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Parental polymorphism survey and phenotyping of recombinant inbred lines for reproductive stage drought tolerance parameters in rice

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ABSTRACT

Drought is the major abiotic constraint leading to yield loss in rainfed areas covering 38% of total rice area. The reproductive stage is the most sensitive to water deficiency as compared to vegetative stage of rice. There are many primary as well as secondary traits for drought tolerance reported leading to linkage of multiple genes/QTLs with the traits. Still the genetic advancement in development of tolerant lines is slow and limited. Hence, in the present investigation, we aimed to study the polymorphism between two contrasting parents for drought tolerance and phenotyping for reproductive stage drought tolerance traits. A highly tolerant germplasm line CR143-2-2 and a susceptible high yielding popular variety of Andhra Pradesh state of India, Krishnahansa were used in the present study. Seventy seven markers (38.3%) distributed in all chromosomes except chromosome 4 and 5 showed polymorphism between the two genotypes. These markers were linked to 19 phenotypic traits like grain yield under drought, days to 50% flowering, harvest index, biomass, drought response index, canopy temperature, plant height, flag leaf length, leaf rolling, leaf drying, panicle exsertion, root length, 1000- seed weight, spikelet fertility, panicle weight, percentage of filled grain, seed number per panicle, root volume, root number and root penetration. The markers like RM12091, RM279, RM104, RM263 and RM523 were linked to multiple traits. The polymorphic markers obtained in the present investigation will be used to validate the developed recombinant inbred lines (RILs) and mapping of QTLs for drought tolerance.

Key words: *Drought stress tolerance, simple sequence repeat (SSR) polymorphism, recombinant inbred lines*

Rice is the major cereal crop grown extensively worldwide. Drought is the major constraint for rice production and yield loss in rainfed areas that occupies 38% of total rice production area (Haefele and Hijmans 2007). Depending on severity, timing and duration, the whole biomass production of rice crop decreases due to water availability at any of its growth stage (Turner *et al*. 1986). Particularly, during reproductive stage, plants are very highly sensitive to water deficiency as compared to vegetative stage (IRRI 1980). Development of drought-resistant rice varieties with higher yields suitable for water-limiting environments will be a key to improve drought stress tolerance thereby increasing rice production and ensure food security. The progress in genetic improvement of rice for water-limiting environments has been slow and limited (Evenson and Gollin 2003). This is because of poor understanding of the genetic mechanisms of tolerance and lack of efficient techniques for screening breeding materials for drought tolerance (Khush 2001). Drought resistance is improved either if the crop is able to access more water or if it can use available water more efficiently (higher transpiration efficiency) (Passioura 2006).

Recent studies have shown that direct selection for grain yield in populations derived from crosses of such drought-tolerant popular high yielding varieties has proven beneficial for combining high yield potential and drought tolerance (Kumar *et al*. 2009; Venuprasad *et al*. 2007; Manjappa *et al*. 2015; Mall *et al*. 2016). So, it is important to confirm

about the genes/QTLs that has direct effect on drought tolerance at reproductive stage. This provides an opportunity for selection of donor lines for improving high yielding popular varieties for their drought tolerance. Confirmation of contrasting donor lines with respect to their response to drought stress will be useful for mapping the genes/QTLs responsible for drought tolerance.

There are many primary, integrative and secondary traits reported for selecting drought stress tolerance in rice (Kamoshita *et al*. 2008). The primary traits include root length, root thickness and penetration ability whereas integrative traits includes grain yield under drought, biomass, harvest index, drought response index, spikelet fertility, % of filled grain, seed number per panicle, days to 50% flowering, seed density, 1000-seed weight, panicle weight and the secondary traits are canopy temperature, leaf rolling, leaf drying, plant height, flag leaf length and panicle exsertion (Kamoshita *et al*. 2008). Majority of the secondary traits *viz*., leaf water potential, epicuticular wax, osmotic adjustment etc. have moderate to high heritability under stress indicating the possibility of incorporating them into breeding programme (Kumar *et al*. 2008). Some secondary traits such as leaf water potential (LWP) has good correlation with yield under stress condition (Jongdee *et al*. 2006). There are number of major and minor QTLs/ genes, present throughout the genome in all 12 chromosomes, have been reported that are linked to the primary or secondary traits (Yue *et al*. 2008; Dixit *et al*. 2012, Steele *et al*.2006; Lin *et al*. 2007; Vikram *et al*. 2011; Matsumoto *et al*. 2005). Many marker based approaches have been used to identify these genes/QTLs of which use of specific simple sequence repeats (SSR) markers played a significant role. Due to the technical simplicity, the small amount of starting DNA requirement, the relatively low cost, rapid turn-around time, and high power of genetic resolution these makers contributed a major role in the area of molecular breeding and replace traditional restriction fragment length polymorphism (RFLP) markers. Microsatellite markers based on simple sequence repeats (SSR) have been developed in many crop species including rice (Wu and Tanksley 1993; Panaud *et al*. 1996; Akagi *et al*. 1996). Around 29,000 SSR markers data are present in gramene data base (www.gramene.org).

In rice, micro-satellites have been classified into two different groups based on length of SSR motif and their potential as informative markers. Class I microsatellites are SSRs >20 nucleotides in length and Class II contains SSRs >12 nucleotides and <20 nucleotides in length. The Class I markers were reported to be highly variable (Cho *et al*. 2000), whereas Class II SSRs are less variable (Temnykh *et al*. 2001). A total of 18,828 Class I microsatellite markers have been identified till now (IRGSP 2005). SSR markers have been proved to be more useful than other markers for parental polymorphism study and are being widely used (Ilango and Sarla 2010). This is a prerequisite for any mapping population study.

The germplasm line CR143-2-2 which is highly tolerant to drought stress and a susceptible high yielding popular variety, Krishnahamsa were used in the present study to identify the polymorphism pattern for drought related genes/QTLs. Two hundred one SSR markers, spread over 12 chromosomes, specific for multiple traits (primary as well as secondary traits) for drought stress tolerance along with markers not reported to be linked to the traits were used to study the polymorphism between the two genotypes. This will lead to mapping and validation of developed RIL population to identify the major/minor genes/QTLs responsible for drought stress tolerance in rice.

MATERIALS AND METHODS

Plant material

Rice genotypes CR143-2-2 and Krishnahamsa constituted the experimental material. The seeds were collected from gene bank of ICAR-National Rice Research Institute, Cuttack, Odisha, India. Seeds were grown under controlled condition of RGA-cum-phytotron facility in different pots following standard disease and pest management procedures. CR143-2-2 is an early duration germplasm line, with high drought stress tolerance, developed by ICAR-National Rice Research Institute for upland ecology. Krishnahansa is a high yielding popular variety of Andhra Pradesh, India. It is an irrigated rice variety usually grown in boro season. It shows high susceptibility to drought stress.

Table 1. List of 201 SSR markers used in the study

Location of the markers	Marker Name*
Chromosome1	RM6703, RM11943, RM212, RM3825, RM237, RM488, RM259, RM5, RM522, RM12091, RM431, RM315, RM472, RM543, RM8085, RM6702, RM403, RM246, RM495, RM428, RM3360, RM5443, RM1349, RM1003, RM1198.
Chromosome 2	RM1367, RM3212, RM573, RM555, RM324, RM5614, RM520, RM263, RM327, RM530, RM262, RM3549, RM279, RM12868, OSR17, RM2634, RM250, RM452, RM521, RM526, RM497, RM13600, RM112, MGR2762, RM221.
Chromosome 3	RM523, RM231, RM7332, RM517, RM411, RM545, RM135, RM85, RM22, RG1356, RM5761, RM454, RM569, RM16030, RM416, RM15780, RM60, RM104, RM232, RM293, RM571.
Chromosome 4	RM518, RM17435, RM131, RM8213, RM471, RM142, RM273, RM252, RM537, RM5586, RM349, RM142, RM476B.
Chromosome 5	RM421, RM509, RM430, RM274.
Chromosome 6	RM510, RM3, MGR4371, RM314, RM170, RM240, RM276, RM5371, RM527, RM253, RM528, RM541, RM136, RM176, RM494.
Chromosome 7	RM429, RM248, RM295, RM481, RM72, RM125, MGR4499.
Chromosome 8	RM256, RM149, RM337, RM339, RM210, RM25, RM331, RM502, RM342A, RM407, RM3231.
Chromosome 9	RM566, RM219, RM189, RM215, RM434, RM278, RM24350, RM24390, RM321, RM464, RM24421, RM444, RM160, RM316, RM343, RM257, RM242, RM201, RM213.
Chromosome 10	RM304, RM216, RM302, RM228, RM258, RM311, RM244, RM596, RM474, RM271, RM467, RM171, RM484.
Chromosome 11	RM206, RM254, RM144, RM229, RM21.
Chromosome 12	RM28099, RM28199, RM28089, RM511, RM28166, RM1261, RM28048, RM28130, RM28050, RM28051, RM28057, RM28059, RM28060, RM28064, RM28067, RM28069, RM28070, RM28075, RM28076, RM28078, RM28079, RM28082, RM28083, RM28088, RM28081, RM28090, RM28095, RM28112, RM28148, RM28157, RM17, RM512, RM179, RM83, RM101, RM463, RM519, RM313, RM309, RM277, RM260 , RM341, RM20A.

* The details of the primers like sequences, Tm, repeat motifs, etc. are available at www.gramene.org

Phenotyping

The phenotyping for reproductive stage drought tollerance was conducted under rain-out shelter at ICAR-National Rice Research Institute, Cuttack during *kharif* season 2013 and 2014. The two parents CR143-2-2 and Krishnahamsa along with 190 RILs were studied for agro-morphologic traits. Primary traits like leaf rolling and leaf drying along with secondary traits like leaf colouration and leaf angle for drought stress were recorded following the IRRI Standard Evaluation System of rice. Canopy temperature was recorded by the instrument VarioCAM, Infrared-Thermal Imager (Jenopetik) under stress condition.Whereas other agro-morphological and yield attributing traits like days to 50% flowering, panicle exsertion, 1000-seed weight, biomass, harvest index, grain yield under drought, % of fertility, leaf length, leaf width, grain length, grain width and LB ratio were observed in the rain-out shelter stress condition.

DNA extraction

Leaves of 20-25 days plants were collected aseptically from respective pots for extraction of total genomic DNA.Extraction procedure starts with the homogenization of leaves with the help of liquid nitrogen in micro centrifuge tubes using pre warmed (65^0C) Cetyltrimethyl ammonium bromide (CTAB) extraction buffer (2% CTAB, 100 mM Tris pH 8, 20 Mm Ethylene diamine tetra acetate (EDTA) pH 8, 1.3 M NaCl) followed by chloroform isoamyl alcohol extraction, RNase treatment and ethanol precipitation (Murray and Thomson 1980). The quality and quantity of extracted DNA was checked by comparing it with λ-DNA on 1% agarose gel. Also, DNA quantification and purity was checked by measuring the O.D at 260 and 280 nm using a UV visible spectrophotometer. The samples were diluted accordingly to approximately 30ng/μL.

Polymerase chain reaction (PCR)

The polymerase chain reaction was carried out in thermal cycler (Applied-Biosystems) using 201 SSR primers (Table 1). The PCR reaction mix included 1 μl of genomic DNA 30 ng/μl; 10 X buffer; 10 mM dNTPs; 50 mM $MgCl_2$, 10 μM each of forward and reverse primers. The thermal profile starts with initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30s, primer annealing 55°C for 1 min and extension 72°C for 1.30 min; final extension at 72°C for 10 min. After completion of

amplification, PCR products were stored at -20°C and the amplified products were analyzed by electrophoresis using 3.5% agarose gel. The DNA fragments were then visualized by using Ethidium bromide dye and the banding pattern was documented using gel documentation unit (Syngene G Box).

Data analysis

The two genotypes were scored according to their base pairs and the data were entered into data matrix which was subjected to further analysis. The polymorphism information content (PIC) was calculated using the formula, PIC = 1- $\sum$ Pij^2, where Pij is the frequency of ith allele for the jth locus and summation extends over n alleles (Anderson *et al.* 1993). In order to find the efficiency of SSR markers for differentiation of genotypes, the discriminating power (D) of each marker loci was calculated following formula, Dj = 1-Cj = 1- $\sum$ Pi (NPi-1)/(N-2), where Dj is discriminating power of jth locus, Pi is frequency of ith allele, Cj confusion probability of jth locus (Tessier *et al*. 1997). Further, in order to know minimum number of marker loci required to identify and differentiate genotypes from each other, total number of non-differentiated pairs (Xj) of genotypes were calculated for the jth locus using formula, Xj ={N(N-1)/2}Cj.

RESULTS

In the present investigation, CR143-2-2, an established reproductive stage drought donor and Krishnahamsa, an irrigated variety were taken as tolerant and susceptible parent, respectively to study the polymorphism pattern and terminal drought phenotypic parameters to be used for drought screening, mapping and validation of developed RILs generated from the parents. Confirmation of parents for presence or absence of tolerant genes is a prerequisite to begin any breeding program and also any genotyping experiment of recombinant inbred lines. The identification of the polymorphism for the trait of interest in the parents can further be used in confirmatory selection of RIL plants carrying the traits of interest.

The two parents CR143-2-2 (tolerant) and Krishnahamsa (susceptible) were observed to be distinct for various parameters like leaf rolling, leaf drying, biomass, fertility percentage, harvest index, etc. under drought stress (Table 2). These two parents also showed difference for various agro-morphologic traits studied (Table 2). One hundred ninety RILs were developed from the cross between these two parents. The RILs were classified into five groups based on two primary traits for drought tolerance *i.e.,* leaf drying and leaf rolling (Table 3 and Table 4) following the IRRI Standard Evaluation System for rice. For leaf drying trait, 143 lines were highly tolerant with SES score of 0-1 and 24 lines were tolerant with SES score of 1.1-3,

Table 2. Phenotypic performance of drought tolerant and drought susceptible parents under stress condition

Sl no.	Phenotyping traits	CR 143-2-2 (Tol. Parent)	Krishnahamsa (Sus. Parent)
1	Canopy temperature	32.18^{0}C	33.78^{0}C
2	1000-seed weight	2293.5 g	2025 g
3	Biomass	2.54 g	1.25 g
4	Leaf rolling	0.5 (SES Score)	6 (SES Score)
5	Leaf drying	0.5 (SES Score)	5.5 (SES Score)
6	Plant height	66 cm	74 cm
7	Harvest index	0.990	0.957
8	Days to 50% flowering	69 days	84 days
9	% of panicle emergence	95	93
10	Spad meter reading	31.88	31.64
11	Flag leaf length	27.96 cm	29.43 cm
12	Flag leaf width	1.29 cm	0.86 cm
13	% of fertility	84.62	55.10
14	Panicle length	18.80 cm	20.93 cm
15	Leaf colouration stage 1	2 (SES Score)	5 (SES Score)
16	Leaf colouration stage 2	4 (SES Score)	7 (SES Score)
17	Leaf colouration stage 3	8 (SES Score)	9 (SES Score)
18	Leaf angle	1 (SES Score)	2 (SES Score)
19	Grain length	7.859 mm	9.356 mm
20	Grain breadth	2.595 mm	2.061 mm
21	L/B ratio	3.21	4.79

Table 3. Leaf drying score of the 190 Recombinant inbred lines (RILs)

Scoring	Number of lines	Scale
5.5	Krishnahamsa	Drought susceptible parent
0.5	CR 143-2-2	Drought tolerant parent
0-1	143	Highly drought tolerant lines
1.1-3	24	Drought tolerant lines
3.1-5	17	Moderately drought tolerant lines
5.1-7	5	Moderately drought susceptible lines
7.1-9	1	Highly drought susceptible lines

Table 4. Leaf rolling score of the 190 Recombinant inbred lines (RILs)

Scoring	Number of lines	Scale
6	Krishnahamsa	Drought susceptible parent
0.5	CR 143-2-2	Drought tolerant parent
0-1	69	Highly drought tolerant lines
1.1-3	70	Drought tolerant lines
3.1-5	32	Moderately drought tolerant lines
5.1-7	16	Moderately drought susceptible lines
7.1-9	3	Highly drought susceptible lines

whereas rest of the lines were moderately tolerant to highly susceptible types (Table 3). Sixty nine RILs were categorized as highly tolerant, seventy as tolerant and rest were moderately tolerant to highly susceptible with leaf rolling score of 0-1, 1.1-3, 3.1-9, respectively (Table 4).

Genotypic confirmation of the parents for various primary and secondary traits for drought traits using linked markers is a prerequisite to validate the RILs. A total of 201 SSR markers linked to twenty two different phenotypic traits like grain yield under drought, days to 50% flowering, harvest index, biomass, drought response index, canopy temperature, plant height, flag leaf length, leaf rolling, leaf drying, panicle exsertion, seed density, root length, 1000-seed weight, spikelet fertility, panicle weight, % of filled grain, seed number per panicle, root thickness and penetration ability, relative water content, root volume and root number, root penetration were taken for the polymorphism study which are distributed in all over the 12 chromosomes were used for the purpose (Table 1). The polymorphism study showed that 19 phenotypic traits out of 22 were found to be linked with their respective polymorphic markers (Table 5; Figure 1) with respect to the parents CR143-2-2 and Krishnahamsa. Only 38.3% of the used markers *i.e.,* 77 markers showed polymorphism. A representative electrophoregram of the polymorphism study has been presented in figure 2. These 77 markers are distributed in all the chromosomes except chromosome number 4 and 5 (Table 6). Figure 1 depicts the 19 phenotypic traits and the respective polymorphic markers *i.e.,* grain yield under drought (50), days to 50% flowering (8), harvest index (3), biomass (1), drought response index (2), canopy temperature (2), plant height (6), flag leaf length (3), leaf rolling (6), leaf drying (4), panicle exsertion (1), root length (1), 1000-seed weight (2), spikelet fertility (5), panicle weight (1), % of filled grain (1), seed number per panicle(1), root volume and root number (1) and root penetration (1). There were certain markers linked to multiple traits *i.e.,* RM12091 linked to grain yield under drought and plant height; RM279 linked to grain yield under drought, drought response index, canopy temperature and flag leaf length; RM263 with grain yield under drought, harvest index; RM104 with plant height, panicle exsertion; RM523 with flowering, flag leaf length, plant height (Table 6). This result shows that the chromosome 12 has highest polymorphic markers (21) whereas the chromosome 1, 2, 3, 6, 7, 8, 9, 10 and 11 have 12, 14, 20, 5, 4, 8, 7, 6 and 2 linked markers, respectively

Table 5. The phenotypic traits and their linked markers showing polymorphism between CR143-2-2 and Krishnahamsa

Sl. no.	Phenotypic traits	Total no. of markers used for the trait	Total number of polymorphic markers detected	Number polymorphic markers detected on individual chromosome
1	Yield	120	50	CH-1 (5), CH-2 (9), CH-3 (7),CH-6 (4), CH-8 (4), CH-9 (2),CH-10 (1), CH-12 (18)
2	Days to 50% flowering	26	8	CH-3 (4),CH-7 (1), CH-8 (1), CH-10 (1), CH-12 (1)
3	Harvest index	7	3	CH-2 (2),CH-12 (1)
4	Biomass	5	1	CH-3 (1)
5	Drought response index	10	2	CH-2 (1), CH-8 (1)
6	Canopy temperature	8	2	CH-2 (1), CH-8 (1)
7	Plant height	13	6	CH-1 (1), CH-3 (3), CH-6 (1), CH-7 (1)
8	Flag leaf length	10	3	CH-2 (1), CH-3 (2)
9	Leaf rolling	16	6	CH-1 (1), CH-8 (1), CH-9 (2), CH-10 (1), CH-11 (1)CH-7 (1),CH-11 (1)
10	Leaf drying	9	4	CH-1 (1),CH-3 (1)
11	Panicle exsertion	10	1	CH-3 (1)
12	Root length	6	1	CH-9 (1)
13	1000 seed weight	5	2	CH-10 (1), CH-12 (1)
14	Spikelet fertility	11	5	CH-1 (1), CH-7 (1), CH-9 (1), CH-10 (1)
15	Panicle weight	4	1	CH-1 (1)
16	% of filled grain	4	1	CH-10 (1)
17	Seed number per panicle	2	1	CH-3 (1)
18	Root volume and root number	3	2	CH-1 (2)
19	Root penetration	2	1	CH-9 (1)

Table 6. The observed polymorphic markers, their linked traits, QTLs and observed alleles in the tolerant and susceptible parents

Sl. no.	Primer Name	Linked QTL/ Position	Trait	Reference	Observed Tolerant Parent allele (bp)	Observed Susceptible Parent allele (bp)
1	RM259	qDTY1.1	Grain yield under drought	Lin *et al.* 2007	150	160
2	RM5	qDTY1.1	Grain yield under drought	Yue *et al.* 2008	110	120
3	RM12091	qDTY1.1	Grain yield under drought, Plant height	Vikram *et al.* 2011	145	150
4	RM5443	qDTY1.1	Panicle weight	Lin *et al.* 2007	155	145
5	RM488	MQTL1.1	Grain yield under drought	Swamy *et al.* 2011	200	190
6	RM1003	Chromosome 1	Root volume & root number	Salunkhe *et al.* 2011	115	105
7	RM6703	Chromosome 1	Grain yield under drought	McCouch *et al.* 2002	200	180
8	RM3825	Chromosome 1	Leaf rolling, Leaf drying	Salunkhe *et al.* 2011	140	150
9	RM495	Chromosome 1	Spikelet fertility	Yue *et al.* 2008	145	155
10	RM8085	Chromosome 1	Root volume and root number	Salunkhe *et al.* 2011	200	170
11	RM262	qDTY2.1	Grain yield under drought	Dixit *et al.* 2012	150	165
12	RM3549	qDTY2.1	Grain yield under drought	Dixit *et al.* 2012	170	160
13	RM327	qDTY2.1	Grain yield under drought	Lin *et al.* 2007	240	220
14	RM324	qDTY2.1	Grain yield under drought	Dixit *et al.*2012	140	150
15	RM279	qDTY2.2	Grain yield under drought, Drought response index, canopy temperature, flag leaf length	Yueet *et al.* 2008	165	150
16	OSR17	qDTY2.2	Grain yield under drought	Dixit *et al.* 2012	170	145
17	RM13600	qDTY2.3	Harvest index	Vikram *et al.* 2011	160	185
18	RM250	qDTY2.3	Grain yield under drought	Mishra *et al.* 2013	160	150
19	RM263	qDTHI2.3	Grain yield under drought, Harvest index	Vikram *et al.* 2011	160	175
20	RM530	qDTY2.3	Grain yield under drought	Vikram *et al.* 2011	190	180
21	RM85	qDTY3.1	Spikelet number per panicle	Lin *et al.* 2007	110	105
22	RM411	qDTY3.1	Grain yield under drought	Temnykh *et al.* 2001	260	230
23	RM15780	qDTY3.1	Grain yield under drought	Pachauri *et al.* 2013	145	135
24	RM104	MQTL3.1	Plant height, Panicle rxertion	Yue *et al.* 2008	210	230
25	RM523	qDTY3.2	Flowering, flag leaf Length, plant height, leaf drying	Yue *et al.* 2008	280	250
26	RM231	qDTY3.2	Grain yield under drought, Flowering	Yue *et al.* 2008	200	170
27	RM7332	qDTY3.2	Grain yield under drought	Salunkhe *et al.* 2011	400	360
28	RM517	qDTY3.2	Grain yield under drought, flowering	Vikram *et al.* 2011	300	270
29	RM135	qDTY3.2	Plant height	Yue *et al.* 2008	250	230
30	RM22	qDTY3.2	Grain yield under drought, biomass	Vikram *et al.* 2011	180	200
31	RM16030	MQTL3.2	Grain yield under drought	Swamy *et al.* 2011	100	140
32	RM571	Chromosome 3	Flag length length	Yue *et al.* 2008	195	185
33	RM276	qDTY6.1	Grain yield under drought	Yue *et al.* 2008	105	145
34	RM527	qDTY6.2	Grain yield under drought, plant height	Lin *et al.* 2007	220	210
35	RM3	qDTY6.2	Grain yield under drought	Salgotra *et al.* 2015	135	155
36	RM528	qDTY6.2	Grain yield under drought	Mohammadi *et al.* 2013	255	245
37	MRG4499	Chromosome 7	Leaf drying, flag leaf	Yue *et al.* 2008	250	220

continued.....

			width, plant height, spikelet fertility			
38	RM337	MQTL8.1	Grain yield under drought	Swamy *et al.* 2011	150	200
39	RM25	qDTY8.1	Grain yield under drought	Lin *et al.* 2007	135	150
40	RM72	qDTF8.1	Leaf rolling, flowering	Lin *et al.* 2007	155	165
41	RM210	MQTL8.2	Grain yield under drought	Swamy *et al.* 2011	135	130
42	RM256	Chromosome 8	Grain yield under drought	Chen *et al.* 1997	150	115
43	RM342A	Chromosome 8	Drought response index, canopy temperature	Yue *et al.* 2008	140	150
44	RM215	qDTY9.1	Grain yield under drought	Yue *et al.* 2008	150	145
45	RM464	qDTY9.1	Grain yield under drought	Dixit *et al.* 2012	230	220
46	RM316	Chromosome 9	Leaf rolling	Yue *et al.* 2008	195	205
47	RM257	Chromosome 9	Spikelet fertility	Yue *et al.* 2008	115	145
48	RM242	Chromosome 9	Root length	Steele *et al.* 2006	185	205
49	RM213	Chromosome 9	Root penetration	Steele *et al.* 2006	185	235
50	RM219	Chromosome 9	Leaf rolling	Yue *et al.* 2008	200	210
51	RM216	qDTY10.1	Grain yield under drought	Vikram *et al*. 2011	135	145
52	RM228	qDTY10.1	Leaf rolling	Lin *et al*. 2007	105	155
53	RM311	qDTY10.1	% Filled grain	Lin *et al*. 2007	305	320
54	RM271	qDTF10.1	Flowering	Vikram *et al*. 2011	110	120
55	RM171	qDTY10.1	1000 Seed weight	Lin *et al*. 2007	355	365
56	RM484	Chromosome 10	Spikelet fertility	Yue *et al*. 2008	160	150
57	RM21	Chromosome 11	Leaf drying	Yue *et al*. 2008	130	145
58	RM28199	qDTY12.1	Grain yield under drought	Mishra *et al*. 2013	170	160
59	RM28089	qDTY12.1	Grain yield under drought	Dixit *et al*. 2012	210	200
60	RM28048	qDTY12.1	Grain yield under drought	Dixit *et al*. 2012	290	270
61	RM28059	qDTY12.1	Grain yield under drought	Matsumoto *et al*. 2005	400	380
62	RM28064	qDTY12.1	Grain yield under drought	Matsumoto *et al*. 2005	280	270
63	RM28067	qDTY12.1	Grain yield under drought	Matsumoto *et al*. 2005	195	190
64	RM28070	qDTY12.1	Grain yield under drought	Matsumoto *et al*. 2005	170	160
65	RM28079	qDTY12.1	Grain yield under drought	Matsumoto *et al*. 2005	350	300
66	RM28082	qDTY12.1	Grain yield under drought	Matsumoto *et al*. 2005	275	260
67	RM28083	qDTY12.1	Grain yield under drought	Matsumoto *et al*. 2005	100	135
68	RM28088	qDTY12.1	Grain yield under drought	Matsumoto *et al*. 2005	250	235
69	RM28090	qDTY12.1	Grain yield under drought	Matsumoto *et al*. 2005	310	300
70	RM519	qDTY12.1	Grain yield under drought	Yue *et al*. 2008	140	150
71	RM313	qDTY12.1	Grain yield under drought	Temnykh *et al*. 2000	135	130
72	RM309	qDTY12.1	Grain yield under drought	Temnykh *et al*. 2000	180	185
73	RM511	qDTY12.1A	Grain yield under drought	Dixit *et al*. 2012	130	135
74	RM28166	qDTY12.1B	Grain yield under drought	Mishra *et al*. 2013	195	200
75	RM1261	qDTY12.1B	Grain yield under drought	Dixit *et al*. 2012	170	155
76	RM20A	Chromosome 12	Leaf rolling, harvest Index, flowering	Lin *et al*. 2007	235	250
77	RM341	Chromosome 12	1000-seed weight	Lin *et al*, 2007	130	160

showing polymorphism. The mean PIC value observed between the genotypes was 0.375.

DISCUSSION

There are many agronomic/phenotypic traits found in rice breeding that are significantly linked with one or more than one gene/ QTL (Jena *et al*. 2008). For an appropriate screening, validation and mapping of gene(s)/QTL(s) in a mapping population, selection of parents and its validation is important and basic requirement. Primary as well as secondary traits are reported that contribute towards drought tolerance in rice. Many genes and QTLs responsible for secondary characters are reported for tolerance to drought stress (Yue *et al*. 2008; Lin *et al*. 2007; Vikram *et al*. 2011). Two hundred one SSR markers distributed all over the 12 rice chromosomes and reported to be linked with various drought stress characters were selected to study the polymorphism between drought tolerant (CR143-2-2) and susceptible (Krishnahamsa) parents. Also, the parents and RILs developed showed high variation for various drought parameters under rainout shelter.

The two parents were observed to be distinct for various parameters like leaf rolling, leaf drying, biomass, fertility percentage, harvest index, days to 50% flowering, plant height, flag leaf length and width under drought stress as expected (Table 1). There was clear difference for various agro-morphologic traits studied (Table 2). Leaf rolling and leaf drying are two important parameters to assess the drought tolerance of rice lines. The present study could classify the 190 RILs developed into five categories *i.e.*, highly tolerant, tolerant, moderately tolerant, moderately susceptible and highly

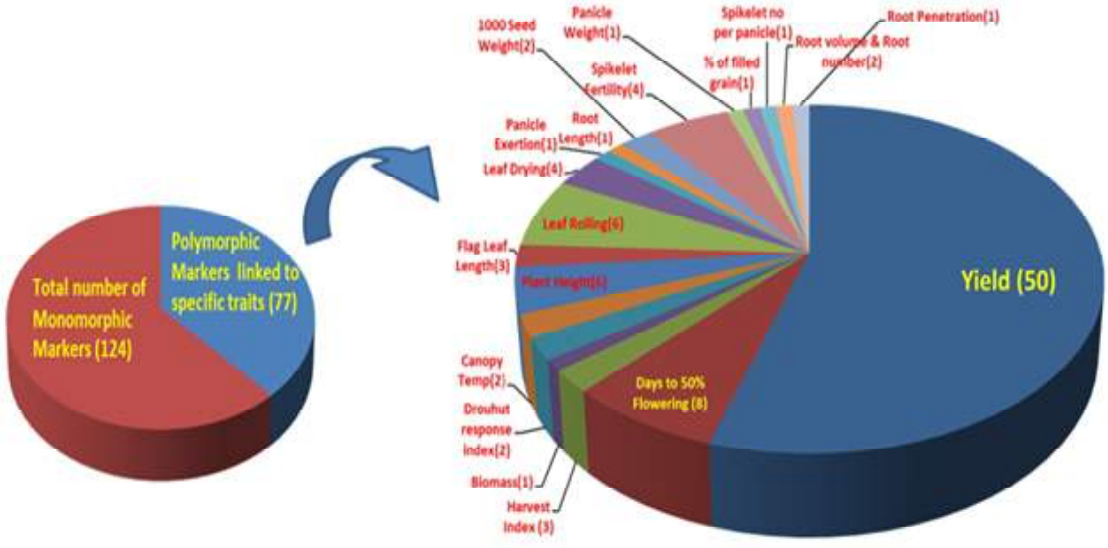

Fig. 1. Percentage of the polymorphic markers linked to their respective phenotypic traits

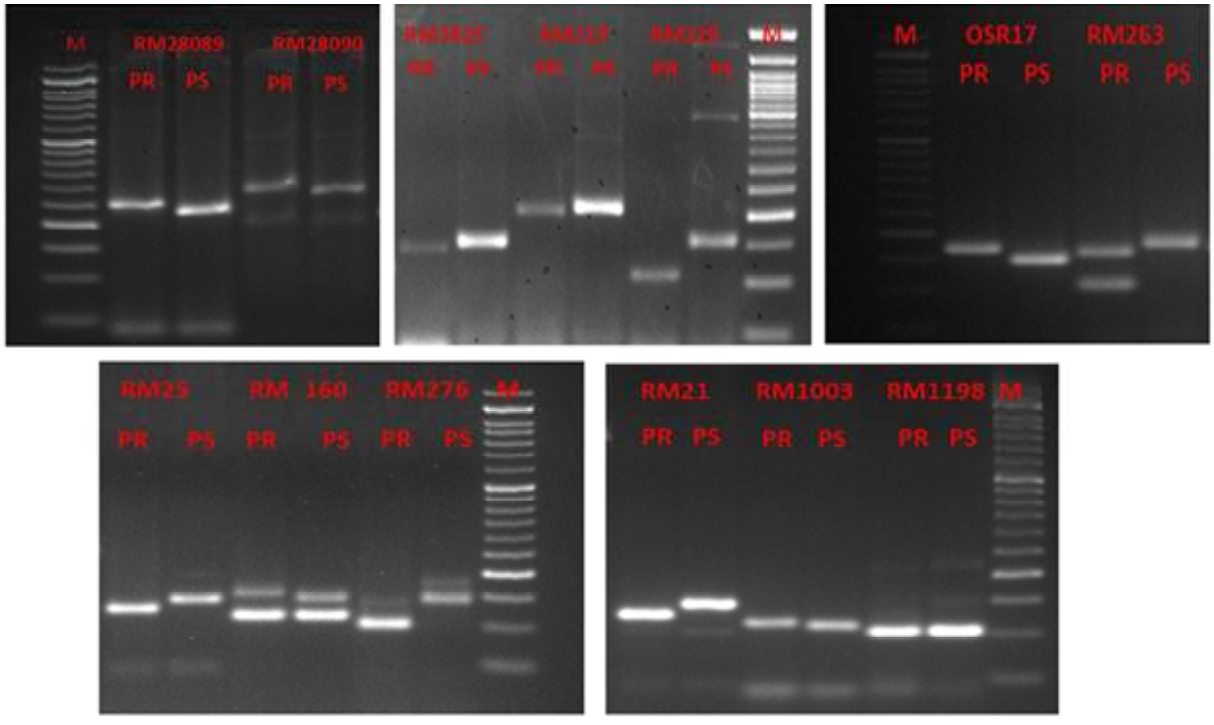

Fig. 2. Representative electrophoregram for polymorphism study (M: 50 bp DNA ladder, PR: Resistant Parent and PS: Susceptible parent)

susceptible based on these two traits (Table 3 and Table 4). Kar and Singh (2016) also categorised the RILs developed from the crosses between BPT5204 and Sahabhagidhan into four groups like highly tolerant, moderately tolerant, moderately susceptible and highly susceptible to drought on the basis of these two traits.

In the present investigation, 94% of the total markers showed clear amplification, while 38% of markers showed polymorphism between the two lines CR143-2-2 and Krishnahamsa (Table 5; Figure 1). As the selected primers belongs to Class I microsatellites that are highly polymorphic in nature showed low PCR failure rate with increased probability of SSR expansion when compared to Class II microsatellites (Cho *et al*. 2000; Temnykh *et al.* 2001; Narsimulu *et al*. 2010).

The 77 markers showing polymorphism are distributed in all the chromosomes except chromosome 4 and 5 (Table 6). The 19 phenotypic traits and the respective polymorphic markers *i.e.,* grain yield under drought (50), days to 50% flowering (8), harvest index (3), biomass (1), drought response index (2), canopy temperature (2), plant height (6), flag leaf length (3), leaf rolling (6), leaf drying (4), panicle exsertion (1), root length (1), 1000-seed weight (2), spikelet fertility (5), panicle weight (1), % of filled grain (1), seed number per panicle (1), root volume and root number (1) and root penetration (1) were observed to be polymorphic whereas the rest did not show either polymorphism or amplification (Figure 1). Earlier studies of Ilango and Sarla (2010) and Kumar *et al.* (2013) showed SSR polymorphism between two or few contrasting parents with an objective to use the polymorphism for selection of target traits or recurrent genome recovery. The markers linked to multiple traits like RM279 linked to yield under drought, drought response index, canopy temperature and flag leaf length; RM263 with grain yield under drought, harvest index observed in the present study indicates the traits are co-inherited and governed by common physiological pathways or gene(s)/QTL(s).

Krishnahamsa is a popular variety of irrigated ecology, whereas CR143-2-2 is a culture for irrigated as well as upland ecology. There may exist many similarities between these genotypes except the drought tolerance. A moderate level of PIC (0.375) was observed possibly may be due to presence of single or few gene pairs for the trait. There are many reports where the PIC ranged from low (Ramakrishnan *et al*. 2016) to high values (Gu *et al*. 2005; Seetharam *et al*. 2009).

These two genotypes CR143-2-2 and Krishnahamsa can be used for developing mapping population for drought stress characters as well as a gene pyramiding programme for improving the drought tolerance character of the popular variety Krishnahamsa. The RILs developed from these two parents that were phenotyped as highly tolerant to highly susceptible are suitable population to map new genes/QTLs for drought stress tolerance. The polymorphic markers obtained in the present investigation will be used to validate the developed RILs from these two parents and mapping of QTLs for drought tolerance. Also, in a gene pyramiding programme, these markers can be useful to select the particular traits with which they are linked.

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