Original Research Article

Molecular analysis of drought tolerance in rice varieties using SSR markers

Abstract

The motive of this study was to learn concerning the abiotic stress of the rice plant. Using the well-distributed simple sequence repeat (SSR) markers RM327, RM206, RM23, and RM1075 were 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.

Keywords: CTAB: Cetyl-trimethyl ammonium bromide, EDTA: Ethylene diamine tetra acetate, EtBr: Ethidium bromide, TAE: Tris acetate EDTA, TEMED: N,N,N',N'-Tetra methyl ethyelene 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. 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. 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 in order 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 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 once. The major goals of the current study to test seedlings of different rice genotypes in drought and control condition 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, Total genomic DNA from fresh leaves of rice varieties were extracted using CTAB method given by Murray and Thompson (1980). CTAB method was used for 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 NaCI, 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 TE buffer was adjusted to 8.0. Ethidium Bromide was stored in 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 TAE buffer was adjusted to 8.0

The steps of DNA isolation are: **[1]** 100 mg fresh leaves of each sample of rice were taken and grind in liquid nitrogen with the help of mortar and pestle. Powdered leaves were transferred into centrifuge tube. [**2]** 4ml pre-heated (65^0c) extraction buffer added in the centrifuge tubes and kept it in water bath at 65 ^{0}C for 1hr. [**3]** Intermittingly, shake the tubes during 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 miture at 6000 rpm for 15 minutes at 4 ^{0}C. [**8]** Supernatant was transferred in fresh eppendrof tubes. [**9]** In supernatant, equal volume of isopropanol and half volum of 5 M NaCl were added and stored it at 4 ^{0}C for overnight. [**10]** Then, eppendrof tubes were centrifuged at 8000 rpm for 15 minutes. Supernatant was discarded and pellet was washed with 70% ethanol. [**11]** Pellet was re-suspended in 50 µl of TE buffer and stored at 4 ^{0}C.

Purification of genomic DNA

Following steps were used for purification of the DNA: **[1]** RNase solution (10 mg/ml) @ 50 µg/ml was added to 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]** Mixture obtained in step 2 was spinned at 10000 rpm for 2 minute at room temperature; aqueous phase was taken out and transferred to a fresh microfuge tube. Extracted twice with equal volume of chloroform: isoamyl alcohol (24:1), centrifuged and taken out the aqueous phase. **[4]** Volume of 3M sodium acetate (pH 4.8) was added to 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 minute in a microfuge tube to obtain the pellet. Supernatant was removed carefully; 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 casted in 1X TAE buffer containing ethidium bromide (2 µl). After solidification of gel, 10 µl of genomic DNA with 5 µl of loading dye were properly mixed. DNA sample were loaded in the well of gel properly. Gel was run at constant voltage (40 V for three hours). Gel was then visualized on U.V. using 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 wave length. **[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.

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

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) 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 presented below.

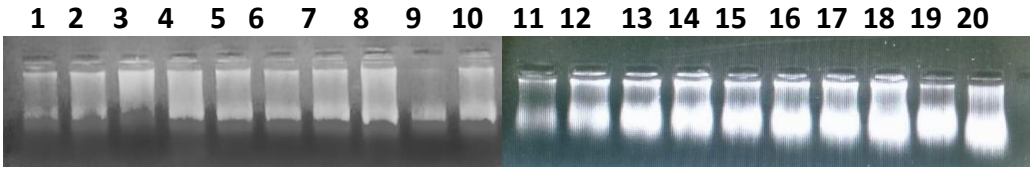


Fig 1. *DNA isolation using rice leaf*

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. And 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 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.

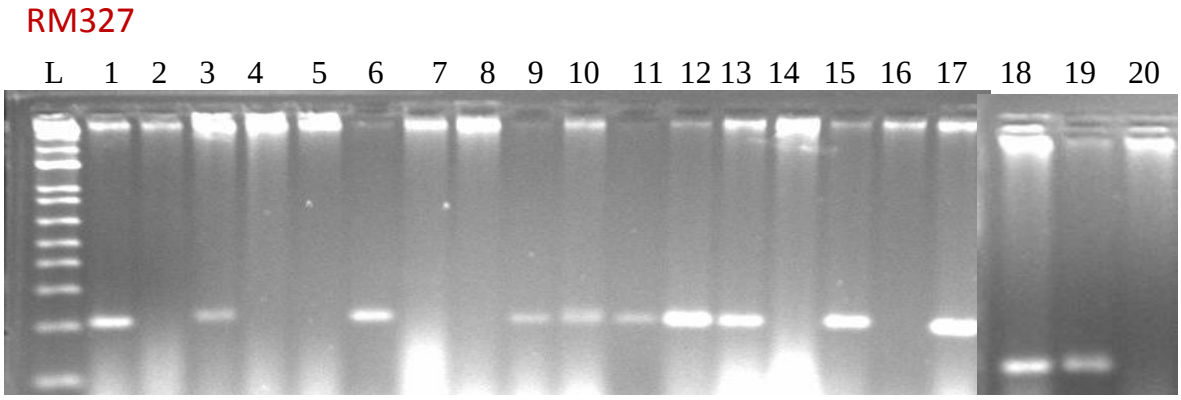


Fig 2. *Gel showing PCR amplification using SSR primer RM327*

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

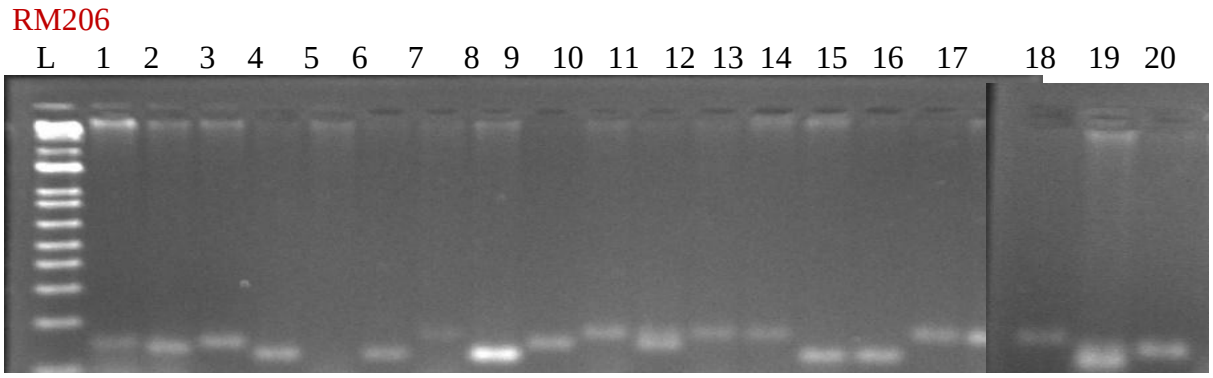
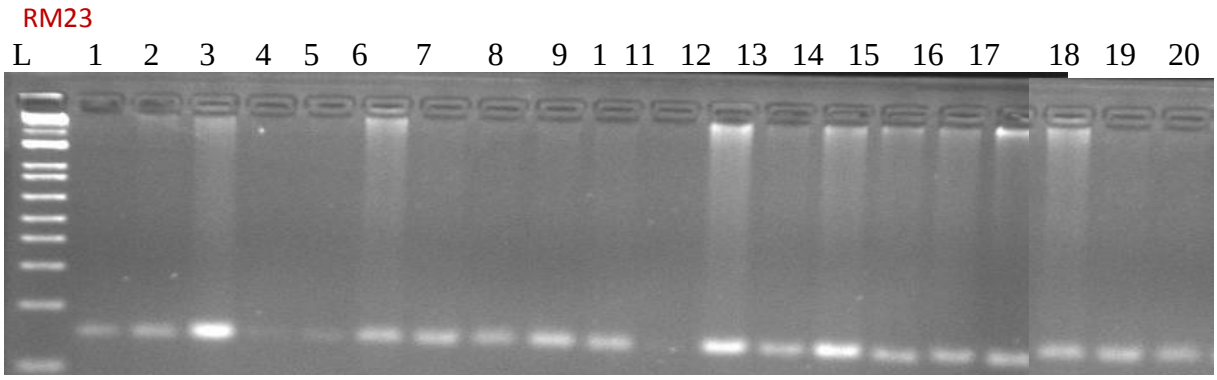


Fig 3. Gel showing PCR amplification using SSR primer RM206

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. *All the twenty varieties are appearing different sized band. Sahabhagi is showing distinct pattern compared to other varieties.*



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

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

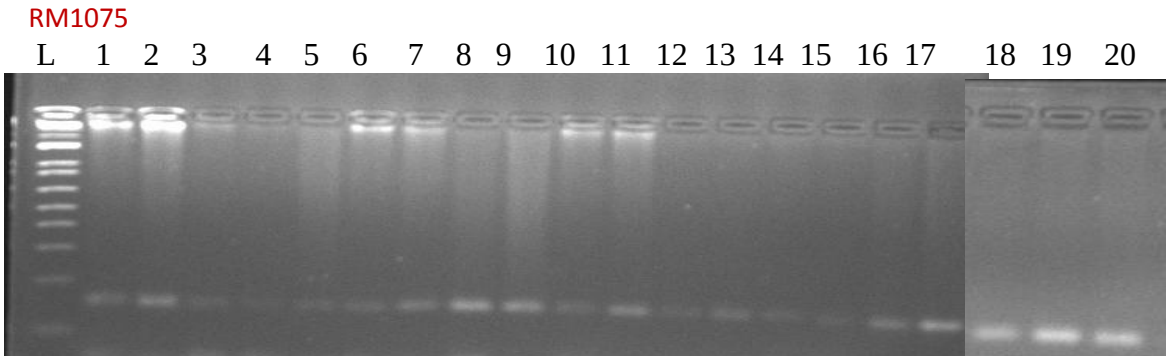


Fig 5. Gel showing PCR amplification using SSR primer RM1075

Note : 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. *SSR primer1075 showing monomorphism*

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

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 further divided in to two groups A and B. Here, group comprises of RM23 and group B separated in to two groups C and D. group C consists RM1075 and group D Involved RM206.

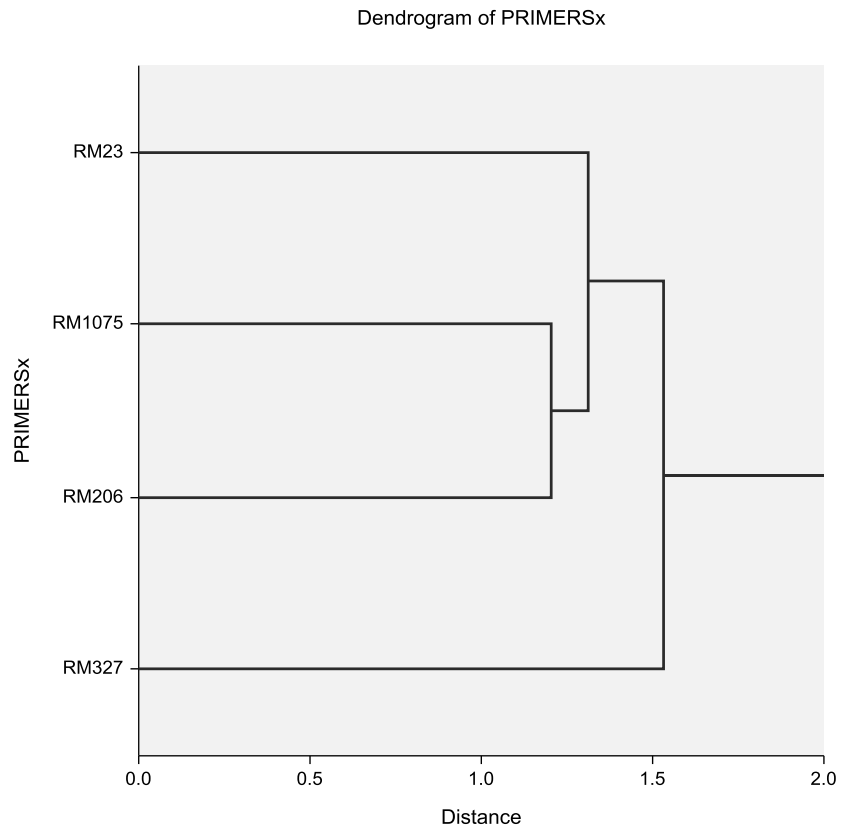


Fig 6. Dendrogram of hierarchical cluster analysis depicting grouping in rice varieties based on the different SSR primers.

Rice varieties based on the different SSR primers were analysed by Dendrogram of hierarchical clustering by grouped in to two groups I and II. Group I further divided in to two groups A and B. in group A two more types are presented as group A1 and A2 comprised varieties were NDR-6039 and HUR-3022. Only one the group B had Sahabhagi. under group II, Group C and D were divided in to four types C1, C2, D1, and D2. Here group C1 showed HUR-1309. C2 further to 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 distance at 1.8. group A and B ploted at 1.5 distance, which represents depicting grouping in rice varieties.

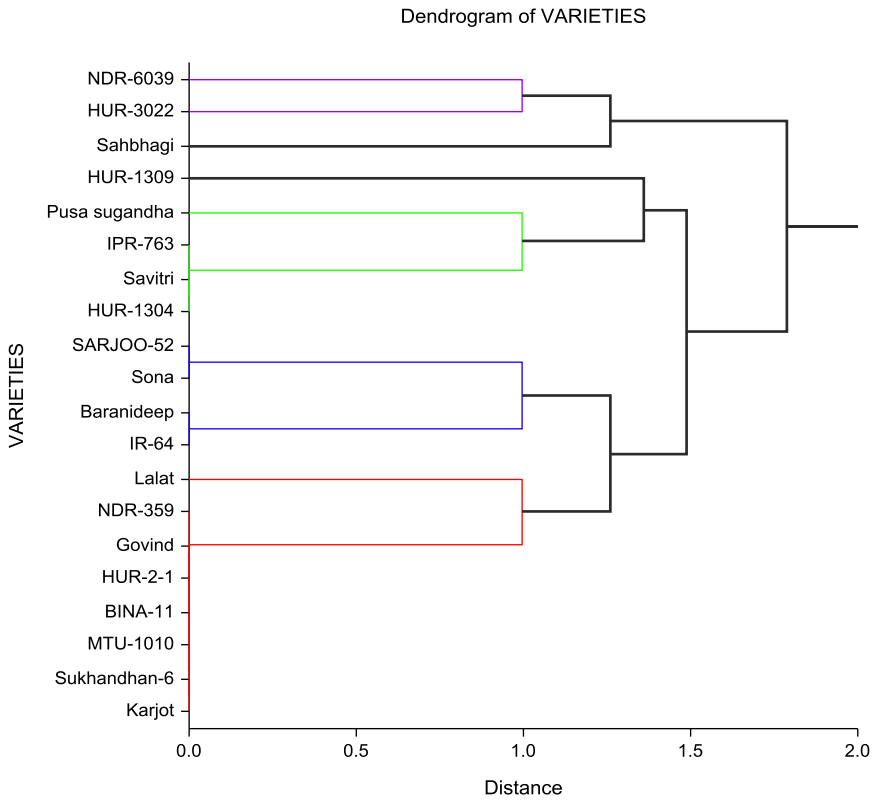


Fig 7. Dendrogram of hierarchical cluster analysis depicting grouping in rice varieties based on the different SSR primers.

Conclusion

This study was carried out to differentiate and identify rice varieties by means of SSRs 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 distance at 1.8. group A and B ploted at 1.5 distance, which represents depicting grouping in rice varieties.

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