Available online at www.sciencedirect.com

ScienceDirect

Rice Science, 2020, 27(2): 124−132

Research Paper

Genetic Relationship and Structure Analysis of Root Growth Angle for Improvement of Drought Avoidance in Early Mid-Early Maturing Rice Genotypes

Elssa PANDIT[1, #], Rajendra Kumar PANDA[2, #], Auromeera SAHOO[1], Dipti Ranjan PANI[3], Sharat Kumar PRADHAN[1]

([1]*Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha 753006, India;* [2]*Orissa University of Agriculture & Technology, Bhubaneswar 751003, India;* [3]*ICAR-National Bureau of Plant Genetic Resource Base Center, National Rice Research Institute, Cuttack, Odisha 753006, India;* [#]*These authors contributed equally to this work*)

Abstract: Deeper rooting 1 (*Dro1*) and Deeper rooting 2 (*Dro2*) are the QTLs that contribute considerably to root growth angle assisting in deeper rooting of rice plant. In the present study, a set of 348 genotypes were shortlisted from rice germplasm based on root angle study. Screening results of the germplasm lines under drought stress identified 25 drought tolerant donor lines based on leaf rolling, leaf drying, spikelet fertility and single plant yield. A panel containing 101 genotypes was constituted based on screening results and genotyped using *Dro1* and *Dro2* markers. Structure software categorized the genotypes into four sub-populations with different fixation index values for root growth angle. The clustering analysis and principal coordinate analysis could differentiate the genotypes with or without deeper rooting trait. The dendrogram constructed based on the molecular screening for deep rooting QTLs showed clear distinction between the rainfed upland cultivars and irrigated genotypes. Eleven genotypes, namely Dular, Tepiboro, Surjamukhi, Bamawpyan, N22, Dinorado, Karni, Kusuma, Bowdel, Lalsankari and Laxmikajal, possessed both the QTLs, whereas 67 genotypes possessed only *Dro1*. The average angle of *Dro* positive genotypes ranged from 82.7º to 89.7º. These genotypes possessing the deeper rooting QTLs can be taken as donor lines to be used in marker-assisted breeding programs.

Key words: root growth angle; *Dro1*; *Dro2*; deeper rooting; upland rice; drought avoidance

Rice is the most important food crop for more than half of the world population. The global population is increasing and expected to reach 9 billion by the middle of the twenty-first century. The targeted food production needs to be increased even from the drought-prone areas with a hike of 40% from this crippled ecosystem by 2025 (Pennisi, 2008). Drought is a major limitation in obtaining higher productivity from rainfed rice cultivation (Bernier et al, 2009; Panda et al, 2016; Barik et al, 2018). It affects the crop at vegetative and reproductive growth stages. Under water stress, genotypes showing delay in leaf rolling and faster recovery from the stress are required for drought breeding (Singh and Mackill, 1991). During vegetative stage, leaf rolling and leaf drying are good criteria of screening drought tolerance (Chang et al, 1974; Farooq et al, 2010; Singh et al, 2012). Drought stress during reproductive stage is most critical as it causes low yield due to higher proportion of unfilled grains in the panicles (Hsiao et al, 1976; Kumar et al, 2006; Davatgar et al, 2009; Singh et al, 2012; Kumar et al, 2015; Torres and Henry, 2018; Barik et al, 2019).

Rice plant is a shallow rooting type relative to other cereal crops and hence is sensitive to moisture stress

Received: 25 September 2018; **Accepted:** 22 December 2018

Corresponding author: Sharat Kumar PRADHAN (pradhancrri@gmail.com)

Peer review under responsibility of China National Rice Research Institute

http://dx.doi.org/10.1016/j.rsci.2020.01.003

in the soil. Deep rooting may assist plants to avoid drought-induced stress by extracting water from deep soil layers (Yoshida and Hasegawa, 1982; Fukai and Cooper, 1995). Kondo et al (2000) showed low moisture stress to rice plants under vertical root distribution in upland condition. The genetic control of deep rooting in rice was confirmed by Uga et al (2011). To improve drought avoidance in rice, introducing the deep-rooting characteristic into shallow-rooting cultivars is considered one of the most promising breeding strategies. To date, only one quantitative trait locus (QTL) responsible for deep rooting namely *Dro1* on chromosome 9 has been cloned in rice (Uga et al, 2011, 2013a). *qSOR1*, a QTL for soil surface rooting on chromosome 7 (Uga et al, 2012), *Dro2* on chromosome 4 (Uga et al, 2013b) and *Dro3* on chromosome 7 (Kitomi et al, 2015) have been mapped. Genotypic variation contributes considerably to root growth angle in rice cultivars with functional *Dro1* allele along with other major QTLs such as *Dro2*, *Dro4* and *Dro5*, and with several additional minor QTLs (Kitomi et al, 2015). Hence, screening of germplasm lines with deep rooting and subsequent utilization as donor in the superior background is essential for upland rice improvement program.

The effects of climate change are considerably harse to rice crop particularly on soil moisture stress. As per earlier estimate, about 50% of global rice production is affected by drought (Bouman et al, 2005). The intensity and distribution of rainfall is erratic, and as a result, rice crop faces drastic soil moisture stress in the course of production (Kang et al, 2009; McKersie, 2015; Yang et al, 2018). Deployment of identified donors and molecular markers in marker-assisted breeding for deeper rooting trait will be benificial in avoiding soil moisture stress. In addition, irrigated rice consumes a lot of water for rice production. Deep rooted high yielding varieties will require less water compared to shallow rooted irrigated rice varieties.

Dro1 gene was cloned and identified by comparing the putative region (LOC_Os09g26840) of Kinadong Patong with that of IR64 revealing a single 1-bp deletion within exon 4 in IR64 leading to introduction of premature stop codon (Uga et al, 2013a). Therefore, this gene loses its phenotypic expression for root growth angle in IR64. *Dro1* gene is an early-auxin response gene regulated by auxin response factors and is negatively regulated by auxin. It expresses around the root meristem tissues enhancing the cell elongation at the root tip, causing asymmetric root growth and downward root bending in response to gravity controlling the root angle. Over-expression of *Dro1* increases deeper rooting. *Dro1* explains around 66% of phenotypic variance for ratio of deep rooting (RDR). In addition to *Dro1*, another QTL *Dro2* contributes around 32% to 56% of the total variance in different populations (Uga et al, 2013a, b). Incorporating these two QTLs into swallow rooting cultivar can be useful for increasing the deeper rooting trait. Although *Dro3*, *Dro4* and *Dro5* were reported for root growth angle, extensive study has not been done for their consistency in different populations. However, *Dro1* and *Dro2* are reported to be consistent among populations conferring effect towards root growth angle and gravitropic curvature. Hence, the present investigation aimed to characterize 348 diverse rice germplasm lines for selection of drought avoidance donors through root growth angle study using *Dro1* and *Dro2* QTLs and further for classifying of the panel population into different sub-populations using various population genetic analyses for the trait.

MATERIALS AND METHODS

Seed materials and field screening

A set of 348 genotypes from rice germplasm were shortlisted based on early and mid-early maturing genotypes, which were acquired from eight rice growing countries of the world (Supplemental Table 1). Traits namely leaf rolling and leaf drying during vegetative stage as well as spikelet fertility and single plant yield at reproductive stage were investigated. The 348 germplasm lines were direct seeded in an augmented block design with 8 blocks and 4 check varieties (N22, Dular, IR64 and IR20) with three rows of 4.5 m length each at spacing of 20 cm × 15 cm between hills during dry season of 2015. The experimental materials were exposed to a minimum of 15 and 7 d rainless during vegetative and reproductive stages, respectively for drought scoring. IRRI standard evaluation system (IRRI, 2013) was followed to score the genotypes. From the 348 genotypes again, a panel for genotyping and precise phenotyping for root growth angle was prepared based on leaf rolling and leaf drying during vegetative stage, and spikelet fertility and single plant yield during reproductive stage of the germplasm lines.

Genomic DNA extraction

Genomic DNA was extracted after crushing young leaves in liquid nitrogen using CTAB extraction buffer

(100 mmol/L Tris-HCl with pH of 8.0, 20 mmol/L EDTA with pH of 8.0, 2% CTAB and 1.3 mol/L NaCl) and chloroform-isoamyl alcohol extraction, followed by RNAase treatment and ethanol precipitation. DNA concentration was estimated by agarose gel electrophoresis taking lambda DNA as standard. Each sample was diluted to approximately 25 ng/μL.

Polymerase chain reaction for amplification of Dro markers

DNA amplification was performed in a Gradient Thermal Cycler (Verity, Applied BioSciences, USA) with a reaction volume of 20 μL, containing 1.5 mmol/L Tris-HCL (pH 8.75), 50 mmol/L KCl, 2 mmol/L $MgCl_2$, 0.1% TrotonX-100, 200 μmol/L each of dATP, dCTP, dTTP, dGTP, 4 pmol of each forward and reverse primers, 1 unit of *Taq* polymerase and 30 ng of genomic DNA. The reaction mixture was first denatured for 4 min at 94 ºC and then subjected to 35 cycles of 1 min denaturation at 94 ºC, 1 min annealing at 57 ºC–62 ºC, and 1 min extension at 72 ºC; and then a final extension for 10 min at 72 ºC. A set of nine specific and flanking markers for *Dro* QTLs were used (Supplemental Table 2). Four flanking markers, ID07_14 and RM24393 on upstream and ID7_17 and RM7424 on downstream of the *Dro1* QTL, were deployed for the locus. In addition to these flanking markers, four specific markers for *Dro1* gene, viz. Dro1-CAPS5, Dro1-INDEL, SNP02-KP and SNP02-IR64, were used.

Visualization of PCR product

DNA products from PCR amplification were loaded in 2.5%–3.0% gel containing 0.8 g/mL ethidium bromide for electrophoresis in 1× TBE (pH 8.0). One lane was loaded with 50 bp DNA ladder. The gel was run at 2.5 V/cm for 4 h and photographed using a Gel Documentation System (SynGene, UK).

Data analysis

Data were scored for computer analysis on the basis of the presence or absence of the amplified products for each genotype-primer combination. The data entry was done into a binary data matrix as discrete variables. Dendrogram was constructed with unweighted pair group method arithmatic average (UPGMA) algorithm using DARwin5 software (Perrier and Jacquemoud-Collet, 2006). STRUCTURE 2.3.4 software was used for data analysis to obtain possible population structure (Pritchard et al, 2000). The model choice criterion to detect the most probable value of K was ΔK, an ad-hoc quantity related to the second-order change of the log probability of data with respect to the number of clusters inferred by STRUCTURE (Evanno et al, 2005). Structure Harvester was used for estimation of the ΔK value (Earl and Von, 2012). The principal coordinate analysis was performed following Pandit et al (2016) and Pradhan et al (2016).

Phenotyping for root angle in rice

The primary root angle was measured by using agarose-glass box method following Uga et al (2013a) with minor modifications. The de-husked seeds were pre-soaked for 3–4 h. These seeds were carefully transferred to 0.4% agarose in semi solid state in a glass box and then the agarose was allowed to solidify, after which the boxes were kept in dark at 30 ºC. The root angle was measured at 24 and 48 h. The glass boxes were rotated at 60º angle for 4–6 h to check the gravitropic effect.

RESULTS

Field screening of germplasm lines for drought avoidance and tolerance under moderate drought stress condition

The susceptible checks showed a score of ≥ 7 for leaf rolling and leaf drying during vegetative stage, and spikelet fertility (< 20%) and single plant yield (< 5 g) were considered for primary screening. Field screening results indicated that 29 germplasm lines were with score 1, 121 with score 3 and 114 with score 5 for leaf rolling (Fig. 1 and Supplemental Table 1). For leaf drying, 45 were with score 1, 161 with score 3 and 92 with score 5 (Fig. 1 and Supplemental Table 1). It was observed that spikelet fertility varied from 6.12% to 83.22% under moderate drought stress condition. Genotypes N22, Ac10914, Ac39973, Ac39890, Ac11261, Ac10984, CR2340-2, Ac39790, IR10C-167 and CR143-2-2 were with score 1 under the stress. Single plant yield varied from 1.26 g (IR20) to 14.42 g (Sahabhagi Dhan) under the stress, and 86 lines showed single plant yield more than 10 g (Fig. 1 and Supplemental Table 2). Screening results showed that 25 genotypes namely CR143-2-2, Dular, Satyabhama, Sahabhagi Dhan, IR83141-B-32-B, IR10C-161, Dinorado, IR64197-3B-15-2, N22, CR Dhan 202, Surjamukhi, Kutiarasi, Ac39973, Ac10976, Ac10925, Bowdel, Serety, Ac11261, Habigonj Boro 6, Anjali, IR10G-

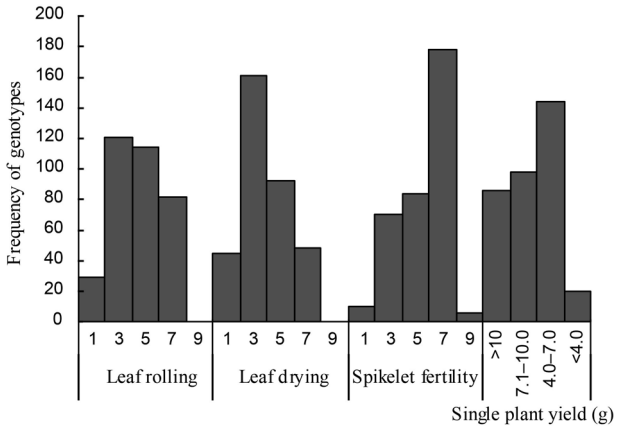

Fig. 1. Frequency distributions of leaf rolling, leaf drying, spikelet fertility and single plant yield among 348 genotypes under moderate drought stress.

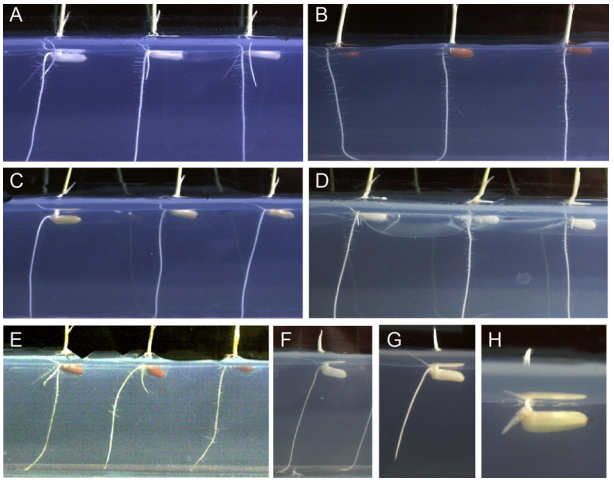

Fig. 2. Representative pictures of gravitropic response of primary roots of Dro positive genotypes by using agarose-glass box method along with negative check IR64.

A, KalingaIII. B, Lalsankari. C, Dular. D, N22. E, Kasalath. F–H, IR64.

103, Ac39843, Ac10914, IR10C-103 and Karni are drought tolerant based on leaf rolling, leaf drying, spikelet fertility and single plant yield.

Phenotyping for root angle trait in rice

The gravitropic responses of the primary roots of the Dro positive genotypes along with the negative check were evaluated by using agarose-glass box method. The primary root angle in glass box containing 0.4% agarose indicated that genotypes Bowdel, Lalsankari, N22, Dular, Tepiboro and Surjamukhi had almost vertical roots while the angle was much less in susceptible variety, IR64 (Fig. 2). The average primary root angle in the Dro positive genotypes varied from 65º to 90º. However, the negative check IR64 exhibited an average root angle of 54º, whereas IR20 showed root angle of 56º. These results revealed that our candidate genotypes may contain *Dro1* QTL(s). After the trays were rotated 60º, the majority of the Dro positive genotypes continued to extend and bend in the direction of gravity within 4–6 h. The gravitropic response of the genotypes is presented in Fig. 3-A. The mean root angle of the genotypes having both *Dro1* and *Dro2* ranged from 82.7º to 89.7º (Fig. 3-B). The *Dro* positive genotypes showed similar root angle in response to gravitropic effect as observed in case of primary root angle. However, sometimes the seminal roots of the *Dro* negative genotypes were irregular before and after rotation. These results suggested that the primary roots of the *Dro* positive genotypes showed normal gravitropic response.

Genotyping of panel population using deeper rooting markers

Nine markers (Supplemental Table 2) were used to amplify *Dro1* and *Dro2* loci in the panel containing 101 genotypes including negative checks. The popular variety IR64 was taken as the negative check for both *Dro* loci (Uga et al, 2011). Four flanking markers and four specific markers for *Dro1* gene were used for amplification of 67 genotypes to be positive for the QTL (Supplemental Table 3). The marker ID07-14 showed the target band of 174 bp in 84 genotypes (Supplemental Fig. 1), whereas ID7-17 showed 220 bp band in 59 genotypes (Supplemental Table 3). A 557-bp amplicon for *Dro1* was obtained with an SSR marker RM24393 in 70 genotypes, whereas RM7424 amplified the expected band of 82 bp in 83 genotypes. The marker SNP02-KP which is specific for Kinadong Patong *Dro1* could be observed in 76 genotypes and these genotypes were negative for SNP02-IR64 confirming the presence of *Dro1* gene. These genotypes were also observed to be positive for Dro1-INDEL and Dro1-CAPS5 markers (Supplemental Table 3). Considering all these marker data, a total of 67 genotypes were found to possess *Dro1* QTL (Supplemental Table 3).

The major QTL *Dro2*, reported for deeper rooting, was used for screening the panel population using a closely linked marker RM6089. The expected size of the amplicon i.e. 170 bp was obtained in 12 genotypes (Supplemental Table 3 and Supplemental Fig. 2). The IR64 allele of 165 bp was obtained in 89 genotypes. Eleven genotypes viz., Dular, Tepiboro, Surjamukhi, Bamawpyan, N22, Dinorado, Karni, Kusuma, Bowdel, Lalsankari and Laxmikajal, were positive for both

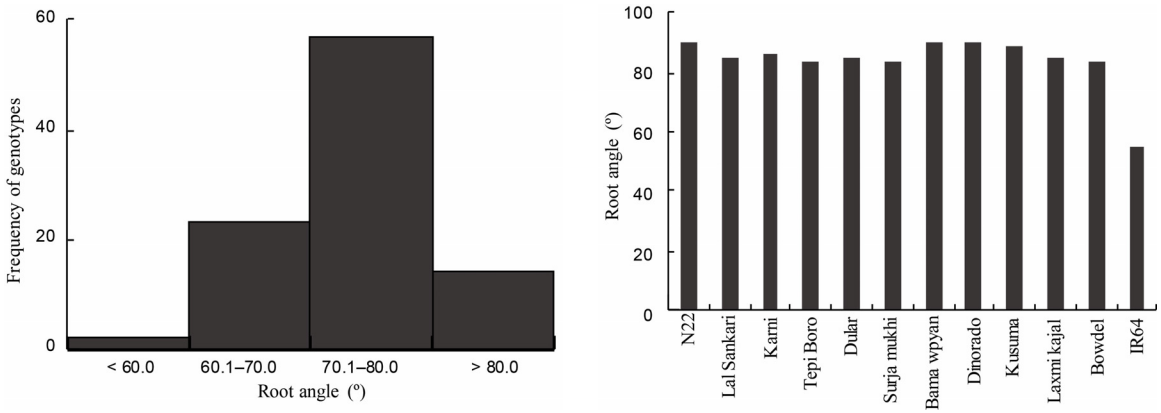

Fig. 3. Frequency distribution of mean root angle of the panel population in response to gravitropic effect (A) and primary mean root angle of genotypes having both *Dro1* and *Dro2* along with negative check IR64 (B).

Dro1 and *Dro2* loci (Supplemental Table 3).

Population structure, principal coordinate and clustering analyses

The population structure of the genotypes was estimated by using STRUCTURE 2.3.4 software following Bayesian clustering approach (Pritchard et al, 2000). By taking higher ΔK-value, four sub-populations were observed (Fig. 4). The four sub-populations were obtained at ΔK value of 43.3 with K = 4 (Fig. 4-A). Maximum allele frequency divergence between the two sub-populations (net nucleotide distance) was 0.1425, 0.843, 0.1425 and 0.9838 for clusters 1 and 3, clusters 1 and 4, clusters 2 and 3, and clusters 3 and 4, respectively. The average distances (expected heterozygosity) between individuals in clusters 1, 2, 3 and 4 were 0.0004, 0.0004, 0.0013 and 0.0148, respectively. The four sub-populations showed fixation index values (*Fst*) of 0.966, 0.965, 0.962 and 0.957, respectively. A lower value of α (0.0271) was detected in the studied panel population. The distribution pattern of α-value in the panel population showed a leptokurtic symmetry while distribution of *Fst* values in the sub-populations were in symmetric shape with almost identical to the left and right from the centre.

Principal coordinate analysis (PCA) was performed to determine the genetic relatedness among the genotypes with respect to the *Dro* loci (Fig. 5-A). The first two components (PC1 and PC2) accounted for 29.11% and 13.49% of total inertia, respectively. The 101 genotypes were distributed in all the four quadrants showing two major groups (Fig. 5-A). The 1st, 2nd, 3rd and 4th quadrants consisted 37, 31, 18 and 15 genotypes, respectively. The genotypes having *Dro1* or *Dro2* locus or both were placed in the 1st (top right) and 2nd (bottom right) quadrants, whereas the negative genotypes were placed in the rest two quadrants, i.e. 3rd and 4th quadrants (Fig. 5-A). However, Hongzui El was placed along with the negative ones in the 4th quadrant, whereas Dhobosankari and Basaramatia were placed in the 2nd quadrant along with the positive genotypes. These three genotypes showed inconsistent response for *Dro1* specific markers.

The clustering analysis of the genotypes based on the molecular screening for *Dro1* and *Dro2* could separate out the tolerant rainfed upland cultivars and the irrigated ecosystem genotypes. The irrigated genotypes IR64 and IR20 that had been taken as negative check together formed a distinct branch in dendrogram (Fig. 5-B). Eight genotypes namely Tepiboro, Bamawpyan, Surjamukhi, N22, Karni, Bowdel, Lalsankri and Laxmikajal, positive for both *Dro1* and *Dro2*, formed distinct cluster (green), whereas Dular, Dinorado and Kusuma having both the QTLs were placed along with the other genotypes. The genotypes having *Dro1* gene only formed two distinct clusters (red) accommodating 49 genotypes (Fig. 5-B). Seven genotypes namely Khaodaw, Vanaprabha, Menkasala, Hazaridhan, Khandagiri, Blackgora and Tawadhan were placed separately from the aforesaid 49 genotypes having *Dro1* gene.

DISCUSSION

This study provides a novel survey for presences of *Dro1* and *Dro2* among diverse rice varieties and landraces. Better donor lines having deeper rooting system were identified for drought stress tolerance. The markers employed showed that eleven genotypes

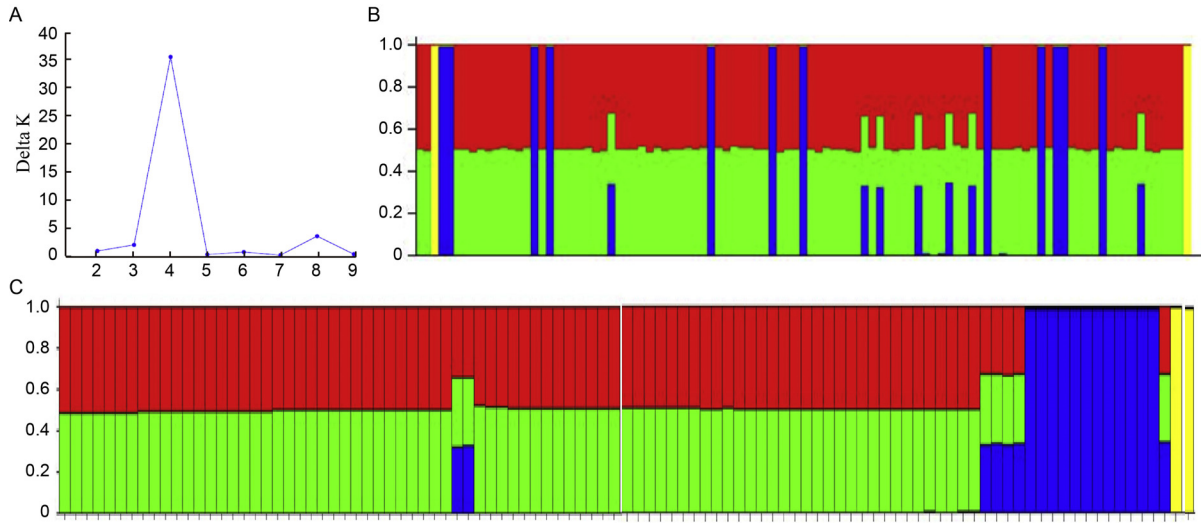

Fig. 4. Graph of *K* value, an ad-hoc statistic related to the rate of change in the log probability of data between successive K values (A), and population structure of 101 germplasm lines based on membership probability fractions of individual genotypes (B) and population structure of 101 germplasm lines sorted based on serial number of the genotypes (C).

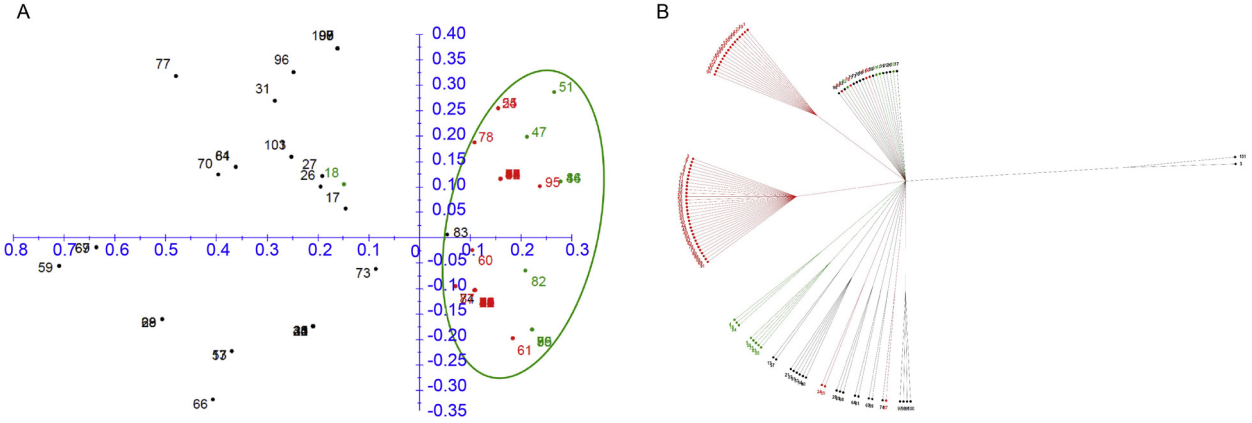

Fig. 5. Principal coordinate analysis (A) and unweighted pair group method arithmatic average (UPGMA) algorithm (B) obtained with the molecular markers for *Dro1* and *Dro2* loci of 101 genotypes.
The dot numbers in the figure represent the serial number of the genotypes.

possessed both the QTLs. These genotypes were drought tolerant at both vegetative and reproductive stages based on our field screening trial (Supplemental Table 1). All these eleven landraces are very good donors for upland rice breeding program. Besides, these donor lines are also being cultivated in some areas of the country.

Deep rooting is a beneficial strategy against drought stress in rice (Yoshida and Hasegawa, 1982; Fukai and Cooper, 1995). Under drought stress, rice plant shows leaf rolling, leaf senescence, reduced plant height, stomatal closure, lower dry matter production and decreased leaf elongation (Farooq et al, 2010; Kumar et al, 2015). Previous study on root angle reveals the existence of genetic variation and its genetic control in rice (Uga et al, 2011). In our study, a majority (71.88%) of the genotypes were with *Dro1* QTL on chromosome 9. Another major QTL *Dro2* had been reported on chromosome 4 from Kinandang Patong, from which *Dro1* was reported (Uga et al, 2013a). *Dro2* is reported to account for up to 56% of phenotypic variance for ratio of deep rooting in rice. RM6089 showed better linkage to *Dro2* locus and consistency in different populations according to Uga et al (2013b). Hence, the present study used RM6089 for screening the genotypes. Only 12 genotypes, namely Laxmikajal, Tepiboro, Surjamukhi, Bamawpyan, Hongzui El, N22, Dular, Dinorado, Karni, Kusuma, Bowdel and Lalsankari, showed Kinandang Patong allele. This result revealed that *Dro2* is not well

distributed throughout the upland genotypes as the QTL *Dro1*. Dular was reported for deep rooting character with high drought tolerance (Kato et al, 2006; Henry et al, 2011). The present study confirmed the presence of both *Dro1* and *Dro2* in Dular. *Dro1* is present in genotypes like Sahabhagidhan, Pyari, Sadabahar, RR20, RR51-1, Habigonj boro 6 and RR354-1, indicating that deeper rooting trait can be incorporated in high yielding rice backgrounds.

The dendrogram constructed based on the molecular screening for deep rooting QTLs showed clear distinction between the rainfed upland cultivars and irrigated genotypes. Although there is no such previous reports on classification of genotypes on the basis of Dro markers, similar study was conducted by Chin et al (2011) and Pandit et al (2018) where they had classified the upland, irrigated and lowland genotypes based on the phosphorous uptake 1 (*Pup1*) QTL specific markers. A few irrigated or aerobic genotypes like Kalinga-III, CR Dhan 204 and CR Dhan 202 were grouped separately whereas genotypes Pyari, CR Dhan 201 and Sahabhagidhan were grouped along with the upland genotypes. As dendrogram was constructed based on the presence of the Dro specific markers, it can be inferred that the aerobic ecosystem and some irrigated ecosystem genotypes may also possess these QTLs. The genotypes RR347-466, CR Dhan 204 and CR Dhan 202 were of negative inconsistency for most of the markers whereas IR64 and IR20 were negative for all.

The panel population showed four sub-populations by structure analysis where the negative checks IR64 and IR20 formed a sub-group (SP4) (Fig. 4). The genotypes in sub-population 3 (SP3) included all the genotypes having *Dro1* and *Dro2* QTLs. SP1 and SP2 inferred ancestry values were almost equally distributed among the rest of the genotypes, which may be due to the presence of inconsistent positive/negative genotypes for *Dro1* markers. However, the PCA analysis clearly distinguished the Dro positive and Dro negative/ inconsistent genotypes. These analyses were able to identify the potential donor lines into separate groups. The genotypes possessing the two deeper rooting QTLs *Dro1* and *Dro2*, grouped under SP3 in structure analysis, can be used as donor lines in breeding programs. More specifically, genotypes amplified by all the studied markers should be characterized and utilized in future. The α-value obtained for the panel population by structure analysis also revealed the domestication of *Dro* QTLs from common ancestor for adaption in rainfed ecosystem. Similar trend of evolution for high and low temperature stress are reported in rice (Pradhan et al, 2016; Pandit et al, 2017).

The gravitropic responses of the primary roots of the Dro positive genotypes along with the negative check indicated that genotypes Bowdel, Lalsankari, N22, Dular, Tepiboro and Surjamukhi had almost vertical roots, and their average angle ranged from 82.7º to 89.7º, while the angle was much less (54º) in the negative check IR64. These results suggested that our candidate genotypes may contain *Dro* QTL(s). IR64 NILs containing *Dro1* QTL show almost vertical roots as compared to the normal one (Uga et al, 2013a; Kitomi et al, 2015), which is in agreement with our results. This indicated the potential of these genotypes to be used as donors in breeding programs.

Upland rice ecosystem is generally deficient in phosphorus content. Dular, Tepiboro, Surjamukhi, Bamawpyan, N22, Dinorado, Karni, Bowdel and Lalsankari contain QTL *Pup1* (Pandit et al, 2015, 2018). The three QTLs *Dro1*, *Dro2* and *Pup1* are important for upland rice breeding and they need to be incorporated into a single high yielding genetic background. Kasalath is reported to contain *Pup1* locus (Wissuwa, 2001; Chin et al, 2010; Pandit et al, 2018). Moreover, in this study, we detected *Deeper rooting1* locus, too. Bowdel, Lalsankari, Karni, Dinorado, N22, Bamawpyan, Tepiboro, Dular and Surjamukhi, can be used as common donor for upland breeding programs for improving both deeper rooting (*Dro1* and *Dro2*) and phosphorous uptake (*Pup1*) in rice.

Deep rooting may assist rice plants to avoid drought-induced stress by utilizing water from deeper soil. *Dro1* and *Dro2* are the QTLs that contribute considerably to the root growth angle assisting in deeper rooting of rice plant. Screening of 348 early and mid-early maturing germplasm lines for drought stress identified 25 drought tolerant germplasm lines. The representative panel containing 101 genotypes showed four sub-populations with different *Fst* values for root growth angle. The clustering analysis and principal coordinate analysis could differentiate the genotypes with or without deeper rooting trait. The dendrogram constructed based on the molecular screening for deep rooting QTLs showed clear distinction between the rainfed upland cultivars and irrigated genotypes. Eleven genotypes namely Dular, Tepiboro, Surjamukhi, Bamawpyan, N22, Dinorado, Karni, Kusuma, Bowdel, Lalsankari and Laxmikajal

possessed both the QTLs, whereas 67 genotypes possessed only *Dro1* QTL. The average angle of Dro positive genotypes ranged from 82.7º to 89.7º. These genotypes possessing the deeper rooting QTLs can be taken as donor lines to be used in marker-assisted breeding programs for incorporation of these QTLs into high yielding popular rainfed rice varieties.

ACKNOWLEDGEMENTS

The authors are highly grateful to the Director, ICAR-National Rice Research Institute, and Head, Crop Improvement Division, National Rice Research Institute, Cuttack, India, for providing all the necessary facilities, and Department of Science and Technology, Govt. of India for providing financial support.

SUPPLEMENTAL DATA

The following materials are available in the online version of this article at http://www.sciencedirect.com/science/journal/16726308; http://www.ricescience.org.

Supplemental Table 1. Leaf rolling, leaf drying, spikelet fertility and single plant yield of 348 early and mid-early duration germplasm lines under moderate drought stress.

Supplemental Table 2. List of markers used in the study.

Supplemental Table 3. Response of panel genotypes to *Dro1* and *Dro2* QTL/gene(s) using gene specific and flanking markers.

Supplemental Fig. 1. Representative electrophoregram obtained with ID07_14 showing target band of 174 bp.

Supplemental Fig. 2. Amplicons obtained with RM6089.

REFERENCES

Barik S R, Pandit E, Pradhan S K, Singh S, Swain P, Mohapatra T. 2018. QTL mapping for relative water content trait at reproductive stage drought stress in rice. *Ind J Genet*, **78**(4): 401–408.

Barik S R, Pandit E, Pradhan S K, Mohanty S P, Mohapatra T. 2019. Genetic mapping of morpho-Physiological traits involved during reproductive stage drought tolerance in rice. *PLoS One*, **14**(12): e0214979.

Bernier J, Serraj R, Kumar A, Venuprasad R, Impa S, Gowdaa R P V, Oane R, Spaner D, Atlin G. 2009. The large-effect drought-resistance QTL *qtl12.1* increases water uptake in upland rice. *Field Crops Res*, **110**(2): 139–146.

Chang T T, Loresto G C, Tagumpay O. 1974. Screening of rice germplasm for drought resistant. *Sabrao J*, **6**(1): 9–16.

Chin J H, Lu X C, Haefele S M, Gamuyao R, Ismail A, Wissuwa M, Heuer S. 2010. Development and application of gene-based markers for the major rice QTL phosphorus uptake 1. *Theor Appl Genet*, **120**: 1073–1086.

Chin J H, Gamuyao R, Dalid C, Bustamam M, Prasetiyono J, Moeljopawiro S, Wissuwa M, Heuer S. 2011. Developing rice with high yield under phosphorus deficiency: *Pup1* sequence to application. *Plant Physiol*, **156**(3): 1202–1216.

Davatgar N, Neishabouri M R, Sepaskhah A R, Soltani A. 2009. Physiological and morphological responses of rice (*Oryza sativa* L.) to varying water stress management strategies. *Int J Plant Prod*, **3**(4): 19–32.

Earl D A, von Holdt B M. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Res*, **4**(2): 359–361.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol Ecol*, **14**(8): 2611–2620.

Farooq M, Kobayashi N, Ito O, Wahid A, Serraj R. 2010. Broader leaves result in better performance of *indica* rice under drought stress. *J Plant Physiol*, **167**(13): 1066–1075.

Fukai S, Cooper M. 1995. Development of drought resistant cultivars using physio-morphological traits in rice. *Field Crops Res*, **40**(2): 67–86.

Hsiao T C. 1973. Plant responses to water stress. *Ann Rev Plant Physiol*, **24**: 519–570.

Henry A, Gowda V R P, Torres R O, McNally K L, Serraj R. 2011. Variation in root system architecture and drought response in rice (*Oryza sativa*): Phenotyping of the *Oryza* SNP panel in rainfed lowland fields. *Field Crops Res*, **120**(2): 205–214.

IRRI (International Rice Research Institute). 2013. SES (Standard Evaluation System for Rice). International Network for Genetic Evaluation of Rice. Los Baños, the Philippines: International Rice Research Institute.

Kang Y H, Khan S, Ma X Y. 2009. Climate change impacts on crop yield, crop water productivity and food security: A review. *Prog Nat Sci*, **19**: 1665–1674.

Kato Y, Abe J, Kamoshita A. Yamagishi J. 2006. Genotypic variation in root growth angle in rice (*Oryza sativa* L.) and its association with deep root development in upland fields with different water regimes. *Plant Soil*, **287**: 117–129.

Kitomi Y, Kanno N, Kawai S, Mizubayashi T, Fukuoka S, Uga Y. 2015. QTLs underlying natural variation of root growth angle among rice cultivars with the same functional allele of deeper rooting. *Rice*, **18**: 16.

Kondo M, Murty M V R, Aragones D V. 2000. Characteristics of root growth and water uptake from soil in upland rice and maize under water stress. *Soil Sci Plant Nutr*, **46**(3): 721–732.

Kumar R, Sarawgi A K, Ramos C, Amarante S T, Ismail A M, Wade L J. 2006. Partioning of dry matter during drought stress in rainfed lowland rice. *Field Crops Res*, **96**: 455–465.

Kumar R, Sreenu K, Singh N, Jain N, Singh N K, Rai V. 2015. Effect of drought stress on contrasting cultivars of rice. *Int J Trop Agric*, **33**(2): 1559–1564.

McKersie B. 2015. Planning for food security in a changing

climate. *J Exp Bot*, **66**: 3435–3450.

Pandit E, Sahoo A, Panda R K, Mohanty D P, Pani D R, Anandan A, Pradhan S K. 2016. Survey of rice cultivars and landraces of upland ecology for Phosphorous uptake 1 (*Pup1*) QTL using linked and gene specific molecular markers. *Oryza*, **53**(1): 1–9.

Pandit E, Panda R K, Pani D R, Chandra R, Singh S, Pradhan S K. 2018. Molecular marker and phenotypic analyses for low phosphorus stress tolerance in cultivars and landraces of upland rice under irrigated and drought situations. *Ind J Genet*, **78**(1): 59–68.

Pennisi E. 2008. The blue revolution, drop by drop, gene by gene. *Science*, **320**: 171–173.

Perrier X, Jacquemoud-Collet J P. 2006. DARwin software. http://darwin.cirad.fr/darwin.

Pradhan S K, Barik S R, Sahoo A, Mohapatra S, Nayak D K, Mahender A, Meher J, Anandan A, Pandit E. 2016. Population structure, genetic diversity and molecular marker-trait association analysis for high temperature stress tolerance in rice. *PLoS One*, **11**(8): e0160027.

Pritchard J K, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, **155**(2): 945–959.

Singh H, Mackill K T. 1991. Senstivity of rice to water deficit at different growth stages. *Phil Crop Sci*, **16**: S11.

Singh C S, Kumar B, Mehandi S, Chandra K. 2012. Effect of drought stress in rice: A review on morphological and physiological characteristics. *Trends Biosci*, **5**(4): 261–265.

Torres R O, Henry A. 2018. Yield stability of selected rice breeding lines and donors across conditions of mild to moderately severe drought stress. *Field Crops Res*, **220**: 37–45.

Uga Y, Okuno K, Yano M. 2011. *Dro1*, a major QTL involved in deep rooting of rice under upland field conditions. *J Exp Bot*, **62**(8): 2485–2494.

Uga Y, Hanzawa E, Nagai S, Sasaki K, Yano M, Sato T. 2012. Identification of *qSOR1*, a major rice QTL involved in soil-surface rooting in paddy fields. *Theor Appl Genet*, **124**(1): 75–86.

Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, Inoue H, Takehisa H, MotoyamaR, Nagamura Y, Wu J, Matsumoto T, Takai T, Okuno K, Yano M. 2013a. Control of root system architecture by deeper rooting 1 increases rice yield under drought conditions. *Nat Genet*, **45**(9): 1097–1102.

Uga Y, Yamamoto E, Kanno N, Kawai S, Mizubayashi T, Fukuoka S. 2013b. A major QTL controlling deep rooting on rice chromosome 4. *Sci Rep*, **3**: 3040.

Wissuwa M, Noriharu A. 2001. Further characterization of two QTLs that increase phosphorous uptake of rice (*Oryza sativa* L.) under phosphorous deficiency. *Plant Soil*, **237**(2): 275–286.

Yang M Z, Xiao W H, Zhao Y, Li X D, Huang Y, Lu F, Hou B D, Li B Q. 2018. Assessment of potential climate change effects on the rice yield and water footprint in the Nanliujiang catchment, China. *Sustainability*, **10**(2): 242.

Yoshida S, Hasegawa S. 1982. The rice root system: Its development and function. *In*: Drought resistance in crops with emphasis on rice. Los Banos, the Philippines: International Rice Research Institute: 97–114.

(Managing Editor: FANG Hongmin)