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Research Article

Molecular characterization of rice (*Oryza sativa* L.) genotypes for drought tolerance using two SSR markers

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Abstract
Plant growth as well as productivity of rice (*Oryza sativa* L.), the major cereal and staple food of millions, is significantly affected by numerous biotic and abiotic stresses. Among these, drought stress is one of the major constraints for production and yield stability of rice in rainfed ecosystem. Hence rice varieties/genotypes with drought tolerance must be identified urgently. The main objectives of the present study were screening of seedlings of various rice genotypes for drought tolerance by conventional method and molecular characterisation of selected rice genotypes for drought tolerance using microsatellite markers. Twenty one rice genotypes obtained from the Plant Breeding Division, RARS, Pattambi, were used for the study. Initially, seedlings of these genotypes were subjected to conventional drought screening with PEG-6000 (6 MPa and 8 MPa). Out of the 21 rice genotypes, only fourteen genotypes were selected based on seedling vigour index and used for molecular characterisation. DNA extraction of samples was performed by CTAB method followed by PCR amplification with two specific SSR markers, *viz.*, RM103 and RM212. Monomorphic bands were observed with RM103 for all the fourteen genotypes; hence it cannot be considered as a suitable marker for drought tolerance in these genotypes. Polymorphic bands were observed in the studied genotypes for the marker RM212. However, linkage between RM212 marker with the seedling vigour index under drought stress was not significant. Hence, further research with other linked SSR markers must be carried out for marker assisted selection for drought tolerance in these rice genotypes.

Key words
Drought, DNA extraction, MAS, molecular, seedling, conventional, PCR, PEG, SSR, tolerance

Introduction

Rice (*Oryza sativa* L.) is the primary source of food for more than 60% of world's population. Plant growth as well as productivity of rice is significantly affected by numerous biotic and abiotic stresses. Sensitivity of rice plants to various abiotic stresses was reported by Gao *et al*. (2007). Among these, drought is one of the major constraints for production and yield stability of rice, especially in rainfed ecosystem. Hence rice varieties /genotypes with drought tolerance must be identified urgently, which can be used for direct cultivation and also as donors in breeding programmes for developing high yielding varieties with drought tolerance.

Molecular markers are useful for detecting high degree of polymorphism in rice (Ni *et al*., 2002, Okoshi *et al.*, 2004) and hence are ideal for studies on genetic diversity. Drought is difficult to manage through conventional phenotypic selection and is one of the most ideal traits for improvement through MAS (Collins *et al*., 2008). The recent identification of major QTLs governing grain yield under drought (Kumar *et al*., 2007; Venuprasad *et al*., 2009) has made possible the use of marker assisted selection (MAS) for improving drought tolerance. RM212 marker was reported to be linked to root length (Boopathi, 2004), osmotic adjustment (Robin *et al*., 2003), deep root mass and plant height (Hittalmani *et al*., 2003).

The main objectives of the present study were screening of seedlings of various rice genotypes for drought tolerance by the conventional method with PEG and molecular characterisation of selected rice genotypes for drought tolerance using SSR markers.

Material and Methods

The present study was conducted at the Division of Plant Breeding, RARS, Pattambi, India during April-June, 2016.

Conventional screening for drought tolerance with PEG: Twenty one rice genotypes obtained from the Division of Plant Breeding and Genetics, Regional Agricultural Research Station (RARS) Pattambi were used for the study (Table 1.)

Ten seeds each of the 21 rice genotypes were placed on germination paper, moistened with required strength of Poly Ethylene Glycol (PEG-6000) to induce drought stress (Fig. 1). Two concentrations of PEG-6000 *viz*., 6 MPa and 8 MPa were used, along with a control. Seeds on the germination paper were daily sprayed with respective solution. Details of germination and seedling growth (length of root and shoot) were observed on fifth day, tenth day and fifteenth day. Germination percentage and seedling length were calculated and seedling vigour index was estimated as detailed below:

Seedling Vigour Index = Germination percentage x Seedling length (cm)

where,
Germination percentage = (No. of germinated seeds/ Total no. of seeds) ×100

Seedling length (cm) = Root length (cm) + Shoot length (cm)

Rice genotypes selected based on seedling vigour index were used for further analysis.

Molecular characterization: DNA of the selected rice genotypes was isolated by CTAB method. 0.1g of plant tissue of all samples was grinded to a fine paste in approximately 1ml of pre-warmed CTAB buffer using a sterile mortar and pestle, and added small amount of PVP while grinding. CTAB/plant extract mixture was transferred to a microfuge tube and incubated at 65˚C for 30min in a waterbath. After cooling, equal volume of chloroform : isoamyl-alcohol (24:1) was added and mixed the solution. The samples were centrifuged at 15,000 rpm for 15 min. at 4˚C. The aqueous layer was collected and added 2.5µl RNase (25µg/ml) for 0.5ml crude DNA, mixed and incubated for 1hr at 37˚C. Then, 300-400µl of chloroform : isoamyl-alcohol was added, mixed by inverting for 15min. and centrifuged at 10,000 rpm for 2min at 4˚C. The supernatant was collected, double the volume of absolute alcohol (100%) was added and centrifuged at 10,000 rpm for 5min at 4˚C. Supernatant was removed, washed the pellet with 70% ethanol and centrifuged at 13,000 rpm for 10min at 4˚C and air dried the pellet. 50µl of sterile distilled water was added and stored the microfuge tubes at 4˚C. Quality of the isolated DNA was confirmed by Agarose gel electrophoresis method.

DNA samples were then subjected to PCR amplification with the respective primers of two SSR markers *viz*., RM103 and RM212 (Table 2) at prescribed conditions. Then the PCR products were subjected to Agarose gel electrophoresis (2%).

Results and Discussion

Use of PEG for inducing stress was suggested by several researchers (Pandey *et al*., 2004; Chutia and Borah, 2012). PEG-based *in vitro* screening was effective in screening large sets of germplasm for drought tolerance with good accuracy (Kulkarni *et al.*, 2007).

Notable level of drought tolerance was observed in the early-maturing *aus* and *indica* varieties traditionally grown in the plateau region of Eastern India, such as N22 and Dehula (Lafitte and Courtois, 2002). Among the *O. glaberrima* accessions evaluated for drought tolerance, 32 were highly resistant and 217 were moderately resistant (Jones *et al*., 1997).

After keeping on germination paper, germination of all the genotypes was observed on fifth day, tenth day and fifteenth day (Plate 1). Germination percentage and seedling length (cm) were calculated and seedling vigor index was estimated (Table 3).

Seedling vigour index was observed to be high in IET 23354, IET 23996, IET 25104, IET 25112, IET 25123, IET 24677, Aiswarya, Harsha, Jyothi, Kanchana, Swarnaprabha and Uma while the index was low in IET 25135, Rasi x Jaya/2, 6RN, 5M, Swetha. Sampada x Jaya/2 was selected as the positive control while IET 23216 was the susceptible check genotype.

Thus, fourteen rice genotypes were selected for molecular characterisation (Table 4). DNA extraction of the selected fourteen genotypes was done by CTAB method. Confirmation of DNA quality was done by Agarose gel electrophoresis. After quantification of DNA, all samples were subjected to PCR process with primers RM103 and RM212 and the PCR products of fourteen genotypes were run on Agarose gel electrophoresis (Fig. 2).

During Gel electrophoresis with RM 103 marker, two bands *i.e*., one lower band (between 200-300bp) and another upper band (between 300-400bp) were formed for all the fourteen genotypes (Plate 2). The banding pattern exhibited by RM103 revealed that this primer is monomorphic to all the fourteen rice genotypes under study. Hence RM103 could not be considered as a suitable marker for the characterisation for drought tolerance among these genotypes.

RM212 is located on chromosome 1 of rice between 135.8 and 143.7 cM (McCouch *et al*., 2002). Wang *et al*. (2005) identified 48 candidate genes on rice chromosome 1 between markers RM212 and RM319, of which 16 were suggested for their potential role in drought tolerance. RM212 is linked to drought resistance traits and may be useful in marker assisted breeding for drought resistance in rice (Kanagaraj *et al*., 2010, Prince *et al*., 2015).

In the present study, the bands formed with RM212 showed that this marker is polymorphic among the fourteen rice genotypes (Fig. 3). Lower band (118 bp) was observed in IET 23354, IET 25112, IET 25123, Sampada x Jaya/2, IET 24677 and Aiswarya. Upper band (143 bp) was observed in IET 23996, IET 25104, Kanchana, Harsha, Jyothi, Swarnaprabha and Uma. Both upper and lower bands were observed in IET 23216 rice genotype. Thus RM212 appeared as a better marker for the characterisation of drought tolerance among these genotypes, due to its

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polymorphic nature. However, on further analysis, linkage between RM212 marker with the seedling vigour index under drought stress was not significant (P=0.65). Hence further research with other linked SSR markers for drought tolerance must be carried out in these rice genotypes.

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Table 1. Rice genotypes used for conventional screening for drought tolerance

Sl. No.	Genotypes	Sl. No.	Genotypes	Sl. No.	Genotypes
1.	IET 23354	8.	Sampada x Jaya/2	15.	Aiswarya
2.	IET 23392	9.	Rasi x Jaya/2	16.	Jaya
3.	IET 23996	10.	MTU 1010	17.	Jyothi
4.	IET 25104	11.	IET 23216	18.	Swarnaprabha
5.	IET 25112	12.	IET 24677	19.	Harsha
6.	IET 25123	13.	Swetha	20.	Kanchana
7.	IET 25135	14.	Ponmani	21.	Uma

Table 2. Details of SSR markers used for molecular characterization

Marker	Forward primer	Reverse primer
RM103	CTTCCAATTCAGGCCGGCTGGC	CGCCACAGCTGACCATGCATGC
RG212	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG

Table 3. Seedling vigour index of rice genotypes under stress and control conditions

Genotypes	6 Mega Pascal			8 Mega Pascal			Control		
	5th day	10th day	15th day	5th day	10th day	15th day	5th day	10th day	15th day
IET 23354	1444	2506	2616	909	1514	1614	1787	2479	2641
IET 23392	903	2140	2370	995	1517	1645	1732	2242	2381
IET 23996	1001	2390	2590	1357	1748	1906	1897	2605	2734
IET 25104	1397	2061	2473	1282	2032	2459	2062	2338	2549
IET 25112	665	1811	2473	829	1456	1830	1481	2104	2490
IET 25123	890	1593	1881	1615	2043	2490	1892	2221	2511
IET 25135	1329	1744	1833	1197	1727	1841	1552	1756	1837
Sampada x Jaya/2	1367	1965	2030	1191	1759	1998	1647	2041	2076
Rasi x Jaya/2	1260	1703	2053	1215	1813	2045	1649	2006	2058
MTU 1010	942	2180	2203	1489	2096	2202	2039	2408	2422
IET 23216	856	1584	1990	892	1648	1745	1758	1946	2053
IET 2467	1464	1890	1200	1322	1999	2864	2236	2626	2954
Aiswarya	2017	2618	2834	1849	2548	2710	2851	3000	3029
Harsha	1816	2323	2384	1619	2137	2295	2296	2365	2403
Jaya	1587	2027	2186	1425	1970	2182	2541	2619	2632
Jyothi	1216	1885	2515	1353	1830	2258	1828	2469	2639
Kanchana	1754	2562	3223	1837	2435	2941	2436	2766	3230
Ponmani	1667	2157	2166	1603	1903	2072	2223	2424	2431
Swarnaprabha	1746	2660	1200	1273	2094	2245	2521	2766	2918
Swetha	1596	2333	2434	1381	2218	2252	1662	1818	2083
Uma	1536	2136	2250	1664	2155	2381	1762	2346	2404
Mean	**1355**	**2108**	**2234**	**1348**	**1935**	**2189**	**1993**	**2350**	**2499**

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Table 4. Rice genotypes selected for molecular characterization

Sl. No.	Genotypes
1.	IET 23354
2.	IET 23996
3.	IET 25104
4.	IET 25112
5.	IET 25123
6.	Sampada x Jaya/2
7.	IET 23216
8.	IET 24677
9.	Aiswarya
10.	Harsha
11.	Jyothi
12.	Kanchana
13.	Swarnaprabha
14.	Uma

Fig. 1. Variation in seedling growth of rice genotypes under PEG induced drought stress and control

a) 6 Mpa PEG

b) 8 Mpa PEG

a) Control

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Fig. 2. Amplification pattern of Rice genotypes with RM 103 marker

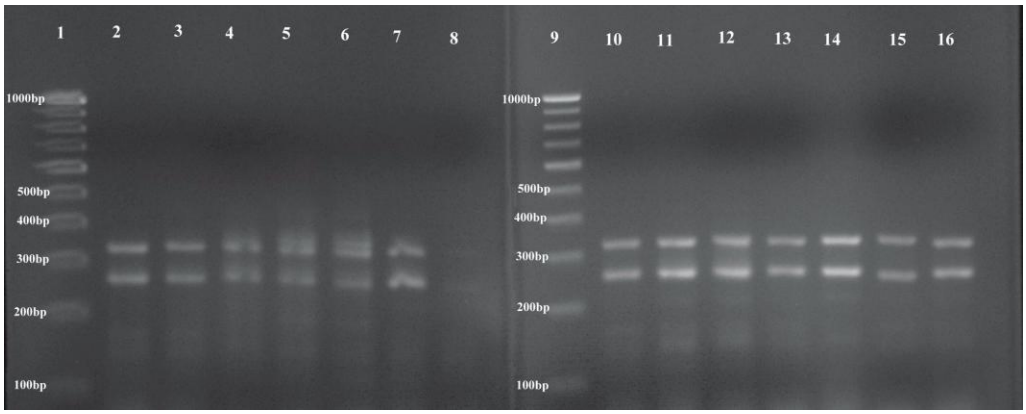
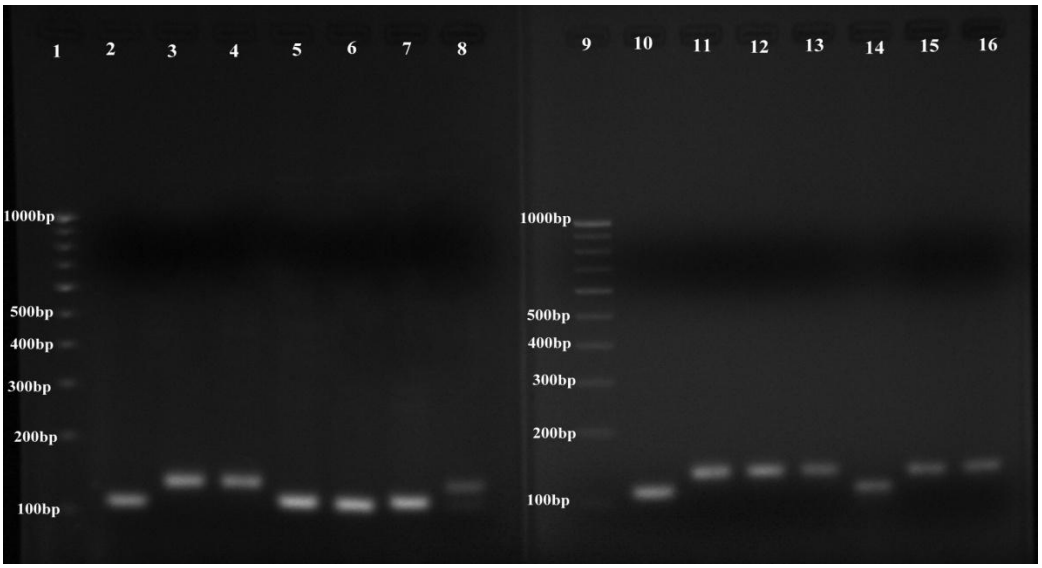


Fig. 3. Amplification pattern of Rice genotypes with RM212 marker



Lane 1	100 bp ladder	Lane 9	100 bp marker
Lane 2	IET 23354	Lane 10	IET 24677
Lane 3	IET 23996	Lane 11	Kanchana
Lane 4	IET 25104	Lane 12	Harsha
Lane 5	IET 25112	Lane 13	Jyothi
Lane 6	IET 25123	Lane 14	Aiswarya
Lane 7	Sampada x Jaya/2	Lane 15	Swarnaprabha
Lane 8	IET 23216	Lane 16	Uma