



Genetic diversity analysis among rice accessions differing in drought tolerance using molecular markers

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Abstract

The extent of genetic diversity among 46 *indica* ecotypes was assessed by fingerprinting using 40 random amplified polymorphic DNA (RAPD) markers. Out of the total (401) bands generated, 376 were found to be polymorphic (93.8%) and informative. Cluster analysis based on RAPD data showed that the drought tolerant and susceptible accessions were grouped in two different clusters. The rice accessions were also screened for drought tolerance under field conditions. Considerable variation was observed for drought resistance traits among the rice accessions. Norungan and TKM1 were found to perform better under water stress as compared to other accessions and may be useful in mapping genes associated with drought resistance. Correlation studies indicated that selection based on higher leaf chlorophyll stability index and proline under stress and thicker roots will be useful for improving drought tolerance in rice.

Key words: Drought resistance, genetic diversity, RAPD loci, rice accessions.

Introduction

Rice (*Oryza sativa* L.) is the principal food for more than one-third of the world's population¹ and is cultivated globally on more than 148 million hectares under wide range of agro-ecological conditions. About 25% of the world's rice area is under rainfed lowlands². Water stress is the serious threat to sustainable rice production in the rainfed ecosystem. Developing rice cultivars with inbuilt drought resistance mechanism(s) will increase rainfed rice production. However, conventional breeding has met with little success due to complexity of stress tolerance and low heritability of yield under stress. Putative traits conferring drought resistance in rice have been proposed³ but incorporation of these traits in breeding programme is limited due to difficulty in screening large germplasm for these secondary traits.

Recent advances in DNA marker technology together with the concept of marker aided selection (MAS) offer solutions to breeding for complex traits such as drought resistance. Quantitative trait loci (QTL) associated with various drought resistance components have been identified in rice⁴. However, most of these studies have been conducted in populations derived from *indica* and *japonica* parents. These two ecotypes are grown in entirely diverse environments and a trait that is useful in one environment may not be useful in another environment⁵. Further, *japonica* alleles may not express under lowland conditions⁶. Focusing on the variation within *indica* ecotypes might instead hasten progress towards breeding for drought resistance in rice.

There are several drought resistance landraces in rice, which are traditionally grown in rainfed ecosystems. The extent of genetic diversity among these accessions has not yet been fully documented especially at molecular level. Genetic diversity analysis using morphological traits was found to be difficult, because they are highly influenced by environment and only

limited number of morphological traits are available for these studies. Assessments of genetic diversity with molecular markers overcome this limitation because these molecular markers have virtually no environmental component. The discovery of polymerase chain reaction-based markers had facilitated the marker assisted selection (MAS) of desirable genotypes, variability studies, phylogenetic analysis, development of marker based gene tags, map based cloning of agronomically important genes⁷. Various molecular markers are being used for fingerprinting such as restriction fragment length polymorphism (RFLP)⁸, random amplified polymorphic DNA (RAPD)⁹, microsatellites¹⁰ and amplified fragment length polymorphism (AFLP)¹¹. Some of these techniques are robust and reliable e.g., RFLP and AFLP, while some are quick, e.g., RAPD and some others are quick and reliable, e.g., microsatellites. The use of RFLP and AFLP involves radioactive chemicals and therefore, their use is restricted. PCR based markers such as microsatellites and RAPD have been of great use in genetic diversity analysis, but microsatellite markers need prior sequence information. RAPD markers offer many advantages such as higher frequency of polymorphism, rapidity, technical simplicity, use of fluorescence, requirement of a few nanograms of DNA, no requirement of prior information of the DNA sequence and feasibility of automation¹². The use of RAPD for identification of rice accession was suggested by Fukuoka et al.¹³. Xie and Zhou¹⁴ studied the phylogenetic relationship of genus *Oryza* by RAPD analysis. However, there is little information on the extent of genetic diversity at molecular level among rice accessions grown in different hydrological habitat. Thus, the study was undertaken with an objective for evaluating the extent of variation at molecular level among rice accessions from India.

Materials and Methods

Plant materials: The rice accessions used in the present study include landraces (Varanel, Puzhudikar, Rajashree, Nelmani, Norungan, Kallurundaikar, Lunishree, Dharmapuri local, Jaldidan-3) and improved cultivars (Adibya, ASD 17, W 1263, ASD 19, Asha (MO 8), CO 43, CO 45, CSR 13, CSR 27, CSR 30, CST-7-1, Heera, IET 13652, IR 20, IR 64, IR 72, Kairali (PTB-49), Kanchana (PTB-50), MDU 5, Peta, Pisini, PMK 1, PMK 2, Pokkali, Ponmani, Prasanna, Sornavari, Suraksha, TKM 1, TKM 2, TKM10, TKM11, TKM 12, TPS 1, TRY 2, Tulasi, Vaidehi, Vandana) derived from different hydrological habitat.

Field trial: Field trial was conducted under upland condition in Tamil Nadu Agricultural University, Coimbatore during dry season (February-June, 2003). The rice accessions were evaluated under water stress with 3 replications. Plot size was 2.5 m x 0.2 m with 20 cm x 10 cm spacing between and within rows, respectively. Seeds were hand-dibbled into dry soil. Fertilizers, insect and weed control measures were applied periodically as required. All plots were surface irrigated to field capacity once a week, except when water stress was imposed by withholding irrigation to stress plots from 60 days after sowing (DAS).

Field measurements: There was a continuous rain-free period for 18 days. During peak stress, changes in soil moisture and penetration resistance were monitored periodically in stress plots with a Thetaprobe and penetrometer, respectively. Leaf rolling and drying scores were taken at midday 15 days after stress on using 1 to 7 scale standardized for rice¹⁵. Leaf relative water content (RWC) was determined at mid day, 16 days after stress¹⁶. Canopy temperature was measured 17 days after stress¹⁷. Leaf samples for estimation of chlorophyll stability index¹⁸ and proline¹⁹ were taken 17 days after stress. Stress was relieved 18 days after stress and recovery score was made 3 days after rewatering. Following this both control and stress plots were regularly irrigated until harvest. The plants were harvested at 120 DAS and biomass was recorded.

DNA isolation and RAPD DNA loci amplification: The genomic DNA was isolated from all the rice accessions and diluted to 25 ng/ μ L. Forty random primers viz., OPG 03, 04, 09, 11, 12, 13, 14, 15, 16, 17, 19, OPAG 04, 06, 07, 10, OPF 04, 05, 08, 09, 10, 15, 16, OPR 07, 16, 20, OPC 03, 06, 10, 12, 14, 17, 19, OPAK 06, 14, 16, 17, 18, 19, OPS 12 and 13 (Operon Technologies Inc., USA) were used to amplify the genomic DNA. All the primers had a GC content of 60-70%. PCR reactions were done by essentially following Williams et al.²⁰. Agarose gel electrophoresis stained with ethidium bromide was carried out and the gel was documented. From the gel profile, bands of DNA were scored for their presence or absence.

Data analysis: Analysis of variance and simple correlation were performed for drought resistance component traits using the formula suggested by Singh and Choudhary²¹. The amplified products were scored separately for each RAPD primer for all the rice accessions. Presence or absence of individual bands was denoted as 1 and 0, respectively. The scores of individual bands were used to create a data matrix as described by Rohlf²² and dendrogram was constructed based on Jaccard's similarity coefficient²³ with unweighted pair group method (UPGMA)²⁴

using the NTSYS-pc version 2.02 (Exeter Software, New York, USA).

Results and Discussion

Genetic variation and correlation among drought resistance

component traits: Significant variation in various drought tolerance traits was observed among the rice accessions (Table 1). Phenotypic and genotypic correlation coefficients among physio-morphological traits and biomass under stress among the rice accessions were also worked out (Table 2). Leaf rolling is one of the visible physiological responses to plant water deficit²⁵. Among the rice accessions, TKM1, TKM10, Norungan, Sornavari and W1263 showed lesser leaf drying/rolling score. Leaf rolling and leaf drying under stress were positively correlated with canopy temperature. This result is in conformity with findings of Babu et al.²⁶. Many researchers have used canopy temperature as a means of determination of water stress due to inadequate soil moisture^{30, 31}. The present study showed wide genetic variation among rice accessions for canopy temperature under stress. Varanel, Vaidehi, TKM10, Norungan and Rajashree maintained relatively cooler canopy temperatures. Canopy temperature was positively correlated with leaf rolling ($r_p=0.47^{**}$) and leaf drying ($r_p=0.6^{**}$). Canopy temperature showed significant negative correlation with root thickness. These results were in accordance with Blum et al.³² and Babu et al.²⁶. Relative water content (RWC) is considered as an alternative measure of plant water status, reflecting metabolic activity in tissues and used as a most meaningful index for dehydration tolerance²⁷. In the present study, ASD19, TRY2, Kairali, Jaldidan-3 and Ponmani recorded higher RWC as compared to others. The difference in RWC among the rice accessions causes difference in their survival under drought stress conditions²⁸. RWC was negatively correlated with proline under stress and these results were in accordance with Michael Gomez and Rangasamy²⁹.

Chlorophyll stability index (CSI) is a measure of integrity of cell membrane and heat stability of pigments under stress conditions¹⁸. Green pigments are thermosensitive and their degradation occurs when subjected to higher temperature. In the present study, Varanel, Jaldidan-3, Norungan, TKM11, TKM2 showed higher CSI under stress as compared to others. CSI was positively and significantly correlated with root thickness ($r_p=0.57^{**}$) and proline ($r_p=0.88^{**}$) under stress. Similar results were reported by Michael Gomez and Rangasamy²⁹. Water stress triggers the accumulation of proline in a wide variety of plant species³³, indicating that proline contributes to maintain in proper balance between extracellular and intracellular osmolarity under water stress³⁴. The present study reveals a greater pool of free proline in Varanel, Jaldidan-3, Norungan, TKM11 and TKM2. This accumulated proline may contribute towards osmotic adjustment, which plays a major role in maintaining turgor³⁵. Proline under stress was negatively correlated with RWC ($r_p=-0.09$) and this was in accordance with Michael Gomez and Rangasamy²⁹. Root morphology and rooting patterns directly affect the amount of water available to a crop and increased width, depth and branching of root system have been shown to decrease plant water stress in rice³⁶. The ability of rice to penetrate compacted soil is linked with the capacity to develop thick and long root axes³⁷ and this has been shown to contribute to

Table 1. Variation in physio-morphological traits under water stress among the rice accessions.

Variety	LR	LD	RWC (%)	CT (°C)	CSI (%)	Proline mg/g	SR	RT (mm)	Biomass g/m ²
Adibya	6.3	5.6	52.8	42.7	68.8	0.67	5.6	0.72	149.1
Jaldidan-3	4.6	3.6	65.3	42.9	78.1	0.87	1.6	1.27	676.4
Asha	4.6	4.3	50.8	44.2	67.7	0.73	3.3	1.23	305.3
ASD17	3.6	4.0	33.4	41.7	42.2	0.63	3.6	0.61	422.0
ASD19	6.6	5.6	79.6	45.7	28.6	0.47	6.6	0.69	66.67
CO45	6.0	5.3	24.6	45.2	38.5	0.57	4.6	0.64	173.3
CSR13	6.6	6.6	59.4	44.7	30.4	0.62	6.6	0.41	68.00
CSR 27	6.6	6.6	47.9	44.8	35.3	0.46	7.0	0.74	95.5
CSR30	6.6	5.6	57.6	41.4	58.6	0.69	4.3	0.55	136.6
CST-7-1	6.6	6.3	42.8	47.6	61.1	0.73	5.6	0.78	83.53
Heera	6.0	5.6	57.0	42.1	49.2	0.65	6.0	0.77	129.3
IET-13652	6.6	6.3	43.2	48.7	31.0	0.57	6.6	0.68	120.0
Kairali	5.0	4.3	65.9	42.0	57.3	0.71	1.6	0.88	455.0
Kanchana	6.6	6.0	51.4	40.3	58.1	0.69	5.3	0.85	178.0
Lunishree	6.3	5.6	45.6	41.3	67.4	0.76	4.6	1.27	124.3
MDU-5	6.6	6.3	37.3	45.9	32.0	0.57	6.0	0.75	116.9
Nelmani	5.6	5.3	56.7	42.5	42.2	0.65	3.6	0.91	121.8
Peta	6.6	4.0	51.6	40.2	65.1	0.73	1.3	0.98	622.8
Pisini	5.0	3.6	36.2	40.7	42.7	0.62	1.0	1.18	716.0
PMK-1	4.6	4.3	22.0	44.8	55.7	0.77	3.6	0.90	408.0
Ponmani	6.3	6.3	63.3	40.5	42.3	0.61	5.3	0.99	90.47
Pokkali	6.6	6.6	37.6	45.2	35.7	0.64	6.3	0.67	118.6
Prasanna	6.6	5.3	50.3	40.9	58.8	0.69	4.6	0.79	161.5
Puzhudikar	6.6	4.6	47.0	40.7	50.7	0.69	2.0	1.19	411.3
Rajashree	6.3	5.3	48.4	39.0	51.2	0.70	4.0	0.89	272.2
Sornavari	4.3	2.6	34.9	41.3	50.4	0.70	1.0	1.14	1183.1
Suraksha	3.6	3.3	50.0	41.1	46.8	0.67	1.3	1.20	540.4
TPS-1	6.6	5.6	43.2	44.4	55.5	0.75	5.0	0.89	32.0
Tulasi	6.0	5.6	56.5	44.8	64.7	0.78	3.3	0.88	182.2
TKM-1	3.6	2.6	52.5	39.7	63.7	0.77	1.0	1.27	746.4
TKM-2	4.6	3.6	31.3	40.0	74.4	0.85	2.0	0.85	370.6
TKM-10	3.6	2.6	43.8	38.7	53.1	0.72	1.0	1.16	513.8
TKM 11	5.6	3.6	31.4	46.8	76.7	0.81	4.3	0.98	346.6
TKM-12	4.6	5.6	49.0	46.2	37.3	0.61	5.0	0.70	138.2
Vaidehi	3.6	4.3	47.2	38.6	72.3	0.74	3.3	1.01	562.0
Vandana	6.6	6.3	60.8	42.9	54.6	0.70	5.3	0.68	103.7
Varanel	4.6	3.6	35.1	38.4	79.9	0.92	1.6	1.11	561.3
IR-20	6.6	6.6	55.2	45.2	26.2	0.40	7.0	0.56	48.8
IR-64	6.6	6.6	46.9	6.0	34.9	0.42	6.6	0.51	38.6
IR-72	4.6	3.6	48.4	42.2	42.9	0.51	1.3	0.95	271.7
CO-43	6.6	6.3	27.4	42.9	42.0	0.65	5.0	0.62	112.8
TRY-2	6.3	5.6	71.0	42.5	39.1	0.61	5.0	0.93	76.8
PMK-2	4.0	4.0	30.9	39.8	53.0	0.69	2.3	1.13	297.3
Norungan	4.3	3.3	51.1	38.9	77.9	0.97	1.0	1.21	876.6
W1263	5.6	3.3	27.3	39.7	33.5	0.46	2.0	0.75	746.4
Kallurundaikar Dharmapuri local	4.3	3.3	52.0	39.0	54.2	0.80	1.3	1.16	724.6
Mean	5.6	4.9	47.2	42.5	52.25	0.68	3.8	0.89	319.8
Range	0.41-1.27	38.4-48.7	22.0-79.6	26.3-79.9	0.4-0.97	3.7-6.7	2.67-6.7	1-7	32-1183.1
CD (0.01 P)	0.79**	0.87**	12.38**	1.02**	2.69**	0.01**	1.01**	0.10**	146.29**

** Significant at 0.01% probability level , LR-Leaf rolling , CT-Canopy temperature , RT-Root thickness , LD-Leaf drying
CSI-Chlorophyll stability index , SR-Stress recovery , BM-Biomass , CSI-Chlorophyll stability index , RWC-Relative water content

drought resistance. Considerable genetic variation was observed in the present study among the rice accessions for root thickness. Jaldidan-3, Lunishree, TKM1, Asha and Norungan had thicker roots with a diameter of more than 1.2 mm each. Root thickness was positively correlated with CSI. The capacity for plant recovery, which is often referred to as 'survival' is a very common phenomenon in the plant kingdom. Plant recovery from desiccation is primarily a function of capacity for maintaining RWC during desiccation³². In the present study, Pisini, Sornavari, TKM1, TKM10 and Norungan showed good stress recovery.

Recovery from drought is considered as a very important trait in cultivars for rainfed systems as cyclic stress is a general phenomenon³⁸. The maintenance of better dry matter during stress may be viewed as one of the criteria to decide drought tolerance. In the present study, chlorophyll stability index ($r_p=0.39^{**}$), proline under stress ($r_p=0.45^{**}$) and root thickness ($r_p=0.64^{**}$) exhibited positive and significant correlation with biomass. Norungan, TKM1, W1263 and Kallurundaikar recorded higher biomass under stress. These rice accessions would serve as donors for the future drought tolerance breeding in rice.

Table 2. Phenotypic and genotypic correlation coefficients among physio-morphological traits and biomass under stress among the rice accessions.

Traits		LD	RWC	CT	CSI	Pr	SR	RT	BM
LR	r _p	0.81**	0.22	0.47**	-0.33**	-0.39**	0.72**	-0.57**	-0.68**
	r _g	0.86**	0.27	0.52**	-0.35**	-0.42**	0.78**	-0.64**	-0.79**
LD	r _p		0.25	0.60**	-0.48**	-0.48**	0.92**	-0.71**	-0.87**
	r _g		0.34	0.67**	-0.51**	-0.52**	0.99**	-0.78**	-0.99**
RWC	r _p			0.00	-0.05	-0.09	0.18	0.00	-0.23
	r _g			0.02	-0.06	-0.10	0.22	0.00	-0.27
CT	r _p				-0.41**	-0.41**	0.68**	-0.54**	-0.58**
	r _g				-0.42**	-0.42**	0.72**	-0.56**	-0.61**
CSI	r _p					0.88**	-0.50**	0.57**	0.39**
	r _g					0.88**	-0.53**	0.59**	0.41**
Pr (s)	r _p						-0.54**	0.59**	0.45**
	r _g						-0.56**	0.61**	0.46**
SR	r _p							-0.75**	-0.84**
	r _g							-0.79**	-0.89**
RT	r _p								0.64**
	r _g								0.68**
BM	r _p								
	r _g								

** Significant at 0.01% probability level
 LR - Leaf rolling, LD - Leaf drying, RWC - Relative water content, CT - Canopy temperature,
 CSI - Chlorophyll stability index,
 Pr - Proline under stress, SR - Stress recovery, RT - Root thickness, BM - Biomass.
 r_p = Phenotypic correlation coefficient
 r_g = Genotypic correlation coefficient

Cluster analysis using RAPD marker data: In the present study, DNA extracted from rice accessions was amplified with 40 decamer primers and a total of 401 scorable RAPD bands were generated. Out of these, 376 (93.8%) were polymorphic. RAPD profile of one of the representative primer is shown (Fig. 1). The maximum number of amplified products was generated by the primer OPG09 (18) followed by OPAK16 (17) and OPF 16 (15). The GC content of all the primers varied between 60-70%, and the primers with GC content more than 40% gave higher polymorphism²⁰. In case of rice, it is found that the number of amplicon products tended to increase with increasing GC content¹³. A dendrogram was generated using data matrix from RAPD primers (Fig. 2). It was seen that the 46 rice accessions were grouped into two major clusters namely A and B. The major cluster A was divided into two minor clusters namely, A₁ and A₂. The minor cluster A₁ was further divided into A₃ and A₄. Subcluster A₃ consisted of 14 accessions namely Adibya, Prasanna, ASD17, CSR27, CSR13, CST 7-1, Heera, IET13652, Kanchana, Lunishree, Pokkali, Kairali, MDU5 and ASD19. Subcluster A₄ consisted of CSR30 alone. CSR13, CSR27, CST-7-1, MDU5, Pokkali, Kanchana, Kairali, Adibya, Lunishree, which are saline tolerant, were grouped into one minor cluster whereas CSR30, a saline tolerant variety, was grouped into different cluster. The minor cluster A₂ was further subdivided into A₅ and A₆. Subcluster A₅ consisted of Puzhudikar, Rajashree, TKM10, Sornavari and Suraksha. Subcluster A₆ consisted of 18 accessions viz., TPS1, Tulasi, TKM12, IR20, CO43, IR64, IR72, TRY2, PMK2, Vandana, TKM1, TKM2, Norungan, TKM11, Vaidehi, Varanel, Kallurundaikar and W1263. The rice accessions TKM1, TKM2, TKM10, TKM11, TKM12 were grouped into a minor cluster A₂ and these are improved cultivars grown in rainfed areas. All the IR rice accessions viz., IR20, IR64 and IR72 were grouped together and these accessions were drought susceptible and suitable for irrigated ecosystem only. Most of the landraces viz., Norungan, Kallurundaikar, Varanel and W1263 were grouped in the subcluster A₆. Puzhudikar, Rajashree, TKM10, Sornavari and Suraksha were grouped into another minor cluster A₅. The major cluster B was divided into two minor clusters B₁ and B₂. Minor cluster B₁ had Jaldidan, Pisini, Peta, Ponmani and PMK1. Minor cluster B₂ had

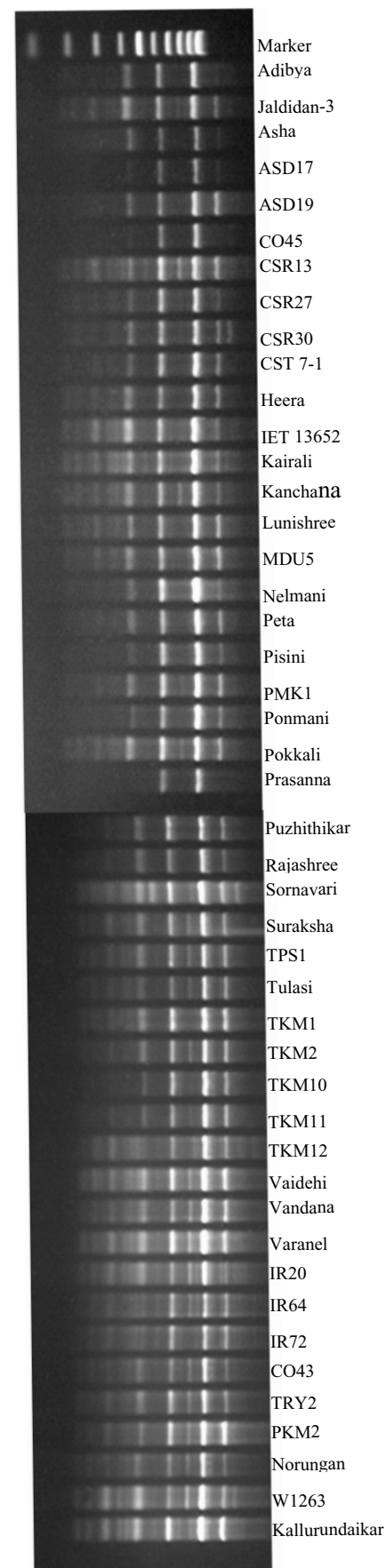


Figure 1. RAPD profile for the primer OPR07 in rice accessions

Asha, CO45 and Nelmani. Most of these rice accessions are drought tolerant or suited for rainfed areas, but they have been grouped into different clusters due to their pedigree. Cluster analysis based on similarity index (SI) generated from RAPD data showed that Asha and Nelmani are distantly related to IR64 and IR72. Asha and Nelmani are from acidic/saline rainfed ecosystems of Kerala, India. IR 64 and IR 72 are from irrigated lowland ecosystems of Philippines. Diversity in hydrological habitat and the geographical location of these rice accessions are well brought out by molecular diversity analysis in the present study. The study helped to identify diverse lines (Norungan, TKM 1, W 1263 and Kallurundaikar) for developing mapping population for tagging QTLs for drought resistance in rice.

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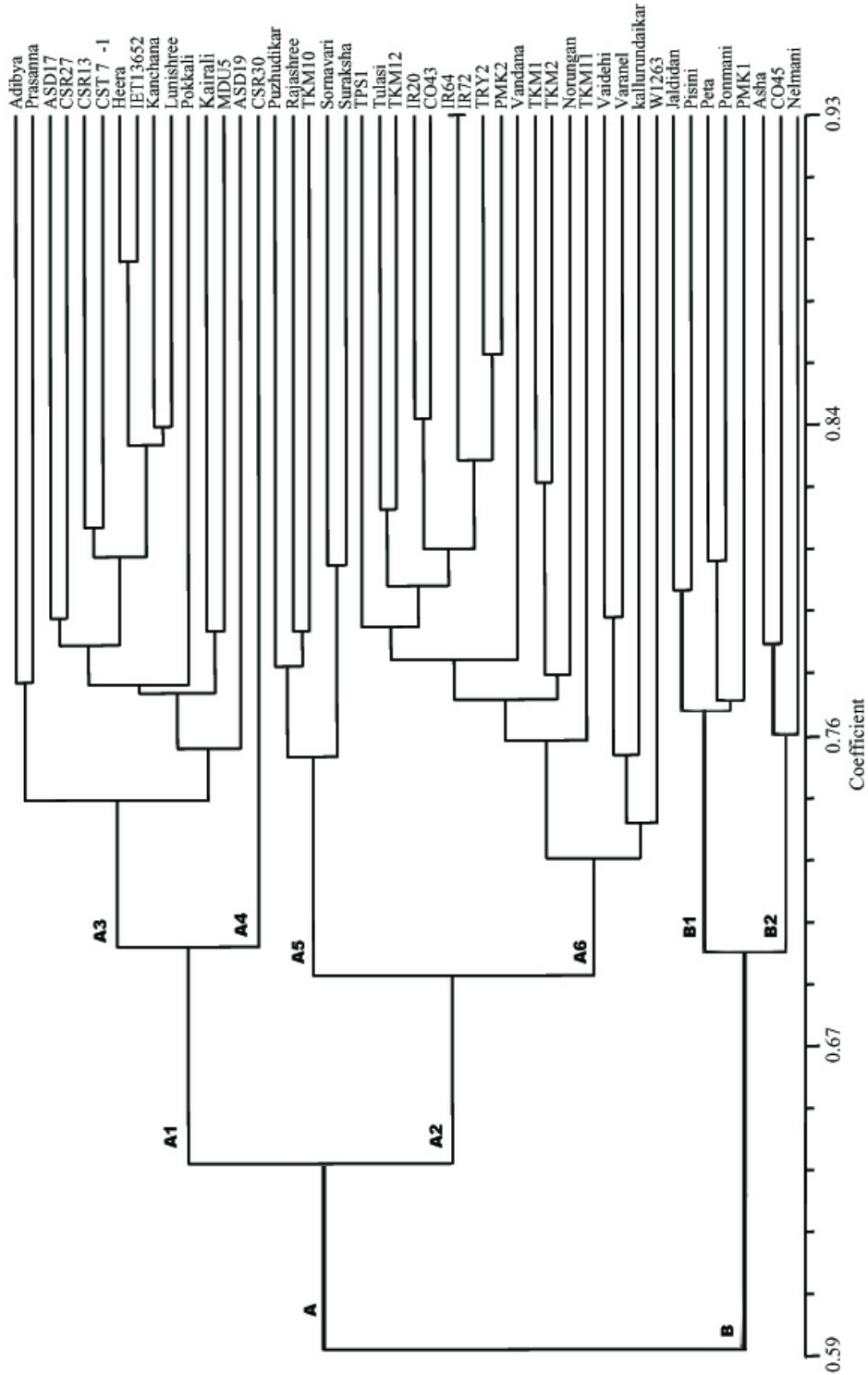


Figure 2. Dendrogram for 46 rice accessions based on data matrix from 40 RAPD primers.