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# Molecular Marker-Based DNA Fingerprinting and Diversity Study of Rice Landraces Differing Salinity Tolerance

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**Abstract:** The purpose of the research was to conserve the identity through molecular marker based DNA fingerprinting. Eight pair simple sequence repeat (SSR) primer and 7 randomly amplified polymorphic DNA (RAPD) primers were used to identify position of allele as well as to study genetic diversity among 35 genotypes differing salinity tolerance. All markers showed polymorphism with a mean number of 12.5 and 6.87 alleles per locus for SSR and RAPD respectively. When the DNA of genotypes were analyzed with SSR markers, tolerant landraces showed similar allelic position with salinity tolerant genotypes like Pokkali, FL478 or FL378 and salinity susceptible landraces showed similar position to popular salinity sensitive cultivar IR-29 or BRRI dhan29. From the SSR analysis, polymorphism information content (PIC) values ranged from 0.7334 (AP3206) to 0.8957 (RM336) where the highest gene diversity (0.9029) was observed in loci RM336 and the lowest gene diversity (0.7576) was observed in loci AP3206 with a mean diversity of 0.8604. In the mean time, RAPD analysis showed the highest gene frequency (1) among 5 loci viz. OPA04-5, OPB10-5, OPC02-8, OPC05-4 and OPC02-8 and the lowest gene frequency (0.0571) among 2 loci viz. OPB10-4 and OPC02-9 with mean gene diversity 0.3039. Cluster analysis based on SSR was more effective than RAPD markers in grouping landraces based on their salt tolerance due to co-dominance of SSR markers. The similarity and distinction between the landraces chosen for the study can be used as a precious tool and also recommend an appropriate selection of parents for mapping or breeding purposes.

**Key words:** DNA fingerprinting, Genetic diversity, landrace, Salinity, Rice

## Introduction

Rice is a major cereal crop, as long as staple food to more than half (FAO, 2010) of the world population living in Asia, Africa, Latin America and West Indies. In Bangladesh about 2.8 million ha covering 30% of total cultivable land is affected by salinity (BBS, 2013) which cause plant death as an upshot of severe growth retardation and molecular injure and become a major obstacle in attaining potential rice yields worldwide (Ashraf and Foolad, 2013)

Long period of cultivation and farmer selection has resulted in the production of a large number of landraces of rice, of which Nearly 10,000 landraces may have thrived in Bangladesh (Gupta, 2011, Wang *et al*., 2013). Actually, the green revolution has resulted in the widespread introduction of around 300 high-yielding varieties in the rice fields all over the world, accompanied

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by severe erosion of the traditional landraces (Hassan *et al*., 2012). But landraces afford "adaptability genes" for unambiguous environmental circumstances like salinity which is the second most risk for rice cultivation worldwide (Das *et al.,* 2013). The mechanisms of salt tolerance in landraces found in saline prone area, may take place at three levels: the whole plant, cellular and molecular levels which include salt exclusion (plants do not take up excess salt by selective absorption); salt reabsorption (plant absorb excess salt but it is reabsorbed from the xylem and $Na^+$ is not translocated to the shoot); root-shoot translocation (salinity tolerance is associated with a high electrolyte content in the roots and a low content in the shoot; salt translocation (plants have the ability to translocate a lesser proportion of $Na^+$ to the shoot); salt compartmentation (excess salt is transported from younger to older leaves); tissue tolerance (plants absorb salt but are properly compartmentalized in vacuoles within leaves in order to lower the harmful effects on plant growth) and salt dilution (plants take up salt but dilute it by fast growth rate and high water content in the shoot) (Bhaskar and Bingru, 2014; Deepa *et al*. 2013; Ashraf and Foolad 2013).

Although a few landraces have been incorporated into modern breeding programs, majority of traditional Bangladeshi landraces remains uncharacterized and underutilized (Das *et al.,* 2013). Besides this kind of important rice genetic resources are endangering day by day due to lack of conservation, as well as cultivation of popular high yielding variety. Researchers have long been used morphological, pigmentation, quality or other characteristics to characterize and distinguish plant genotypes within a species but the assessment of deviation endure from many drawbacks such as influence of environment on trait expression, epistatic interaction, pleiotropic effects etc (Zhu *et al.,* 2012). Furthermore, the scarcity of sufficient number of these descriptors for definite classification of increasing number of reference collection of varieties enforces to look for alternatives.

Study of genetic diversity has long been a major goal in evolutionary biology. Information on the genetic diversity within and among closely related rice genotypes is vital for a rational use of genetic resources. The analysis of genetic variation both within and among elite breeding materials is of fundamental interest to plant breeders. It contributes to monitoring germplasm and can also be used to predict potential genetic gains (Chakravarthi1 and Naravaneni, 2006).

DNA fingerprinting is a more practical strategy that would be to limit the comparisons to specific regions of the genome, which frequently differ between individuals (Sajib, 2012).

Besides evaluation of genetic diversity using molecular marker technology is non-destructive, requires small amount of samples and does not require large experimental setup and equipment's for measuring physiological parameters.

Among molecular marker systems SSR and RAPD are fastest and simplest as well as have been extensively used for genetic diversity analysis because they detect high level of allelic diversity. Besides both markers occur frequently throughout plant genomes, and are easily assayed by PCR. In rice Kanawapee *et al.* (2011), Rahman *et al.* (2010), Davla *et al*. (2013) and Chakravarthi1 and Naravaneni, (2006) and Zhu *et al*. (2012) were successfully used SSR and RAPD marker for DNA fingerprinting and genetic diversity study.

Thus the objective of this research was to find out information of DNA fingerprinting and genetic diversity of selected 35 rice genotypes using SSR and RAPD markers.

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## Results and Discussion

As molecular markers are unlimited in numbers and are not affected by environmental factors in the developmental stages of the plant, the molecular markers may arise from different classes of DNA mutations such as substitution mutation, rearrangements or errors in replication of tandemly repeated DNA (Rahman *et al.* 2010). The overall information has described in this section.

**DNA fingerprinting database and estimated genetic diversity based on SSR markers**

All 35 genotypes were successfully fingerprinted with the 8 SSR primer pairs where markers were found to be polymorphic and revealing a total of 100 alleles with an average number of 12.5 alleles per locus (Table 2). At the RM336 locus, a total of 17 different alleles were identified among the 35 rice genotypes ranging in size from 129 to 135 bp. Likewise, 16 alleles (size range 225-240 bp), 9 alleles (size range 354-374 bp), 8 alleles (size range 154-161bp), 14 alleles (size range 200-216 bp), 13 alleles (size range150-169bp), 12 alleles (size range 141-159 bp) and 11 alleles (size range 201-215 bp) were detected at the locus RM585, AP3206, AP3206f, RM493, RM7075, SalT1 and RM80943 respectively. The accurate positions of alleles are enlisted in Table 3 and banding patterns generated by selected primer pairs are shown in Fig. 1.

As a measure of the informative nature of SSR, the PIC values ranged from 0.7334 (AP3206) to 0.8957 (RM336) with a mean value 0.8467 that showed a significant, positive correlation with the number of alleles and allele size range evaluated in this study (Table 2). Major allele is defined as the allele with the highest frequency and also known as most common allele at each locus. The frequency of the most common allele at each locus ranged from 0.1429 (SalT1) to 0.4286 (AP3206) with a mean frequency of 0.2393 (Table 2). An allele observed in less than 5% of the 35 accessions was considered to be rare which was observed at all of the SSR loci with a mean of 1.5 and a total of 12 across all the loci (Table 2). In this study, marker RM336 detected the highest number of alleles (25) and rare alleles (3).

The highest gene diversity (0.9029) was observed in loci RM 336 and the lowest gene diversity (0.7576) was observed in loci AP3206 with a mean diversity of 0.8604 (Table 2). It was observed that marker detecting the lower number of alleles showed lower gene diversity than those which detected higher number of alleles which revealed higher gene diversity.

SSR Genetic distance refers to the genetic divergence among populations, which can be measured by a variety of parameters in relation to the frequency of a particular trait. Pair-wise comparison value of (Nei's, 1973) genetic distance between genotypes was computed from data of 8 primers and ranged from 0.3750 to 1.0000 where highest genetic distance (1.0000) was observed between various genotype pair. Among salinity tolerant and moderately tolerant landraces Kajol Shail, Jota Balam, Bazra Muri, Ghunshi, Hamai, Mondeshor, Nona kochi, Tal Mugur, Nona Bokhra, Kashrail, JolKumari and Pokkali showed highest genetic distance (1.0000) with high yielding IR-29 and BRRI dhan29 those could be utilized as parent in marker assisted backcrossing (MAB) program to develop salt tolerant and high yielding new rice varieties with all desirable characters.

The UPGMA-based dendrogram was also obtained from the binary data deduced from the DNA profiles of the samples analyzed where the genotypes that are derivatives of genetically

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similar types clustered together. The cluster analysis led to the grouping of the 35 genotypes in two major clusters (Fig 3). Cluster 1 comprised of salinity susceptible landraces- Hati Bajore, Kalmilata, Dudh Kalam, Rupessor, Mota Aman, Chap Shail, Kali Boro and Kali Boro 138/2 along with well known susceptible rice variety IR-29 and BRRI dhan29. Cluster 2 had two sub cluster (S.C-2.1 and S.C-2.2) where all of the tolerant and moderately tolerant genotypes were found. The position of well known salinity tolerant rice genotypes FL378, FL478, Binadhan-8 and Pokkali in same cluster also indicated that those partners must have a salinity tolerant gene. The dendrogram revealed that the genotypes that are the derivatives of genetically similar type clustered together. Based on the above result, it may be concluded that, the highly tolerant and moderately tolerant genotypes grouped in same cluster due to lower genetic distance.

**DNA fingerprinting database and estimated genetic diversity based on RAPD markers**

All of the 7 RAPD primers (OPA04, OPB04, OPB08, OPB10, OPC02, OPC04 and OPC05) were effectively used to amplify genomic DNA of all 35 rice genotypes. Each primer produced well-separated multiple band profiles (Fig 2) with a number of DNA fragment varying from 5 to 9 (Table 4) with a mean alleles per locus of 7.57. The primer OPB10 gave the minimum number of fragments (5), while the highest number of fragments 9 was amplified with primers OPA04 and OPC02. A total 53 alleles were detected, of these, 48 were polymorphic (90.39%) and 5 were monomorphic (9.61%) where the lowest and highest number of polymorphic bands were 4 (OPB10) and 9 (OPB08) respectively. The primer, which showed the least polymorphism (75%), produced 8 bands of which only 6 was polymorphic. The primer OPB08 yielded 9 bands that were all polymorphic (100%).

The overall gene frequencies of loci positions are enlisted in Table 5 as a DNA fingerprinting database based on respective RAPD markers where the highest value of gene frequency was obtained 1 among 5 loci viz. OPA04-5, OPB10-5, OPC02-8, OPC05-4 and OPC02-8 while the lowest gene frequency was obtained 0.0571 among 2 loci viz OPB10-4 and OPC02-9. Here gene frequency values less than 1(<1) were considered as polymorphic. Among 53 loci, 48 had a value less than 1. Besides mean total number of allele was 1.9057 and mean effective number of allele was 1.5053. Thus, the results obtained from RAPD analysis suggest that there are high degrees of genetic variations among the local land races differing salinity tolerance. Moreover, average gene diversity **(**h) and Shannon's Information index (I) across all primer for all loci were estimated 0.3039 and 0.4569 respectively (Table 5) where the higher level of gene diversity value and Shannon's Information index was found in locus OPA04-1, OPA04-4, OPA04-7, OPA04-8, OPA04-9, OPB04-10, OPB04-4, OPB04-5, OPB08-3, OPB08-4, OPB08-5 OPB08-6, OPB08-8, OPB08-9, OPB10-3 OPC02-2, OPC02-5 and OPC02-6. The highest genetic distance (0.7122) was found between Chapshail vs. FL378. Among salinity tolerant landraces Jamai Naru, Patnai, Bazra Muri, Ghunshi, Kalo Mota, Mondeshor showed relatively higher genetic distance with high yielding IR-29 and BRRI dhan-29 those could be utilized as parent in marker assisted backcrossing (MAB) program to develop salt tolerant and high yielding new rice varieties with all desirable characters.

The genetic similarity coefficients among 30 rice genotypes based on the RAPD fragments were

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used to construct a dendrogram by UPGMA analysis. Basically there were 2 cluster, one of which Cluster 1(C.1) separated into 2 group like sub cluster 1(S.C. 1) and sub cluster 2 (S.C. 2) where S.C. 1 also divided into 2 group like S.C.1.1 and S.C.1.2.

S.C.1.1 comprised of 15 genotypes where moderately tolerant Kajol Shail, salinity tolerant Jamai Naru and Patnai belonged to S.C.1.1A. Another group S.C.1.1BI comprised of all moderately tolerant landraces except Kalmilata but S.C.1.1BII contains Rupessor, Mota Aman, Kali Boro and BRRI dhan29 which were salinity susceptible. On the other hand S.C.1.2 comprised of 2 group where susceptible genotypes like Nona Kochi, Karengal, Kalo Mota, Kali Boro 138/2 and IR-29 were belonged to S.S.1.2A and salinity tolerant genotypes including Pokkali, Tal Mugur, Binadhan-8, FL378, Khashrail and FL478 were belonged to S.S.1.2BI, besides salinity susceptible Dudh Kalam and moderately tolerant Bhute Shalot also belonged to same location of dendrogram. Furthermore, except salinity tolerant landrace Ghunsi, another two member of S.S.2 were moderately salinity tolerant. In addition Hogla and Chap Shail belonged to cluster 2 (C-2) where first one was salinity tolerant and last one was salinity susceptible.

**Comparison between SSR and RAPD marker analysis**

By using SSR and RAPD markers, this study showed that there was a high level of genetic diversity among 35 rice germplasm, the percentages of polymorphic bands examined by SSR and RAPD were 76.11% and 90.39%, respectively (Table 6). The existing average gene diversity was 0.8604 for SSR while and 0.30 for RAPD in selected rice gene pool. Among salinity tolerant landraces Jamai Naru, Ghunshi, Hogla, Patnai, Kalo Mota, Khashrail, Nona Bokhra and Pokkali showed relatively higher genetic distance with high yielding IR-29 and BRRI dhan29 in the both case of RAPD and SSR markers.

This research supports and extends earlier studies of Kanawapee et al. (2011), Rahman et al. (2010), Rahman et al. (2009), Rahman et al. (2007), Davla et al (2013) and Chakravarthi1 and Naravaneni, (2006) and Zhu et al. (2012) and Das *et al*., (2013).

## Materials and methods

Seeds of the rice genotypes were collected from Biotechnology Division of Bangladesh Institute of Nuclear Agriculture (BINA). The genotypes were grown and DNA fingerprinting were done in the Biotechnology laboratory of the same institute during the period June/2013-December/ 2013. Among 35 genotypes 30 were landraces from highly saline prone area Satkhira, Potuakhali and Noakhali district of Bangladesh, 3 were modern commercial varieties and 2 were IRRI developed salt tolerant improved line (Table 1). According to previous study, among 30 land races, 10 were tolerant, 12 were moderately tolerant and 8 were susceptible to saline condition at electrical conductivity (EC) 12 dS/m in seedling stage and 8 dS/m in reproductive stage.

**Raising of Seedlings and isolation of genomic DNA**

10-12 seeds of each variety were selected randomly and dried in oven at 54°C for 48 hrs for better germination. After germination in the wet blotting paper in petridishes, the seedlings were grown in small plastic pots. Bulked DNA was isolated from 2-5 juvenile culms of 21-day-old seedlings of each of 35 rice genotypes following the protocol described by Aljanabi and Martinez (1997) and also used by Rahman *et al*., (2007) with some modifications. Excluding usage of

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CTAB, the modified protocol included digestion with homogenization buffer (Solution: Tris-50 mM, EDTA-25 mM, NaCI-300 mM, 1% SDS and deionized water) at 65°C for 30 min, extraction with phenol: chloroform: isoamyl alcohol (25:24:1), precipitation with ice-cold and extra pure isopropyl alcohol and purification with absolute ethanol (Plus sodium acetate, 3M) and 70% ethanol chronologically. Finally, DNA sample of each rice variety dissolving in 30-40µl of TE buffer within 1.5 ml eppendorf tube was preserved separately at -20°C. Presence of genomic DNA was confirmed on 1% agarose gel qualitatively in a minigel (OWL-OSP300-2Q, USA) and quantification was accomplished using a spectrophotometer (Spectronic® Genesis™, Spect- ronic Instruments Inc., USA).

**PCR amplification**

Eight primer pairs for salinity screening rice SSR markers such as AP3206, AP3206f, RM585, RM336, RM8094, SalT1, RM7075 and RM493 were used in this purpose. The PCR conditions especially the annealing temperatures, were optimized for different loci as necessary to produce scorable amplification products (Table 2). The annealing temperature was $55^0$C for AP3206, AP3206f, RM336, SalT1, and RM585, $50^0$C for RM7075, $59^0$C for RM493 and $48^0$C for RM8094. PCR reactions were carried out on a Thermocycler-13 (Biometra). The reaction volume was 10 µL containing 2 uL genomic DNA (100 ng/ µL), 1 ul of each primer (2 µM), 1 µL of dNTPs (0.25 mM each), 1 ul 10X *Taq* buffer A (mixed with $MgCl_2$), 0.2 µL *Taq* DNA polymerase (1Unit) and 3.8 µL double distilled water. The temperature profile consisted of 5 minutes initial denaturation at $94^0$C followed by 35 cycle of 1 minute at $94^0$C, 1 minute at the respective annealing temperature and 2 minute at $72^0$C, ending with 7 minute final extension.

Seven RAPD primers such as OPA04, OPB04, OPB08, OPB10, OPC02, OPC04 and OPC05 were used where PCR conditions especially the annealing temperatures were same for these selected primers. The annealing temperature was $40^0$C and PCR reactions were carried out on a Thermocycler-13 (Biometra). The reaction volume was 10 ul containing 2.5 µL genomic DNA (100 ng/ µL), 2 µl of primer (2µM), 1ul of dNTPs (0.25mM each), 1 µL 10X *Taq* buffer A (mixed with $MgCl_2$), 0.2 µL *Taq* DNA polymerase (1Unit) and 3.3 µL double distilled water. The temperature profile consisted of 3 minutes initial denaturation at $95^0$C followed by 30 cycle of 1 minute at $94^0$C, 1 minutes at the $40^0$C annealing temperature and 2 minutes at $72^0$C, ending with 7 minutes final extension.

**Electrophoretic separation of the amplified products and visualization**

The PCR products of SSR makers were separated on a 8% denaturing polyacrylamide gel containing 9.45ml 40% acrylamide solution Fluka Analytical), 0.211 g bisacylamide powder (SIGMA, Life science), 5 mL 10X TBE, 500ul Ammonium per sulphate (APS) and 70 µL TEMED (SIGMA, Life science). Electrophoresis was conducted using ELITE 300 PLUS (Wealtec) vertical polyacrylamide gel electrophoresis system. It was run for about 1.5-3.5 hr (running time depends on the expected PCR value) at 80 Volts.

However PCR products of RAPD marker were analyzed directly on 1.5% agarose containing 3 g Agarose powder (Agarose NEEO Ultra-qualitet/ ROTH) and 200 mL 0.5X TBE buffer using a horizontal agarose gel electrophoresis system. It was run for about 1.5 hour at 80 volts. Both kind of DNA fragments amplified by RAPD and SSR primers were visualized after ethidium bromide

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staining by UV transilluminator (ASUS, Maxdat, Biometra).

## Scoring and statistical analysis

The patterns of bands obtained after amplification with the SSR primers were scored using ALPHA VIEW version 3.2.8 to identify the size of allele of DNA band comparing with DNA ladder. Number of alleles and mean alleles per locus were estimated for all SSR loci using POWER MARKER version 3.23 (Kejun and Spencer, 2005). Whilst RAPD band patterns were scored manually by three peoples independently where observed number of alleles and their status of polymorphism were found using software POPGENE Version 1.31 (Yeh *et al.,* 1999). UPGMA (Unweighted Pair Group Method with Arithmetic Means) dendrogram from SSR and RAPD marker were constructed using the software TREEVIEW 1.1 (Alok, 2004).

## Conclusion

The study could successfully locate allele for respective landraces from the analysis of both polymorphic SSR and RAPD markers where elementary DNA fingerprinting databases were constructed. As these markers can verify the landraces uniquely, they can be used in crop identity protection in breeding programs to improve salinity tolerant rice varieties. Genetic variation is important in maintaining the developmental stability and biological potential of an organism. In essence, the present work revealed ample genetic variation and relatedness among the rice landraces. Low levels of genetic variation and high levels of genetic relatedness were found among salinity tolerant as well as salinity susceptible landraces. The polymorphic markers used in the study were evaluated by PIC score which is a measure of polymorphism of the markers. This score is valuable for choosing highly polymorphic markers for linkage analysis and variety selection for breeding program of rice genotype. Significant genetic variation at maximum number of loci between landraces indicates rich genetic resources in rice. Information on genetic variation from the present study might be useful for breeders in making decision for improvement of landraces through selective breeding and cross breeding programmes. Besides, breeders could make a strategy for conservation of landraces having diverse gene pools. As literature on genetic analysis of Bangladeshi rice is very scarce, present study could help the researchers in this regard in near future. However, there were some lacunae in the present study like only 35 individuals and in total 15 primers were used in both SSR and RAPD analysis that reduces the chance to obtain a reliable knowledge precisely about the genetic structure of each landraces of rice. Further studies involving large number of samples and primers need to be conducted to get more precise information.

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**Table 1:** Performance of 35 rice genotypes under salinity stress at seedling stage

| Sl. no. | Name of genotypes | Reaction to Salinity | Place of Collection | Category |
|---|---|---|---|---|
| 1 | Hogla | Tolerant | Satkhira | Landrace |
| 2 | Jamai Naru | Tolerant | Satkhira | Landrace |
| 3 | Dakh Shail | Tolerant | Satkhira | Landrace |
| 4 | Patnai | Tolerant | Satkhira | Landrace |
| 5 | Pokkali | Tolerant | INDIA | Landrace |
| 6 | FL-378 | Tolerant | IRRI | Improved line |
| 7 | Khak Shail | Tolerant | Satkhira | Landrace |
| 8 | Tal Mugur | Tolerant | Satkhira | Landrace |
| 9 | Nona Bokhra | Tolerant | Satkhira | Landrace |
| 10 | Ghunsi | Tolerant | Satkhira | Landrace |
| 11 | Binadhan-8 | Tolerant | Satkhira | Modern variety |
| 12 | FL478 | Tolerant | BINA | Improved line |
| 13 | Kasrail | Tolerant | IRRI | Landrace |
| 14 | Kajol Shail | Moderately Tolerant | Satkhira | Landrace |

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| | | | | |
|---|---|---|---|---|
| 15 | Bazra muri | Moderately Tolerant | Satkhira | Landrace |
| 16 | Hamai | Moderately Tolerant | Satkhira | Landrace |
| 17 | Karengal | Moderately Tolerant | Satkhira | Landrace |
| 18 | Kalo Mota | Moderately Tolerant | Potuakhali | Landrace |
| 19 | Holde Gotal | Moderately Tolerant | Satkhira | Landrace |
| 20 | Jota Balam | Moderately Tolerant | Satkhira | Landrace |
| 21 | Mondeshor | Moderately Tolerant | Satkhira | Landrace |
| 22 | Nona Kochi | Moderately Tolerant | Noakhali | Landrace |
| 23 | Jolkumari | Moderately Tolerant | Noakhali | Landrace |
| 24 | Bhute Shalot | Moderately Tolerant | Satkhira | Landrace |
| 25 | Kute Patnai | Moderately Tolerant | Satkhira | Landrace |
| 26 | Dudh Kalam | Susceptible | Potuakhali | Landrace |
| 27 | Hati Bajore | Susceptible | Potuakhali | Landrace |
| 28 | Kalmilata | Susceptible | Potuakhali | Landrace |
| 29 | Chap Shail | Susceptible | Satkhira | Landrace |
| 30 | Rupessor | Susceptible | Potuakhali | Landrace |
| 31 | Mota Aman | Susceptible | Potuakhali | Landrace |
| 32 | Kali Boro | Susceptible | Potuakhali | Landrace |
| 33 | Kali Boro138/2 | Susceptible | Potuakhali | Landrace |
| 34 | BRRI dhan29 | Susceptible | BRRI | Modern variety |
| 35 | IR-29 | Susceptible | IRRI | Modern variety |

**Table 2:** Allele variation, PIC values and gene diversity for SSR markers identified in 35 rice gerplasms.

| Primer Name | RM | Ta ($^0$C) | NA | EPV | SR (bp) | PIC values | Rare allele | FMA | Gene diversity |
|---|---|---|---|---|---|---|---|---|---|
| **RM336** | $(CTT)_{18}$ | 55 | 17 | 154 | 129-165 | 0.8957 | 3 | 0.2000 | 0.9029 |
| **RM585** | $(TC)_{45}$ | 55 | 16 | 233 | 225-240 | 0.8915 | 2 | 0.1714 | 0.8996 |
| **AP3206** | $(GAA)_8$ | 55 | 9 | 375 | 354-374 | 0.7334 | 1 | 0.4286 | 0.7576 |
| **AP3206f** | $(AC)_{18}$ | 55 | 8 | 167 | 154-161 | 0.7487 | 1 | 0.3714 | 0.7771 |
| **RM493** | $(CTT)_9$ | 59 | 14 | 211 | 200-216 | 0.8874 | 1 | 0.1714 | 0.8963 |
| **RM7075** | $(ACAT)_{13}$ | 50 | 13 | 155 | 150-169 | 0.8594 | 2 | 0.2286 | 0.8718 |
| **Sal T1** | $(ACAA)_{15}$ | 55 | 12 | 159 | 141-159 | 0.8908 | 1 | 0.1429 | 0.8996 |
| **RM8094** | $(CT)_{18}$ | 48 | 11 | 209 | 201-215 | 0.8664 | 1 | 0.2000 | 0.8784 |
| **Mean** | | | **12.5** | | | **0.8466** | **1.5** | **0.2393** | **0.8604** |

**Ta= Annealing Temperature, RM=Repeat Motif, NA= Number of allele, EP= Expected PCR value, SR=size ranges and FMA=Frequency of major allele**

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**Table 3:** DNA fingerprinting database of selected rice genotypes based on 8 SSR marker analysis

| Genotypes | Accurate position (bp) of selected SSR Marker | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AP3206 | AP3206f | RM336 | RM493 | RM585 | RM7075 | RM8094 | SalT1 |
| Kajol Shail | 372 | 161 | 154 | 216 | 239 | 154 | 215 | 153 |
| Hogla | 372 | 160 | 152 | 209 | 249 | 162 | 209 | 147 |
| Jamai Naru | 372 | 159 | 155 | 208 | 247 | 168 | 209 | 147 |
| Dakh Shail | 372 | 159 | 150 | 212 | 240 | 154 | 209 | 150 |
| Patnai | 372 | 158 | 154 | 208 | 247 | 155 | 209 | 149 |
| Bhute Shalot | 372 | 157 | 153 | 207 | 238 | 167 | 208 | 153 |
| Kute Patnai | 371 | 157 | 150 | 207 | 242 | 154 | 207 | 153 |
| Khak Shail | 371 | 157 | 150 | 207 | 243 | 154 | 208 | 150 |
| Holde Gotal | 372 | 156 | 157 | 206 | 243 | 154 | 207 | 153 |
| Jota Balam | 372 | 156 | 158 | 213 | 254 | 160 | 209 | 149 |
| Bazra Muri | 371 | 157 | 158 | 206 | 250 | 162 | 210 | 149 |
| Ghunshi | 373 | 156 | 159 | 206 | 251 | 168 | 209 | 149 |
| Hamai, | 372 | 156 | 155 | 208 | 243 | 152 | 209 | 150 |
| Karengal, | 372 | 155 | 156 | 206 | 248 | 169 | 208 | 149 |
| Kalo Mota | 372 | 156 | 151 | 204 | 248 | 169 | 202 | 151 |
| Mondeshor | 372 | 156 | 150 | 204 | 260 | 169 | 211 | 150 |
| Nona kochi | 372 | 155 | 150 | 205 | 250 | 164 | 201 | 151 |
| Tal Mugur | 374 | 155 | 152 | 206 | 240 | 155 | 201 | 151 |
| Nona Bokhra | 373 | 154 | 152 | 206 | 240 | 155 | 201 | 151 |
| Binadhan-8 | 373 | 154 | 152 | 210 | 247 | 155 | 0/0 | 141 |
| FL-478 | 372 | 154 | 150 | 208 | 236 | 161 | 0/0 | 154 |
| Kashrail | 363 | 154 | 150 | 205 | 240 | 153 | 202 | 155 |
| JolKumari | 372 | 154 | 165 | 214 | 240 | 155 | 0/0 | 159 |
| Pokkali | 371 | 157 | 152 | 204 | 247 | 155 | 0/0 | 141 |
| FL378 | 371 | 155 | 153 | 205 | 239 | 161 | 0/0 | 155 |
| Dudh Kalam | 367 | 155 | 139 | 200 | 241 | 162 | 202 | 157 |
| Hati Bajore | 369 | 157 | 139 | 200 | 248 | 150 | 206 | 154 |
| Kalmilata | 370 | 155 | 140 | 202 | 240 | 196 | 203 | 155 |
| Rupessor | 369 | 155 | 142 | 201 | 244 | 196 | 207 | 159 |
| Mota Aman | 369 | 155 | 139 | 200 | 251 | 196 | 207 | 159 |
| Chap Shail | 367 | 155 | 138 | 200 | 251 | 196 | 206 | 155 |
| Kali Boro | 367 | 155 | 130 | 201 | 247 | 196 | 207 | 159 |
| Kali Boro 138/2 | 367 | 155 | 130 | 200 | 247 | 196 | 211 | 156 |
| BRRI dhan29 | 367 | 155 | 139 | 201 | 237 | 196 | 201 | 158 |
| IR-29 | 354 | 155 | 129 | 201 | 248 | 196 | 206 | 156 |

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**Table 4:** RAPD primers with corresponding polymorphic bands scored in 35 rice genotypes.

| Primer codes | Sequence (5′-3′) | Total number of bands scored | Number of polymorphic bands | Percentage of polymorphic loci (%) |
|---|---|---|---|---|
| OPA04 | AATCGGGCTG | 9 | 8 | 88.89 |
| OPB04 | GGACTGGAGT | 6 | 6 | 100 |
| OPB08 | GTCCACACGG | 9 | 9 | 100 |
| OPB10 | CTGCTGGGAC. | 5 | 4 | 80.00 |
| OPC02 | GTGAGGCGTC | 9 | 8 | 88.89 |
| OPC04 | CCGCATCTAC | 7 | 7 | 100.00 |
| OPC05 | GATGACCGCC | 8 | 6 | 75.00 |
| Total | | 53 | 48 | 632.78 |
| **Average** | | **7.57** | **6.87** | **90.39** |

**Table 5:** DNA fingerprinting database based on respective RAPD markers

| Loci | Size (bp) | Gene frequency | na* | ne* | h* | I* | Ht |
|---|---|---|---|---|---|---|---|
| OPA04-1 | 1293 | 0.5429 | 2 | 1.9854 | 0.4963 | 0.6895 | 0.4963 |
| OPA04-2 | 1137 | 0.9429 | 2 | 1.1208 | 0.1078 | 0.219 | 0.1078 |
| OPA04-3 | 1007 | 0.9143 | 2 | 1.1859 | 0.1567 | 0.2925 | 0.1567 |
| OPA04-4 | 904 | 0.6857 | 1 | 1.7575 | 0.4310 | 0.6225 | 0.4310 |
| OPA04-5 | 774 | 1.0000 | 2 | 1.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPA04-6 | 625 | 0.7429 | 2 | 1.6182 | 0.3820 | 0.5700 | 0.3820 |
| OPA04-7 | 489 | 0.4000 | 2 | 1.9231 | 0.4800 | 0.6730 | 0.4800 |
| OPA04-8 | 250 | 0.6286 | 2 | 1.8760 | 0.4669 | 0.6597 | 0.4669 |
| OPA04-9 | 151 | 0.3143 | 2 | 1.7575 | 0.4310 | 0.6225 | 0.4310 |
| OPB04-1 | 1275 | 0.3143 | 2 | 1.7575 | 0.4310 | 0.6225 | 0.4310 |
| OPB04-2 | 1150 | 0.7714 | 2 | 1.5448 | 0.3527 | 0.5375 | 0.3527 |
| OPB04-3 | 1025 | 0.9714 | 2 | 1.0588 | 0.0555 | 0.1297 | 0.0555 |
| OPB04-4 | 650 | 0.7143 | 2 | 1.6897 | 0.4082 | 0.5983 | 0.4082 |
| OPB04-5 | 575 | 0.6857 | 2 | 1.7575 | 0.4310 | 0.6225 | 0.4310 |
| OPB04-6 | 425 | 0.1714 | 2 | 1.3968 | 0.2841 | 0.4581 | 0.2841 |
| OPB08-1 | 2008 | 0.1143 | 2 | 1.2538 | 0.2024 | 0.3554 | 0.2024 |
| OPB08-2 | 1627 | 0.8000 | 2 | 1.4706 | 0.3200 | 0.5004 | 0.3200 |
| OPB08-3 | 1400 | 0.4857 | 2 | 1.9984 | 0.4996 | 0.6927 | 0.4996 |
| OPB08-4 | 1250 | 0.2857 | 2 | 1.6897 | 0.4082 | 0.5983 | 0.4082 |
| OPB08-5 | 1125 | 0.5714 | 2 | 1.9600 | 0.4898 | 0.6829 | 0.4898 |
| OPB08-6 | 1105 | 0.3429 | 2 | 1.8202 | 0.4506 | 0.6429 | 0.4506 |
| OPB08-7 | 1000 | 0.8571 | 2 | 1.3243 | 0.2449 | 0.4101 | 0.2449 |
| OPB08-8 | 800 | 0.5714 | 2 | 1.9600 | 0.4898 | 0.6829 | 0.4898 |
| OPB08-9 | 650 | 0.4286 | 2 | 1.9600 | 0.4898 | 0.6829 | 0.4898 |
| OPB10-1 | 1365 | 0.0857 | 2 | 1.1859 | 0.1567 | 0.2925 | 0.1567 |
| OPB10-2 | 1328 | 0.8857 | 2 | 1.2538 | 0.2024 | 0.3554 | 0.2024 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| OPB10-3 | 1181 | 0.5714 | 2 | 1.9600 | 0.4898 | 0.6829 | 0.4898 |
| OPB10-4 | 1132 | 0.0571 | 2 | 1.1208 | 0.1078 | 0.219 | 0.1078 |
| OPB10-5 | 739 | 1.0000 | 1 | 1.0000 | 0.0000 | 0.000 | 0.0000 |
| OPC02-1 | 1396 | 0.2571 | 2 | 1.6182 | 0.3820 | 0.5700 | 0.3820 |
| OPC02-2 | 1241 | 0.5714 | 2 | 1.9600 | 0.4898 | 0.6829 | 0.4898 |
| OPC02-3 | 1085 | 0.9143 | 2 | 1.1859 | 0.1567 | 0.2925 | 0.1567 |
| OPC02-4 | 852 | 0.9429 | 2 | 1.1208 | 0.1078 | 0.2190 | 0.1078 |
| OPC02-5 | 541 | 0.7143 | 2 | 1.6897 | 0.4082 | 0.5983 | 0.4082 |
| OPC02-6 | 538 | 0.2857 | 2 | 1.6897 | 0.4082 | 0.5983 | 0.4082 |
| OPC02-7 | 505 | 0.4857 | 2 | 1.9984 | 0.4996 | 0.6927 | 0.4996 |
| OPC02-8 | 463 | 1.0000 | 1 | 1.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPC02-9 | 450 | 0.0571 | 2 | 1.1208 | 0.1078 | 0.2190 | 0.4800 |
| OPC04-1 | 1729 | 0.6000 | 2 | 1.9231 | 0.4800 | 0.6730 | 0.1078 |
| OPC04-2 | 1402 | 0.9429 | 2 | 1.1208 | 0.1078 | 0.2190 | 0.4963 |
| OPC04-3 | 1205 | 0.9214 | 2 | 1.9854 | 0.4963 | 0.6895 | 0.1567 |
| OPC04-4 | 1058 | 0.9143 | 2 | 1.1859 | 0.1567 | 0.2925 | 0.4310 |
| OPC04-5 | 960 | 0.3143 | 2 | 1.7575 | 0.4310 | 0.6225 | 0.2024 |
| OPC04-6 | 812 | 0.8857 | 2 | 1.2538 | 0.2024 | 0.3554 | 0.4506 |
| OPC04-7 | 542 | 0.3429 | 2 | 1.8202 | 0.4506 | 0.6429 | 0.2527 |
| OPC05-1 | 1363 | 0.2286 | 2 | 1.5448 | 0.3527 | 0.5375 | 0.2841 |
| OPC05-2 | 1307 | 0.8286 | 2 | 1.3968 | 0.2841 | 0.4581 | 0.2449 |
| OPC05-3 | 1049 | 0.1429 | 2 | 1.3243 | 0.2449 | 0.4101 | 0.0000 |
| OPC05-4 | 872 | 1.0000 | 1 | 1.0000 | 0.0000 | 0.0000 | 0.4082 |
| OPC05-5 | 534 | 1.0000 | 1 | 1.0000 | 0.0000 | 0.0000 | 0.4082 |
| OPC05-6 | 333 | 0.2857 | 2 | 1.6897 | 0.4082 | 0.5983 | 0.4082 |
| OPC05-7 | 275 | 0.7143 | 2 | 1.6897 | 0.4082 | 0.5983 | 0.0555 |
| OPC05-8 | 201 | 0.9714 | 2 | 1.0588 | 0.0555 | 0.1297 | 0.3039 |
| Mean | | | **1.9057** | **1.5193** | **0.3039** | **0.4569** | 0.3039 |
| St. Dev | | | **0.2951** | **0.3483** | **0.1710** | **0.2262** | **0.0293** |

*na = Observed number of alleles, *ne = Effective number of alleles, *h = Nei's (1983) gene diversity, *I = Shannon's Information index, $H_t$ = Hardy-Weinberg average heterozygoity expected in sub-population and St. Dev= Standard Deviation

**Table 6:** Pearson correlation among information of SSR and RAPD analysis

| **Among 35 rice genotypes** | **SSR** | **RAPD** |
|---|---|---|
| Number of primer used | 8 | 7 |
| Total amplified band | 142.00 | 53.00 |
| Total polymorphic band | 108.00 | 48.09 |
| Percentage of polymorphic band | 76.11 | 90.39 |
| Mean of polymorphic band | 13.50 | 6.87 |
| Highest value of Nei's genetic distance | 0.90 | 0.71 |

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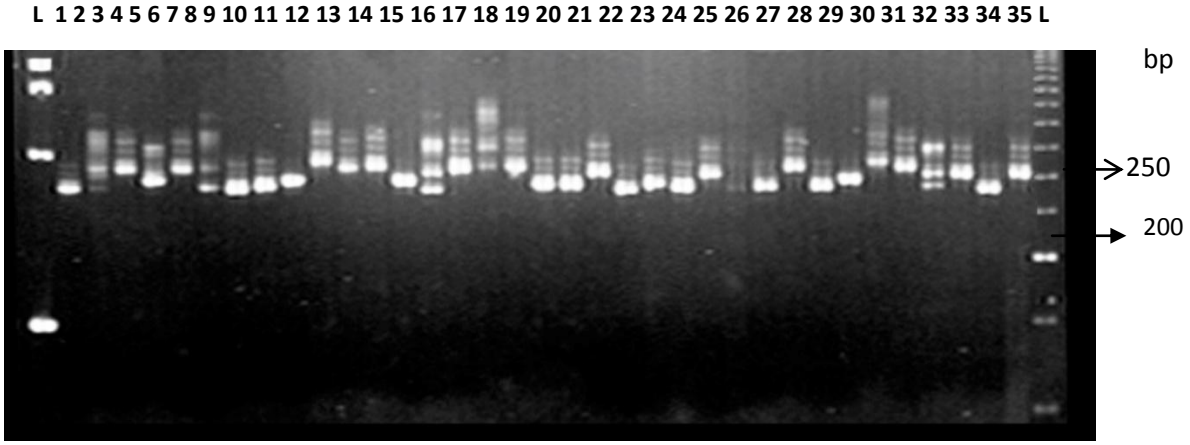
| | | |
|---|---|---|
| Average gene diversity | 0.92 | 0.30 |
| Pearson Correlation (r) | | |
| SSR | | 0.77* |
| RAPD | 0.77* | |

*Correlation is significant at the p<0.01 level

***A)***




***B)***

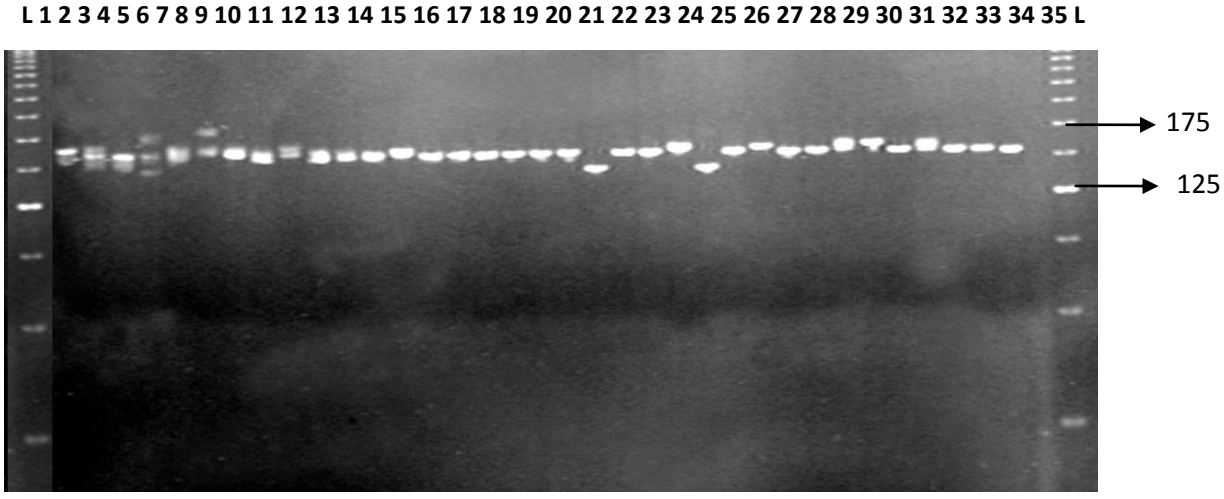



**Fig. 1:** SSR profile of 35 rice germplasm using Primer RM585**(A),** SalT1 **(B)** and AP3206**(C),** AP3206f **(D),** RM7075 (**E),** RM493**(F),** RM336 **(G),** and RM8094 **(H).** (1-Kajol Shail,2-Hogla, 3-Jamai Naru, 4- Dakh Shail, 5- Patnai,6- Bhute Shalot, 7-Kute Patnai, 8- Khak Shail, 9- Holde Gotal, 10- Jota Balam, 11- Bazra Muri, 12-Ghunshi, 13- Hamai, 14- Karengal, 15-Kalo Mota, 16- Mondeshor, 17- Nona kochi, 18- Tal Mugur, 19-Nona Bokhra, 20- Binadhan-8,21- FL-478, 22- Kashrail, 23- JolKumari, 24-Pokkali, 25-FL378, 26- Dudh Kalam, 27- Hati Bajore, 28-Kalmilata, 29-Rupessor,30- Mota Aman, 31-Chap Shail,32- Kali Boro, 33- Kali Boro 138/2, 34-BRRI dhan29, 35- IR-29 and L-25bp ladder ).

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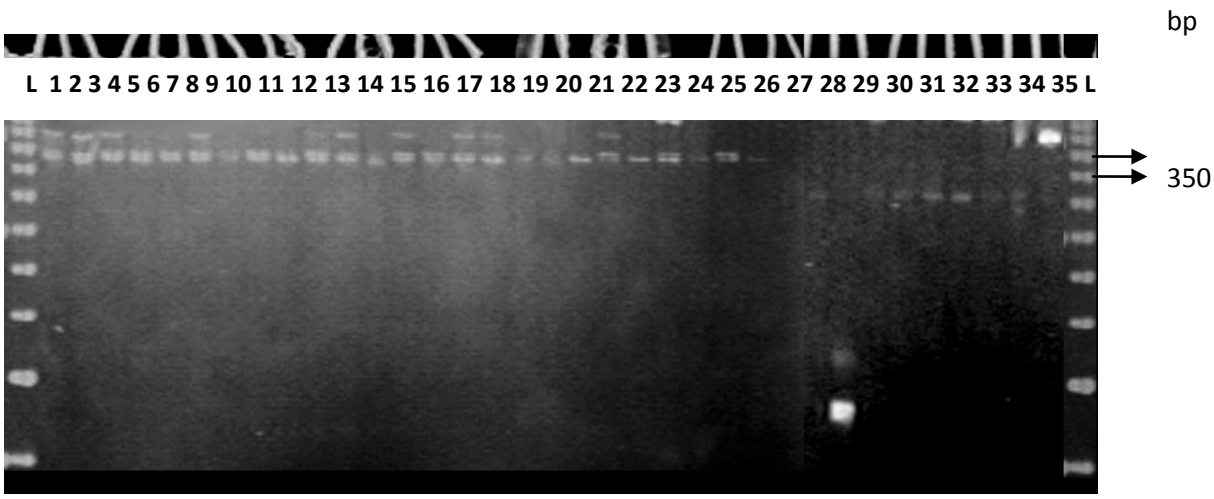
*C)*




*D)*

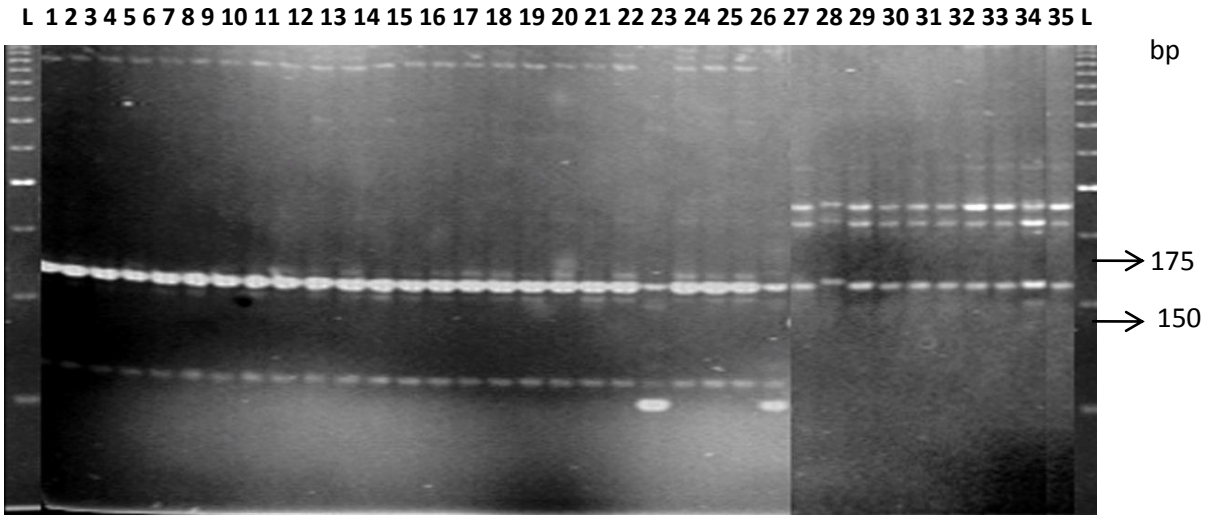
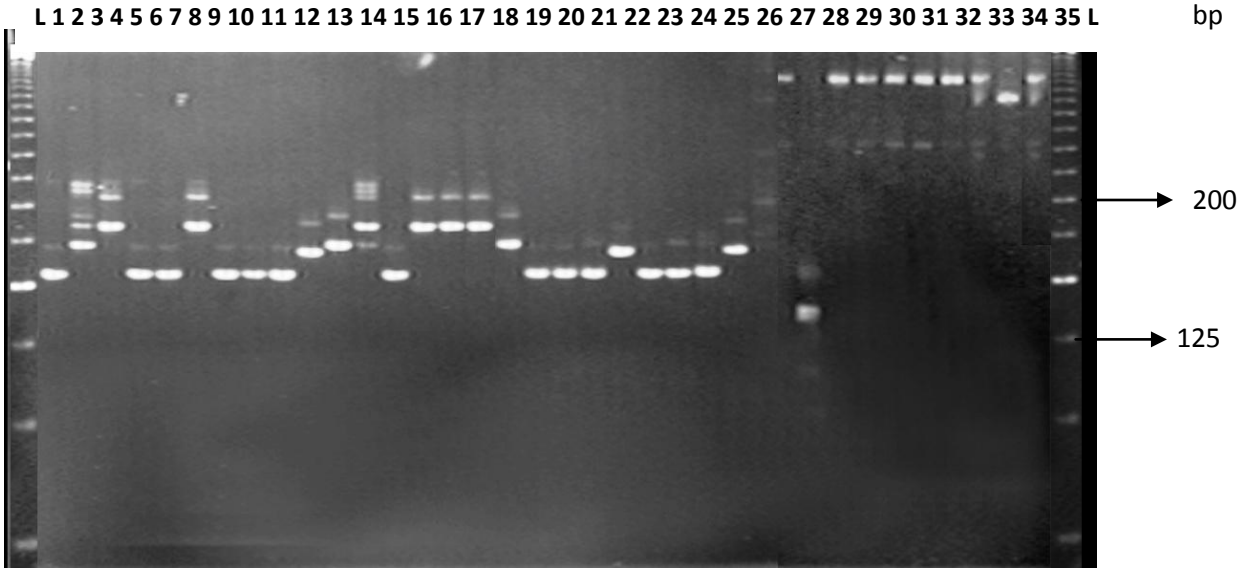



**Fig. 1:** (contd.)

*E)*



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*F)*

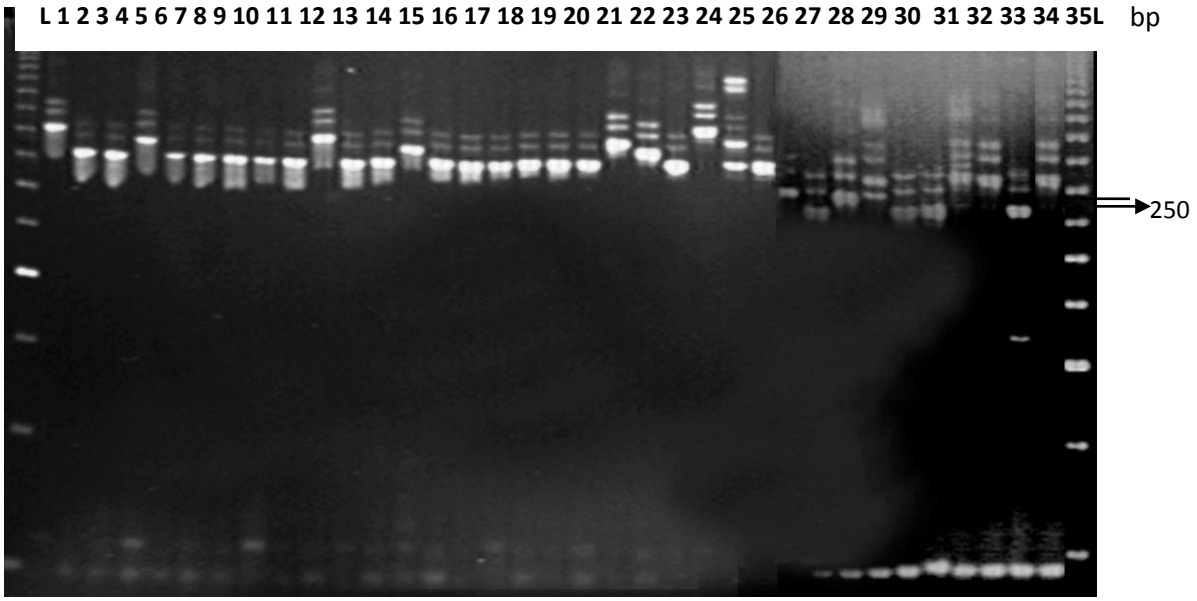
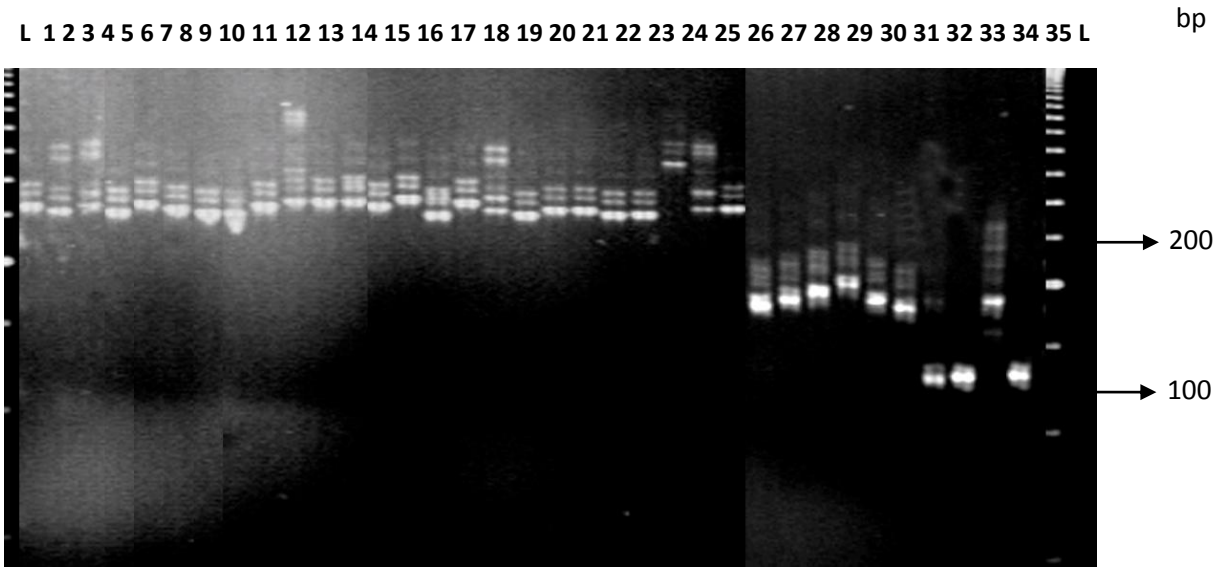



**Fig. 1:** (contd.)

*G)*



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***H)***

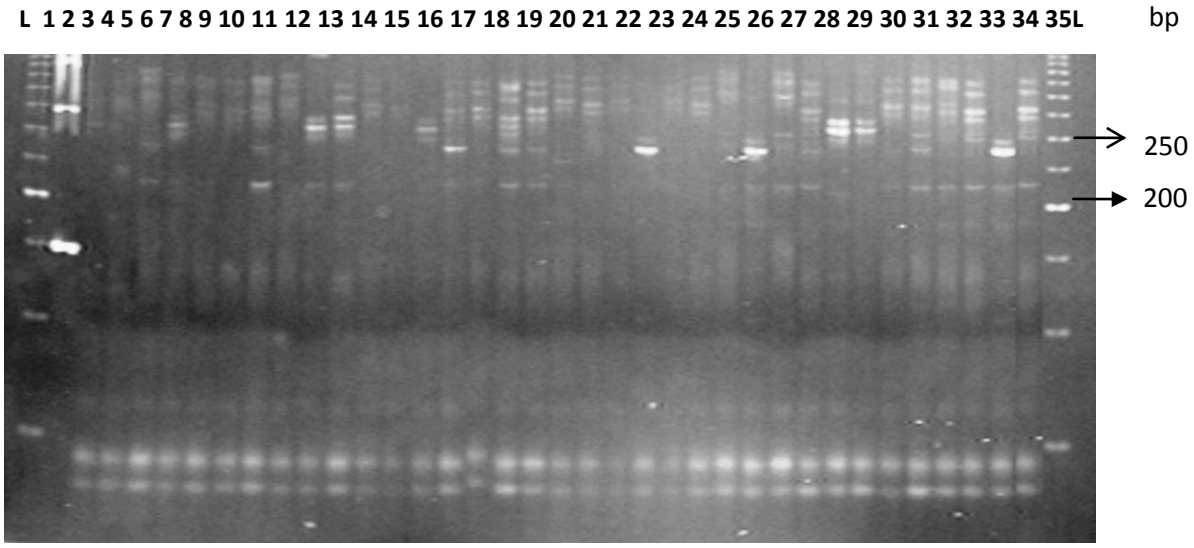
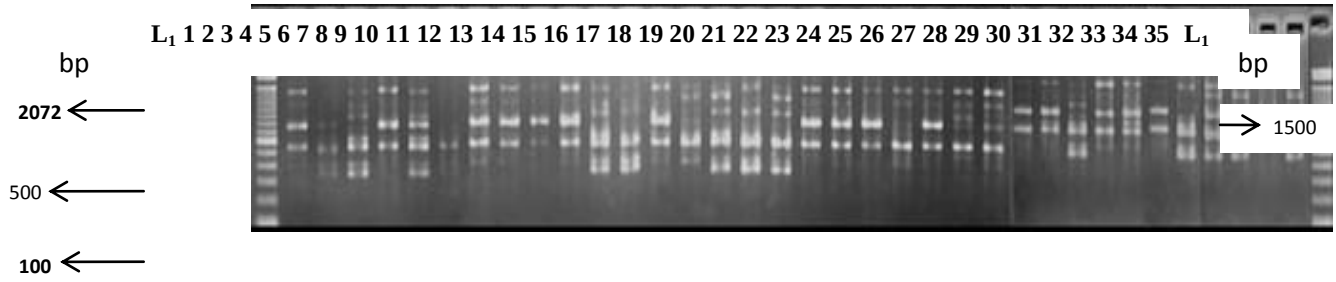
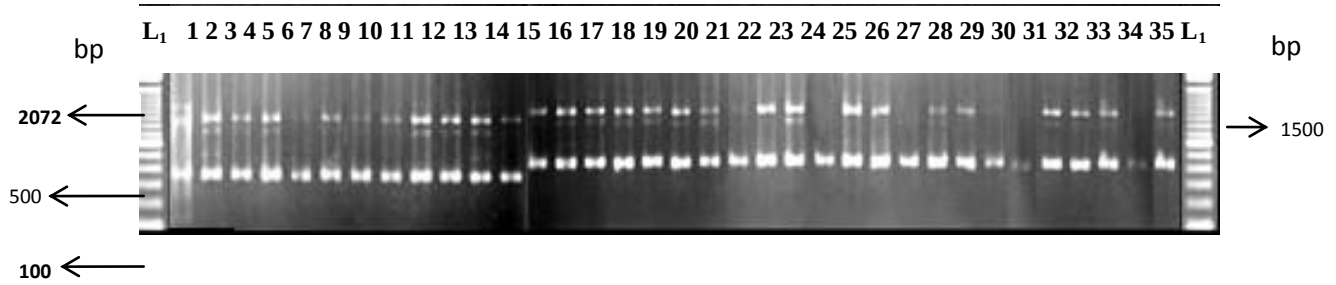



**Fig. 1:** (contd.)

***A)***




***B)***




***C)***

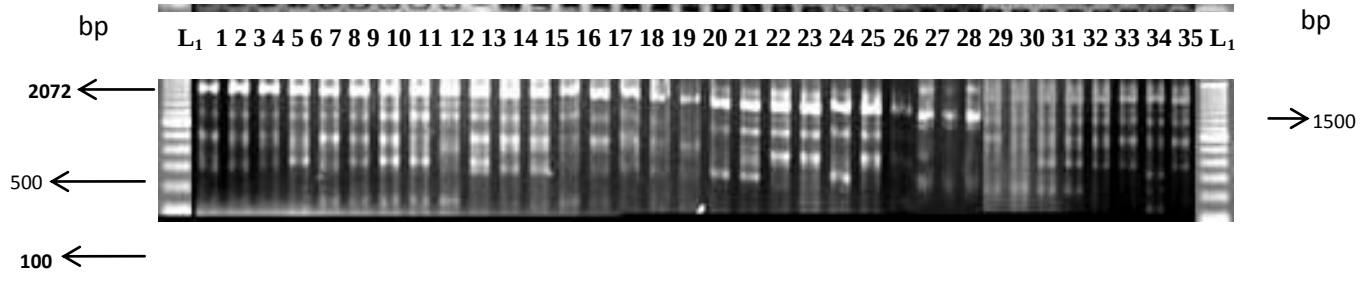


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**Fig. 2:** RAPD profile of 35 rice germplasm using Primer OPB08 **(A),** OPB10 **(B)** and OPA04 **(C),** OPB04 ***(D),*** OPC02 (**E),** OPC04 **(F)** and OPC05 **(G).** (1-Kajol Shail,2-Hogla, 3-Jamai Naru, 4- Dakh Shail, 5-Patnai,6- Bhute Shalot, 7-Kute Patnai, 8- Khak Shail, 9- Holde Gotal, 10- Jota Balam, 11- Bazra Muri, 12-Ghunshi, 13- Hamai, 14- Karengal, 15-Kalo Mota, 16- Mondeshor, 17- Nona kochi, 18- Tal Mugur, 19-Nona Bokhra, 20- Binadhan-8,21- FL-478, 22- Kashrail, 23- JolKumari, 24-Pokkali, 25-FL378, 26- Dudh Kalam, 27- Hati Bajore, 28-Kalmilata, 29-Rupessor,30- Mota Aman, 31-Chap Shail,32- Kali Boro, 33- Kali Boro 138/2, 34- BRRI dhan29, 35- IR-29 and $L_1$-100 bp ladder).

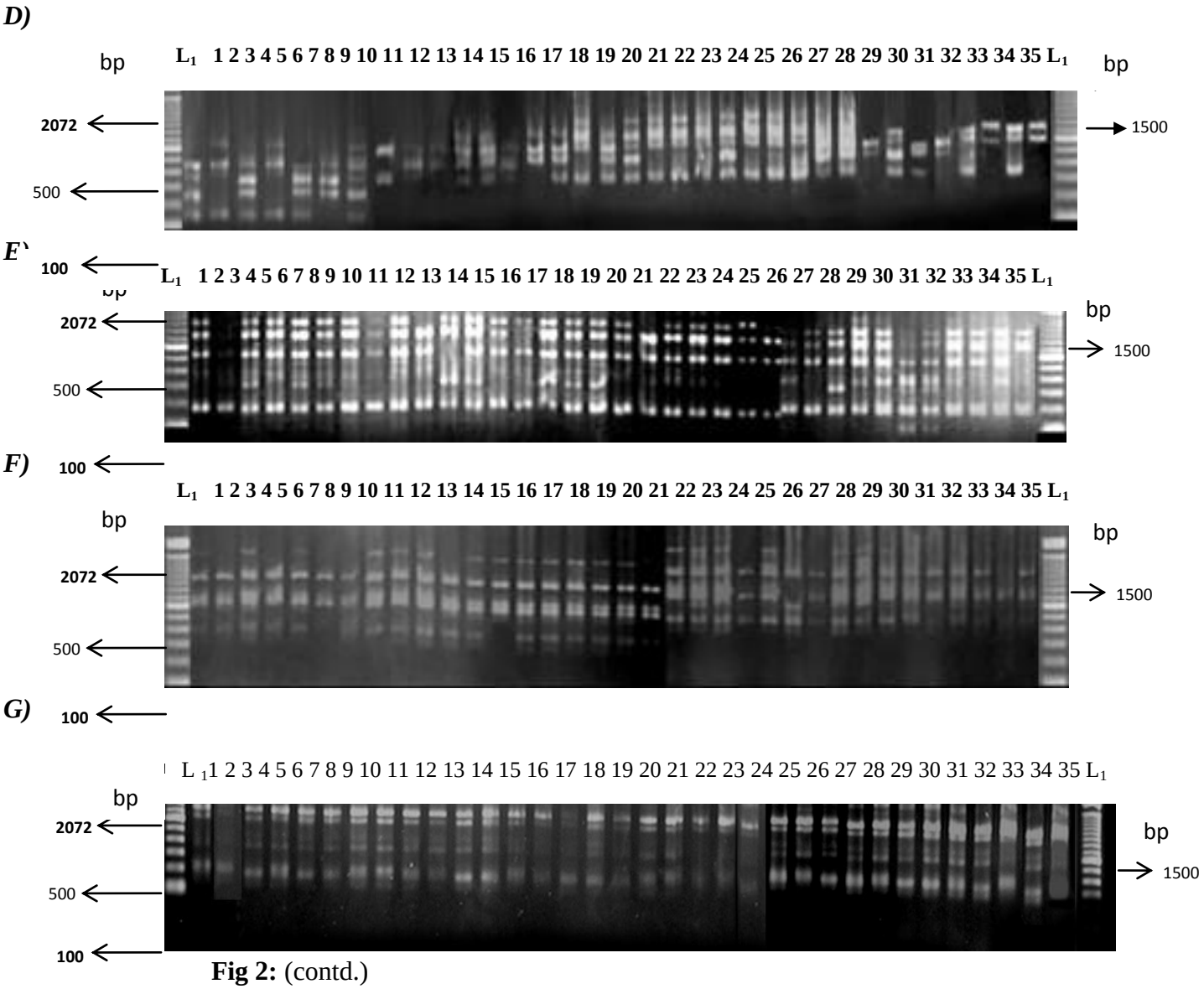


**Fig 2:** (contd.)

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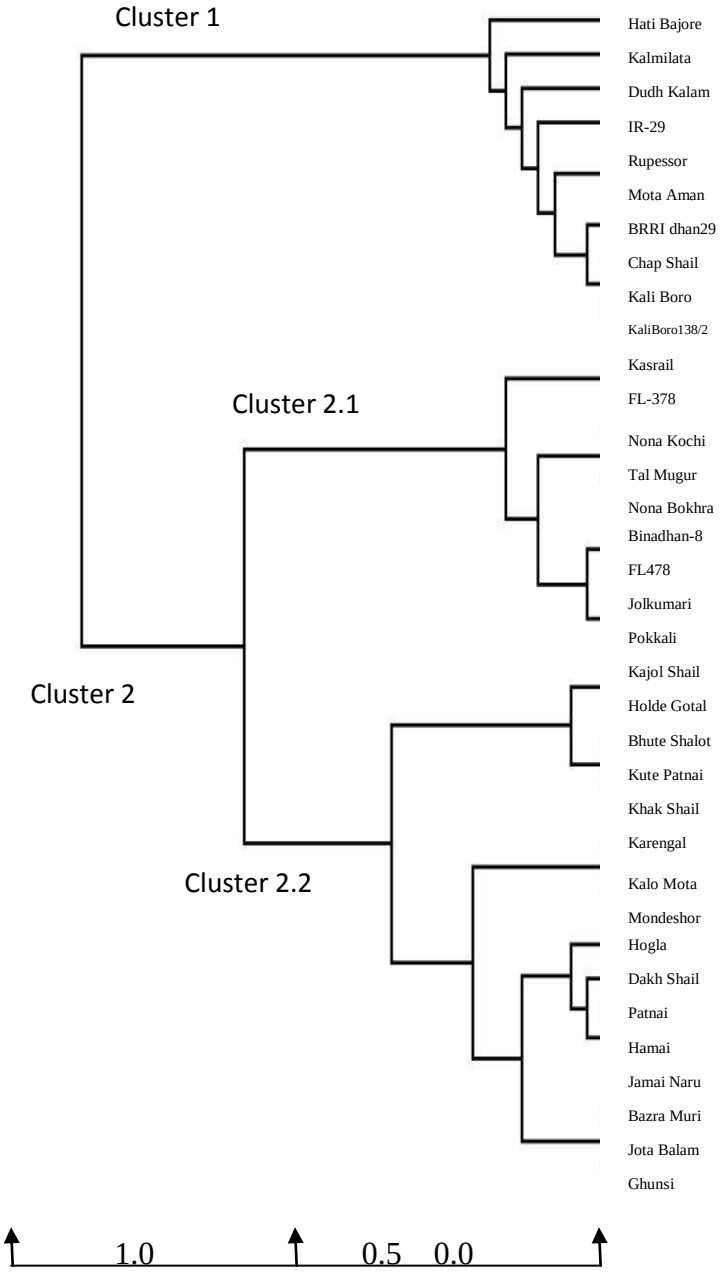


**Fig. 3:** UPGMA dendogram based on Nei's (1973) genetic distance summarizing the data on differentiation among 35 rice genotypes according to SSR analysis. Arrow line indicates the scale of genetic distance (0.00-1.00).

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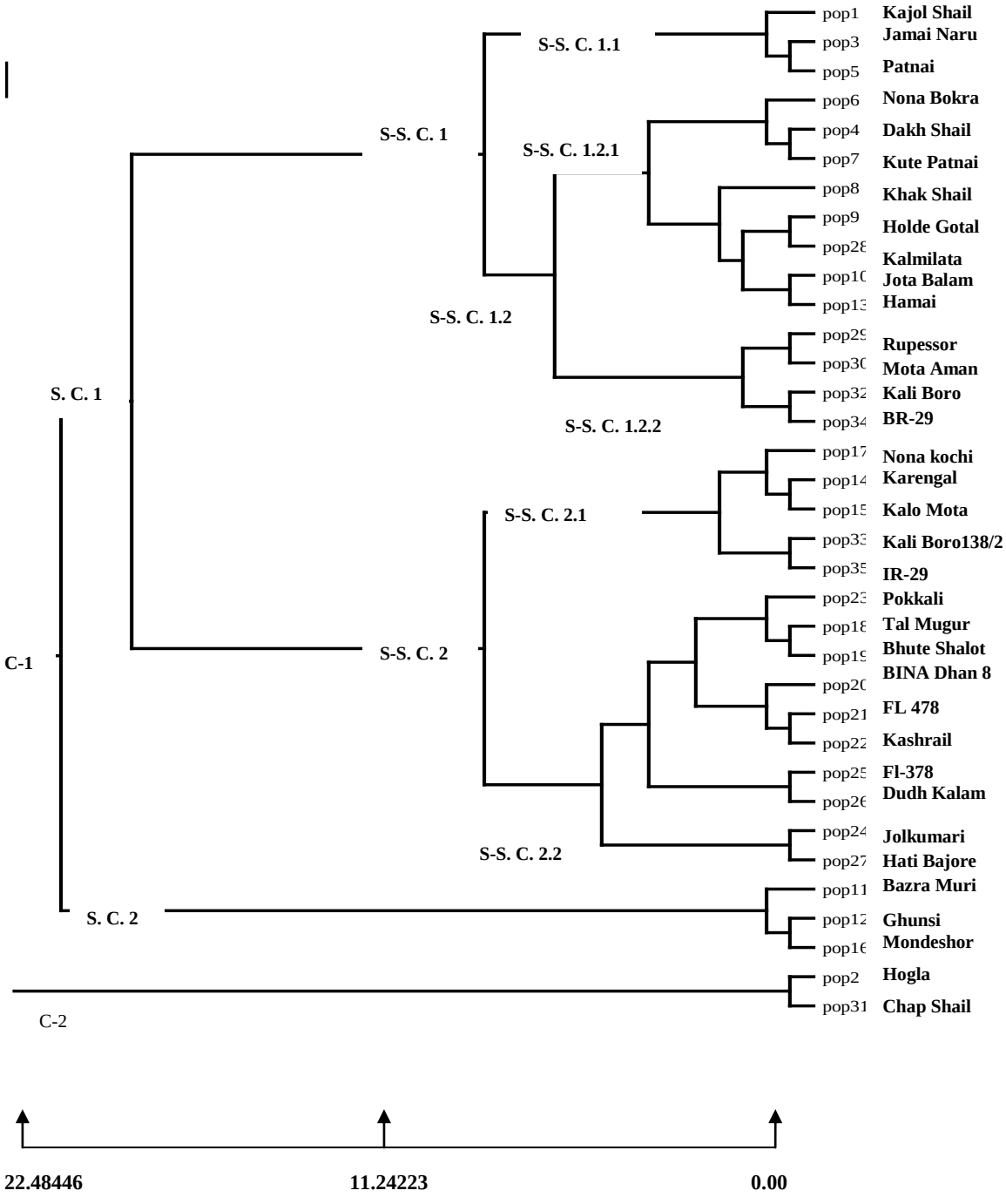


**Fig. 4:** UPGMA dendogram based on Nei's (1983) genetic distance summarizing the data on differentiation among 35 rice genotypes according to RAPD analysis. Arrow indicates the scale of genetic distance (0.00-22.48).