International Journal of Plant & Soil Science

Volume 35, Issue 22, Page 275-284, 2023; Article no.IJPSS.109521
ISSN: 2320-7035

Evaluation for Parental Polymorphism and Identification of Microsatellites Linked to Drought Tolerance in Rice (*Oryza sativa* L.)

Shankar C. [a*], Abida P. S. [a], Jiji Joseph [b], Rose Mary Francies [c], Deepu Mathew [a] and Beena R. [d]

[a] Centre for Plant Biotechnology and Molecular Biology, Kerala Agricultural University, Thrissur, Kerala–680656, India.
[b] Department of Plant Breeding and Genetics, Kerala Agricultural University, Thrissur, Keral–680656, India.
[c] Agricultural Research Station, Mannuthy, Thrissur, Kerala, India.
[d] Department of Plant Physiology, Kerala Agricultural University, Vellayani, Kerala, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors SC, APS, JJ, RMF, DM and BR conceived and planned this research. Author SC wrote the first draft and performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i224134

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/109521

Original Research Article

Received: 11/09/2023
Accepted: 16/11/2023
Published: 21/11/2023

ABSTRACT

Aims: To identify the ready-to-use polymorphic microsatellite markers associated with drought tolerance for marker-assisted backcross breeding through a polymorphism survey between Jyothi and Chuvannamodan rice varieties.
Place and Duration of Study: Centre for plant biotechnology and molecular biology, Thrissur, Kerala, India, during January to May 2023.

**Corresponding author: E-mail: shankarperu55@gmail.com;*

Methodology: The genomic DNA of Jyothi and Chuvannamodan was isolated by following CTAB (Cetyltrimethyl Ammonium Bromide) method with modification. Isolated DNA from both varieties was subjected to PCR amplification using 208 Simple Sequence Repeats (SSRs) primer pairs distributed in 12 chromosomes. The amplified PCR products were electrophoresed in 3% agarose gel and separated fragments were visualized and documented in the Gel Documentation System. Different allele sizes produced by the same SSRs primers between two varieties are identified as polymorphic markers. Polymorphism per-cent was calculated, and frequency distribution and chromosome distribution of polymorphic markers were analyzed.

Results: A total of 208 SSR primers were surveyed for the parental polymorphism. Out of which, 85 SSR primers exhibited clear polymorphism between Jyothi and Chuvannamodan and the remaining 123 were monomorphic primers. The amplicon size ranged from 83bp (RM430) to 495bp (RM18919) among the different primers. The survey revealed maximum parental polymorphism on chromosome 4 (69.23%), followed by chromosome 5 (64.28%), and minimum polymorphism on chromosome 8 (21.73%). The average per cent of polymorphism between the parents was 40.86%. Among the 85 polymorphic markers, 66 had dinucleotide repeats, 17 had trinucleotide repeats, and 1 had tetranucleotide repeats.

Markers with dinucleotide repeats showed higher level of polymorphism. A group of 28 polymorphic markers were identified to be linked with traits including root-related traits, grain yield, leaf rolling and leaf drying under drought conditions.

Conclusion: The polymorphic markers identified in the present study form the basis for tagging drought-tolerant QTLs/genes, fine mapping of those genes, and subsequently in marker-assisted breeding programs. The polymorphic markers linked with the QTLs for grain yield under drought can be pyramided in Jyothi through marker-assisted backcross breeding.

Keywords: Simple sequence repeats; chuvannamodan; jyothi; parental polymorphism; marker-assisted backcrossing.

1. INTRODUCTION

Rice botanically known as *Oryza sativa* L., is a staple food crop for the majority of the world's population. It is a widely grown crop, in Asian nations alone accounting for more than 90% of total production worldwide [13]. It is a member of the Poaceae family, which also includes 12 other genera [43]. Jyothi is the ruling variety of Kerala released from the Regional Agricultural Research Station, Pattambi, India in 1974. This variety has a poor spikelet fertility percentage under drought, which shows that it is highly susceptible to drought. Traditional rice Chuvannamodan is a short-duration variety of 105 to 110 days with an average yield of 2200 kg ha^{-1}. A study evaluated 15 cultivars for drought tolerance and found Chuvannamodan performed well for drought-tolerant-related traits like transpiration rate, stomatal conductance, and membrane stability [2]. Furthermore, proteome analysis indicated that Chuvannamodan was highly tolerant to drought. Among 80 traditional and high-yielding cultivars, Chuvannamodan was found as one of the highly drought-tolerant varieties [4,19].

The advent of genetic markers has significantly boosted the efficiency of plant breeding by accurately following the inheritance of various important traits. They have proven to be useful tools for assessing genetic diversity and understanding genetic relationships within and across species [6]. There are several genetic markers for classifying genotypes and are not influenced by the environment. Marker data helps to analyze the genetic similarities and differences between the genotypes which will be of great interest in crop breeding programs [12]. The differences are known as molecular markers because they are often linked with specific genes and can act as a signpost to those genes. When such markers are very tightly linked to genes of interest, they can be used for the indirect selection of desirable alleles, which is known as marker-assisted selection [44,46]. These markers are being adapted by researchers as an effective tool for crop breeding programs like selecting suitable plants for hybridization, gene tagging, QTL mapping, DNA fingerprinting, etc.

Simple Sequence Repeat (SSR) markers are short, tandemly repeating DNA sequences of 2 to 6 base pairs. These microsatellites are the type of a variable number of tandem repeats (VNTR) that are abundant, multi-allelic, highly polymorphic, and co-dominant in the genome. Polymerase Chain Reaction (PCR) amplifications with primers designed from flanking regions of

these variable number of tandem repeat (VNTR) enable targeted amplification of their locus. The size of the PCR products amplified would depend on the number of repetitive DNA units in the VNTR alleles. Due to the variation in the number of repeat motifs, the amplicons from diverse genotypes will show length polymorphism [7]. Molecular markers, when combined with linkage maps and genomics, aid in altering and improving the useful traits in plants [31].

In Marker-assisted breeding (MAB), foreground selection and background selection are two important aspects performed in either concurrent or consecutive generations. The markers used in MAB must be polymorphic for efficient and accurate selection [16]. The use of markers in MAB considerably saves time as well as increases efficiency compared to conventional backcrossing. Hence, the present study was conducted to identify the polymorphic SSR markers between the rice varieties Jyothi and Chuvannamodan.

2. MATERIALS AND METHODS

2.1 Rice Varieties Used in the Study

Jyothi is one of the high-yielding rice varieties, mostly suitable for direct sowing or transplanting in the Kole and Kuttanad regions of Kerala, India. Traditional rice Chuvannamodan is a short-duration variety but tolerant to drought conditions.

2.2 Isolation of Genomic DNA

The genomic DNA was isolated from the young leaves of Jyothi and Chuvannamodan by using the CTAB method with modification [25,38]. 100mg of leaf tissues were cryogenically grounded in pestle and mortar with 2% PVP and preheated 2X DNA extraction buffer (2% CTAB, 100mM Tris Hcl, 20mM EDTA (Ethylene Diamine Tetraacetic Acid), and 1.4M NaCl) at 65^{o} C. The quality of isolated DNA was assessed by 0.8% of agarose gel electrophoresis. The purity and the quantity of the DNA were checked at optical density (OD) values of A260 and A280 nm by Nano spectrophotometer.

2.3 Parental Polymorphism Survey

A total of 208 SSR markers were used to generate polymorphism between Jyothi and Chuvannamodan. All the RM primer sequences and chromosome locations were retrieved from the https://www.gramene.org/ database covering 12 chromosomes of rice. Primers were synthesized with the help of Vision Scientific and used in the parental polymorphic study. Amplification of genomic DNA was performed in the Biorad T100 thermal cycler. PCR reactions were carried with 10 µl volume containing sterile water of 4.9 µl; 10X Taq buffer with 15mM $MgCl_2$ of 1.0 µl; 10mM dNTPs of 1.0 µl; 3U Taq DNA polymerase of 0.1 µl; 10 µM forward primer of 0.5 µl; 10 µM reverse primer of 0.5 µl and 50 ng DNA template of 2 µl. The template DNA was amplified with a PCR reaction program a) initial denaturation at 95^{o}C for 3 minutes, b) denaturation at 94°C for 50 seconds, c) primer annealing at 55°C for 30 seconds, d) primer elongation at 72°C for 1 minute, e) final extension at 72°C for 10 min., and a final hold at 4 °C until removal. 30 cycles of steps b) to d) were used to amplify template DNA. 3% agarose gel electrophoresis was used to examine the amplified PCR products. The amplified products were then visualized using the gel documentation unit. The banding pattern was observed and recorded for further analysis.

3. RESULTS AND DISCUSSION

The present study was conducted to identify the informative polymorphic microsatellite (SSR) markers. Parental polymorphism was generated between Jyothi and Chuvannamodan by using SSR primers for the marker-assisted backcross breeding (MABB) program.

Out of 208 SSR primers, 85 were found to be polymorphic between Jyothi (drought susceptible) and Chuvannamodan (drought tolerant), and 123 primers were monomorphic. The amplicon size ranged from 83bp (RM430) to 495bp (RM18919) among the different RM primers. RM212, RM490, RM3412, RM339, RM493, RM10871, RM10745, RM12091, RM264 are the some of polymorphic markers (Fig. 1). Among the polymorphic markers, ten were on chromosome 1, six on chromosome 2, seven on chromosome 3, nine on chromosome 4, nine on chromosome 5, eleven on chromosome 6, five on chromosome 7, five on chromosome 8, eight on chromosome 9, five on chromosome 10, six on chromosome 11 and four on chromosome 12.

The survey revealed 40.86 per-cent average polymorphism between the Jyothi and Chuvannamodan. The percentage of polymorphism depends on the number of SSR primers surveyed on the two varieties. The

maximum polymorphic percentage was found on chromosomes 4 (69.23 %) followed by on chromosome 5 (64.28%) while minimum polymorphic percentage was found on chromosome 8 (21.73%) followed by on chromosome 7 (29.41%) (Table 1). Similar to the findings, a study reported 108 polymorphic SSR primers between Pratikshya and CR Dhan 801 with average parental polymorphism at 21.28 per-cent [21]. A polymorphism study found 31.7 per-cent by using 230 SSRs between IR5541904 and Super Basmati for utilization in the identification of QTLs under drought condition [32]. Another study reported 40.5 per-cent of polymorphism levels between *O. sativa* cv. IR64 and *O. glaberrima* parents using 464 SSRs for marker-assisted introgressing drought tolerant traits through backcross breeding [5].

SSR-based polymorphism is defined by the nature of repeat motifs like di, tri, and tetra in the genome. In the present study, dinucleotide repeats in the microsatellite region showed greater polymorphism than tri and tera-nucleotide repeats. It may be due to the abundance of dinucleotide repeats distributed through the genome of rice. The repeats can be differentiated based on highly repetitive sequences and moderately repetitive sequences for a possible polymorphism. Among 85 polymorphic markers, 66 markers contained dinucleotide repeats, 17 markers had trinucleotide repeats and 1 had tetranucleotide repeats. The frequency distribution of repeat motifs revealed that among the 66 dinucleotides, the GA motif occurred more frequently (20 times) than others, accounting for 33.33 per-cent, followed by CT repeats of 18 times, accounting for 30 per-cent (Fig. 2). Similar to the result, 36 per-cent of SSR primers correspond to poly (GA) motifs in developing 2740 microsatellite markers for rice [22]. The prevalence of dinucleotide repeats in parental polymorphism was reported by previous researchers [15, 21,23]. Out of 17 trinucleotide repeats, CTT and AAG repeated 8 and 3 times, respectively. The frequency of CTT accounted for 9.41 per-cent of total polymorphism. Other trinucleotide repeats AAT, TAA, GCT, CGA, CTC, and ATC were repeated 1 time. The RM markers with GA, AT, ATT, and CTT repeat motifs will show the greatest variation in allele size. In this regard, our findings are also analogous to previously published SSR diversity data in rice [7, 8] which revealed a wide range of allelic variations in size for markers containing GA, AT, ATT, and CTT repeat motifs. Only one marker (RM10745) with a TATG repeat motif was found polymorphic in the study. The chromosome-wise distribution of all the polymorphic markers found in the study with physical position [22] is given in the form of a physical map (Fig. 3).

Out of 85 polymorphic markers, 28 markers were reported previously to be linked with drought-tolerant traits. A polymorphic marker RM208 on chromosome 2 was reported to be tightly linked with yield under drought [5]. On chromosome 3, a novel genomic region was identified to be flanked by RM168 and RM520 for the majority of the root-related traits [32], those markers are polymorphic in our study. The development of a root system is regarded as a key characteristic in rice for drought mitigation. In the same study, RM168 was also found to be associated with total water uptake which will help to overcome stress. A study revealed three polymorphic

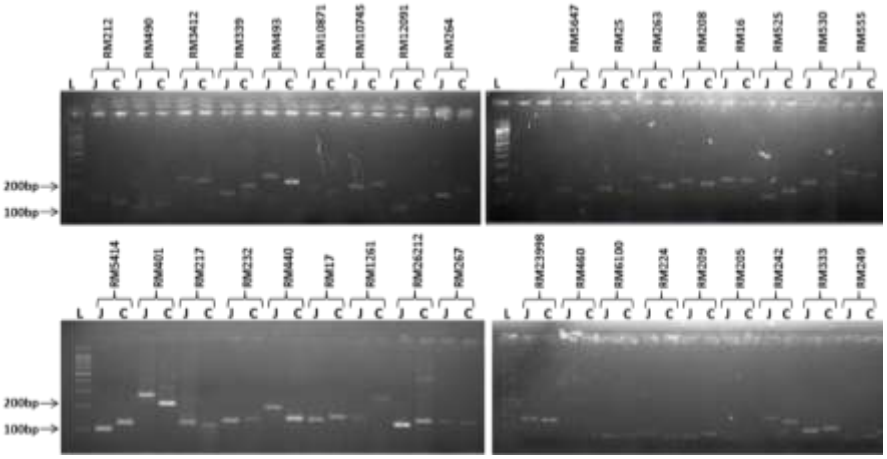
Table 1. Chromosome-wise polymorphic primers and polymorphic per-cent

Chromosome	No. of SSR markers used	No. of identified polymorphic markers	Polymorphism per cent
1	32	10	31.25
2	17	6	35.29
3	16	7	43.75
4	13	9	69.23
5	14	9	64.28
6	22	11	50.00
7	17	5	29.41
8	23	5	21.73
9	19	8	42.10
0	11	5	45.45
1	11	6	54.54
2	13	4	30.76
Total	**208**	**85**	**40.86**

Table 2. Association of identified polymorphic markers with different traits under drought condition

S.No	Polymorphic markers	Linkage group	Traits linked	Donor	Recipient
1	RM212	1	No. of grains per panicle [45] Biomass [24]	Zhenshan 97 Nootripathu	Minghui 63 IR20
2	RM3825	1	Leaf rolling and leaf drying [35] Panicle length [36]	Nootripathu Banglami	IR20 Ranjit
3	RM431	1	Grain yield [9] Grain yield [40]	Vandana ARC 10372	Way Rarem Ranjit
4	RM297	1	Harvest index [10]	Vandana	Way Rarem
5	RM490	1	Seedlings germination [26]	Ahlamitarum	Neda
6	RM493	1	Leaf rolling [47]	-	-
7	RM12091	1	Grain yield [14]	.Dhagaddeshi	Swarna and IR64
8	RM302	1	leaf rolling and leaf drying [35]	Nootripathu	IR20
9	RM263	2	Grain yield and days to flowering [34]	Kali Aus/2	IR64
10	RM208	2	Yield per plant Panicle fertility [5]	*O. glaberrima*	IR64
11	RM266	2	Grain weight [48]	TQ line	IRBB line
12	RM555	2	Grain yield [9]	Apo	Swarna
13	RM22	3	Grain yield [33]	WAB 450-I-B-P-157-2-1	Swarna
14	RM520	3	Grain yield [39]	Apo	IR64
15	RM168	3	Deep root length[32]	IR55419-04	Super Basmati
16	RM279	3	Grain weight[37]	-	-
17	RM518	4	Grain yield [27]	Kali Aus	IR64 and MTU1010
18	RM586	6	Grain yield [11]	IR55419-04/2	TDK1
19	RM587	6	Grain yield [11]	IR55419-04/2	TDK1
20	RM3	6	Grain yield [11,20]	IR55419-04/2 Milyang 23	TDK1 Taichung 189
21	RM339	8	Grain yield[42]	Swarna	Basmati334
22	RM566	9	Grain yield[10]	Aday sel	IR64
23	RM242	9	Root traits [17]	AERON1	MRQ74

S.No	Polymorphic markers	Linkage group	Traits linked	Donor	Recipient
24	RM257	9	Relative water content [3]	CR 143-2-2	Krishnahamsa
25	RM216	10	Grain yield [41]	N22	MTU1010
26	RM209	11	Grain yield [29]	Norungan	IR62266-42-6-2
27	RM17	11	Relative water content [30]	TKM9	Norungan
28	RM1261	12	Grain yield [1]	IR84984-83-15-481-B	FUNAABOR-2



L - 100bp ladder; J - Jyothi; C - Chuvannamodan

Fig. 1. Amplification profile of polymorphic markers

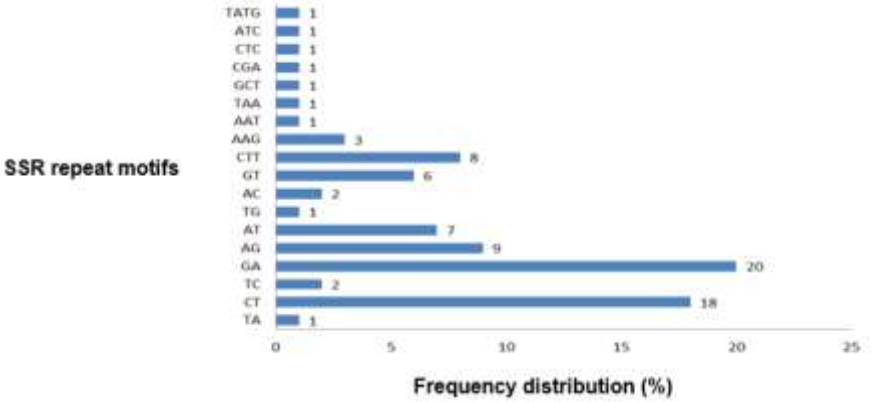


Fig. 2. Frequency distribution of polymorphic SSR repeat motifs

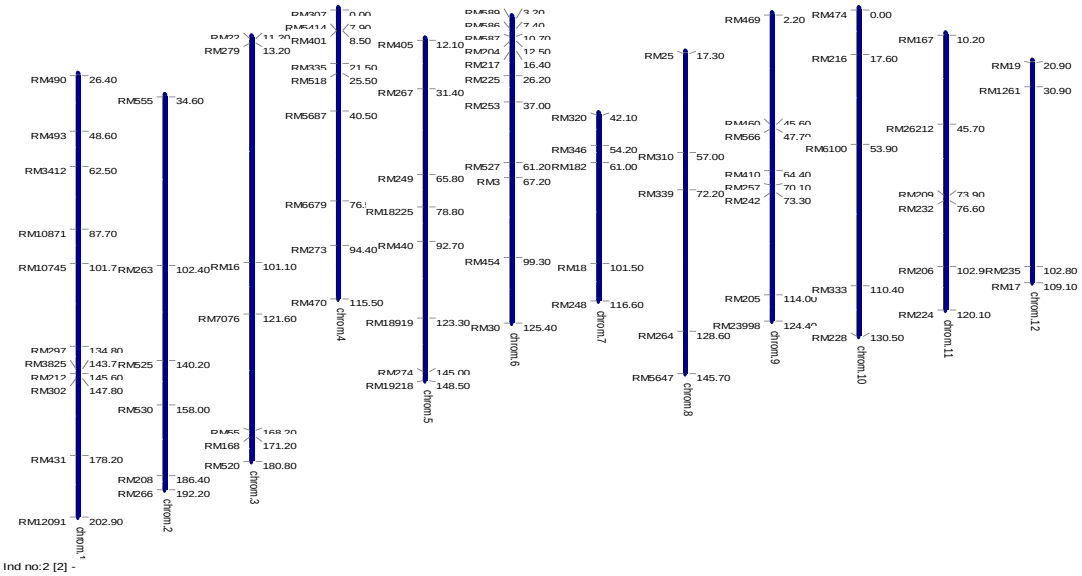


Fig. 3. Chromosome-wise distribution of 85 polymorphic SSR markers

markers namely, RM212, RM302, and RM3825 on chromosome 1 linked to drought resistance QTLs like deep root mass, grain yield, deep root to shoot ratio, relative water content, and leaf drying [18,28]. The polymorphic markers associated with yield and other traits under drought were reported in the previous study listed in the Table 2.

4. CONCLUSION

All the identified polymorphic markers in the present study between Jyothi and Chuvannamodan may or may not be associated with the drought-tolerant traits. Hence, QTL analysis for various traits should be performed in mapping populations developed from these parents to identify the putative markers for different traits under drought condition. The identified 85 polymorphic SSR markers can also be used in diversity analysis, linkage studies, and bulk segregant analysis. Jyothi as the recurrent parent and Chuvannamodan as the donor parent can be used for developing the backcross population to introduce the drought tolerant traits from the donor to the recurrent parent. These polymorphic markers can be used in foreground and background selection during marker-assisted backcross breeding programs.

ACKNOWLEDGEMENTS

The authors express their acknowledgement to Centre for Plant Biotechnology and Molecular Biology, Kerala Agricultural University for providing infrastructural and support for carrying out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anyaoha CO, Fofana M, Gracen V, Tongoona P, Mande S. Introgression of two drought QTLs into FUNAABOR-2 early generation backcross progenies under drought stress at reproductive stage. Rice Sci. 2019;26(1):32-41.
2. Babu PN. Identification and characterisation of traditional rice genotypes for drought tolerance through proteomic approach. M.Sc. (ag.) [thesis]. Thrissur: Kerala Agricultural University. 2014;75.
3. Barik SR, Pandit E, Pradhan SK, Singh S, Swain P, Mohapatra T. QTL mapping for relative water content trait at reproductive stage drought stress in rice. Indian J Genet Plant Breed. 2018;78(04):401-8.
4. Beena R, Praveenkumar VP, Vighneswaran V, Narayankutty MC. Bulked line analysis: a useful tool to identify microsatellite markers linked to drought tolerance in rice. Indian J Plant Physiol. 2018;23(1):7-15.
5. Bimpong IK, Serraj R, Chin JH, Ramos J, Mendoza EMT, Hernandez JE, et al. Identification of QTLs for drought-related traits in alien introgression lines derived from crosses of rice (*Oryza sativa* cv. IR64) × O. glaberrima under lowland moisture stress. J Plant Biol. 2011;54(4):237-50.
6. Chakravarthi BK, Naravaneni R. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L.). Afr J Biotechnol. 2006;5(9).
7. Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theor Appl Genet. 1997;95(4):553-67.
8. Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, McCouch S, R, Park W, D, Ayres N, Cartinhour S. Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). Theor. Appl. Genet. 2000;100(5):713-722.
9. Dixit S, Mallikarjuna Swamy BP, Vikram P, Bernier J, Sta Cruz MT, Amante M, et al. Increased drought tolerance and wider adaptability of qDTY 12.1 conferred by its interaction with qDTY 2.3 and qDTY 3.2. Mol Breeding. 2012;30(4):1767-79.
10. Dixit S, Swamy BP, Vikram P, Ahmed HU, Sta Cruz MT, Amante M, et al. Fine mapping of QTLs for rice grain yield under drought reveals sub-QTLs conferring a response to variable drought severities. Theor Appl Genet. 2012; 125(1):155-69.
11. Dixit S, Singh A, Sta Cruz MT, Maturan PT, Amante M, Kumar A. Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. BMC Genet. 2014;15:16.
12. Düzyaman E. Phenotypic diversity within a collection of distinct okra (*Abelmoschus esculentus*) cultivars derived from Turkish land races. Genet Resour Crop Evol. 2005; 52(8):1019-30.
13. Fukagawa NK, Ziska LH. Rice: importance for global nutrition. J Nutr Sci Vitaminol (Tokyo). 2019;65;Suppl:S2-3.
14. Ghimire KH, Quiatchon LA, Vikram P, Swamy BPM, Dixit S, Ahmed H et al. Identification and mapping of a QTL (qDTY1.1) with a consistent effect on grain yield under drought. Field Crops Res. 2012;131:88-96.
15. Grover A, Aishwarya V, Sharma PC. Biased distribution of microsatellite motifs in the rice genome. Mol Genet Genomics. 2007; 277(5):469-80.

16. Jiang GL. Molecular markers and marker-assisted breeding in plants. Pl. breed. 2013;3:45-83.
17. Jalil MA, Juraimi AS, Yusop MR, Uddin MK, Hakim MA. Introgression of root trait genes for drought tolerance to a Malaysian rice variety by marker-assisted backcross breeding. Int J Agric Biol. 2018;20(1):119-26.
18. Kanagaraj P, Prince KSJ, Sheeba JA, Biji KR, Paul SB, Senthil A et al. Microsatellite markers linked to drought resistance in rice (*Oryza sativa* L.). Curr Sci. 2010;836-9.
19. Kurata N, Nonomura KI, Harushima Y. Rice genome organization: The centromere and genome interactions. Ann Bot. 2002;90(4):427-35.
20. Lin MH, Lin CW, Chen JC, Lin YC, Cheng SY, Liu TH, et al. Tagging rice drought-related QTL with SSR DNA markers. 2007;4(1):65-76.
21. Majhi PK, Singh SK, Anandan A, Khaire AR, Korada M, Habde SV, et al. Parental polymorphism survey for evaluation and selection of contrasting parents for drought tolerance in rice (*Oryza sativa* L.) by using SSR markers. Int J Environ Clim. 2022;12(11):2507-19.
22. McCouch SR, Chen X, Panaud O, Temnykh S, Xu Y, Cho YG, et al. Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Mol Biol. 1997;35(1/2):89-99.
23. McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M et al. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res. 2002;9(6):199-207.
24. Michael Gomez S, Manikanda Boopathi N, Satheesh Kumar S, Ramasubramanian T, Chengsong Z, Jeyaprakash P et al. Molecular mapping and location of QTLs for drought-resistance traits in indica rice (*Oryza sativa* L.) lines adapted to target environments. Acta Physiol Plant. 2010;32(2):355-64.
25. Murray DC, Pearson SG, Fullagar R, Chase BM, Houston J, Atchison J et al. High-throughput sequencing of ancient plant and mammal DNA preserved in herbivore middens. Qua. Sci Rev. 2021;58:135-45.
26. Noryan M, Hervan IM, Sabouri H, Kojouri FD, Mastinu A. Drought resistance loci in recombinant lines of Iranian Oryza sativa L. in germination stage. BioTech (Basel). 2021;10(4).
27. Palanog AD, Swamy BPM, Shamsudin NAA, Dixit S, Hernandez JE, Boromeo TH et al. Grain yield QTLs with consistent-effect under reproductive-stage drought stress in rice. Field Crops Res. 2014;161:46-54.
28. Prathi N, B. Identification and characterization of traditional rice genotypes for drought tolerance through proteomic approach [Ph.D. thesis]. Thrissur: Kerala Agricultural University. 2015;163.
29. Rajurkar AB, Muthukumar C, Ayyenar B, Thomas HB, Chandra Babu R. Mapping consistent additive and epistatic QTLs for plant production traits under drought in target populations of environment using locally adapted landrace in rice (*Oryza sativa* L.). Plant Prod Sci. 2021;24(3):388-403.
30. Ramchander S, Raveendran M, Robin S. Mapping QTLs for physiological traits associated with drought tolerance in rice (*Oryza sativa* L.). J Investig Genom. 2016;3(3);00052.
31. Ribaut JM, de Vicente MC, Delannay X. Molecular breeding in developing countries: Challenges and perspectives. Curr Opin Plant Biol. 2010;13(2):213-8.
32. Sabar M, Shabir G, Shah SM, Aslam K, Naveed SA, Arif M. Identification and mapping of QTLs associated with drought tolerance traits in rice by a cross between Super Basmati and IR55419-04. Breed Sci. 2019;69(1):169-78.
33. Saikumar S, Kalmeshwer Gouda PK, Saiharini A, Varma CMK, Vineesha O, Padmavathi G, et al. Major QTL for enhancing rice grain yield under lowland reproductive drought stress identified using an O. sativa/O. glaberrima introgression line. Field Crops Res. 2014;163:119-31.
34. Sandhu N, Singh A, Dixit S, Sta Cruz MT, Maturan PC, Jain RK, et al. Identification and mapping of stable QTL with main and epistasis effect on rice grain yield under upland drought stress. BMC Genet. 2014;15:63.
35. Salunkhe AS, Poornima R, Prince KSJ, Kanagaraj P, Sheeba JA, Amudha K et al. Fine mapping QTL for drought resistance traits in rice (*Oryza sativa* L.) using bulk segregant analysis. Mol Biotechnol. 2011;49(1):90-5.
36. Sharma V, Verma RK, Dey PC, Chetia SK, Baruah AR, Modi MK. QTLs associated with yield attributing traits under drought stress in upland rice cultivar of Assam. ORYZA- An Internatio Journ on Rice. 2017;54(3):253-7.
37. Tabkhkar N, Rabiei B, Samizadeh Lahiji H, Hosseini Chaleshtori M. Genetic variation and association analysis of the SSR markers linked to the major drought-yield QTLs of rice. Biochem Genet. 2018;56(4):356-74.
38. Tiwari S, Tomar RS, Tripathi MK, Ahuja A. Modified protocol for plant genomic DNA isolation. Indian Res J Genet Biogr. 2017;9(4):478-85.
39. Venuprasad R, Bool ME, Dalid CO, Bernier J, Kumar A, Atlin GN. Genetic loci responding to two cycles of divergent selection for grain yield under drought stress in a rice breeding population. Euphytica. 2009;167(2):261-9.
40. Verma RK, Chetia SK, Dey PC, Sharma V, Baruah AR, Modi MK. Development of advanced breeding lines for high grain yield under drought stress in elite rice genetic background. Res Crops. 2017;18(4):705-10.
41. Vikram P, Swamy BM, Dixit S, Ahmed HU, Teresa Sta Cruz M, Singh AK, et al. qDTY 1.1, a major QTL for rice grain yield under reproductive-stage drought stress with a

consistent effect in multiple elite genetic backgrounds. BMC Genet. 2011;12(1):1-15.
42. Vikram P, Swamy BPM, Dixit S, Ahmed H, Cruz MTS, Singh AK, et al. Bulk segregant analysis: "An effective approach for mapping consistent-effect drought grain yield QTLs in rice". Field Crops Res. 2012;134:185-92.
43. Wei X, Huang X. Origin, taxonomy, and phylogenetics of rice. Rice. 2019:1-29.
44. Wilcox M. Notes on Atriplex (Amaranthaceae) species and hybrids, particularly A. littoralis and the hybrid A. littoralis x A. prostrata (A. x hulmeana). Brit. IRI. Bot. 2022;42.
45. Xing W, Zhao H, Zou D. Detection of main-effect and epistatic QTL for yield-related traits in rice under drought stress and normal conditions. Can J Plant Sci. 2014;94(4): 633-41.
46. Yu J, Wang J, Lin W, Li S, Li H, Zhou J et al. The genomes of Oryza sativa: A history of duplications. PLOS Biol. 2005;3(2):e38.
47. Zhang G, Hou X, Wang L, Xu J, Chen J, Fu X, et al. Photo-sensitive leaf rolling 1 encodes a polygalacturonase that modifies cell wall structure and drought tolerance in rice. New Phytol. 2021;229(2):890-901.
48. Zhang H, Zhu YJ, Zhu AD, Fan YY, Huang TX, Zhang JF et al. Fine-mapping of qTGW2, a quantitative trait locus for grain weight in rice (*Oryza sativa* L.). PeerJ. 2020;8:e8679.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/109521