

Original Research Article

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Genetic Diversity Analysis of Rice Cultivars Differing in Dormancy Based on SSR Markers

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ABSTRACT

Keywords

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Seed dormancy is an important trait affecting grain yield and quality in cereal crops. It is controlled by its own complicated genetic and environmental factors. The polymorphism pattern study of 24 rice genotypes for RM primers used in the present molecular study were clearly classified the rice genotypes into dormant (Cluster I, Cluster IIA) and non-dormant (Cluster II B, Cluster III). In the present study, a total of 40 alleles were detected across the 24 rice genotypes by 11 polymorphic SSR markers. The number of alleles generated per locus by each marker ranged from 2 to 6. Maximum number of alleles (6) produced by RM 520. The PIC values among the SSR loci tested are ranged from 0.1411 (RM 480) to 0.7025 (RM 520) with an average of 0.495 per locus. Among the polymorphic SSR markers detected, RM 520 showed maximum numbers of alleles (6) as well as the highest PIC value (0.709). The markers showed average PIC value of 0.4954 that almost showed 49 % polymorphism, which confirms that SSR markers used in this study were highly informative for genetic studies and are extremely useful in distinguishing the polymorphic rate of a marker at a specific locus.

Introduction

Dormancy enabled seeds to survive long periods of environmental conditions unfavorable for germination (Li *et al.*, 2012). The seeds have evolved to postpone germination until a time and place that not only supports germination, but also maximizes

seedling establishment and growth (Cao *et al.*, 2014). Because dormant seeds don't germinate, dormancy was, for many years, considered to be some sort of seed defect or inactivity that meant that even a viable seed simply could not germinate (Garces *et al.*, 2014; Mortensen and Grasser 2014; Tiwari *et al.*, 2016).

Seed dormancy is the transitory inability of a viable seed to germinate even under condition favourable for germination (Li and Foley, 1997). It is an important stage of plant life cycle and a feature that is also useful in cultivated plants, as it can avoid pre-harvest sprouting (PHS) in the field conditions that are favourable for germination, such as warm and humid weather and the consequent poor grain quality and lower yield (Harlan *et al.*, 1973). The degree of seed dormancy is affected by genetic factors controlling germination including the mechanical hardness of seed coats, the morphological or physiological immaturity of embryos and the activity of specific metabolic systems (Bewley, 1997; Baskin and Baskin 1998 and 2004; Bentsink *et al.*, 2007). However, seed dormancy can be a disadvantage in plant breeding when it is necessary to sow successive generations as soon as possible after harvest so as to increase the number of selection rounds in a short period (Roberts, 1961). Dormancy is one of the traits among the cereal crops that have undergone domestication and could be a desirable trait as it can help prevent preharvest sprouting hence improved grain yield and quality (Harlan and de Wet, 1973; Gubler *et al.*, 2005).

Dormancy in cereals seeds has been reported by several workers over the years (Das and sinha, 1965; Vaesey *et al.*, 2004; Nirmala, 2006, Sooganna *et al.*, 2012, Shiratsuchi *et al.*, 2017). Recently a number of genetic studies on seed dormancy were carried out using molecular markers in various plant species such as rice (Seshu *et al.*, 1986; Takahashi 1997; Wan *et al.*, 1997; Dong *et al.*, 2002; Miura *et al.*, 2002; Gu *et al.*, 2004; Kumar *et al.*, 2009; Shiratsuchi *et al.*, 2017), in barley (Oberthur *et al.*, 1995; Larson *et al.*, 1996; Prada *et al.*, 2004), sorghum (Lijavetzky *et al.*, 2000) and wheat (Anderson *et al.*, 1993; Kulwal *et al.*, 2004), and Arabidopsis (Schaar *et al.*, 1997).

Microsatellites are tandem repeats of DNA sequences of only a few base pairs (1-6 bp) in length, the most abundant being the dinucleotide repeats (Morgante and Olivieri, 1992). Wu and Tanksley (1993) showed that simple sequence repeats such as (GA)_n and (GT)_n is not only present in the mammalian genome but also in rice genome. Yang *et al.*, (1994) reported that because of the greater resolving power and the efficient production of massive amount of SSR data may be particularly useful for germplasm assessment and evolutionary studies of crop plants. SSRs have been characterized in many plant genomes such as rice, sorghum, maize etc.

A vast number of SSRs have been published in rice (Mc Couch *et al.*, 2002) Because of their merits such as abundance, even genomic distribution and high level of polymorphism, SSRs are considered to be the markers of choice for DNA fingerprinting and genetic analysis studies.

Several major genes and Quantitative Trait Loci (QTL) controlling agronomically important traits have been mapped with tightly linked DNA markers in several crop species including rice, facilitating marker-assisted selection (MAS) for the traits. MAS have been successfully used in selecting for the trait of interest irrespective of the plant growth stage. Information on QTLs associated with dormancy would be of great help to the plant breeders so that this trait can be tailored in their breeding programmes in developing dormant and non-dormant rice varieties.

Materials and Methods

Plant materials

The experimental material consisted of 24 rice genotypes collected from ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, India (Table 1).

Genomic DNA isolation

Extraction of bulk DNA was carried out by using maxiprep DNA isolation protocol described by Kumar *et al.*, (2007) in rice crop. The purity and concentration of the isolated bulk DNA samples was estimated by UV-absorption spectrophotometer (Beckman DU 650 model) described. The ratio of absorbance at 260 nm and 280 nm was used as an indicator of DNA purity. The ratio between 1.4 and 1.9 was considered as relatively pure DNA samples as it did not show any effect on PCR reaction. The bulk genomic DNA concentration was measured by using the formula: DNA concentration in $\mu\text{g}/\mu\text{l}$ = OD 260 x 50 μg x dilution factor.

Polymorphism survey through SSR markers

The polymorphism survey was carried out with 11 SSR markers (Table 2). The PCR plate was labeled with respect to sample number and 2 μl (i.e. 50-100 ng) of template DNA was added to the respective wells. The master mix consisted of 0.5 μl forward primer, 0.5 μl reverse primer, 0.5 μl dNTP's, 0.6 μl *Taq* DNA polymerase (M/s Bangalore Genei Pvt. Ltd.), 1.0 μl of 10 x PCR buffer (Tris with 1.5 mM Mg Cl₂) and 4.9 μl of sterile distilled water was added to make up the volume to 10 μl . Then the master mix (8.0 μl) was dispensed to the PCR plate with template DNA. The PCR plate was covered with a sealing mat. It was then placed in a programmable thermal cycle (M/s Applied Biosystem, USA) for DNA amplification.

The thermal cycling programme is as follows: initial denaturation of 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55°C for 30 sec, and extension at 72 °C for 1 min, followed by final extension at 72 °C for 7 min and hold at 4 °C.

After completion of the PCR, the plate was stored at -20°C and later checked for amplification on a 3% agarose gel stained with Ethidium bromide. The PCR products were checked for amplification by electrophoresis using a 3 % agarose gel (M/s SeaKem LE Agarose, Lonza, USA) in a Submarine Horizontal Electrophoresis Unit (M/S. Biorad, USA). About 10.5 g of agarose was weighed and transferred into a reagent bottle containing 350 ml of 0.5X TBE (Tris-borate EDTA) buffer and mixed well. The content was then boiled gently in a microwave oven with intermittent mixing. The process was followed until agarose melts completely and the solution becomes clear. In the meanwhile, the gel-casting tray was washed with water and wiped with ethanol. Then it was sealed with cello tape. The agarose gel solution was cooled down to bearable heat (~40°C) and 18 μl of ethidium bromide (10 mg/ml) was added to it.

The content was mixed thoroughly and poured into the gel-casting tray where the combs were arranged in the slots on the gel-casting tray. The gel was allowed to solidify at room temperature for 20-30 min. Care was taken so that no air bubble was present. Later the gel was transferred to a Submarine Horizontal Electrophoresis Unit containing 0.5 χ TBE buffer. Before loading, amplified products were mixed with 1/6th volume of gel loading dye (40% sucrose; 0.25% bromophenol blue) and loaded into the wells with help of micro tips.

The electrodes were connected to power pack and samples were run at 120 V for 1 hr. 100 bp ladder (Fermentas Generuler - 0.1 $\mu\text{g}/\mu\text{l}$) was loaded in first well to determine the size of amplified fragments. The amplicons were visualized under UV-transilluminator and documented using ALPHA IMAGER gel documentation system (M/s. Alpha Innotech).

Statistical analyses

For each SSR marker, the total number of alleles were noted and the allelic status was converted in to binary (0, 1) data. ‘Polymorphism information content’ (PIC) was determined as described by Senior *et al.*, (1998). PIC is a measure of allele diversity at a locus and is equal to $1 - \sum(P_{ij}^2)$, where P_{ij} is the frequency of j^{th} allele for i^{th} locus summed across all alleles in the locus. PIC is synonymous with the term “gene diversity” as described by Weir (1996).

The PIC values ranging from ‘0’ (monomorphic) to ‘1’ (very highly discriminative, with many alleles in equal frequencies) provide an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at locus, but also the relative frequencies of those alleles in the genotypes under study. For example, a marker locus that reveals five alleles, but where one allele is found in very high frequency (for example, freq. = 0.9), has overall less discriminative capability than a locus that also has five alleles, but in which those alleles are found in more equal frequencies.

Mean PIC value was calculated based on PCR values obtained across various SSRs loci, excluding a few primers that revealed a higher frequency of nulls, as latter could inflate the PIC value.

Cluster analysis

The clustering of accessions was done by using Numerical Taxonomy and Multivariate Analysis System (NTSYSpc. Rohlf, 1998) based on a similarity matrix using an Un weighted Pair Group Method with Arithmetic average (UPGMA) algorithm following Sequential Agglomerative Hierarchical and Nested (SAHN) module.

Results and Discussion

Number of alleles

In the present study, a total of 40 alleles were detected across the 24 rice genotypes by 11 polymorphic SSR markers. The number of alleles generated per locus by each marker ranged from 2 to 6. Maximum number of alleles (6) produced by RM 520. In the present study, the PIC values among the SSR loci tested are ranged from 0.1411 (RM 480) to 0.7025 (RM 520) with an average of 0.4954 per locus. Among the polymorphic SSR markers detected, RM 520 showed maximum numbers of alleles (6) as well as the highest PIC value (0.7095). The amplification profiles of polymorphic SSR markers are given in the Figure 1. Polymorphism information content (PIC) value is the reflection of allele diversity and frequency among the genotypes. To measure the informativeness of each SSR marker, PIC value was calculated. The PIC value is the indicator in predicting the usefulness of DNA markers for gene mapping, molecular breeding and germplasm evaluation. Markers with higher PIC values possess greater potential to reveal allelic variation. The average PIC value of SSR markers of different crops tested by different researchers varied based on number of SSR markers used and number of genotypes tested. The markers showed average PIC value of 0.4954 that almost showed 49% polymorphism, which confirms that SSR markers used in this study were highly informative for genetic studies and are extremely useful in distinguishing the polymorphic rate of a marker at a specific locus.

Cluster analysis

The cluster analysis resolved the 24 rice genotypes into three major clusters at 0.72% similarity. Dendrogram based on UPGMA

analysis grouped the 24 genotypes into three clusters. Cluster I divided at 0.70 similarity coefficient consists of only one genotype (MTU2077) which is dormant and cluster II

consists of both dormant and non-dormant genotypes and cluster III is the major one consists of 17 non-dormant genotypes.

Table.1 List of rice genotypes and seed dormancy status

Varieties	Trait
Mandyavijaya	Non-dormant
Manasarovar	Non-dormant
Akshayadhan	Non-dormant
RP Bio-226	Non-dormant
Suraksha	Non-dormant
Sampada	Non-dormant
Mahasuri	Non-dormant
Vikramarya	Non-dormant
Swarnadhan	Non-dormant
Phalguna	Non-dormant
Dhanarasi	Non-dormant
Varadhan	Non-dormant
Mugadhasugandha	Non-dormant
DRR Dhan 38	Non-dormant
Jaya	Non-dormant
Sugandhamati	Non-dormant
Sona	Non-dormant
Sonasali	Non-dormant
DRR Dhan 39	Non-dormant
Jarava	Non-dormant
MTU1001	Dormant
MTU2067	Dormant
MTU2077	Dormant
Jeerakasala	Dormant

Table.2 Number of alleles and Polymorphism information content (PIC) values of 11 SSR markers

MARKER	FORWARD PRIMER SEQUENCE	REVERSE PRIMER SEQUENCE	NUMBER OF ALLELES	PIC	HETEROZYGOSITY
RM 346	CGAGAGAGCCCATAACTACG	ACAAGACGACGAGGAGGGAC	4	0.6142	0.6759
RM 237	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC	2	0.3318	0.42
RM 271	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC	2	0.2859	0.3457
RM 520	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG	6	0.7025	0.74
RM 564B	CATGGCCTTGTGTATGCATC	ATGCAGAGGATTGGCTTGAG	5	0.6707	0.7143
RM 164	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC	4	0.5806	0.6281
RM 3602	TGAAAAGCCACTCAGATGCG	TGGTGAAAGGGTCAGAACTG	5	0.5396	0.6
RM 231	CCAGATTATTCCTGAGGTC	CACTTGCATAGTTCTGCATTG	4	0.6506	0.7072
RM 480	GCTCAAGCATTCTGCAGTTG	GCGCTTCTGCTTATTGGAAG	2	0.1411	0.1528
RM 234	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	3	0.4898	0.5805
RM 421	AGCTCAGGTGAAACATCCAC	ATCCAGAATCCATTGACCCC	3	0.4427	0.4965

Figure.1 Amplification profiles of RM421, RM234, RM231 and RM 480 SSR primers from 24 rice genotypes. Lane 1 is 100 bp ladder

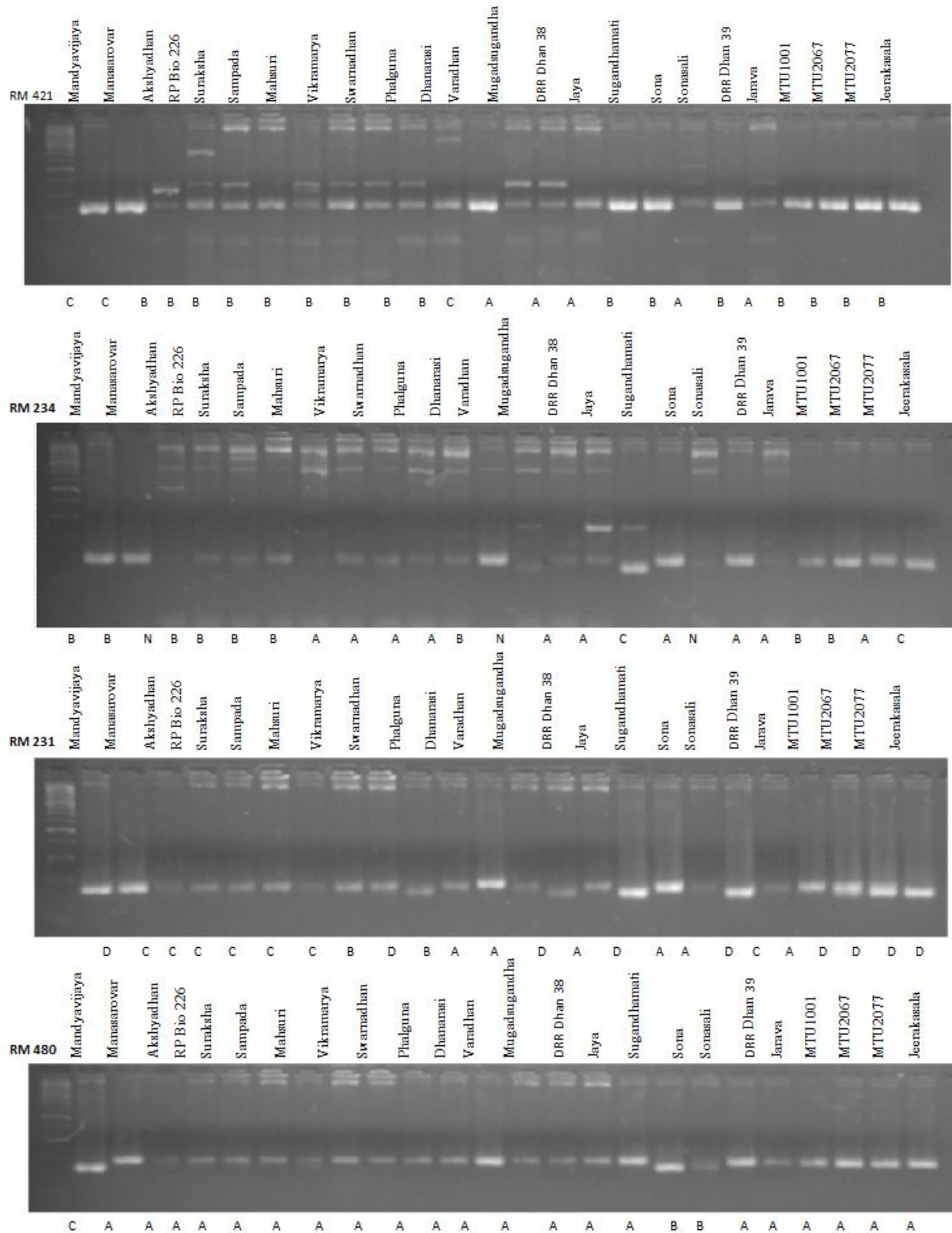
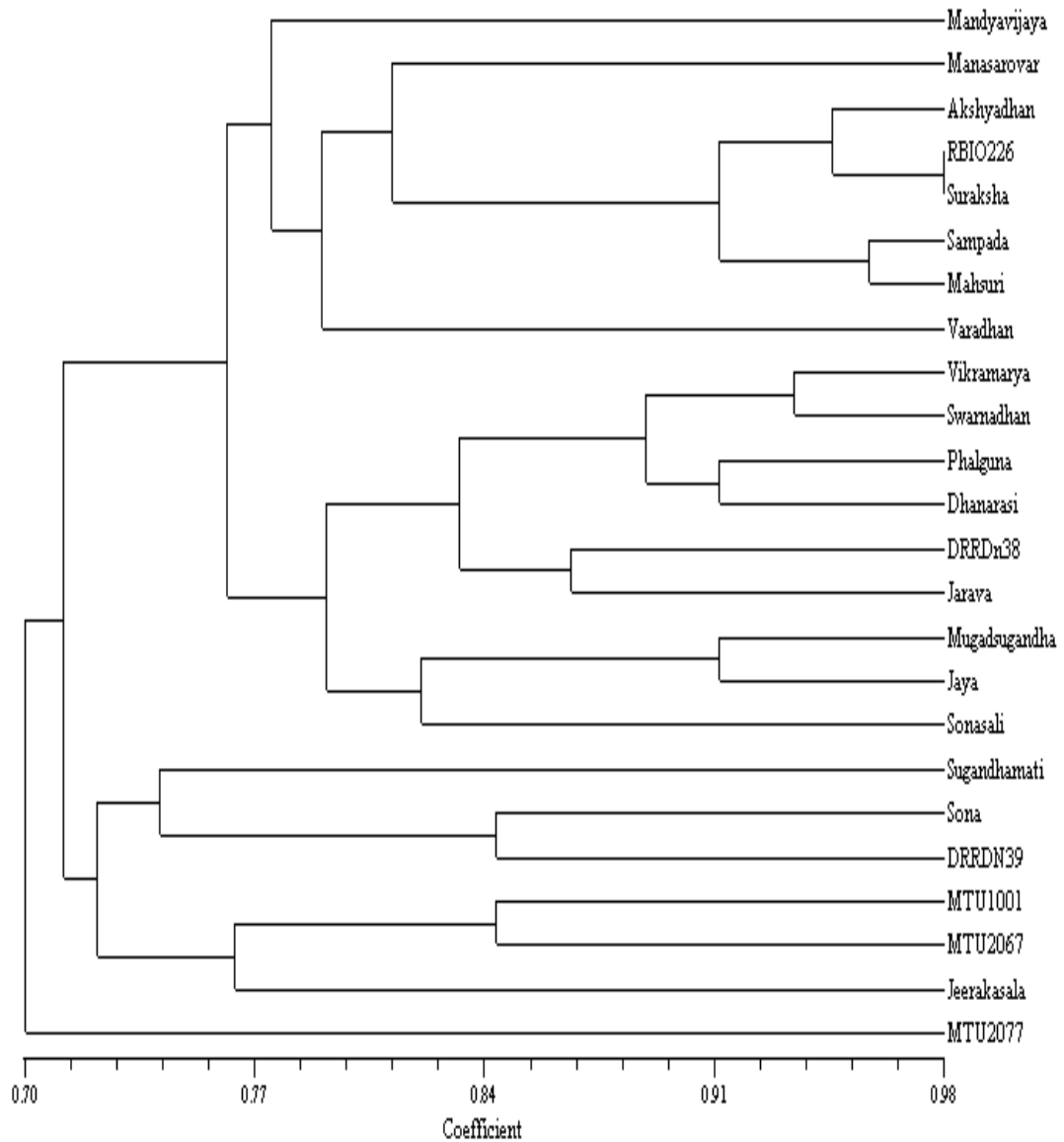


Figure.2 Dendrogram based on UPGMA analysis grouped the 24 rice genotypes



The cluster II was divided into two sub clusters, cluster II-A and cluster II-B at similarity coefficient 0.76 and 0.73 respectively. The cluster II-A consists of three genotypes which are dormant in nature and cluster II-B consists of three genotypes which are non-dormant. The cluster III is the major one consisting of 17 genotypes which are non-dormant. It was divided in to three sub clusters viz., Cluster III-A, cluster III-B and

cluster III-C at similarity coefficient 0.81, 0.83 and 0.77 respectively (Fig. 2).

The primers used in the present study were clearly classified the genotypes into dormant and non-dormant. The similar results were reported by Heng Ye *et al.*, (2011) for primers. RM 3602 was linked with the qSdn-1 on chromosome 1. RM 520, RM 164 were linked with qSdn-3, qSdn-5 respectively.

Similarly RM 234, RM346 were also linked with qSdn-7 on chromosome 7. The primer RM 480 was linked with the qSdn-5 on chromosome 5 Lu *et al.*, (2011). The similar findings were also reported by Langbiao Guo *et al.*, (2004) that RM 237 and RM 164 were associated with qSdn-1 and qSdn-5 respectively.

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