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QTL mapping for relative water content trait at reproductive stage drought stress in rice

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Abstract

Drought is the main constraint for rice production in rain-fed ecologies. Low relative water content (RWC) at reproductive stage is the most important limiting factor for the reduction of yield and its parameters. Identifying QTLs linked to RWC at reproductive stage can provide more opportunity to the researchers in maker-assisted breeding approach. For QTL associated to RWC in rice, two contrasting parents CR 143-2-2 and Krishnahamsa for drought tolerance were utilized for the development of recombinant inbred lines (RILs). Significant variation for RWC was observed among the RILs and parental lines in both the years of study. Bulked segregant analysis was utilized for genotyping of the mapping population. Two hundred and one SSR markers were selected and tested for parental polymorphic analysis from which 77 polymorphic markers were used for genotyping the RILs. Inclusive composite interval mapping detected, *qRWC*$_{9.1}$ with LOD value of 4.27 and phenotypic variation (PVE%) of 60.87 within the marker interval of RM316-RM257 on chromosome 9. Consistent effect of this RWC QTL for drought tolerance traits was detected in both the years with the same marker interval. Hence, this QTL may be useful for marker-assisted improvement at reproductive stage drought tolerance in rice.

Key words: Drought, molecular mapping, relative water content, novel QTL

Introduction

Rice (*Oryza sativa* L.), is one of the major staple cored crops in the world producing 758.9 mt of paddy from 162 mha area (FAO, 2017). Rice is consumed worldwide and the major food source of south and south-east Asian countries. Drought is the major environmental factor that has adverse effect and responsible for drastic crop loss. Low water availability and erratic rain fall pattern are the main causes for drought in rain-fed ecologies. As water resources for agronomic use is limited, it's a challenge for the researchers to develop new varieties which have tolerance to drought. Various agronomic, morphological and physiological traits affect rice plant growth and its improvement on exposure to moisture stress. Not only the performance of yield and its component traits but also some secondary adaptive traits play an important role in drought tolerance. The progress in identifying such genes/QTLs for the secondary physiological traits is slow due to the poor understanding of its role in under stress environment.

Recent studies indicate that direct selection of yield *per se* and its component traits has been studied well by many researchers but there are so many integrative and secondary traits are responsible for drought stress tolerance mechanism in rice (Kamoshita et al. 2008). Due to poor availability of data on the physiological traits and their interaction, it's important to find out the genes/QTLs comtrolling their traits and their mapping. These findings might provide an ample opportunity for the researchers regarding these novel genes/QTLs and their interaction with the integrative or secondary traits. Confirmation of the identified QTLs with respect to their physiological traits under drought stress will be useful for selection of donor(s) and to point our molecular breading strategies. Under this investigation, leaf relative water content is an important parameter to be considered in water limiting situation of rice plant growth. Plant water status can differ significantly among cultivars in a same period of water exclusion resulting changes in leaf rolling, leaf drying and leaf water potential (O'Toole and Moya 1978). In rice, these

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differences lead to variations in stomatal conductance for transpiration in drought stress conditions (Dingkuhn et al. 1989a). Yield reduction due to water deficit has strongly influenced physiological traits like leaf water potential, RWC and cell membrane stability (Courtosis et al. 2000). The RWC is one of the useful methods to measure tissue water status (Sinclare and Ludlow 1985). However, RWC is closely related to leaf water potential. A significant correlation among leaf rolling and leaf water content was reported with existence of genotypic differences (Dingkuhn et al. 1989b).

The genetic improvements of rice in water limiting conditions are much slower due to environmental effects (Evenson and Gollin 2003) and lack of efficient techniques for screening (Khush 2001), whereas, phenotypic selections for drought tolerance contributing genes is also a difficult and labour intensive process. Therefore, it is necessary for researchers to develop molecular markers controlling different drought related traits for easy identification in a short time (Nguyen et al. 1997). When a QTL is identified for a particular trait, it can be used for improving that particular trait in breeding program using marker-assisted selection (MAS). QTLs for several drought tolerance traits have been mapped in rice (Li and Xu 2007; Kamoshita et al. 2008). To identify the molecular markers for gene/ QTL of interest, different strategies like selective genotyping (Sun et al. 2010; Navabi et al. 2009) and bulked segregant analysis (BSA) (Michelmore et al. 1991) were adapted. Previously, BSA has been adapted to tag genes controlling qualitative trait, whereas now it can be used to analyse more complex traits like drought tolerance. Mapping of genes for drought stress using BSA approach has already been applied in crops like barley (Altinkut et al. 2003), wheat (Altinkut and Gozukirmizi 2003) and maize (Quarrie et al. 1999). Also linked markers for yield under drought in rice have been identified earlier using BSA (Venuprasad et al. 2009). The present investigation was taken up with the objective of mapping QTLs for relative water content under reproductive stage drought stress using recombinant inbred lines (RILs) in a managed stress environment (MSE).

Material and methods

Plant materials

One hundred and ninety recombinant inbred lines (RILs) along with parents CR 143-2-2 (tolerant) Krishnahamsa (susceptible) were taken for the study. For the mapping study, RILs were phenotyped for RWC under rain-out shelter (ROS) of ICAR-National Rice Research Institute, Cuttack, Odisha during wet seasons, 2014 and 2015. The tolerant parent, CR143-2-2 is an early upland breeding line possessing high reproductive stage drought tolerance. The susceptible parent Krishnahansa is an irrigated rice variety, usually grown in dry season, a high yielding and popular cultivar of Andhra Pradesh, India. Hybridization and subsequent generation advancement till F_{7-8} RILs development was followed as per single seed descent method.

Phenotyping for relative water content

Field experiment was taken up in alpha lattice design with two replications. All the RILs were arranged in six blocks accommodating 34 entries per block. Both the parents were also included in the total entries. The two parents CR143-2-2 and Krishnahamsa along with 190 RILs were phenotyped for RWC under reproductive stage drought stress. To measure RWC the leaves at extreme day condition at about 12 noon were collected and stored in separate plastic bags. Instant fresh weight of these leaves was taken by a standardized weighing balance (Sartorius) and then dipped into water in separate petri dishes for 24h aseptically. Turgid weight was calculated by weighing the wet leaves. Finally these particular leaves were packed and exposed to heat in an oven at about 45^0C for 3-4 days for estimation of dry weight. Per cent RWC for different recombinant lines along with their respective parents was estimated by using the standard formula (Schonfeld et al. 1988).

$$[(\text{Fresh weight}) - (\text{Dry weight})/(\text{Turgid weight}) - (\text{Dry weight})] \times 100$$

DNA extraction and polymerase chain reaction

Recombinant inbred lines along with the parents were grown in RGA-cum-Phytotron facility chamber. About 400mg of fresh leaf samples were collected from 20-25 days seedlings and the DNA was extracted as (Murray and Thomson 1980). One percent agarose gel and spectrophoto neither was used to test quality and quantity of the DNA. Each samplewas further diluted to a specific uniform concentration of approximately 30ng/μL. Polymerase chain reaction was carried out in thermal cycler using selected SSR primers. A specific thermal profile considered with initial denaturation at 94°C for 4 min, 35 cycles of denaturation at 94°C for 30s, primer annealing at 55°C for 1 min and extension at 72°C for 1.30 min; final extension at 72°C for 10 min. Amplified products were analyzed by electrophoresis in 3.5% agarose gel and

visualized by using gel documentation unit. Bulked segregant analysis was used as per Wang et al. (1994).

Statistical analyses

To determine the co-efficient of variation and least square deviation for RWC estimates of 190 RILs and their parents, two years data were considered (wet season, 2014 and 2015). Cropstat v7.2 software (IRRI, 2007) was used for the data analysis. Frequency distribution normal curve, skewness and kurtosis were calculated using SPSS software (Version 20.0, Chicago, USA). Phenotypic covariance (PC), genotypic covariance (GC), environmental covariance (EC), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and heritability (broad-sense) among the recombinant lines were calculated using software SPAR v2.0 (http:// iasri.res.in/spar).

Genetic mapping, QTL and epistatic analysis

Composite interval mapping (CIM) was used to detect the QTL-marker linkage by using the software Inclusive composite interval mapping v4.0 (ICIM v4.0) (Wang et al. 2014). Other than composite interval mapping (CIM), single marker analysis (SMA), additive mapping and epistatic mapping (EPI) were used to calculate the association of phenotypic and molecular proportions for the development of linkage map. The walking speed along chromosomes for all QTLs was 1.0cM and threshold value of LOD 3.0 with 1000 permutation at $P<0.05$ was considered for mapping. The QTLs were named according to the nomenclature guidelines given by McCouch et al. (1997). QTLs with epistatic effect were estimated by QTL ICI-Mapping v4.0 (Wang et al. 2014). Selection of ICIM-EPI with a probability value (PIN) of 0.01 and threshold LOD of 5.0 was considered in the analysis.

Results and discussion

Phenotyping for relative water content of RILs under reproductive stage drought stress

A wide variation for RWC content was observed among the recombinant inbred lines and the parents (Table 1). At optimum stress level, CR143-2-2 showed high level of (90.57%) RWC, whereas Krishnahamsa had relatively low value of 56.78%. Experiment conducted by Dingkuhn et al. (1989b) in aerobic rice suggested that grain yield had a direct impact on RWC in mild stress situation. Balance between water supply through roots and transpiration rate measured in flag leaf directly influence the plant relative water content status (Sinclair and Ludlow1985). The RWC among the RILs varied from 12.08 to 98.16% with mean of 75.52. Significant coefficient of variation and $LSD_{5\%}$ was observed to be 5.9 and 8.74, respectively for this trait. Phenotypic coefficient of variation and genotypic coefficient of variation were found with significant values of 21.45 and 20.63, respectively for RILs and high values of genotypic covariance (242.72) and phenotypic covariance (262.34) and were recorded. A significant genetic advance of 37.37 and high heritability (broad-sense) of 0.93 was also recorded in recombinant lines. Similar variations have been reported earlier in different transgressive segregants for various morpho-physiological and plant production traits under drought stress condition (Blum et al. 1999; Babu et al. 2003; Manickavelu et al. 2006).

Table 1. Statistical parameters used in relative water content study of 190 RILs

Statistical measures	Value
Mean	75.52
Range	12.08-98.16
Standard Deviation	15.89
Variance	252.50
Skewness	0.18
Kurtosis	1.18
Coefficient of variation	5.90
$LSD_{5\%}$	8.74
Genotypic covariance	242.72
Environmental covariance	19.62
Phenotypic covariance	262.34
Phenotypic coefficient of variation	21.45
Genotypic coefficient of variation	20.63
Genetic advance	37.37
Heritability (Broad sense)	0.93
F-value	25.74

Frequency distribution

Distribution of all 190 RILs and parents in accordance to their range for RWC trait are presented in Fig. 1. Both the skewness (0.18) and kurtosis (1.18) were found to be positive and the frequency distribution histogram showed positively skewed leptokurtic distribution towards tolerant parent. Normal distribution graph of RWC for both the years (wet seasons, 2014 and 2015) are presented in Fig. 1. A similar distribution

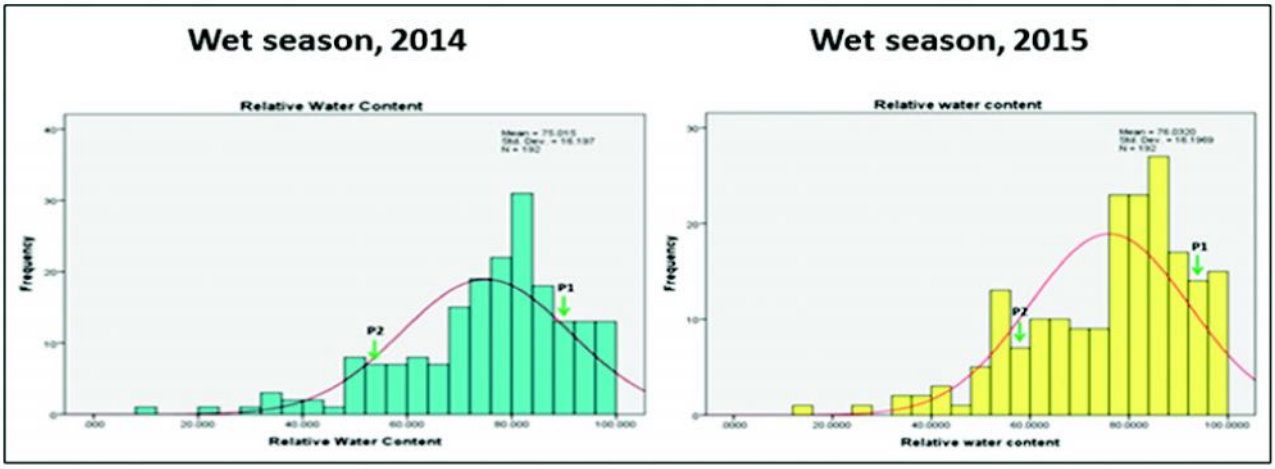

Fig. 1. Histogram showing frequency distribution for RWC in CR143-2-2/Krishnahamsa during wet seasons, 2014 and 2015

for mapping of osmotic adjustment frequency in a BIL (IR62266-42-6-2/IR60080-46A) was reported earlier in rice (Robin et al. 2003).

Molecular analysis of recombinant inbred lines

Out of 201 SSR primers used for polymorphic study, 77 primers showed polymorphism for the contrasting parents. These 77 primers were further checked for polymorphism with the extreme two bulks, high RWC bulk (B1) and low RWC bulk (B2), which showed 21 primers to be polymorphic. These 21 polymorphic SSR primers were used in genotyping of 190 RILs for development of linkage map (Table 2). These 21 primers were distributed among eight chromosomes of rice and the details of the primers are presented in Table 2. Polymorphic pattern was well studied by using these primers for all 190 RILs and parents. Representative gel electrophoresis images are presented in Fig. 2.

QTL mapping

Twenty one polymorphic SSR markers obtained from BSA genotyping were used in linkage map construction. Four markers were found in chromosome 12, whereas 3 markers each were turned linked with chromosomes 1, 2 and 10. Chromosomes 3, 6, 8 and 9 possessed 2 markers each. Following the method of inclusive composite interval mapping (ICIM) parameters like LOD threshold and probability in stepwise regression (PIN) was taken as 3.0 and 0.01, respectively. Walking speed of 1.0cM was considered for the analysis. RWC estimates and genotypic data of RILs were analysed using inclusive composite

Table 2. Microsatellite markers obtained through the polymorphic analysis between CR143-2-2 and Krishnahamsa

Chro-mosome	No. of markers analyzed	Names of the parental polymorphic markers obtained
1	25	RM6703, RM3825, RM488, RM259, RM5, RM12091, RM8085, RM495, RM5443, RM1003
2	26	RM324, RM263, RM327, RM530, RM262, RM3549, RM279, OSR17, RM250, RM341, RM13600
3	21	RM523, RM231, RM7332, RM517, RM411, RM135, RM85, RM22, RM16030, RM15780, RM104, RM571
4	13	-
5	4	-
6	15	RM3, RM276, RM527, RM528
7	7	MGR4499
8	11	RM256, RM337, RM210, RM25, RM342A, RM72
9	19	RM464, RM215, RM219, RM316, RM257, RM242, RM213
10	13	RM216, RM228, RM311, RM271, RM171, RM484
11	5	RM21
12	42	RM28199, RM28089, RM511, RM28166, RM1261, RM28048, RM28059, RM28064, RM28067, RM28070, RM28079, RM28082, RM28083, RM28088, RM28090, RM519, RM313, RM309, RM20A

Markers used in bulk segregant analysis are underlined

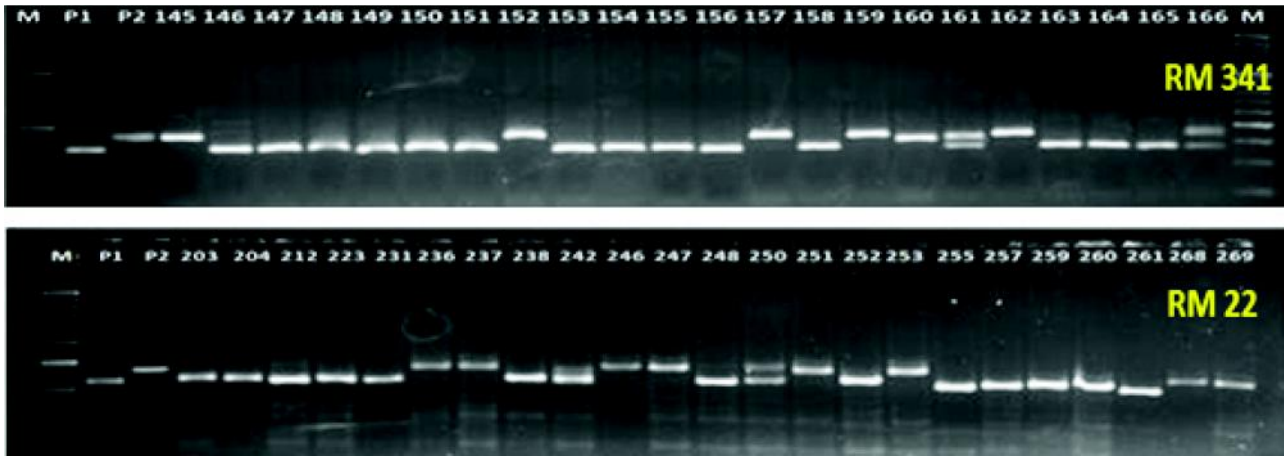

Fig. 2. Representative gel showing polymorphism pattern of SSR primers in different recombinant inbred lines. The numbers represent the different RI line numbers used in mapping. Respective primer names are given in the right top corner position in each gel photos. P1 = Tolerant parent; P2 = Susceptible parent, M = 50bp DNA ladder

interval mapping and detected a significant QTL for RWC on chromosome 9 with a LOD value of ≥ 3.0. Based on the primer location on chromosome 9, QTL for RWC (*qRWC*$_{9.1}$) was found at position of 18.8cM on the basis of wet season 2014 RWC data. A significant peak with LOD 4.13 and PVE% of 59.62 were observed in the marker interval of RM316 and RM257 (Table 3; Fig. 3). On the basis of wet season, 2015 RWC data, the QTL (*qRWC*$_{9.1}$) was again detected within the same marker interval but at a distance of 23.8cM for the trait. LOD value and PVE (%) were found to be 4.78 and 62.65, respectively in the second year (Table 3; Fig. 3). However, from the pooled data analysis, it was confirmed that the QTL significant for RWC was located on chromosome 9 with LOD value and PVE (%) of 4.27 and 60.87, respectively within the same marker interval.

Table 3. QTL identified from inclusive composite interval mapping on chromosome 9 for RWC trait

Year	Position	Left marker	Right marker	LOD	PVE (%)	Add
2014	18.8	RM316	RM257	4.13	59.62	15.14
2015	23.8	RM316	RM257	4.78	62.65	14.52
Pooled	21.8	RM316	RM257	4.27	60.87	14.56

Results representing both main effect QTL and additive effect for two years and pooled are presented in Table 4. The peak showing main effect QTL with the LOD value of 4.13 and the peak value of the additive effect as 15.14 for the QTL in wet season, 2014. Similarly, the additive effect of 14.52 and main effect LOD value of 4.78 were obtained in wet season, 2015. Pooled data from both the years represent the LOD

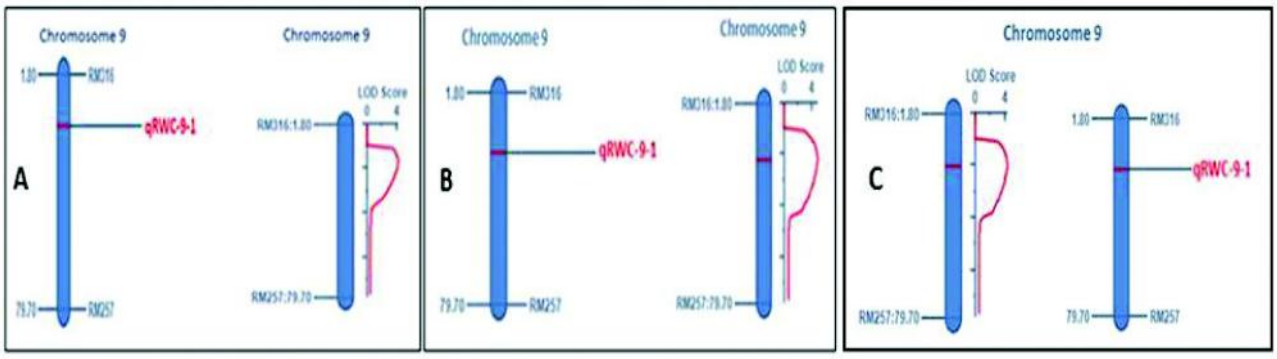

Fig. 3. Main effect QTL and LOD score of *qRWC9.1* detected in chromosome 9 on the basis of (A) RWC estimates of 2014 and (B) RWC estimates of 2015 and (C) Pooled correct is RWC but not RWC estimates for both the years

Table 4. Digenic epistatic effect of RWC from pooled data

Chrom#1	Pos1	LM1	RM1	Chrom#2	Pos2	LM2	RM2	LOD	PVE (%)	Add1	Add2	Add-by-Add
1	67.8	RM495	RM6703	8	30.1	RM337	RM72	7.98	63.1	-8.70	-6.07	-8.07
6	71.2	RM527	RM3	8	40.1	RM337	RM72	6.89	61.94	6.79	-6.40	9.86
2	112.6	RM341	RM263	8	50.1	RM337	RM72	5.56	47.98	7.82	-6.82	9.25
1	82.8	RM495	RM6703	9	46.8	RM316	RM257	5.62	58.86	6.93	6.91	-9.43
3	22.2	RM22	RM517	9	61.8	RM316	RM257	5.35	52.07	5.61	7.40	-11.50
8	45.1	RM337	RM72	9	61.8	RM316	RM257	7.35	58.20	-6.95	7.11	9.42
1	97.8	RM495	RM6703	10	74.4	RM271	RM171	5.93	60.58	-7.70	6.91	7.8
8	35.1	RM337	RM72	10	79.4	RM271	RM171	8.0	63.7	-5.96	7.69	9.22
9	46.8	RM316	RM257	10	79.4	RM271	RM171	6.33	59.89	6.87	7.82	-8.19
8	40.1	RM337	RM72	12	30	RM20A	RM511	6.54	63.0	-6.22	8.95	7.8
9	46.8	RM316	RM257	12	55	RM20A	RM511	5.25	72.37	18.97	-6.41	7.20

*****LM= left marker, RM= right marker

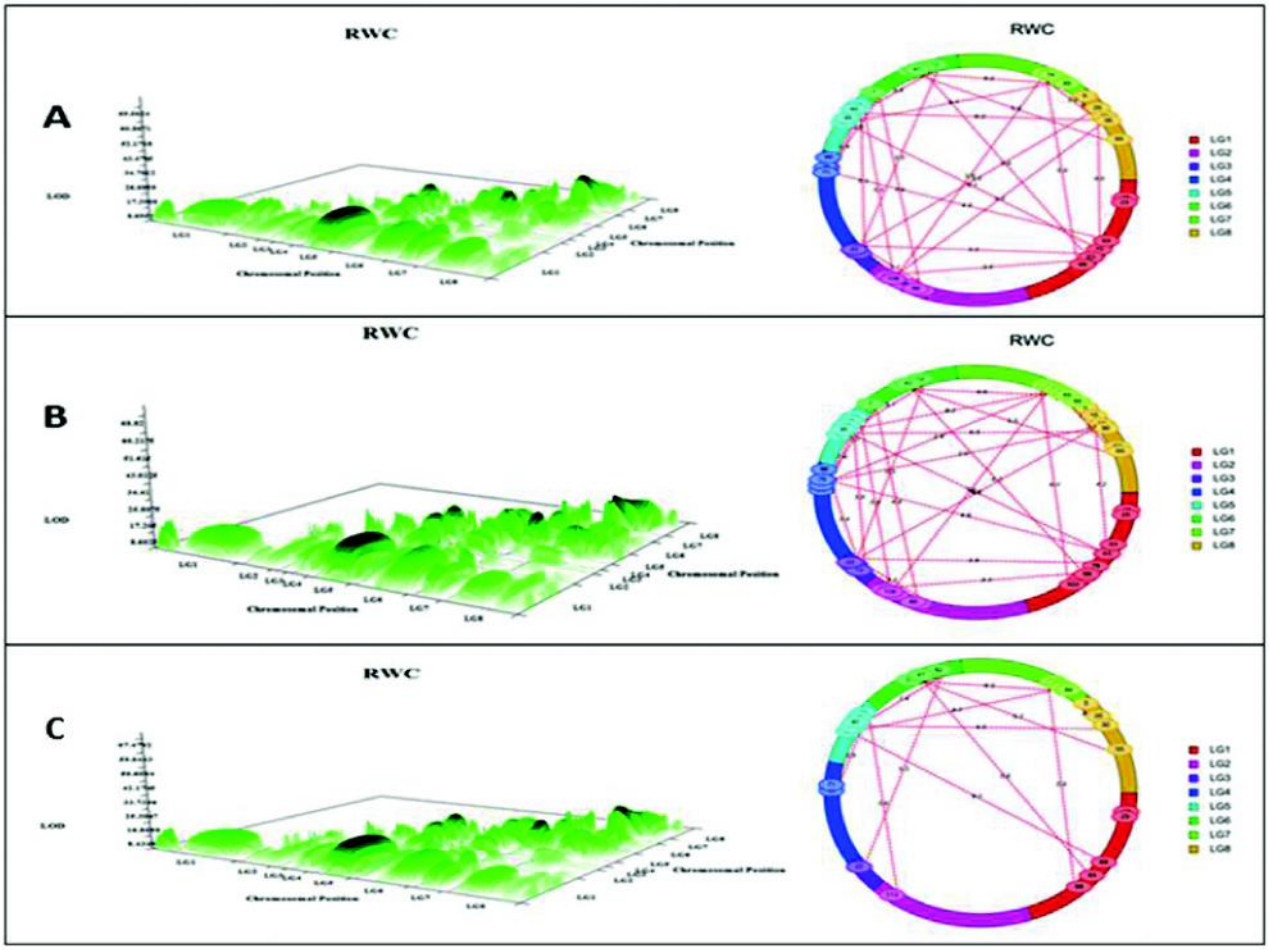

Fig. 4. Digenic epistatic effects of the QTL identified on the basis of (A) RWC estimates of 2014, (B) RWC estimates of 2015 and (C) Pooled correct is RWC but not RMC estimates for both the years showing highest LOD value and linkage association between the linkage groups

and additive effect of 4.27 and 14.56, respectively. Relative water content is an important physiological trait related to growth, development and production of rice (Ludlow 1990; Champoux et al. 1995; Ray et al. 1996). Bernier et al. (2009) revealed that DTY12.1 was responsible for the physiological trait RWC under stress and large effect of this QTL can increase the water uptake capacity of upland rice plants. Robin et al. (2003) detected 14 QTLs located on chromosomes, 1, 2, 3, 4, 5, 7, 8 and 10 with 58% phenotypic variability for water stress in leaves by using an advance backcross population (IR62266-42-6-2/IR60080-46A). Similarly, Courtois et al. (2000) found that, a marker segment of RZ730-R2801-RG810 in chromosome 1 was found to be linked with multiple traits like RWC leaf rolling, leaf drying and relative growth rate by taking 135 double haploid lines derived from IR64/Azucena cross. In present study, QTL for RWC was detected at 21.8cM within the marker interval of RM316-RM257. So far no QTL has been reported for RWC located in chromosome 9 under reproductive stage drought stress, hence the QTL, *qRWC*$_{9.1}$ identified in present study may be a novel QTL controlling RWC in rice at reproductive stage of the crop.

Epistatic effect

QTL epistatic interaction mapping for additive, additive and dominance and epistatic effects in most bi-parental populations have been well studied in QTL ICI-Mapping software (Meng et al. 2015). In the present study, QTL *qRWC*$_{9.1}$ showed significant digenic epistatic effect among the different linkage groups. Fig. 4(A) represents the 3 dimensional view of the peak obtained from the LOD values, whereas the second picture represents a total of 25 interactions detected among the interacting QTLs using the recombinants. Similarly, Fig. 4(B) represents 3D view with highest LOD and 23 interactions in recombinant lines in the year 2015 were detected. However, from the pooled data analysis shown in Fig. 4(C) presenting different LOD values and interactions in 3-dimensional graph. From the pooled data analysis, 11 digenic interactions obtained among which 3 interactions for RWC were found significant in chromosome 9 within the marker interval of RM316-RM257 with add-by-add effect of –9.43-11.5 and 9.42 (Table 4). LOD threshold in multiple environment analysis is much efficient to detect significant QTL through digenic epistatic interaction (Li et al. 2007). In present study, *qRWC*$_{9.1}$ with significant for add-by-add of –11.5, LOD value of 5.35 and PVE (%) of 52.07 was more than the threshold LOD value ($\geq$ 5.0) and observed with significant interactions in 3-dimentional digenic epistatic interaction.

Authors' contribution

Conceptualization of research (TM, SKP); Designing of the experiments (SRB, SKP); Contribution of experimental materials (SRB, SKP); Execution of field/ lab experiments and data collection (SRB, EP, PS); Analysis of data and interpretation (SRB, EP, SS); Preparation of manuscript (SKP, SRB, EP).

Declaration

The authors declare no conflict of interest.

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