only RM178 showed the expected 1:2:1 ratio, while segregation of RM163 did not fit 1:2:1 ratio (table 3).

Table 2. Goodness of fit to 1:2:1 marker segregation in F_2 population of the cross BPT5204/ A67

Marker	**No. of individuals**	**Group**	**Observed**	**Chi square for $1P_1$:2H:$1P_2$**	**Probability**
RM 220	142	GP_1 GH GP_2	33 68 41	1.154	0.50-0.75
RM 253	132	GP_1 GH GP_2	29 69 34	0.651	0.50-0.75

GP_1, Group of F_2 showing BPT5204 banding; GH, Group of F_2 showing Heterozygous banding; GP_2, Group of F_2 showing A67 banding.

Table 3. Goodness of fit to 1:2:1 marker segregation in F_2 population of the cross BPT5204/ Dodiga

Marker	**No. of individuals**	**Group**	**Observed**	**Chi square for $1P_1$:2H:$1P_2$**	**Probability**
RM 178	117	GP_1 GH GP_2	29 61 27	0.282	0.75-0.90
RM 163	131	GP_1 GH GP_2	49 54 28	10.77	0.005

GP_1, Group of F_2 showing BPT 5204 banding; GH, Group of F_2 showing Heterozygous banding; GP_2, Group of F_2 showing Dodiga banding.

The association between F_2 genotype (by SSR markers) and their corresponding F_3 phenotype was studied by single marker analysis (SMA) using IRRISTAT program. The phenotypic data for root and shoot lengths of 109 F_3 families and corresponding marker data of F_2 individuals of BPT5204/A67 was used for analysis (table 4). The comparison of means for the shoot and root lengths at different stages between three groups (two homozygotes and one heterozygote) revealed that the shoot length was associated with the marker RM253 at both stages (0.014 and 0.01). The phenotypic variance explained was 9.6% (SL10) and 10.1% (SL14), respectively.

Table 4. Association between marker and early vigour related traits in F_2:F_3 of BPT5204/ A67 cross

Characters	Group	Marker			
		RM253		RM220	
		Mean	Probability	Mean	Probability
RL10	GP_1 GH GP_2	11.6790 11.5436 11.8933	0.857	11.6700 11.2040 12.0787	0.056
SL10	GP_1 GH GP_2	10.7797 12.6260 12.5550	0.014* (9.6%)	11.5867 12.1433 12.5070	0.655
RL14	GP_1 GH GP_2	13.3758 12.9096 13.7571	0.394	13.3262 12.7423 13.6163	0.082
SL14	GP_1 GH GP_2	13.0050 14.0453 14.7133	0.01** (10.1%)	13.6827 13.8202 14.3687	0.541

GP_1, Group of F_2 showing BPT5204- Low Vigour banding; GH, Group of F_2 showing heterozygous banding; GP_2, Group of F_2 showing A 67 - High Vigour banding;
* & ** Significant at 5% and 1% level of probability
Values in parenthesis indicates coefficient of determination (Phenotypic variance)

In BPT5204/Dodiga, the phenotypic data of 75 F_3 families and corresponding marker data of F_2 individuals was used for assessing association between RM178 and RM163 markers on chromosome 5 with root and shoot lengths at both the stages (10^{th} and 14^{th} DAS) in BPT5204/Dodiga (table 5). The comparison of means for the shoot and root lengths at different stages between three groups (two homozygotes and an heterozygote) revealed the association of RM178 with root length at 10^{th} DAS (0.038) and shoot length at 10^{th} (0.003) and 14^{th} (0.029) DAS. Its contribution to phenotypic variance as measured by R^2 value for shoot length was 18% (SL10) and 12.1% (SL14), respectively. The phenotypic variance was found to be 11.2% for root length at 10^{th} DAS.

Table 5. Association between marker and early vigor related traits in F_2:F_3 of BPT5204/ Dodiga cross

Characters	Group	Marker			
		RM178		RM163	
		Mean	Probability	Mean	Probability
RL10	GP_1	10.35	0.038*	11.83	0.815
	GH	11.67	(11.2%)	11.24	
	GP_2	11.41		11.78	
SL10	GP_1	8.08	0.003**	9.16	0.654
	GH	9.24	(18.0%)	9.31	
	GP_2	9.89		9.85	
RL14	GP_1	14.14	0.096	15.38	0.816
	GH	15.55		14.88	
	GP_2	14.72		15.33	
SL14	GP_1	12.64	0.029*	13.31	0.46
	GH	13.73	(12.1%)	13.87	
	GP_2	14.26		14.39	

GP_1, Group of F_2 showing BPT5204- Low Vigour banding; GH, Group of F_2 showing Heterozygous banding; GP_2, Group of F_2 showing Dodiga - High Vigour banding.
* & ** Significant at 5% and 1% level of probability
Values in parenthesis indicate coefficient of determination (Phenotypic variance)

DISCUSSION

QTL Mapping:

In the present study apart from identification of QTLs, additive weight for each QTL was also computed to know the effect of replacement of a female allele (IR64) by a male (Azucena) allele. The additive effect could be either positive or negative. A positive value indicates increase in effect, i.e., Azucena has positive alleles, while negative value indicates decrease in effect, i.e., IR64 has positive alleles.

The use of linked markers in QTL mapping compensates for recombination between the markers and the QTL and it is considered to be statistically more powerful (Yadav *et al.*, 1997). The QTLs for first and final counts of germination were located on chromosome 1 in the region (RM5-RM306). Interestingly, the alpha-amylase gene *amy1B/A* influencing germination is known to be at 13 cM away from RM5 towards centromere on the long arm of chromosome 1 (Temnykh *et al.*, 2001). The additive effect for the locus RM5-RM306 was positive indicating contribution of positive allele by the japonica variety Azucena. Thomson *et al.* (2003) reported QTL controlling germination in the same region in cross *Oryza rufipogon* and US japonica cultivar *Jefferson* and the positive allele was contributed by Jefferson.

High correlation (0.82) between the first count and rate of germination compared to final count and rate of germination (0.33) also reveals greater contribution of first count towards rate of germination, which may be due to shared QTL. The gene *amy1A/C* contributing to germination is also located on the same arm (long arm) (Temnykh *et al.*, 2001). The QTLs for rate of germination were present on chromosomes 3, 4 and 5 in the present study. Zhang *et al*., (2005b) also reported presence of QTLs for germination on chromosome 4, 5 and 6 in Lemont/Teqing population. All the loci, controlling rate of germination had negative additive effect indicating the contribution of positive alleles by IR64. The female parent IR64 was also found superior for rate of germination compared to Azucena.

The QTL (RM5-RM306) for germination was mapped in the present study were also reported by Temnykh *et al.*, (2001) and Thompson *et al.*, (2003) indicating conservation of genomic regions across genotypes. It indicates that some of the QTLs or genomic regions controlling seedling vigour in rice were commonly detected across very different populations but others were largely dependent on the populations used in the QTL mapping as mentioned by Zhang *et al.*, (2005b).

The six QTLs for shoot length on chromosome 1 with phenotypic variance 10-15 % indicates the trait is controlled by many loci (Li and Rutger, 1980; Redona and Mackill, 1996 a & b) harboring on various chromosomes (Redona and Mackill, 1996b; Zhang *et al.*, 2005b). The additive weight of these QTLs was negative indicating IR64 had positive alleles for shoot length. Total seedling dry weight QTL (RM26-C1447) on chromosome 5 in Zhenshan97/Minghui 63 studied by Cui *et al*., (2002) was located close to RM87-RM334. Main and epistatic QTLs on chromosome 5 for dry matter weight have also been reported by Zhang *et al*. (2005b) in Lemont/Teqing population. The region RM178-RM26 on chromosome 5 falls within RZ70-RZ225 based on IR64/Azucena linkage map (McCouch *et al.*, 2001). The seedling vigour QTL (RZ70-RZ225) reported by Prasad *et al*., (2000) is in agreement with our study. The congruence of QTLs was observed on chromosome 5 in the region RM87-RM334 for three traits, *viz*., rate of germination, seedling dry weight and vigour index may be due to either linkage or pleiotropy. Multiple QTLs controlling seedling vigour on chromosome 5 was reported by (Zhang *et al.*, 2005b).

The additive effect of all the three QTLs at this locus was negative due to the replacement of IR64 alleles by Azucena indicating IR64 has positive alleles. The plural selection efficiency can be increased by selecting markers closely associated with these traits. The vigour index is significantly correlated with rate of germination (r = 0.49**) and seedling dry weight (r = 0.80**). RM87-RM334 region on chromosome 5 was common for vigour index, rate of germination and seedling dry weight. This is in consistent with the earlier observation that phenotypically correlated traits often tend to map together (Abler *et al.*, 1991; Paterson *et al.*, 1991; Shashidhar *et al.*, 1999; Hittalamani *et al.*, 2002).

Molecular marker technology is capable of identifying close relationships and discerning between pleiotropy and tight linkage or overlapping genes. In a given genotype, genes with both positive and negative control would regulate expression of any quantitative trait. Plant breeding is directed towards accumulating favourable genes/alleles for traits by exerting selection. Identifying the undesirable/desirable alleles at different loci is therefore important. Molecular marker technology is potential enough to provide such information, with which selection for desirable alleles at genotype or molecular level can efficiently be exercised.

Validation of markers in new populations:

The markers RM253, RM220, RM178 showed expected 1:2:1segregation, while RM163 showed segregation distortion. Similar observations were also noted earlier by Redona and Mackill, 1996b. The marker RM253 is associated with shoot length in BPT5204/ A67 population. The phenotypic variance explained was 9.6% (SL10) and 10.1% (SL14), respectively. Similarly, this marker was in the vicinity of qSV-6 (C76-RM253) controlling epistatic QTLs for germination rate and shoot length (Zhang et al., 2005b) in Lemont/Teqing population. Shoot length trait is controlled by many QTLs with small contribution from each (Li and Rutger, 1980; Redona and Mackill, 1996 a & b; Zhang *et al.*, 2005b). The shoot length QTLs (5) in IR64/Azucena explained 10-15% of phenotypic variation. In IR64/Azucena (current study) major QTL (RM136-RM3) controlling vigour index was found to be located close to this region. RM220 marker was associated neither with root nor shoot length in BPT5204/A67 population, even though initial screening of marker with ten high and low seedling vigour F_2 plants showed possible association.

The QTL (RM178-RM26) on chromosome 5 was associated with vigour index in IR64/Azucena (current study) and multiple QTLs controlling seedling dry weight, rate of germination and vigour index were also located in the nearby region (RM87-RM334). Cui *et al.* (2002) reported QTLs controlling seedling vigour related traits (germination rate, root dry weight and root activity) in the C246-RM26 region and root length QTL in RM26-C1447 region in Zhenshan 97/Minghui 63 population. The root length QTL in that study also explained variance ranging from 11.4-15%. Significant correlation (0.42) between RL10 and SL10 in BPT5204/Dodiga population could be due to the association of this marker with both the traits. Even though RM163 marker was located on the same chromosome, it was not associated with either root or shoot lengths, which may be due to segregation distortion of the marker.

The information obtained on the new populations (BPT5204/A67 and BPT5204/Dodiga) with respect to markers is limited. About 50% of the 42 markers selected based on other populations showed polymorphism in the new populations and only four exhibited putative association with early vigour trait. Among them only two markers, one each in a population, were useful for identifying early vigour QTLs. Intensive studies (Redona and Mackill, 1996 a & b, Cui *et al.*, 2002; Zhang *et al.*, 2005b) along with the current study on early vigour reveal the existence of many QTLs (20-32) in different populations. Seedling vigour being a complex trait, early generation selection has been ended up with limited success. Therefore, identification of many and common QTLs across genetic background, followed by validation of associated markers to identify active genomic regions controlling early vigour is important for marker-assisted selection to be successful.

ACKNOWLEDGEMENT

We acknowledge financial support from Council of Scientific and Industrial Research (CSIR), New Delhi, India and Mr. Dinesh Joshi, Executive Director, Barwale Foundation, Hyderabad for providing laboratory facility for research work.

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