Original Research Article

Molecular analysis of drought tolerance in rice varieties using SSR markers

Abstract

The objective of this study was to learn about the abiotic stress of the rice plant. Using the well-distributed simple sequence repeat (SSR) markers RM327, RM206, RM23, and RM1075 identified 20 different rice genotypes. The groups of 20 rice cultivars that were cultivated under stress conditions were identified using SSR-based clustering analysis. Dendrogram demonstrated genotype cluster analysis This indicates that there is significant genetic variability within each group, according to the population structure in UP. Rice, Future coordinated association mapping investigations will be made possible by knowledge of population structure and related phenotypic traits recognised by geneticists and breeders.

Key words: Rice, drought tolerance, SSR markers

Abbreviations CTAB: Cetyl-trimethyl ammonium bromide, EDTA: Ethylene diamine tetra acetate, EtBr: Ethidium bromide, TAE: Tris-acetate EDTA, TEMED: N,N,N',N'-Tetra methyl ethylene diamine and NaCl: Sodium chloride.

Introduction

Rice is an edible starchy cereal grain that is produced by the annual grass (*Oryza sativa*, L. Poaceae family). Rice is the primary staple meal for almost half of the world's population, including nearly all of East and Southeast Asia. It serves as the main energy source for more than half of the world's population. The quantity of protein, iron, manganese, fibre, and vitamin B in rice varies depending on the strain. It can thus be quite important to overcome malnutrition. Rice yields suffer greatly from water stress, one of the catastrophic abiotic pressures that affect rainfed rice habitats. Improved cultivars must be developed specifically for rainfed locations to increase both yield and productivity. In this regard, the slow pace of drought breeding is a barrier. An alternative for accelerating drought-resistant crop development is the use of markers. Application of QTLs linked to characteristics relevant to drought might benefit from population-wide validation. Approximately 23% of

the world's calories are consumed by rice, one of the most significant food crops for more than half of the population (Li et al., 2011; Bernier et al., 2008). In Asia, where 90% of the world's rice is farmed, it has been estimated that rice provides 35 to 60% of daily caloric intake (Khush, 1997).

Numerous biotic and abiotic stress have a big impact on plant development as well as the production of rice (*Oryza sativa* L.), the main grain and staple diet of millions. One of these key obstacles to rice productivity and yield stability in a rainfed habitat is drought stress. So it is crucial to find rice varieties and genotypes that are drought tolerant right away. Because rice has a high amount of polymorphism, which aids in establishing relationships among individuals even with a small number of markers, simple sequence repeat (SSR) markers have been widely utilised in genetic diversity research. The major goal of the current study is to test seedlings of different rice genotypes in drought and control conditions by microsatellite markers to characterise chosen rice genotypes for drought tolerance at the molecular level (Shaheen et al. 2017)

Materials and methods

Isolation of genomic DNA from rice leaves and total genomic DNA from fresh leaves of rice varieties were extracted using the CTAB method given by Murray and Thompson (1980) The CTAB method was used for the isolation of DNA from rice leaves. CTAB was used to precipitate the nucleic acid at low salt concentration and low temperature (4^0C). Preparation of buffers and standard solution are 0. 1 M Tris (pH 8.0), 0.5 M EDTA (pH 8.0) and 5 M NaCl in Stock Solution: (100 ml). 3 g of C-TAB, 1 M Tris, 0.5 M EDTA, 5 M NaCl, 2 g of PVP, 2 β-mercaptoethanol, Maintained final volume in Extraction Buffer (pH 8.0), Ethanol: 70% (100 ml), Chloroform: Isoamyl alcohol:: 24:1, TE Buffer:100 ml, Made total volume 100 ml by distilled water. pH of the TE buffer was adjusted to 8.0. Ethidium Bromide was stored in a dark bottle at room temperature. Loading dye made volume 10ml. TAE Buffer (1 x TAE per litre) Total volume was made up to 1000 ml. The pH of the TAE buffer was adjusted to 8.0

The steps of DNA isolation are: **[1]** 100 mg of fresh leaves of each sample of rice were taken and grind in liquid nitrogen with the help of a mortar and pestle. Powdered leaves were transferred into the centrifuge tube. [**2]** 4ml pre-heated (65^0c) extraction buffer was added to the centrifuge tubes and kept in a water bath at 65 ^{0}C for 1hr. [**3]** Intermittingly,

shake the tubes during the incubation period of heating. [**4]** Cooled the tubes at room temperature after incubation periods. [**5]** Added 4 ml of chloroform: iso-amyl alcohol (24:1). [**6**] Mixed properly by inverting centrifuge tubes 25-30 times, [**7]** Centrifuge test tubes containing solution mixture at 6000 rpm for 15 minutes at 4 ^{0}C. [**8]** Supernatant was transferred in fresh Eppendorf tubes. [**9]** In the supernatant, an equal volume of isopropanol and half a volume of 5 M NaCl were added and stored at 4 ^{0}C overnight. [**10]** Then, Eppendorf tubes were centrifuged at 8000 rpm for 15 minutes. The supernatant was discarded and the pellet was washed with 70% ethanol. [**11]** The pellet was re-suspended in 50 µl of TE buffer and stored at 4 ^{0}C.

Purification of genomic DNA

The following steps were used for purification of the DNA: **[1]** RNase solution (10 mg/ml) @ 50 µg/ml was added to the DNA sample and incubated at 37 ^{0}C for one hour. **[2]** Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed gently. **[3]** The mixture obtained in step 2 was spinned at 10000 rpm for 2 minutes at room temperature; the aqueous phase was taken out and transferred to a fresh microfuge tube. Extracted twice with an equal volume of chloroform: isoamyl alcohol (24:1), centrifuged and taken out of the aqueous phase. **[4]** A volume of 3M sodium acetate (pH 4.8) was added to the above mixture and mixed properly. 2.5 times absolute alcohol was added and mixed by quick gentle inversion to precipitate the DNA **[5]** Mixture obtained in step 4 was centrifuged at 9000 rpm for 5 minutes in a microfuge tube to obtain the pellet. The supernatant was removed carefully; the pellet was washed with 70% cold ethanol. Pellets were dried in air and dissolved pellet (DNA) in 50 µl TE buffer.

Agarose Gel electrophoresis: Agarose gel of 0.8% was cast in 1X TAE buffer containing ethidium bromide (2 µl). After the solidification of the gel, 10 µl of genomic DNA with 5 µl of loading dye was properly mixed. DNA samples were loaded in the well of the gel properly. The gel was run at a constant voltage (40 V for three hours). Gel was then visualized on U.V. using a gel documentation system.

Dilution of DNA for PCR: 20 µl of autoclaved TE buffer was taken and 5 µl genomic DNA was added to make 50 to 100 mg per µl in each sample, based on their quantification volume.

Quantification of DNA: [1] Took 1 ml TE buffer in a cuvette and calibrated the spectrophotometer at 260 nm as well as 280 nm wavelength. **[2]** Added 5 µl of DNA mixed properly and recorded the optical density (O.D.) at both 260 and 280 nm. **[3]** Estimated the DNA concentration according to the following formula.

$$\text{Amount of DNA (mg/µl)} = \frac{\text{(OD)260 x 50 x dilution factor}}{100}$$

PCR Amplification

The quantity of genomic DNA was measured using a Nanodrop instrument. The final concentration of DNA was adjusted to 28-30 ng/µl for PCR reaction. The PCR reaction volume was 10 µl. The PCR reaction mixture of 10 µl consists of 0.4 mMdNTPs, 4 mM of MgCl2, 150 mM of Tris-HCl, 10 pmoles of forward and reverse primer and 0.05 U Taq polymerase with 30 ng of DNA. The reagents were mixed thoroughly and then placed in a Thermal Cycler for cyclic amplification using the amplification programme Step 1 (Initial denaturation) at 94 °C for 5 min. Step 2 (Denaturation) 94 °C for 1 min. Step 3 (Annealing) 55 °C for 1 min. Step 4 (Extension) 72 °C for 1 min. Step 5 (Final extension) 72 °C for 5 min. Step 6 (Storage) 4 °C for infinity. Steps 2, 3 and 4 were repeated 35 times.

Result and discussion

DNA Isolation of twenty varieties was done and the image is presented below.

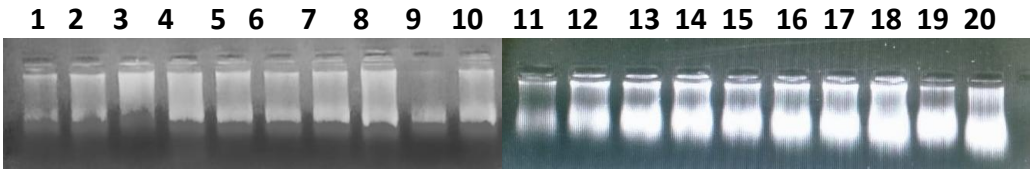


Fig 1. *DNA isolation using rice leaf*

Note: 1. Karjot**, 2.** IR-64**, 3.** Sukhandhan**, 4.** HUR-1304**, 5.** Sahabhagi**, 6.** MTU-1010**, 7.** Lalat**, 8.** Baraneedeep, **9.** Pusa Sugandha, **10.** Sona**, 11.** BINA-11**, 12.** HUR-1309**, 13.** HUR-3022**, 14.** Savitri**, 15.** HUR-2-1**, 16**. NDR-6093, **17.** SARJOO-52**, 18.** Govind, **19.** NDR-359**, 20.** IPR-763.

PCR amplification with primer RM327, RM206, RM23 and RM1075. This primer amplified all the genotypes and also showed polymorphism. RM327 Showed polymorphism except IR-64, sahabhagi, MTU-1010, Lalat, Baranideep, Savitri, NDR-6039 and IPR-763. RM206 Showed polymorphism except sahabhagi. RM23 Showed polymorphism of all genotypes. RM1075 Showed polymorphism of all genotypes..all of the entries had the same band size except RM206. This suggested that genotyping with a few additional markers would be required for a clearer distinction of utilised molecular markers for polymorphism studies in rice. Chakravarthi *et al*. (2006), Ashraf *et al.* (2016), Sruthi *et al.* (2016), Beser *et al*. (2019), Sahoo *et al*. (2019), Pratap *et al*. (2020), Suvi *et al.* (2020), Hoque *et al*. (2021), Vasumathy *et al*. (2021), Deepika *et al*. (2022) and Choudhury *et al*. (2023) showed similar results in their study.

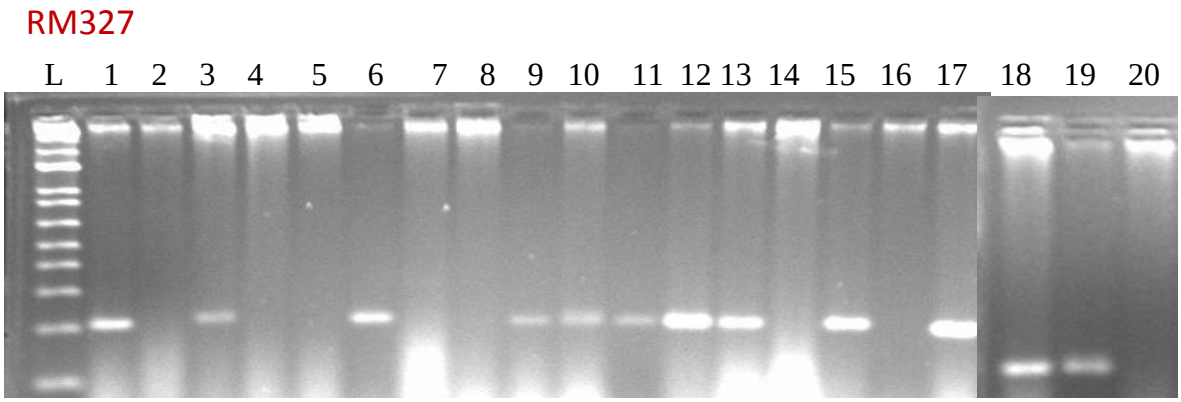


Fig 2. *Gel showing PCR amplification using SSR primer RM327*

Note: *IR-64, that HUR-1304, Sahabhagi, Lalat, Baraneedeep, Savitri, NDR-6039 and IPR-763 are showing distinct patterns compared to other varieties.*

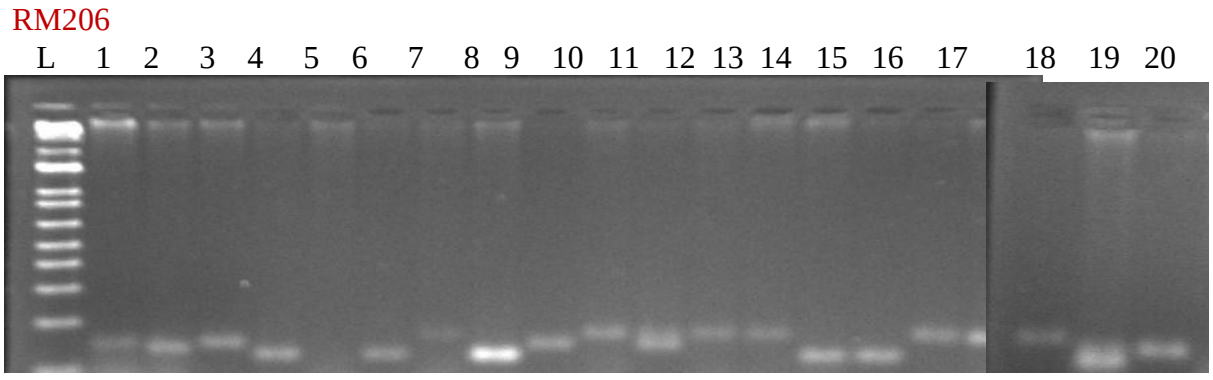
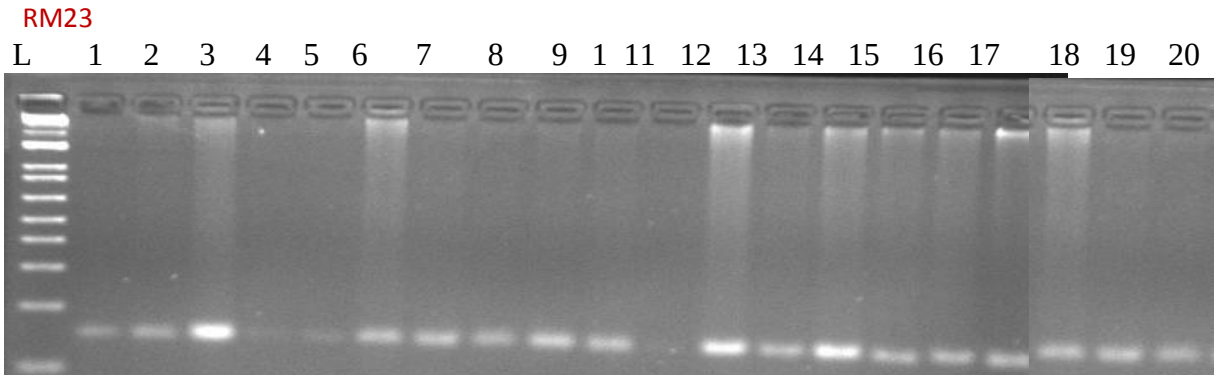


Fig 3. Gel showing PCR amplification using SSR primer RM206

Note :. *All the twenty varieties appear appearing different-sized bands. Sahabhagi is showing a distinct pattern compared to other varieties.*



. Fig 4. Gel showing PCR amplification using SSR primer RM23

Note : *SSR primer RM23 showing monomorphism. HUR-1304, Sahabhagi and BINA-11 are showing slite or no band pattern compared to other varieties.*

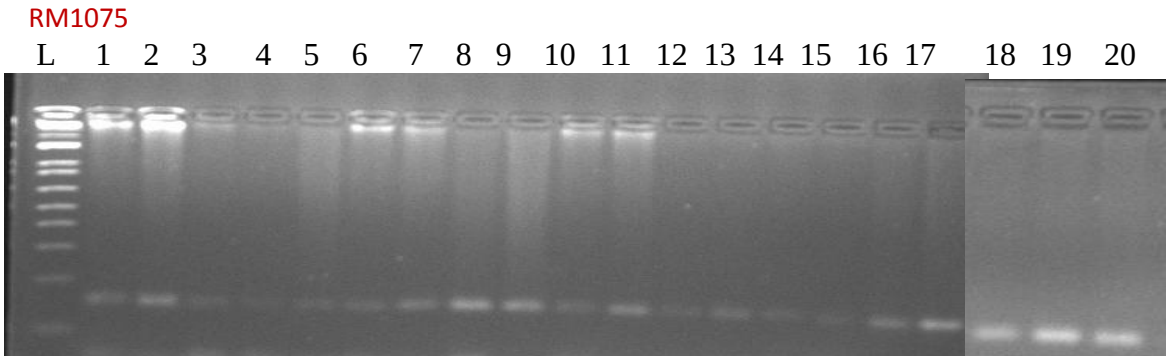


Fig 5. Gel showing PCR amplification using SSR primer RM1075

Note *SSR primer1075 showing monomorphism*

Dendrogram of rice varieties with primers (RM327, RM206, RM23 and RM1075)

The dendrogram of rice varieties with primers RM327, RM206, RM23 and RM1075 explained that group I and group II were formed only group II holds the primer RM327 and group I is further divided into two groups A and B. Here, the group comprises of RM23 and group B separated into two groups C and D. group C consists RM1075 and group D Involved RM206.

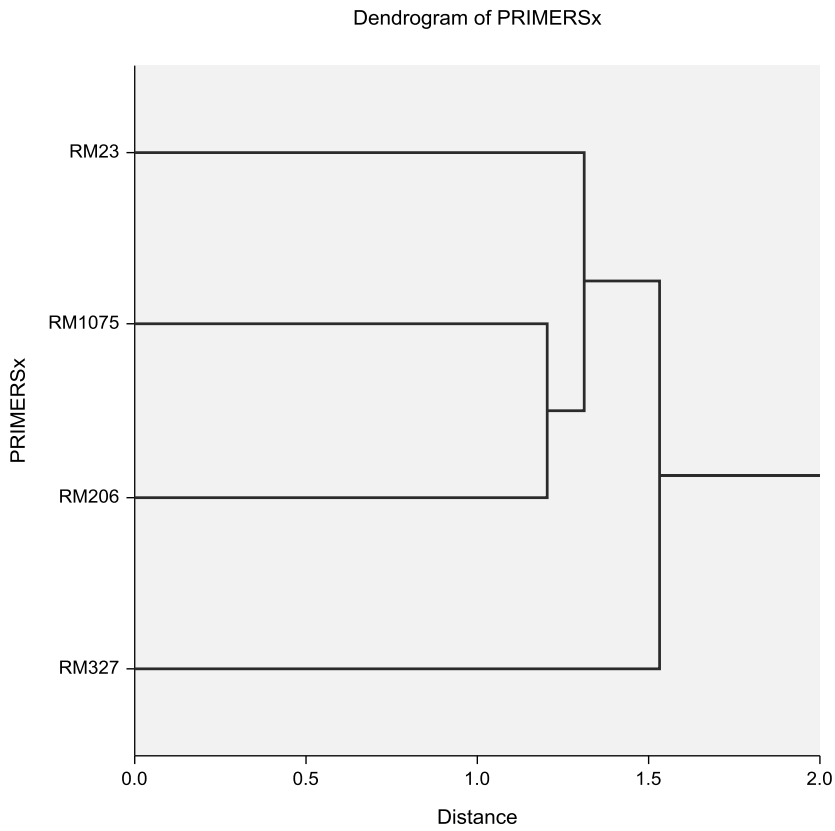


Fig 6. Dendrogram of hierarchical cluster analysis depicting grouping in rice varieties based on the different SSR primers.

Rice varieties based on the different SSR primers were analysed by a Dendrogram of hierarchical clustering by grouped into two groups I and II. Group I further divided into two groups A and B. In group A two more types are presented as groups A1 and A2 comprising varieties were NDR-6039 and HUR-3022. Only one group B had Sahabhagi Under Group II, Groups C and D were divided into four types C1, C2, D1, and D2. Here group C1 showed HUR-1309. C2 further divided as C2a and C2b comprise IPR-763, HUR-1304, Pusa Sugandha and Savitri. Group D separated in to four groups D1a, D1b, D2a and D2b comprises SARJOO-52, Sona, Baraneedeep, IR-64, Lalat, NDR-359, Govind, HUR-2-1, BUNA-11, MTU-1010, Sukhandhan-6 and Karjot. The group I and II were presented dendrogram with a distance of 1.8. Groups A and B placed at 1.5 distance, which represents grouping in rice varieties.

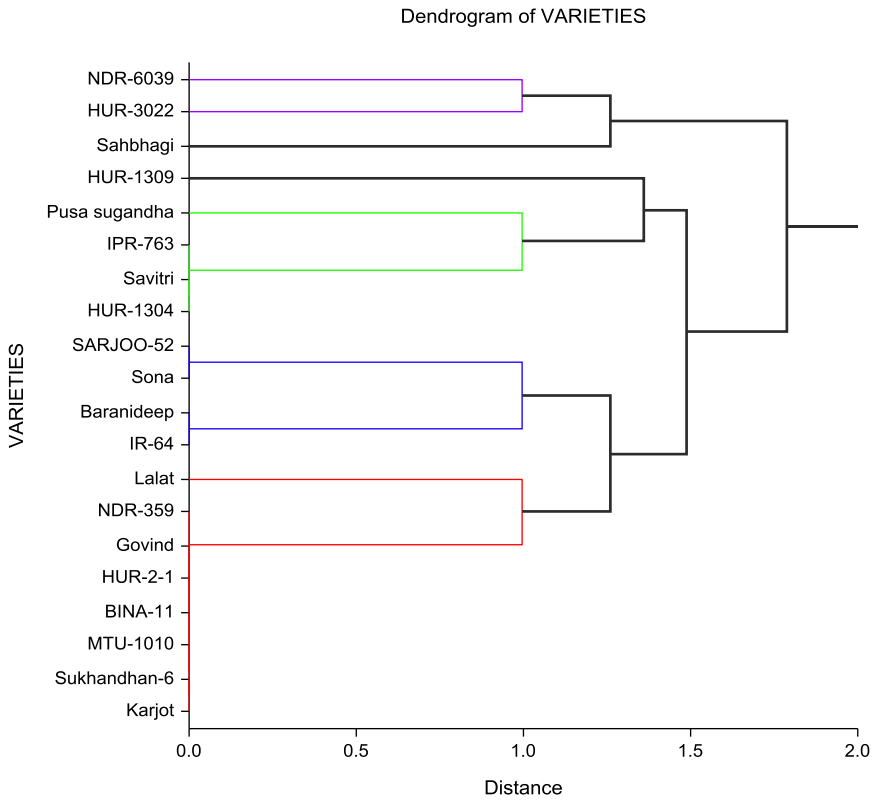


Fig 7. Dendrogram of hierarchical cluster analysis depicting grouping in rice varieties based on the different SSR primers.

Conclusion

This study was carried out to differentiate and identify rice varieties using SSR analysis with using rice varieties in UP. Primer RM327, IR-64, HUR-1304, Sahabhagi, Lalat, Baraneedeep, Savitri, NDR-6039, and IPR-763 exhibit distinctive patterns. Each of the twenty variations has a distinctive size band. Comparing Sahabhagi to other types, a different pattern may be seen. RM23, an SSR primer, exhibits monomorphism. In comparison to other kinds, HUR-1304, Sahabhagi, and BINA-11 have a weak or no band pattern. A monomorphic SSR primer 1075 was observed. Compared to other primers, the RM327 primer had a considerable impact on two clusters. The group I and II were presented dendrogram with a distance of 1.8. Groups A and B plotted at 1.5 distances, which represents depicting grouping in rice varieties.

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