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Genetic diversity analysis of rice cultivars (*Oryza sativa* L.) differing in salinity tolerance based on RAPD and SSR markers

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Abstract Thirty rice cultivars were evaluated for salinity tolerance during the seedling stage and were divided into five tolerance groups including tolerant (T), moderately tolerant (MT), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) which comprised 5, 10, 9, 4 and 2 cultivars respectively. Genetic diversity of all rice cultivars was evaluated using random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) markers. The cultivars were evaluated for polymorphisms after amplification with 20 random decamer primers and 20 SSR primer pairs. A total of 161 RAPD markers and 190 SSR alleles were produced which revealed 68.94% and 89.47% polymorphism respectively. Mean genetic similarity coefficient was 0.82 for RAPD and 0.70 for SSR. Cluster analysis based on RAPD markers was effective in grouping cultivars based on their salt tolerance ability. Group IA_1, IB and IV contained three T, three S and two HS rice cultivars respectively. The MT and MS cultivars which showed similar physiological responses to salinity were resolved into two groups: Group IA_2 and Group II comprising ten and eight MT/MS cultivars respectively. Cluster analysis based on SSR markers separated rice cultivars into groups based on genetic relatedness which did not correspond to salinity tolerance level. The results from this study provided some useful implications for salt tolerance breeding programs. The evaluation of genetic similarity and cluster analysis together with salt tolerance ability provides some useful guides for assisting plant breeders in selecting suitable genetically diverse parents for the crossing program.

Keywords: genetic similarity, RAPD markers, rice, salinity tolerance, SSR markers

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops. About 3 billion people, nearly half the world's population, depend on rice for survival. In Asia as a whole, much of the population consumes rice in every meal. In many countries, rice accounts for more than 70% of human caloric intake. In Thailand, 66% of agricultural area is extensively used for growing rice. However, about 35% of rice-growing area faces varying degrees of soil salinity, which originated from the accumulation of underground salt dome and exacerbated by salt mining, deforestation and irrigation (Akbar, 1986). Therefore, this problem has become a major concern for rice production particularly in coastal regions and inland areas of the tropics.

Soil salinity is a major factor limiting agriculture in the northeast of Thailand. Salt stress or salinity stress occurs when plants uptake overabundant ions, such as Na^+, Ca^{2+}, Mg^{2+}, SO_4^{2-} and Cl^-. Saline soils cause two major problems for plants (1) high concentration of salts decreases soil water potential making water uptake difficult (2) accumulation of high concentration of Na^+ and Cl^- is toxic to plant cells

(Parida and Das, 2005). Salt tolerant plants evolve several mechanisms for salinity tolerance including the modification of membrane characteristics involved in ion absorption, translocation, compartmentation and excretion of salt (Li et al. 2008). These tolerance mechanisms facilitate retention and acquisition of water, protect chloroplast functions, and maintain ion homeostasis. Essential pathways that lead to synthesis of osmotically active metabolites (such as proline, glycinebetaine, sugars, etc.) and certain free radical scavenging enzymes help control ion and water flux and support scavenging of oxygen radicals (Chinnusamy et al. 2005). Physiological characters which have usually been used for screening rice cultivars for salinity tolerance at the seedling stage included plant survival, plant dried weight, leaf injury and Na^+/K^+ ratio (Gregorio et al. 1997; Ali et al. 2004; Bhowmik et al. 2009; Haq et al. 2009). Recently, several authors found that salt-susceptible rice cultivars accumulated higher level of proline under salt stress than the salt-tolerant ones (Renuka Devi et al. 1996; Demiral and Türkan, 2005; Theerakulpisut et al. 2005).

Rice is considered susceptible to salinity particularly during early vegetative and later at the reproductive stages (Mass and Hoffman, 1977; Shannon et al. 1998). Rice genotypes vary considerably in salinity tolerance that is principally due to additive gene effects (Sahi et al. 2006). One major approach to generate salt-tolerant rice cultivars through breeding is to maximize the genetic diversity between parental genotypes that is usually estimated by measurements of morphological and physiological differences. This conventional approach for screening rice genotypes can be costly, space- and time-consuming and labor-intensive, and requires large sample size. Moreover, salt tolerance among genotypes can be altered by other environmental factors other than salinity such as temperature, light or humidity (Yeo et al. 1990). Identifying genetic variations of different salt tolerance genotypes based on DNA polymorphism offers several advantages over measuring physiological traits. Evaluation of genetic diversity using DNA marker technology is non-destructive, requires small amount of samples, is not affected by environmental factors, and does not require large experimental setup and equipments for measuring physiological parameters.

Among molecular marker systems used to identify and assess the genetic diversity and phylogenetic relationships in plant genetic resources, random amplified polymorphic DNA (RAPD) technique developed by Williams et al. (1990) is the fastest and simplest. In rice, RAPD analysis has been extensively used for many purposes including identification and classification of germplasm (Choudhury et al. 2001; Ravi et al. 2003), identification of duplicate rice accession in germplasm collection (Virk et al. 1995) and predicting quantitative variation within germplasm (Virk et al. 1996). Simple sequence repeats (SSR) or microsatellites are simple, tandemly repeated, di- to tetra-nucleotide sequence motifs flanked by unique sequences (Hamada et al. 1982). They have become useful markers for genetic diversity analysis because they detect high level of allelic diversity, occur frequently throughout plant genomes, and are easily assayed by PCR. SSR markers have been extensively used to identify genetic variation among rice species (Ren et al. 2003), analyze genetic structure within the cultivated rice (Garris et al. 2005), evaluate genetic diversity among strains of wild rice (Shishido et al. 2006) and among cultivars of cultivated rice (Yu et al. 2003; Jain et al. 2004; Zeng et al. 2004; Jayamani et al. 2007).

In the present study, both marker systems were employed to provide genetic diversity information for 30 rice genotypes, of which 21 are landraces and improved cultivars from Thailand, 8 are improved cultivars from the International Rice Research Institute (IRRI), the Philippines and one landrace from India. These cultivars were selected based on their differences in the level of salinity tolerance. The information on genetic relationships among these genotypes may provide useful information for selecting appropriate parental genotypes in breeding program for improving salt tolerance of elite cultivars based on genetic similarity and clustering data together with variations in salt tolerance levels.

MATERIALS AND METHODS

Plant materials, salinity treatments and evaluation of salinity tolerance

Seeds of 30 *indica* rice cultivars (Table 1) were kindly provided by Chumpae Rice Research Center at Khon Kaen, Thailand and Rice Gene Discovery Unit and Rice Science Center, Biotec, Thailand. Twenty-one genotypes are landraces or improved cultivars commonly grown in Thailand (Table 1). Cultivars improved at the International Rice Research Center (IRRI) included FL496, FL530, MATATAG2, PSBRc86, IR28, IR29, IR34 and IR64. Pokkali, the salt-tolerant landrace from India was

included for comparison. To test for salt tolerance ability, seeds were surface-sterilized with 1.5% calcium hypochlorite, thoroughly washed and imbibed for 48 hrs. Seedlings (twelve plants per genotype) were grown for 4 days on plastic grids floated on distilled water in a plastic tank. Distilled water was then replaced by Yoshida nutrient solution (Yoshida, 1976) and the seedlings were grown for 10 days. The experiment was replicated three times. Salinity treatment was imposed by replacing the nutrient solution with the one containing NaCl having an electrical conductivity of 12 dS m^{-1}. The pH of nutrient solution was maintained at 5.0-5.5 during the entire growth period. Plants grown in non-salinized solution (*i.e*. control) were also set up for comparison.

Table 1. List of rice cultivars used in this study, their salinity tolerance scores and salt tolerance groups.

No.	Cultivars	Locations	Parental lines / Parental relations	SS	ST
1	Pokkali	India	Indian local	3.16	T
2	Nam Sa-Gui19	Thailand	Improved	3.22	T
3	RD23	Thailand	RD7/IR32/RD1	3.52	T
4	SPR90	Thailand	RD21/IR4422-98-3-6-1//RD11/RD23	3.61	T
5	RD10	Thailand	Derived from RD1	3.73	T
6	Pathum Thani1	Thailand	BKNA6-18-3-2/PTT85061-86-3-2-1	4.50	MT
7	FL496	IRRI	IR29/Pokkali	4.57	MT
8	MATATAG2	IRRI	IR61009-37-2-1-2///IR1561/Utri Merah//IR1561	4.68	MT
9	RD15	Thailand	Derived from KDML105	4.68	MT
10	FL530	IRRI	IR29/Pokkali	4.95	MT
11	IR64	IRRI	IR5657-33-2-1/IR2061-465-1-5-5	5.02	MT
12	Leuang Anan	Thailand	Thai local	5.13	MT
13	Leuang Tahmo	Thailand	Thai local	5.17	MT
14	RD25	Thailand	KDML105/IR2061-213-2-3-3//KDML105/IR26	5.19	MT
15	Sew Mae Jun	Thailand	Improved	5.22	MT
16	Hawm Om	Thailand	Thai local	5.61	MS
17	RD33	Thailand	KDML105/IR70177-76-3-1	5.83	MS
18	Surin1	Thailand	IR61078/IR46329-SRN-18-2-2-2	5.95	MS
19	KDML105	Thailand	Improved	5.97	MS
20	Hawm Nahng Nuan	Thailand	Thai local	6.03	MS
21	Daw Hawm	Thailand	Thai local	6.11	MS
22	Khao Med Laek	Thailand	Thai local	6.13	MS
23	RD6	Thailand	Derived from KDML105	6.50	MS
24	KHSPR	Thailand	SPR84177-8-2-2-2-2/SPR85091-13-1-1-4//KDML105	6.61	MS
25	Pathum Thani60	Thailand	DML70*2/Chinese345	7.00	S
26	IR29	IRRI	R833-6-2-1-1/IR1561-149-1//IR24*4/*O.Nivara*	8.00	S
27	PSBRc 86	IRRI	IR10198-66-2/TCCP266-B-B-B-10-3-1	8.03	S
28	IR28	IRRI	IR833-6-2-1-1/IR1561-149-1//IR24*4/*O.Nivara*	8.18	S
29	Khao Kaset	Thailand	Thai local	8.75	HS
30	IR34	IRRI	IR833-6-2-1-1/IR1561-149//IR24*4/*O.Nivara*	9.00	HS

SS= salinity tolerance score; ST = salinity tolerance group: T = salt-tolerant, MT = moderately salt-tolerant, MS = moderately salt-susceptible, S = salt-susceptible and HS = highly salt-susceptible.

After 10 days in the salinized solution, the visual symptoms of salt injury of individual seedlings were inspected and the salinity tolerance score (SS) of 1-9 were assigned based on the standard evaluation system (SES) with some modifications (Gregorio et al. 1997). Salt injury symptoms are described as follows: score: 1, normal growth, no leaf symptoms; 3, nearly normal growth, only the tips of few leaves whitish and rolled; 5, growth severely retarded, most leaves rolled, but the two youngest leaves are still elongating; 7, complete cessation of growth, all lower leaves dried out, the two youngest leaves started to wilt; 9, the whole plant dried out and dead. For each genotype, SSs were averaged and a genotype was classified as salt-tolerant (T), moderately salt-tolerant (MT), moderately salt-susceptible (MS), salt-susceptible (S) and highly salt-susceptible (HS) when the mean SS obtained were 3 ± 0.75, 4.5 ± 0.75, 6 ± 0.75, 7.5 ± 0.75 and 8.25-9.00, respectively.

Measurement of ion content

Shoots of plants were oven-dried for 3 days at 60ºC. Approximately 0.1 g powder of each dried sample was subjected to chemical analyses by digesting in 10 ml of nitric acid (HNO_3) at 300ºC, 5 ml perchloric acid ($HClO_4$) at 200ºC and 2 ml of 6 M HCl. The concentrations of Na^+ and K^+ were analyzed using a Flame photometer (Model M410 Sherwood).

Measurement of proline

Proline content was analyzed by the modified procedure of Bates et al. (1973). Approximately 0.1 g of fresh weight of shoot was soaked for 3 hrs with 5 ml of 3% aqueous sulfosalicylic acid. Two ml of extract was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid and boiled in a water bath at 100ºC for 1 hr. The reaction was stopped by placing tubes on ice. The solution was extracted with 4 ml of toluene and the absorbance of the toluene fraction was measured at 520 nm. The amount of free proline was evaluated using a standard curve and expressed as µg g^{-1} tissue fresh weight.

Isolation of genomic DNA

For each rice genotype, total genomic DNA was isolated from young leaves of 12 healthy plants which were grown in a greenhouse for 14 days. DNA was extracted using the modified protocol of CTAB method (Doyle and Doyle, 1987). DNA concentration and purity were determined by measuring the absorbance of diluted DNA solution at 260 nm and 280 nm (Milton roy Spectronic 1001 plus, USA). The quality of the DNA was tested by staining DNA with ethidium bromide after electrophoresis in 0.8% agarose gel at 100V for 45 min in 0.5X TBE buffer and the image was visualized with an ultraviolet transilluminator.

RAPD analysis

Twenty different 10-mer oligonucleotide RAPD primers (Operon Technologies Inc., USA) (Table 2) were used. Each polymerase chain reaction (PCR) was carried out in a 25 µl volume containing 25 ng template DNA, 1.5 mM $MgCl_2$, 0.32 mM dNTPs, 1X *Taq* DNA polymerase buffer, 10 pmol decanucleotide primer and 2 units of *Taq* DNA polymerase (iNtRon, Biotechnology Inc., Korea). Amplification was performed in a thermal cycler (Corbett Research, Australia) using the following conditions: denaturation at 95ºC for 3 min; 40 cycles of 1 min denaturation at 94ºC, 1 min annealing at 40ºC and 2 min extension at 72ºC; and a final extension at 72ºC for 7 min. The RAPD-PCR products were analyzed directly on 1.5% agarose gel in 1X TBE buffer. The DNA was stained with 0.5 mg/ml ethidium bromide, visualized and photographed under a UV transilluminator.

SSR analysis

PCR amplification of SSR markers was carried out using twenty primer pairs listed in Table 3. Each reaction tube contained 25 ng of template DNA, 1 x PCR buffer, 2 mM $MgCl_2$, 0.4 mM of dNTPs, 0.4 µM each of forward and reverse primers and 1.6 units of *Taq* DNA polymerase (iNtRon, Biotechnology Inc., Korea). Amplification was performed using the following conditions: denaturation at 94ºC for 5 min; 35 cycles of 1 min denaturation at 94ºC, 1 min annealing at 55ºC, 1 min extension at 72ºC and a final extension at 72ºC for 5 min. The SSR amplification products were separated in a vertical denaturing 4.5% polyacrylamide gel in a Sequi-Gen®GT nucleic acid electrophoresis system (BioRad Laboratories Inc., USA). DNA fragments were revealed using the silver staining procedure.

Table 2. Means of salinity tolerance scores, % survival, Na^+/K^+ ratio and increase in proline of each salinity tolerance group.

Salinity tolerance group	SS	% Survival	Na^+/K^+ ratio	Increase in proline (folds)
Tolerant; T (cv. no. 1-5)	3.44[a]	100[a]	0.77[a]	1.57[a]
Moderately tolerant; MT (cv. no. 6-15)	4.91[b]	94.83[ab]	1.09[ab]	2.82[ab]
Moderately susceptible; MS (cv. no. 16-24)	6.08[c]	81.81[b]	1.20[b]	2.83[ab]
Susceptible; S (cv. no. 25-28)	7.80[d]	45.33[c]	1.83[c]	3.47[ab]
Highly susceptible; HS (cv. no. 29-30)	8.88[e]	6.17[d]	2.08[c]	4.01[b]
Significance[S]	*	*	*	*

SS = salinity tolerance score; means followed by the same letter within each column are not significantly different using Duncan's multiple range test at 95%, [S] Level of significance are represented by (*) at $p < 0.0$.

Data analysis

Polymorphic information content (PIC) values were calculated for each RAPD primer and SSR locus based on Anderson et al. (1993). The amplified bands were scored for each RAPD primer and SSR primer pairs based on the presence or absence of bands, generating a binary data matrix of 1 and 0 for each marker system. Both matrices were then analyzed using the NTSYS pc statistical package version 2.1. The data matrices were used to calculate genetic similarity based on Jaccard's similarity coefficients, and two dendrograms displaying relationships among 30 rice cultivars were constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The Pearson's correlation between similarity coefficients based on RAPD and SSR markers was determined from data among all 30 cultivars.

RESULTS

Evaluation of salt tolerance and physiological responses

Rice seedlings of all cultivars grew normally and exhibited 100% survival when grown under hydroponic solutions in the absence of salt stress. Rice cultivars showed varying degrees of salt injury and growth retardation and were classified into five groups based on the mean salinity tolerance scores (SSs) as shown in Table 1. Among the five cultivars in the salt-tolerant (T) group, Pokkali was the most tolerant with the mean SS of 3.16, the remaining cultivars were decreasing in tolerance level and increasing in SS up to 3.73 for RD10. Even though the growth of these cultivars was slightly retarded and some leaves were injured, all plants could survive 10 days of salinity treatment. Ten cultivars comprising the moderately salt-tolerant (MT) group suffered more injury than those of the T group exhibiting the mean SSs between 4.5 for Pathum Thani1 to 5.22 for Sew Mae Jun. Some seedlings of six cultivars in this group could not tolerate the salinity solution and died after 10 days giving survival percentage between 96.66% for Leuang Anan and 83% for Leuang Tahmo. However, four genotypes namely Pathum Thani1, FL530, RD25 and Sew Mae Jan exhibited 100% survival. Nine cultivars classified in the moderately salt-susceptible (MS) group varied in SSs from 5.61 for Hawm Om to 6.61 for KHSPR, and survival percentage from 94.33% for Surin 1 to 72.66% for KHSPR. Plants of four cultivars classified as salt-susceptible (S) were severely damaged showing the SSs of 7.0, 8.0, 8.03 and 8.18 and survival rate of 83.3, 44.33, 29.33 and 24.33% for Pathum Thani60, IR29, PSBRc86 and IR28, respectively. The two most susceptible genotypes including Khao Kaset and IR34 were classified as highly salt-susceptible (HS). Only 12.33% of Khao Kaset plants survived and 100% of IR34 seedlings had died.

In addition to the visual scoring of injury symptoms, the ratio between Na^+ and K^+ and proline content in the survived seedlings of each genotype were determined. The Na^+/K^+ ratio has long been used as an accurate physiological indicator for salinity tolerance and proline is often implicated as involving in osmotic adjustment in response to salt stress. The proline content was determined in both salt-stressed and non-stressed seedlings and expressed as the folds increase in stressed as compared to non-

stressed seedlings. The SSs, % survival, Na^+/K^+ ratio and folds increase in proline, of all genotypes in each salinity tolerance group were then averaged and summarized in Table 2. The SSs, Na^+/K^+ ratio and folds increase in proline were increasing from the most tolerant group to the most susceptible group, and vice versa for survival percentage.

Polymorphism of RAPD markers and cluster analysis

All of the 20 RAPD primers were effectively used to amplify genomic DNA of all 30 rice cultivars. The most appropriate amount of DNA for RAPD analysis was 25 ng and $MgCl_2$ at a concentration of 1.5 mM produced well-separated bands in the amplification reactions (Figure 1). These primers produced multiple band profiles with a number of amplified DNA fragment varying from 3 to 12 (Table 3), with a mean alleles per locus of 8.1. The primer OPB03 gave the minimum number of fragments (3), while the highest number of fragments (12) was amplified with primers OPA03 and OPA12. A total of 161 alleles were detected. Of these, 111 were polymorphic (68.94%) and 50 monomorphic (31.06%). The lowest and highest number of polymorphic bands were 1 (OPB04) and 10 (OPA12 and OPB12). The primer, which showed the least polymorphism (20%), produced 5 bands of which only 1 was polymorphic. OPA12 and OPB12 showed the highest number of bands, 12 of which 10 (83.33%) and 11 of which 10 (90.91%) were polymorphic, respectively. The primer OPB06 yielded 8 bands that were all polymorphic (100%). The PIC values of 20 random primers ranged from 0.08 (OPA03) to 0.73 (OPJ08) and the average for all primers were 0.38.

Table 3. Twenty RAPD primers, their sequences, total number and size range of amplified bands, the number of polymorphic and monomorphic bands and polymorphic information content obtained for each primer used to amplify the genomic DNA of 30 rice cultivars.

Primers	Primer sequence (5'-3')	AN	Size range of bands (bp)	PM	%	MM	%	PIC
OPA01	CAGGCCCTTC	10	230 – 1400	5	50.00	5	50.00	0.46
OPA02	TGCCGAGCTG	9	450 – 1900	6	66.67	3	33.33	0.34
OPA03	AGTCAGCCAC	12	250 – 1300	5	41.67	7	58.33	0.08
OPA04	AATCGGGCTG	10	230 – 1400	7	70.00	3	30.00	0.41
OPA05	AGGGGTCTTG	8	280 – 1445	6	75.00	2	25.00	0.27
OPA07	GAAACGGGTG	9	220 – 1200	5	55.56	4	44.44	0.36
OPA10	GTGATCGCAG	10	190 – 1400	7	70.00	3	30.00	0.38
OPA12	TCGGCGATAG	12	245 – 1800	10	83.33	2	16.67	0.45
OPA13	CAGCACCCAC	10	350 – 1400	9	90.00	1	10.00	0.25
OPB01	GTTTCGCTCC	8	400 – 1400	6	75.00	2	25.00	0.31
OPB03	CATCCCCCTG	3	480 – 800	2	66.67	1	33.33	0.40
OPB04	GGACTGGAGT	5	400 – 950	1	20.00	4	80.00	0.16
OPB06	TGCTCTGCCC	8	450– 1600	8	100	-	-	0.60
OPB07	GGTGACGCAG	9	300 – 1100	6	66.67	3	33.33	0.36
OPB09	TGGGGGACTC	5	450– 1550	4	80.00	1	20.00	0.32
OPB10	CTGCTGGGAC	6	250 – 1100	4	66.66	2	33.33	0.36
OPB11	GTAGACCCGT	5	480 – 1150	3	60.00	2	40.00	0.27
OPB12	CCTTGACGCA	11	200 – 1900	10	90.91	1	9.09	0.50
OPJ06	TCGTTCCGCA	7	150 – 1630	4	57.14	3	42.86	0.52
OPJ08	CATACCGTGG	4	320 – 1580	3	75.00	1	25.00	0.73

AN: Alleles number; PM: Polymorphic bands; MM: Monomorphic bands; PIC: Polymorphic information content.

The genetic similarity coefficients among 30 rice genotypes based on the RAPD fragments were used to construct a dendrogram (Figure 2) by UPGMA analysis. Pair-wise estimates of genetic similarity among the 30 genotypes ranged from 0.64 to 0.94. The highest genetic similarity revealed was between RD15 and KDML105 (0.94). From the RAPD-based dendrogram, Pokkali (a landrace variety from India) which was the most genetically dissimilar and physiologically distinct was separated from all other cultivars. The remaining cultivars were separated into 4 groups (I-IV). Group I contains 16 cultivars showing 84.4% similarity. Group I could be further divided into two subgroups, IA and IB. Sub-group IA contained 13 cultivars which were all Thai local and improved cultivars that could be further sub-divided into two branches, IA_1 and IA_2. The IA_1 branch consists of three improved T cultivars RD23, SPR90 and RD10. The IA_2 branch consists of 10 cultivars, which were either MT or MS except for Pathum Thani60. For subgroup IB, only salt-susceptible cultivars from IRRI (IR28, IR29 and PSBRc86) were clustered together. Eight cultivars comprising Group II displayed 85% similarity and were either MT or MS. Among these, Pathum Thani1 and RD15 which were both derived from KDML105 formed into sub-group IIA together with KDML105. The remaining five cultivars in the sub-group IIB were all improved cultivars from IRRI, except Surin1. Group III comprised of only two Thai landraces, Khao Med Laek and Daw Hawm, which are both MS with very similar SSs. The two HS cultivars, Khao Kaset (local Thai landrace) and IR34 (improved IRRI cultivar), formed a distinct group separating from the remaining 27 members of group I, II and III at the similarity coefficient of 0.80. Thus RAPD analysis was able to differentiate the 30 rice cultivars into groups according to salt tolerance level. Group I contains three subgroups IA_1, IA_2 and IB corresponding to the T, MT/MS and S respectively. Group II, III and IV corresponded to MT/MS, MS and HS respectively.

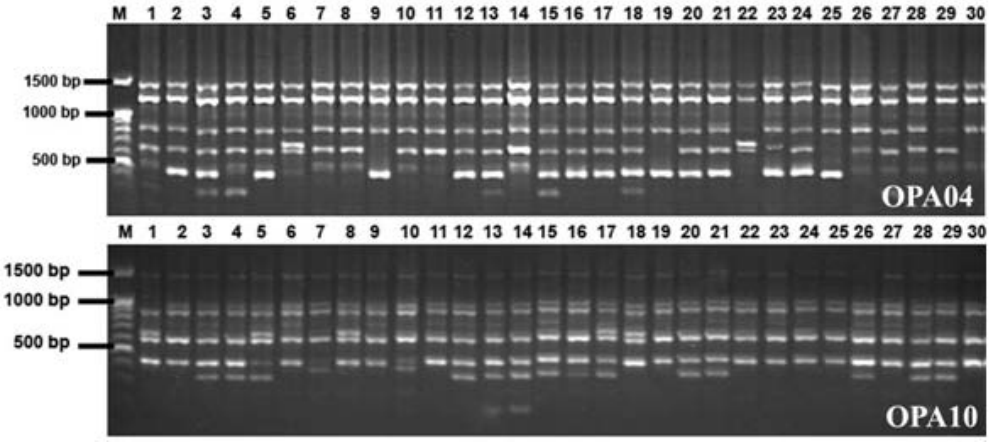

Fig. 1 Representative RAPD profiles of 30 rice cultivars obtained with OPA04 and OPA10 primers. Lane M: 100-bp DNA marker, lanes 1-30 corresponded to rice cultivars listed in Table 1.

Polymorphism of SSR markers and cluster analysis

The 30 rice cultivars were fingerprinted using 20 SSR primer pairs generating a total of 190 alleles of which 20 were monomorphic and 170 polymorphic (89.47%). The sizes of alleles ranged from 73 to 480 bp. Two representative silver-stained gels are shown in Figure 3. The number of alleles detected by a single marker ranged from 5 (RM7 and RM261) to 18 (RM1) with an average of 9.5 alleles per locus. The PIC values for the SSR loci ranged from a minimum of 0.52 (RM20) to a maximum of 0.91 (RM206) with an average of 0.76 (Table 4). The low PIC values were observed from the primer RM11 (0) and RM20 (0.52). The PIC values of the remaining microsatellite loci were all above 0.60. The greatest number of alleles was found at the RM1 and RM5 loci both located on chromosome 1, comprising 18 and 16 alleles, respectively. Moreover, these primers gave highest number of polymorphic bands.

The genetic relationship among rice cultivars was presented in a dendrogram based on informative microsatellite alleles (Figure 4). The 30 rice cultivars were divided into 4 groups. Pokkali was separated from the remaining 29 cultivars at the similarity coefficient of 0.62. Groups I, II and III contained both

Thai and IRRI cultivars. FL496 and FL530, both originated from a cross between IR29 and Pokkali, were most closely related at the similarity coefficient of 0.98. Group I could be further divided into two subgroups IA and IB at the genetic similarity coefficient of 0.72. Group IA contained 4 cultivars all of which are local Thai landraces and group IB could be further divided into two branches (IB_1 and IB_2). The IB_1 branch had 5 Thai cultivars, 3 of which were improved cultivars derived from KDML105. For the IB_2 branch, IR64, Khoa Kaset and IR34 were included. Group II composed of subgroup IIA and IIB had genetic similarity coefficient of 0.735. Subgroup IIA contained only improved rice cultivars from Thailand. Subgroup IIB contained S rice cultivars from IRRI (IR28, IR29 and PSBRc86) and KHSPR from Thailand in one branch. It also contained MT cultivars, FL496 and FL530, in another branch. The improved T cultivar, SPR90 from Thailand was separated from Group II at genetic similarity coefficient of 0.71. Group III and VI included all Thai rice cultivars except MATATAG2 which came from IRRI. Thus, cluster analysis based on SSR markers could not separate rice cultivars into groups according to the salinity tolerance although it could classify cultivars by the location and association with genetic origin.

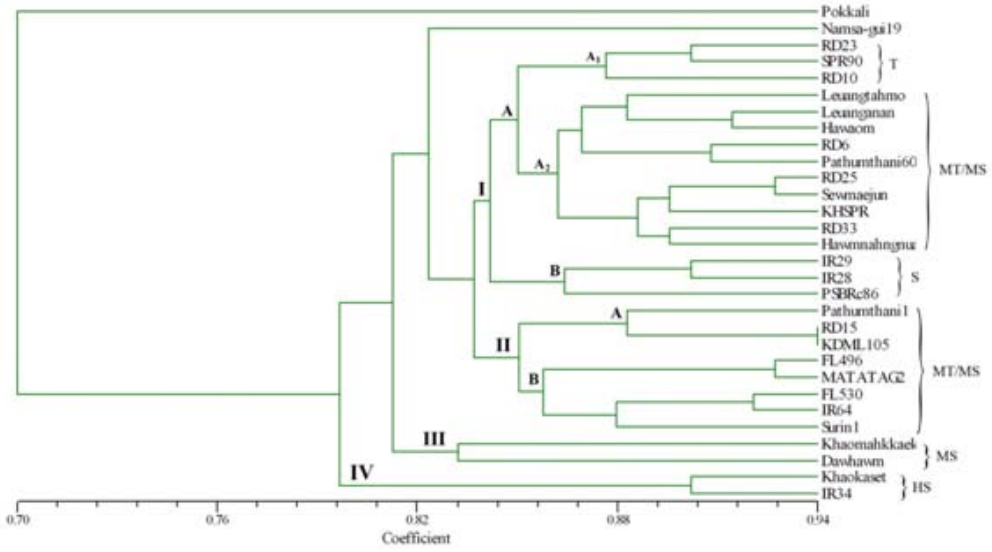

Fig. 2 Dendrogram of 30 rice cultivars revealed by UPGMA cluster analysis based on Jaccard's similarity coefficients generated from RAPD markers.

Genetic diversity within the salinity tolerance groups and comparison of RAPD and SSR markers

By using RAPD and SSR markers, this study showed that there was a high level of genetic diversity between 30 cultivars, the percentages of polymorphic bands examined by RAPD and SSR were 68.94% and 89.47%, respectively (Table 5). The PIC values for SSR were also higher than those of RAPD primers. The highest similarity coefficients from RAPD and SSR data were 0.94 (between RD15 and KDML105) and 0.98 (between FL496 and FL530), and the lowest 0.64 (between Khao Kaset and Pokkali) and 0.54 (between Surin1 and Pokkali), respectively. The average similarity coefficients of 0.82 (for RAPD) and 0.76 (for SSR) indicated the high level of genetic variation that exists in the rice gene pool. Estimated Pearson's correlation between genetic similarity coefficients obtained from analysis of RAPD and SSR markers was low positive ($r = 0.329$) and significant ($p < 0.01$) (Table 5 and Figure 5).

DISCUSSION

A wide range of variation in salt tolerance levels was evident among 30 rice cultivars evaluated at the seedling stage in hydroponic saline solutions. As expected the Indian landrace, Pokkali, was the most

tolerant with SS of 3.16 and exhibited minimum salt injury, whereas FL496 and FL530 which were derived from a cross between Pokkali and the salt-susceptible IR29 were less tolerant showing SSs of 4.57 and 4.95 respectively (Table 1). Along with Pokkali, four improved Thai cultivars *i.e.* Nam Sa-Gui19, RD23, SPR90 and RD10 were included in the T group. Among 21 Thai local and improved cultivars, Nam Sa-Gui19 was the most tolerant showing of SS of 3.22 which is closest to that of Pokkali whereas Khao Kaset was the most susceptible showing the highest SS of 8.75 and 88% plant death. The most economically important Thai aromatic cultivar, KDML105 was moderately susceptible (SS = 5.97). The level of salinity tolerance of Pokkali, FL496, FL530, IR29, RD6, Leuang Tahmo and KDML105 was in accordance with that determined in previous reports which used similar growing conditions and evaluation system (Suriya-arunroj et al. 2004; Theerakulpisut et al. 2005). However, when grown under controlled environmental conditions, KDML105 was determined as being salt tolerant on the basis of physiological performance including chlorophyll and carotenoid content, chlorophyll fluorescence and membrane integrity (Cha-um et al. 2010).

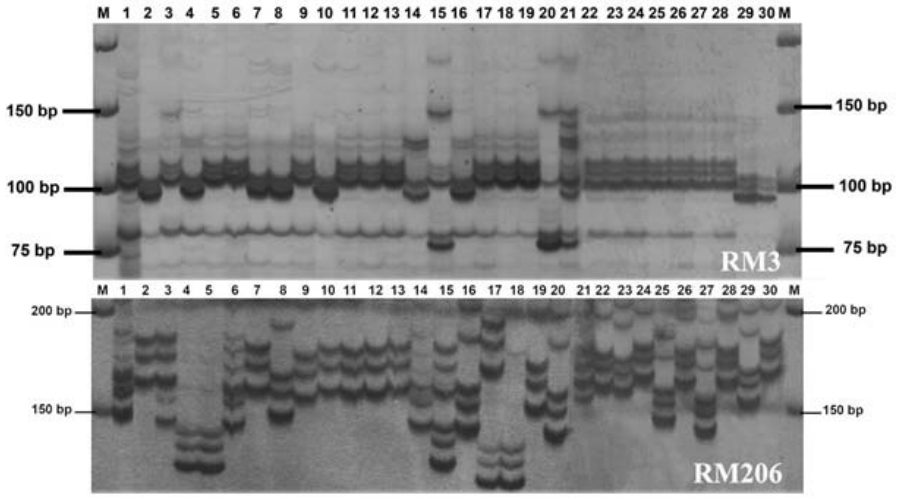

Fig. 3 Amplified products from genomic DNA of 30 rice cultivars using RM3 and RM206 primers. The lanes represent, M: 50-bp DNA marker, 1-30: rice cultivars listed in Table 1.

Evaluation of salinity tolerance score based on IRRI's standard evaluation system focusing on plant injury was able to differentiate this set of rice germplasm into five distinct groups with statistically significant mean SSs (Table 2). Salinity tolerance scores strongly related to plant survival, and two physiological parameters *i.e.* Na^+/K^+ and proline accumulation. It has been well established that concentration of Na^+ and Na^+/K^+ ratio in rice plants are correlated with growth and grain yield (Khatun et al. 1995; Lutts et al. 1995) and suggested as selection criteria for salt tolerance (Yeo and Flowers, 1986). A major QTL, *Saltol*, which was believed to be a major contributor to seedling stage tolerance and act to control shoot Na^+/K^+ homeostasis has been mapped at similar locations on chromosome 1 (Gregorio et al. 1997; Bonilla et al. 2002; Thomson et al. 2010). According to Table 2, the large difference in means Na^+/K^+ between the T and the MS groups corresponded with the significant difference in % survival between these groups. The physiological responses of plants in the MS and MT groups were, however, quantitatively overlapping in terms of Na^+/K^+ and % survival and showed the same level of proline accumulation. It is not surprising then that the MT and MS cultivars were clustered together in the RAPD dendrogram. In a study of genetic diversity of 33 rice genotypes with different adaptation to saline soils, Zeng et al. (2004) classified genotypes into only three groups T, MT and S. As for proline accumulation, only the two extreme groups, T and HS, showed significantly different levels of accumulation. Although the accumulation of proline also displayed a trend of increment from the more tolerant to the more susceptible groups, the mean values were overlapping among the MT, MS and S groups. Proline accumulation may not be as tightly related to salinity tolerance as the Na^+/K^+. Although several reports suggested adaptive roles of proline (Smirnoff and Cumbes, 1989; Hare and Cress, 1997), few reports indicated no correlation between proline accumulation and salt stress resistance (Lutts et al. 1996; Garcia et al. 1997).

Table 4. The list of 20 SSR primers used in the estimation of genetic diversity of 30 rice cultivars. Variation of alleles number, allele size range, monomorphic and polymorphic bands and PIC values are shown.

Primers	CN	SSR motif	Nucleotide sequence	AN	Size of bands (bp)	MM	PM	PIC
RM 1	1	(AG)26	GCGAAAACACAATGCAAAAA (F) GCGTTGGTTGGACCTGAC (R)	18	78 – 170	-	18	0.90
RM 3	6	(GA)2GG (GA)25	GGTTAGGCATCGTCACGG (F) TCACCTCACCACACGACACG (R)	11	73 – 175	2	9	0.69
RM 5	1	(GA)14	TGCAACTTCTAGCTGCTCGA (F) GCATCCGATCTTGATGGG (R)	16	80 – 167	-	16	0.86
RM 6	2	(AG)16	GTCCCCTCCACCCAATTC (F) TCGTCTACTGTTGGCTGCAC (R)	8	160 – 225	1	7	0.65
RM 7	3	(GA)19	TTCGCCATGAAGTCTCTCG (F) CCTCCCATCATTTCGTTGTT (R)	5	165 – 190	1	4	0.61
RM 11	7	(GA)17	TCTCCTCTTCCCCCGATC (F) ATAGCGGGCGAGGCTTAG (R)	6	145 – 210	6	-	0
RM 13	5	(GA) 6-(GA)16	TCCAACATGGCAAGAGAGAG (F) GGTGGCATTCGATTCCAG (R)	6	115 – 163	-	6	0.72
RM 19	12	(ATC)10	CAAAAACAGAGCAGATGAC (F) CTCAAGATGGACGCCAAGA (R)	8	230 – 420	-	8	0.82
RM 20	11	(ATT)14	ATCTTGTCCCTGCAGGTCAT (F) GAAACAGAGGCACATTTCATTG (R)	7	218 – 290	2	5	0.52
RM 25	8	(GA)18	GGAAAGAATGATCTTTTCATGG (F) CTACCATCAAAACCAATGTTC (R)	13	130 – 195	-	13	0.80
RM 44	8	(GA)16	ACGGGCAATCCGAACAACC (F) TCGGGAAAACCTACCCTACC (R)	9	100 – 185	-	9	0.87
RM 164	5	(GT)16TT (GT)4	TCTTGCCCGTCACTGCAGATATCC (F) GCAGCCCTAATGCTACAATTCTTC (R)	11	235 – 440	-	11	0.86
RM 170	6	(CCT)7	TCGCGCTTCTTCCTCGTCGACG (F) CCCGCTTGCAGAGGAAGCAGCC (R)	14	95 – 210	2	12	0.85
RM 189	9	(AG)11	GGGAGTTGAAGTGGTCGGAGAAGG (F) CACGCGACTTCAGTTCGTCTTCC (R)	6	290 – 480	1	5	0.85
RM 206	11	(CT)21	CCCATGCGTTTAACTATTCT (F) CGTTCCATCGATCCGTATGG (R)	13	102 – 195	-	13	0.91
RM 209	11	(CT)18	ATATGAGTTGCTGTCGTGCG (F) CAACTTGCATCCTCCCCTCC (R)	12	132 – 250	1	11	0.73
RM 224	11	(AAG)8 (AG)13	ATCGATCGATCTTCACGAGG (F) TGCTATAAAAGGCATTCGGG (R)	8	120 – 195	-	8	0.74
RM 231	3	(CT)16	CCAGATTATTTCCTGAGGTC (F) CACTTGCATAGTTCTGCATTG (R)	7	162 – 265	1	6	0.72
RM 261	4	C9(CT)8	CTACTTCTCCCCTTGTGTCG (F) TGTACCATCGCCAAATCTCC (R)	5	117 – 175	3	2	0.62
RM337	8	GT)16TT (GT)4	GTAGGAAAGGAAGGGCAGAG (F) CGATAGATAGCTAGATGTGGCC (R)	7	155- 450	-	7	0.89

CN: Chromosome number; AN: Alleles number; MM: Monomorphic bands; PM: Polymorphic bands; PIC: polymorphic information content.

Based on the RAPD fingerprinting data, high levels of polymorphism were observed among this group of rice cultivars. A total of 161 bands were amplified from 20 RAPD primers, of which 111 (68.94%) were polymorphic (5.55 polymorphic bands per primer) (Table 5). The level of polymorphism is comparable to that reported by Choudhury et al. (2001) who estimated genetic similarity among 48 cultivars of Indian rice using 58 RAPD primers and found 67.5% polymorphism. However, other authors reported higher level of polymorphism for rice *i.e.* 80% among 42 Indian elite varieties estimated by using 40 RAPD primers (Davierwala et al. 2000), 72.9% among 7 Egyptian genotypes using 8 RAPD primers (Saker et al. 2005) and 89.4% among 40 cultivars of Pakistani rice using 25 RAPD primers (Rabbani et al. 2008). Nevertheless, it is evident that RAPD can efficiently distinguish all of the rice cultivars in this study. Pokkali, which is the most distant genotype displayed as many as 10 RAPD markers (out of 121 bands) which are genotype-specific *i.e.* not present in any other cultivars, and two markers were present in all other cultivars except Pokkali. The most closely related cultivars, KDML105 and RD15 (which was derived from gamma-irradiation of KDML105), showed 97 common and 9 different bands. The number of polymorphic bands per primer varied from 4 (Devierwala et al. 2000), 5.55 (this study), 6.54 (Choudhury et al. 2001), 7.4 (Rabbani et al. 2008), 7.86 (Saker et al. 2005) and 10.2 (Verma et al. 1999) depending on the selection of RAPD primers and the degree of diversity of genotypes.

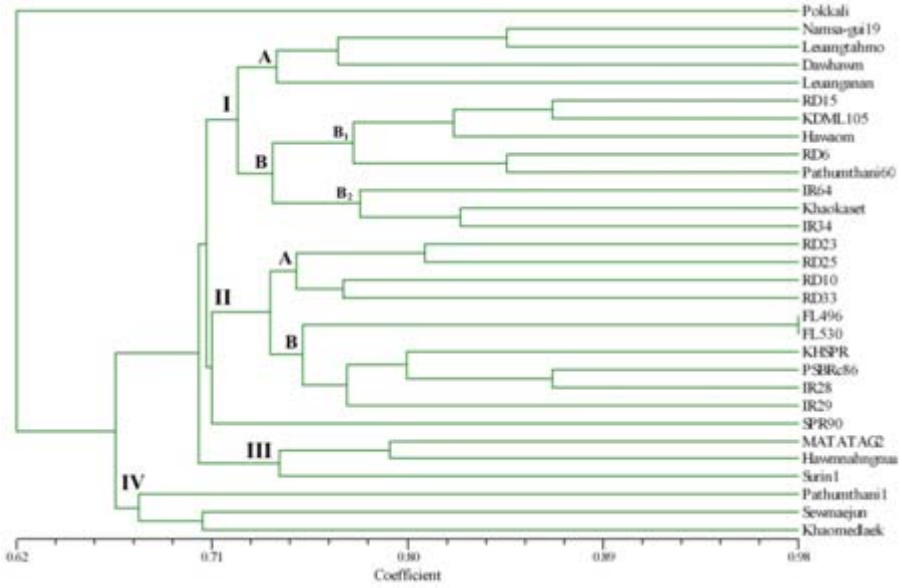

Fig. 4 Dendrogram showing genetic relationship among 30 rice cultivars revealed by UPGMA cluster analysis of Jaccard's similarity coefficients based on SSR markers.

Similarity coefficients among cultivars ranged from 0.64 to 0.94 with an average of 0.82 which were highly similar to the values obtained among 42 Indian elite rice varieties (0.59 to 0.95, average 0.80; Davierwala et al. 2000), 40 varieties of Pakistan rice (0.5 to 0.96, average 0.82; Rabbani et al. 2008) and 45 accessions of the AA-genome *Oryza* species (0.59 to 0.92). The relatively high level of similarity was detected between closely related genotypes derived from common ancestors such as KDML105 and RD15 (0.94), FL496 and MATATAG2 (0.92) and IR28 and IR29 (0.90). Pokkali, on the other hand, generally displayed low genetic similarity with the remaining cultivars ranging from 0.64 to 0.79.

Cluster analysis based on RAPD markers was effective in grouping cultivars based on their salt tolerance ability (Figure 2). Pokkali, which is the most distinct genotype both in relation to genetic background and physiological response to salinity, was separated from all the remaining cultivars suggesting that some of the genotype-specific RAPD markers could possibly be associated with salt responses. Nam Sa-Gui19 which is the second most tolerant cultivar with highly similar physiological parameters to Pokkali separated from the 24 cultivars comprising Group I and II. The two distantly related HS cultivars comprising Group IV, Khao Kaset and IR34, shared three common RAPD markers

(610, 270 and 230 bp from primer OPA01) which are absent from all the remaining cultivars. Moreover, their physiological response values are highly similar *i.e*. 8.75 and 9.0 SS, 12.33% and 0% survival, and 3.63 and 4.38 folds increase in proline, for Khao Kaset and IR34, respectively. Group I was clearly separated into three sub-groups (T, MT/MS and S) according to both genetic relatedness and salt tolerance levels. Although Group II was composed of cultivars of both Thai and IRRI origins, they have similar physiological responses (moderately tolerant and moderately susceptible) and therefore are clustered together. The two MT/MS groups (IA_2 and II) were separated not only because of different pattern of RAPD fingerprints but also the difference in physiological responses. It is interesting to note that the MT cultivars of Group II (two Thai and four IRRI cultivars) were slightly more tolerant (having the average SS 4.73 and Na^+/K^+ 1.05) than those of Group IA_2 (four Thai cultivars; average SS 5.17 and Na^+/K^+ 1.15). Likewise, the MS cultivars of Group II (KDML105 and Surin1) were slightly less susceptible (average SS 5.96 and Na^+/K^+ 1.07) than those of Group IA_2 (five Thai cultivars; score 6.11 and Na^+/K^+ 1.17). RAPD markers have been efficiently used for evaluation of genetic relationship between several rice species (Ren et al. 2003) and among different varieties of cultivated rice in several countries (Ko et al. 1994; Guimarães et al. 1996; Kim et al. 1996; Saker et al. 2005). Only a few studies, however, could relate cluster analysis based on RAPD markers with morphological or physiological traits. Fuentes et al. (2005) demonstrated that genetic diversity estimates based on RAPD data efficiently differentiated rice genotypes into clusters represented by both genetic parentage and agro-morphological traits. Recently, Rabbani et al. (2008), using RAPD analysis, could divide 40 Pakistani rice cultivars into 3 main clusters corresponding to aromatic, non-aromatic and japonica group. Using multiple regression analysis, Virk et al. (1996) was able to identify RAPD markers closely associated with six agronomic traits analyzed from 48 rice accessions from 10 Asian countries. The present study has shown that the genetic variation identified by RAPD markers is associated with variations in complex physiological traits such as salinity tolerance level.

Table 5. Effectiveness of RAPD and SSR markers in detecting polymorphism of 30 rice cultivars.

	RAPD	SSR
Among 30 cultivars		
Total amplified bands	161	190
Polymorphic bands	111	170
Percentage of polymorphic bands	68.94	89.47
Number of primers used	20	20
Mean of polymorphic bands/primer	5.55	9.5
Range of PIC value	0.08 – 0.73	0.52 – 0.91
Mean PIC value	0.38	0.76
Mean Jaccard's similarity coefficient (MS)	0.82	0.70
Range of Jaccard's similarity coefficients	0.64 – 0.94	0.54 – 0.98
Correlation (r)		
RAPD	-	0.329**
SSR	0.329**	-

** Correlation is significant at the $p < 0.01$ level.

In case of SSR compared with the RAPD analysis, higher levels of polymorphism were observed among 30 rice cultivars. A total of 170 polymorphic and 20 monomorphic alleles (89.47% polymorphism) with an average number of alleles of 9.5 per locus (range 5-18 per locus) were recorded (Table 5). These values were comparable to those reported earlier (8.42 alleles per locus; range 3-21) by Giarrocco et al. (2007) that used 26 SSR loci to estimate genetic relationship among 69 Argentine rice accessions. Jayamani et al. (2007) also reported similar values (7.7 alleles per locus; range 3-16) from a fingerprinting study of 178 Portuguese rice accessions at 24 SSR loci. Other reports, however, found either lower or higher allelic diversity. Zeng et al. (2004) and Bounphanousay et al. (2008) reported much lower values at 4.3 alleles per locus (range 2-9) and 3.1 alleles per locus (range 2-7), respectively. In contrast, Brondani et al. (2006) determined 6-22 alleles per locus (average 14.6) from 192 accessions of Brazilian landrace rice. The reason for the wide variation in the number of alleles detected was due to the different sets of germplasm, number of genotypes, number and

distribution of SSR loci and method of gel electrophoretic detection in different studies. The low number of alleles was usually obtained from a collection of breeding lines and closely related cultivars such as those used in Zeng et al. (2004). High number of alleles was expected to be found when a large number of landraces from a wide range of geographical origins is included in the study (Brondani et al. 2006).

Similarity coefficients among various cultivars analyzed ranged from 0.54-0.98 (average 0.7). Similar values of 0.77-0.98 were detected among 16 accessions of traditional, long-grain, scented Iranian rice and 7 cultivars from other countries (Moumeni et al. 2003). Similarity coefficients ranging from 0.36 to 0.96 were obtained among 45 accessions of AA-genome *Oryza* species from various locations suggesting a wider range of genetic variability (Ren et al. 2003). As expected, similarity coefficients among 193 accessions of parental lines used at IRRI obtained from 26 countries were relatively low ranging from 0.22 to 0.68 (Yu et al. 2003).

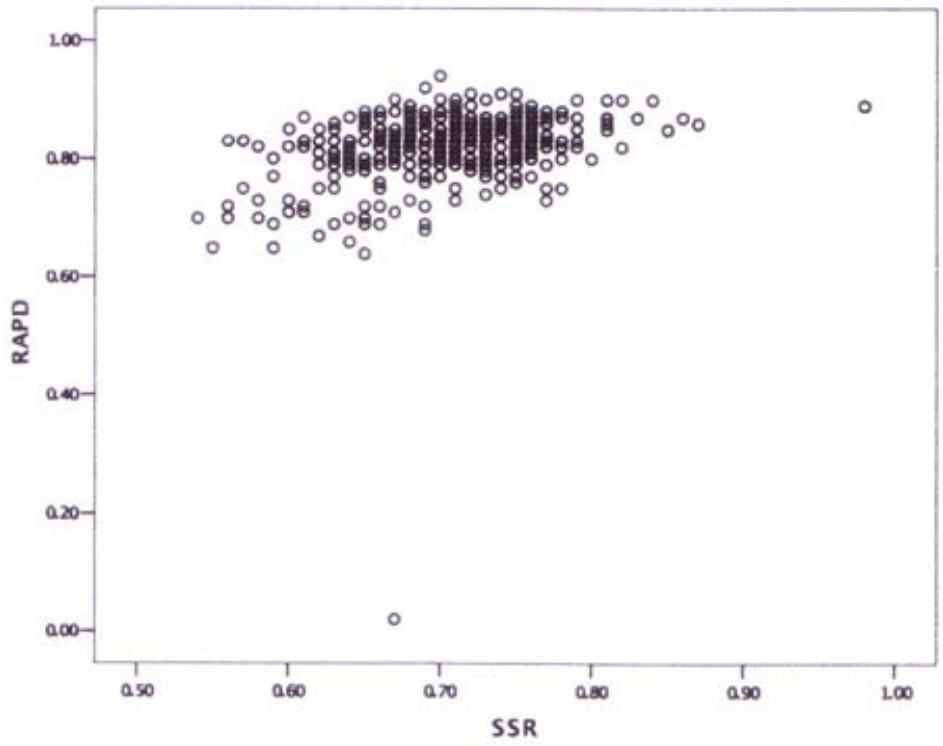

Fig. 5 Correlation between genetic similarity coefficients analyzed by SSR and RAPD markers. Comparison of each pair of rice cultivars for Jaccard's similarity coefficient obtained from analysis of SSR and RAPD markers is represented by the symbol (O).

Cluster analysis based on SSR markers divided rice cultivars into four groups and several sub-groups according to genetic relatedness and, unlike clustering based on RAPD, none of the groupings could be associated with salinity tolerance level (Figure 4). Group I was comprised of almost all Thai rice except IR64 and IR34. In the subgroup IB_1, four (RD15, KDML105, RD6 and Pathumthani60) out of five cultivars are improved cultivars, which were related to each other by being descended from KDML105 or DML70. All cultivars in this subgroup also share a common feature of being aromatic. Group II contained 10 related cultivars and was divided into subgroup IIA which are all improved cultivars (RD23, RD25, RD10 and RD33) descended from crosses among IRRI breeding lines and Thai cultivars including RD1, RD7, KDML105. Subgroup IIB contained six cultivars all descended from IRRI breeding lines. SSR markers have been widely used to characterize rice germplasm and evaluate genetic relationships among cultivars. Allelic variations among 169 SSR loci has been used to evaluate genetic diversity among 234 accessions of rice and clearly detected five distinct groups corresponding to *indica, aus, aromatic, temperate japonica* and *tropical japonica* (Garris et al. 2005). Siwach et al.

(2004) was able to differentiate 24 rice genotypes into two major groups corresponding to Basmati and non-Basmati types based on SSR analysis at 50 loci. Cluster analysis based on 32 SSR loci clearly placed 35 Asian rice cultivars into two major groups *i.e.* aromatic and non-aromatic coarse grain rice (Pervaiz et al. 2009). Analysis based on SSR markers (Davierwala et al. 2000) was able to divide Indian elite rice cultivars into clusters according to complex physiological characters namely early duration maturity, medium duration maturity and semi-deepwater and deep-water rice. Analysis of microsatellite markers at 25 loci among 33 rice genotypes differing in salinity tolerance (tolerant, moderately tolerant and susceptible) could divide rice into two major groups *i.e. japonica* and *indica* (Zeng et al. 2004). Rice genotypes in each group could be further divided into subclusters corresponding to the country of origin but not the levels of salinity tolerance.

Comparison between SSR- and RAPD-based analysis of genetic diversity revealed that the SSR markers detected higher polymorphism (89.47% compared with 68.94%) among this set of rice genotypes and lower average genetic similarity (0.70 compared with 0.82). The dendrograms based on both marker systems separated the 30 rice cultivars into 4 major groups. However, the overall clustering pattern based on RAPD and SSR markers was not congruent. The clustering was conserved only for a small number of genotypes *i.e.* IR34 and Khao Kaset; IR28, IR29 and PSBRc86; RD6 and Pathumthani60; and RD15 and KDML105. The low Pearson's correlation (r = 0.329) between the two similarity matrices indicated that clusters produced based on the two marker systems were not conserved. Similar correlation (r = 0.39) was obtained from a study involving 42 Indian elite rice varieties using 8 RAPD and 9 SSR primers (Davierwala et al. 2000). The higher correlation between RAPD and SSR markers had been obtained by Ravi et al. (2003) who observed a correlation of 0.582 from a genetic diversity analysis among 40 cultivated rice varieties and five wild relatives using 36 RAPD and 38 SSR primers. Other previous studies often gave low correlation coefficient values *i.e.* 0.24, 0.39 and 0.54 for rice, olive and popcorn respectively (Belaj et al. 2003; Cho et al. 2004; Leal et al. 2010). The reasons for the poor correlation between RAPD and SSR data could be explained by (1) the different nature of genetic polymorphism detected by different types of markers; (2) different DNA segments targeted; (3) difference in the number of loci or primers and the genome coverage; (4) the difference in the level of reproducibility of the methods (Pejic et al. 1998; Sun et al. 2001; Wu et al. 2004).

The rice germplasm in this study was characterized at 20 SSR loci distributed over 11 chromosomes with mostly only 1-2 loci on each chromosome. This low number of loci certainly would have a low probability of including salt tolerance genes. It, therefore, was observed that clustering based on SSR markers could not resolve rice cultivars into distinct salt tolerance groups. Diversity analysis based on 111 polymorphic RAPD markers, on the other hand, involved amplification of random genomic fragments some of which could possibly be associated with salt tolerance genes. It was evident, with these RAPD primers, that this set of rice germplasm could be clustered according to four levels of salinity tolerance *i.e*. tolerant (T), moderately tolerant and moderately susceptible (MT/MS), susceptible (S) and highly susceptible (HS). The results from this study provide some useful implications for salt tolerance breeding programs. The evaluation of genetic distance together with salt tolerance ability provides some useful information for assisting plant breeders in selecting suitable genetically diverse parents for the crossing program. One potential strategy is to select genotypes initially by useful agronomic characteristics, and then select from those genotypes subsets, which are mutually dissimilar on the basis of molecular marker data (Beer et al. 1993; Davierwala et al. 2000). For example, salt tolerance of KDML105 could be improved by intercrossing with salt-tolerant genotypes namely Pokkali, Nam Sa-Gui19, RD23 and SPR90, which have relatively, low genetic similarity with KDML105 and placed in different clusters from KDML105. The Indian variety Pokkali has frequently been used as donor for salt tolerance genes but limited success was obtained because this traditional landrace possesses too many undesirable traits often linked to salinity tolerance (Gregorio et al. 2002). According to this result, KDML105 which is low-yielding (1.8 tons/hectare), susceptible to salinity, brown plant hopper, bacterial leaf blight and yellow orange leaf virus, can be crossed with SPR90 which gives higher yield (3.6 tons/hectare), is much higher in salt tolerance and resistant to all mentioned pests and diseases. The cross between these elite cultivars, which were clustered in different groups according to both RAPD and SSR data, could be performed with a goal of creating KDML105-derived progenies that possess high cooking quality and unique aroma, better tolerance of salinity and resistance to brown plant hopper, bacterial leaf blight and yellow orange leaf virus. In conclusion, genetic diversity among rice cultivars has been successfully performed by analysis of RAPD and SSR markers. Clustering based on RAPD could divide rice cultivars into groups according to the level of salt tolerance.

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