RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
46 (1) 1-20, 2014

EVALUATION OF RICE LANDRACES IN VIETNAM USING SSR MARKERS AND MORPHOLOGICAL CHARACTERS

NGUYEN THI LANG[1*], BUI PHUOC TAM[1], NGUYEN VAN HIEU[1], CHAU THANH NHA[1], ABDELBAGI ISMAIL[3], RUSSELL REINKE[3] and BUI CHI BUU[2]

[1] Cuu Long Delta Rice Research Institute, Thoi Lai, Can Tho, Vietnam
[2] Institute of Agricultural Sciences for Southern Vietnam, Vietnam
[3] International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines
[*]Corresponding author's email: ntlang@hcm.vnn.vn

SUMMARY

Information on genetic diversity among traditional varieties is critical in breeding programs as this influences parental selection in varietal development. A total of 100 traditional varieties in the genebank of the Cuu Long Delta Rice Research Institute (CLRRI), Vietnam, were used to explore this diversity using SSR markers. The study aims to evaluate the genetic diversity of traditional rice varieties and involves molecular diversity analysis using 55 polymorphic SSR markers revealed among the 100 varieties. The Vietnam varieties generated four clusters at 0.60 similarity coefficient. Some varieties with similar names were grouped into different clusters as molecular analysis showed that they were actually genetically different. The 100 landrace varieties collected were evaluated phenotypically. In the analysis of quantitative traits, the range of coefficients of variability was high. It varied from 94.38–80.3% (filled grain) to 60.02–5.63% (unfilled grain). This shows that these traits can be considered most stable as exemplified by their coefficients of variability. The highest values seen in unfilled grain indicate that this character is more affected by the environment and farmers' cultural management practices. The mean values of quantitative trait measurements were higher (78.75–139.75 cm). The highest values noted in yield (3.10–105.16 g) and survival (21–30 days) show good prospects to plant breeders. It has remained one of the major breeding objectives in developing rice varieties. Looking at agro-morphology, ANOVA showed highly significant differences among the 100 traditional rice varieties. The standardized Shannon-Weaver diversity indices for the quantitative morphological characters ranged from 0.68 to 0.95 with a mean of H' = 0.79. Cluster analysis using UPGMA grouped the 100 traditional varieties into 3major clusters. Varieties collected from the same site were grouped together in the same cluster.

Keywords: Coefficients of variability, molecular analysis, quantitative morphological characters, traditional varieties

Manuscript received: November 26, 2012; Decision on manuscript: November 22, 2013; Manuscript accepted: March 20, 2014.

Communicating Editor: Bertrand Collard

INTRODUCTION

Landraces are generally considered a rich source of genetic variation. Furthermore, local varieties provide farmers with alternatives in areas where modern crop varieties are not well-adapted, contributing to diversity at the field level. However, for rice, there has been a decrease in the number of traditional varieties being planted and only a few productive and relatively uniform

high-yielding varieties dominate the rice landscape (Tran, 2000). More than 3,000 accessions of traditional rices and 600 accessions of wild rices were collected and evaluated for use as rice breeding materials at the Cuu Long Delta Rice Research Institute (CLRRI) gene bank. The landraces thus offer great potential to transfer genes for tolerance for biotic and abiotic stresses into rice cultivars. CLRRI has generated a series of hybrids and introgression lines from crosses of elite breeding lines of rice with several wild species such as OM50L (IR42/Mot Bui Do). Genes for resistance to brown plant hopper, bacterial leaf blight, and blast and new sources of cytoplasmic male sterility have been transferred from several wild species into rice (Lang *et al*., 2002).

Recent advances in molecular biology, principally the development of polymerase chain reaction (PCR) for amplifying DNA, DNA sequencing, and data analysis have resulted in powerful techniques that can be used for screening, characterization, and evaluation of genetic diversity. With molecular marker techniques, powerful tools have been developed to accurately assess and characterize genetic resources. Several types of molecular markers are available for evaluating the extent of genetic variation in rice (Ni *et al*., 2002). These include restriction fragment length polymorphism (Botstein *et al*., 1980), random amplified polymorphic DNA, amplified fragment length polymorphism, and microsatellites or simple sequence repeats (Mc Couch, 1988; Temnykh *et al*., 2000; Lang *et al*., 2009).

Characterization and evaluation of diversity among traditional varieties will provide plant breeders the information necessary to identify initial materials for hybridization to produce varieties with improved productivity and quality.

The objectives of the study are as follows:

1. To evaluate the genetic diversity of traditional rice varieties in the gene bank of CLRRI, Vietnam, using morphological characters and microsatellite markers
2. To study the correlation among the characters for application in plant breeding for salt tolerance in rice
3. To compare results between morphological characters and molecular markers.

MATERIALS AND METHODS

A total of 100 rice varieties were evaluated (Table 1) and the following quantitative traits were considered:

Panicle length (cm) - length of panicle at maturity measured from the base of the plant to the tip of the panicle (taken from 10 randomly selected primary panicles per accession per replication)

Panicles per plant (number) - total number of panicles per plant (from 10 randomly selected primary panicles per accession per replication)

1000-grain weight (g) - weight of 1000 well-developed grains at 14% moisture content (from 5 randomly selected primary panicles per accession per replication)

Days to maturity - days from seeding when 80% of the grains are fully ripened on a per replication basis

5. Filled grains (number) - obtained from counts of total number of filled grains per panicle (from 5 randomly selected primary panicles per accession per replication)

Unfilled grains (number) - obtained from counts of total number of unfilled grains per panicle (from 5 randomly selected primary panicles per accession per replication)

Yield obtained from the harvested plants in each replication. Harvested grains were threshed, cleaned, dried, and weighed for each accession per replication. Moisture content per plot was determined immediately after weighing using a moisture meter.

Yield = weight of harvested grain (g)/ number of hills harvested x number of possible hills x MF (of the harvested grains)

where $MF = \frac{100 - MC}{86}$

Biomass--weight of 10 plants harvested from each accession per replication. Harvested plants were dried before weighing.

$$\text{Harvest Index} = \frac{\text{Economic yield}}{\text{Biologicalyield}} \text{x100}$$

where economic yield is the total weight of grain harvest from 10 plants per accession per replication and biological yield is the total grain weight and biomass from 10 plants per accession per replication.

Survival days: seedling culture and survival time in saline nutrient solution. Sterilized seeds were germinated on moistened filter paper in petri dishes at 30 ^{0}C for 48 h. Two pregerminated seeds were placed in each well of styrofoam seedling trays floating on distilled water. After 3 days, the seedlings were well established, and the distilled water was replaced by salinized nutrient solution (Yoshida *et al*., 1976). Initially, the saline nutrient solution had an electrical conductivity (EC) of 6 dS/m. Three days later, salinity was increased to 12 dS/m by adding NaCl to the nutrient solution. The solution was renewed every 8 days and pH was adjusted to 5.0 daily. When a seedling was completely yellow and no green tissue was evident, it was considered dead. Days of plant survival were recorded as the time that elapsed from seeding to death (Lang *et al*., 2001).

Table 1. Passport information of the 100 traditional varieties used in the study (Lang *et al.*, 2009).

No.	Accession	Name of variety	Passport information
1	466	Mahsuri	India(CLRRI) genebank
2	1718	Nàng Thơm Đốc	Mekong Delta, Southeast Vietnam, 106^o 48’ 32’’ E longitude and 10^o 35’ 19’’ N latitude
3	750	Nhỏ Thơm	Ben Tre 106 ^{o} 48’ East longitude and 105^o 57’ North latitude
4	1714	Mùa Đốc	Kiengiang, Vietnam, 104^o 40’- 105^o 32’ 40” longitude, 90^o 23’ 50”- 100^o 32’30” latitude
5	786	HTA FR85004	Wetland rice, Thailand, 15 00 N, 100 00 E
6	687	Giá Đen	Mekong Delta, Southeast Vietnam, 106^o 48’ 32’’ E longitude and 10^o 35’ 19” N latitude
7	754	Nàng Thơm Muộn	Longan, Vietnam, 105^o 30' 30" - 106^o 47' 02" longitude and 10^o 23' 40"-11^o 02' 00" latitude, alluvial soil
8	1719	Nàng Thơm Đốc	Mekong Delta, Southeast Vietnam, 106^o 48’ 32’’ E longitude and 10^o 35’ 19’’ N latitude
9	557	Nàng Thơm	Mekong Delta, Southeast Vietnam, 106^o 48’ 32’’ E longitude and 10^o 35’ 19’’ N latitude
10		Mot Bui Do	Camau peninsula, Vietnam, 104080 - 10505 longitude and 8030 - 9010 latitude, saline soil
11	755	Nàng Thơm Muộn	Longan, Vietnam, 105^o 30' 30" - 1060^o 47' 02" longitude and 10^o 23' 40" - 11^o 02' 00" latitude, alluvial soil
12		Mot Bui Lun	Mekong Delta, Southeast Vietnam, 106^o 48’ 32’’ E longitude and 10^o 35’ 19’’ N latitude
13	1722	Nàng Loan Đốc	Mekong Delta, Southeast Vietnam, 106o48’32’’ E longitude and 10^o 35’ 19’’ N latitude
14	1579	Lùn Rằn	Mekong Delta, Southeast Vietnam, 106^o 48’ 32’’ E longitude and 10^o 35’ 19’’ N latitude
15		Tai nguyen Duc	Camau peninsula, Vietnam, 104080 - 10505 longitude and 8030 - 9010 latitude, saline soil

16	1552	Nếp Than	An Giang 104º 70’ east longitude and 105º 50’ North latitudel
17	530	Nàng Hương	Tiengiang, Vietnam, 106º 48’ 32’’ East longitude and 100º 35’ 19’’ North latitude
18	1533	Trắng Tép	Camau peninsula, Vietnam, 104080 - 10505 longitude and 8030 - 9010 latitude, saline soil
19	1701	tai nguyen Trang	Bac Lieu 105º 15’ 00’’ East longitude and 9º 00’ and 9º 37’ 30” North latitude
20	566	Nếp Phụng Tiên	Camau peninsula, Vietnam, 104080 - 10505 longitude and 8030 - 9010 latitude acid suffate
21	727	Xương Gà	Tay ninh
22	674	Biệt Cá Trơn	An Giang 104º 70’ East longitude and 105º 50’ and 100-110 North latitude
23	572	Nàng Hương Chợ Đào	Longan, Vietnam, 105º 30' 30" - 106º 47' 02" longitude and 10º 23' 40" -11º 02' 00" latitude, alluvial soil
24	731	Nàng Thơm	Longan, Vietnam, 105º 30' 30" - 106º 47' 02" longitude and 10º 23' 40" -11º 02' 00" latitude, alluvial soil
25	1555	Hai Hoành	Ben Tre 106º 48’’ East longitude and 10 50º57’ North latitude 9º 48’ -10º 20’
26	749	Nhỏ Thơm	Mekong Delta, Southeast Vietnam, 106º 48’32’’ E longitude and 10º 35’19’’ N latitude, saline soil
27	756	Nàng Thơm Muộn	Longan, Vietnam, 105º 30' 30" - 106º 47' 02" longitude and 10º 23' 40" - 11º 02' 00" latitude, alluvial soil
28	1533	Trắng Tép	Mekong Delta, Southeast Vietnam, 106º 48’ 32’’ E longitude and 10º 35’ 19’’ N latitude
29	541	Nàng Hương	Lua nuoc troi, Longan, Vietnam, 105º 30' 30" -106º 47' 02" longitude and 10º 23' 40" - 11º 02' 00" latitude, alluvial soil
30	1711	Nông Nghiệp Chùm Đốc	An Giang 104º 70’ East longitude and 105º 50’ north latitude
31	701	Rơ Đinh LĐ	Cambodia, 102nd -108th eastern longitude and 10th -15th parallels of Northern latitude.
32	697	Đông Xuân	Ben Tre 106 º 48’ East longitude and 105º 57’ North latitude
33	665	Ba Bụi	Camau peninsula, Vietnam, 104080 - 10505 longitude and 8030 - 9010 latitude, saline soil
34	1576	Lùn Kiên Giang 1	Kiengiang, Vietnam, 104º 40’- 105º 32’ 40” longitude, 90º 23’ 50” – 100º 32’ 30” latitude
35	1702	Nàng Loan Đốc	Can Tho 90º 4’ 43’’ East longitude and 105º 19’ 51’’ North latitude
36	1553	Rắn Lùn	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
37	684	Đỏ Lún	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
38	611	Nang quot	Ben Tre 106º 48’ East longitude and 1050º 57’ North latitude
39	1720	Nàng Loan Đốc	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
40	554	Trắng Hòa Bình	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
41	1536	Trắng Tép	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
42	1573	Lùn Cẩn	Ben Tre 106º 48’’ East longitude and 10 50º 57’ North latitude
43	560	Nàng Hương	Tiengiang, Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude

44	1636	Lúa Thơm Lùn	Lua nuoc troi, Longan, Vietnam, 105030' 30"-106047' 02" longitude and 10º 23' 40"-11º 02' 00" latitude, alluvial soil
45	1562	Nếp Trắng	Lua nuoc troi, Longan, Vietnam, 105º 30' 30" - 106º 47' 02" longitude and 10º 23' 40"-11º 02' 00" latitude, alluvial soil
46	556	Nếp Than	AnGiang
47	790	HTA 88060a	Wetland rice, Thailand, 15 00 N, 100 00 E
48	636	Nếp Nhung	Ben Tre 106º 48’ East longitude and 1050º 57’ North latitude 9º 48’ - 10º 20’
49	567	Nếp Phụng Tiên	Ben Tre 106º 48’’ East longitude and 1050º 57’ North latitude 9º 48’ - 10º 20’
50	704	Tẻ Tép	Ben Tre 106º 48’’ East longitude and 1050º 57’ North latitude 9º 48’ - 10º 20’
51	1567	Trắng Tròn	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
52	698	Đông Xuân	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude Tay Ninh
53	600	Nang thom CD	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
54	671	Bát Ngát	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
55	601	Nang huong	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
56	571	Ngọc Nữ	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
57	668	Chánh Hưng	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
58	602	Nang huong	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
59	700	Rơ Đinh LĐ	Cambodia, 102nd -108th Eastern longitude and 10th -15th parallels of Northern latitude.
60	1585	Trời Cho	Tayninh, Vietnam, 105º 48’ 43” – 106º 22’ 48’’ longitude and 10º 57’ 08’’ - 11º 46’ 36’’ latitude, alluvial soil
61	1642	Nàng Hương	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
62	1572	Trắng Phếu	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’ 19’’ North latitude
63	1541	Nếp Cá Rô	Tayninh, Vietnam, 105º 48’ 43” – 106º 22’ 48’’ longitude and 10º 57’ 08’’ - 11º 46’ 36’’ latitude, alluvial soil
64	1699	Nếp Ruồi Xanh	Lua nuoc troi, Longan, Vietnam, 105º 30’ 30’’ - 106º 47’ 02’’ longitude and 10º 23’40’’ - 110º 2’ 00’’ latitude, alluvial soil
65	554	Trắng Hòa Bình	Deep water rice, Songhau, Western Vietnam, 106º 48’ 32’’ East longitude and 10º 35’19’’ North latitude
66	1614	Nếp Áo Vàng	Quang tri
67	1587	KT15	Kiengiang, Vietnam, 104º 40’- 105º 32’ 40” longitude, 9º 23’ 50 - 10º 32’ 30” latitude
68	1586	Nếp Ba Tập	Kiengiang, Vietnam, 104º 40’- 105º 32’ 40” longitude, 9º 23’ 50 - 10º 32’ 30” latitude
69	739	Nàng Thơm Thanh Trà	Longan, Vietnam, 105º 30’ 30’’ - 106º 47’ 02’’ longitude and 10º 23’ 40’’ -11º 02’ 00’’ latitude, alluvial soil
70	1580	Ba Cô	Tay Ninh

71	580	Nhỏ Thơm	Ben Tre 106º 48'' East longitude and 10º 50' 57'' North latitude
72	1588	Nếp Chuột Chê	Tay Ninh
73	748	Nếp Tiên	Tay Ninh
74	1534	Trắng Lựu	Can Tho 90º 4' 43'' East longitude and 105º 19' 51'' North latitude Can Tho
75	635	Nanh Chồn	Dong Nai
76	752	Mbarbla	Cambodia, 102nd -108th Eastern longitude and 10th - 15th parallels of Northern latitude.
77	1557	Lùn Thống	Kiengiang, Vietnam, 104º 40' - 105º 32' 40 longitude and 9º 23' 50'' – 10º 32' 30" latitude
78	1665	Nàng Quớt	Deep water rice, Songhau, Western Vietnam, 106º 48' 32'' East longitude and 10º 35' 19'' North latitude
79	737	Nàng Thơm Thanh Trà	TPHo Chi Minh
80	1699	Nếp Ruồi Xanh	Tay ninh
81	764	Nàng Thơm Thanh Trà	Longan, Vietnam, 105º 30' 30'' - 106º 47' 02'' longitude and 10º 23' 40''-110º 2' 00'' latitude, alluvial soil Long An Province
82	726	Xương Gà	Tay Ninh
83	581	Nàng Hương Chợ Đào	Longan, Vietnam, 105030' 30''-106047' 02'' longitude and 10023'40''-11002' 00'' latitude, alluvial soil
84	1575	Nàng Tiên Ngọc Nữ	Tra Vinh
85	1610	Vàng Nghệ	Quang Binh Province
86	751	Mbarbla	Camau peninsula, Vietnam, 104080 - 10505 longitude and 8030 - 9010 latitude, deepwater
87	699	Bông Bưởi	Tay ninh Province
88	1543	Nàng Hương	Tien Giang province
89	1563	Một Bụi	Camau peninsula, Vietnam, 104080 - 10505 longitude and 8030 - 9010 latitude, saline soil
90	1587	KT15	Kien Giang province
91	1637	Một Bụi	Camau peninsula, Vietnam, 104080 - 10505 longitude and 8030 - 9010 latitude, saline soil
92	555	Nanh Chồn	BaRia Vung Tau
93	762	Mi Bar Tơ Bô	Cambodia, 102nd -108th eastern longitude and 10th -15th parallels of Northern latitude.
94	670	Bát Ngát	HaTienKien Giang Province longitude and 9º 23' and 10º 32' of Northern latitude.
95	1612	Nếp Áo Vàng	Quang Tri Province
96	552	Tàu Hương	Ben Tre 106º 48'' East longitude and 1050º 57' North latitude 9º 48' - 10º 20'
97	580	Nhỏ Thơm	Ben Tre 106º 48'' East longitude and 1050º 57' North latitudel 9º 48' - 10º 20'
98	761	Mi Bar Tơ Bô	Cambodia, 102nd -108th Eastern longitude and 10th -15th parallels of Northern latitude.
99	1574	Thần Nông Lùn	Kiengiang, Vietnam, 104º 40'- 105º 32'40 longitude and 9º 23' 50'' – 10º 32' 30" latitude
100	791	HAT 88086	Wetland rice, Thailand, 15 00 N, 100 00 E

Data analysis

Analysis of variance (ANOVA)

The agromorphological data collected were initially analyzed using ANOVA to verify genetic variation in the traits measured. The few traits with insignificant genetic variation, based on the F test, were not considered for further analyses.

Shannon-Weaver diversity index

Diversity indices for the various traits were computed using the following formula:

$$H' = \frac{-\sum pi * \log_2(pi)}{\log_2 n}$$

where *n* is the number of phenotypic classes for a character and *pi* is the portion of the total number of entries belonging to the *i* class.

The Shannon -Weaver diversity index was standardized by dividing H' by the $\log_2$ of the total number of phenotypic classes. To estimate phenotypic diversity of varieties, H' was computed in MS Excel for each of the morpho-agronomic descriptors. The mean phenotypic diversity index was computed for the pooled diversity estimates per descriptor. The standardized value ranged from 0 to 1, with 1 indicating maximum diversity.

Correlation analysis

The correlation coefficient (*r*) is a measure of the association between 2 or more variables. It is a measure of symmetrical association between variables and does not measure the dependence of one variable over another. Correlation among agro-morphological traits was calculated by using the SAS program.

Distance matrix

Distance matrix was calculated by means of the Euclidean distance coefficient (Sneath and Sokal, 1973):

$$Eij = [\sum_k (X_{ki} - X_{kj})^2]^{1/2}$$

where *Eij* = 0 to ∞; the larger the value, the more distant the degree of the relationship.

Xi and *Xj* are the standardized values for the ith and jth characters in the kth varieties.

Cluster analysis

Cluster analysis was carried out for agro-morphology-based genetic distance matrix using the UPGMA clustering method in the NTSYS program. The results of the UPGMA were used to draw the dendrogram of the 100 traditional varieties.

Polymorphic information content (PIC), which provides an estimate of the discriminatory power of a locus, by taking into account not only the number of alleles that are expressed but also the relative frequencies of those alleles, was estimated using the formula suggested by Nei (1973):

$PIC = 1-\sum x^2k$

where x^2k represents the frequency of the *k*th allele.

Molecular-based characterization and analysis using SSR

DNA extraction

The 90 varieties were grown in pots. Maximum protection was employed to ensure healthy and disease-free seedlings. The leaves were collected 2-3 weeks after planting for DNA extraction.

Standard molecular grade chemicals and general techniques for preparing stock solutions, buffers, reagents, and equipment were followed according to Sambrook *et al*. (1989). Molecular work was conducted at the Genetics and Plant Breeding Department of the Cuu Long Delta Rice Research Institute, Cantho, Vietnam.

DNA suitable for PCR analysis was prepared using a simplified procedure (McCouch *et al.*, 1988). A piece of a young rice leaf (2 cm) was collected and placed in a labeled 1.5 ml centrifuge tube in ice. The leaf was

ground using a polished glass rod in a well of a spot test plate (Thomas Scientific) after adding 400 µl of extraction buffer. Grinding was done until the buffer turned green, an indication of cell breakage and release of chloroplasts and cell contents. Another 400 µl of extraction buffer was added into the well by pipetting. Around 400 µl of the lysate was transferred to the original tube of the leaf sample. The lysate was deproteinized using 400 µl of chloroform. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA was precipitated using absolute ethanol. DNA was air-dried and re-suspended in 50 µl of TE buffer (Lang, 2002).

DNA quality checks used 1% agarose by melting 3 g of agarose in 300 ml of TAE buffer. The mixture was heated in a microwave for 5-6 min and then cooled to around 55-60 ^{0}C. This was then poured on a previously prepared electrophoresis box with combs. Gels were prepared and the combs removed after about 45 min. Seven microliters of DNA sample plus 3 µl of loading buffer (Tris 1 M pH = 8.0, glycerol, EDTA 0.5 M pH = 8.0, xylene cyanol 0.2%, bromphenol blue 0.2%, and distilled water) was run at 70-80 v, 60 mA for 45 min or until the loading buffer dye moved far away from the wells. The gel was then taken out and stained with ethidium bromide, after which it was observed under UV light.

Microsatellite analysis

The whole microsatellite analysis included PCR assay, polyacrylamide gel electrophoresis, and band detection and scoring.

PCR assay

Microsatellite primers were used to survey polymorphism on the samples. These were randomly selected from the 312 microsatellite primer pairs currently available for rice (Temnykh *et al*., 2000). The PCR reaction was as follows:

Reactions were overlaid with mineral oil and processed in a programmable thermal controller set for 35 cycles of 1 min at 94 ^{0}C, 1 min at 55 ^{0}C, and 2 min at 72 ^{0}C, with a final extension at 75 ^{0}C for 5 min. After amplification, 10 µl of stop solution was added to the PCR product, which was then denatured at 94 ^{0}C for 2 min. Eight microliters of each reaction were run on polyacrylamide gel.

Band detection and scoring

Plates were separated using a plastic wedge and were removed from the tank. The acrylamide gel was soaked in ethidium bromide staining solution for 15 to 20 min. Bands in the ethidium bromide-stained gels were detected and photographed under UV light. Allelic bands were scored as 1 (present) or 0 (absent), respectively. Data were entered directly into an Excel spreadsheet.

Data analysis

Pairwise comparisons of lines based on the presence of unique and shared polymorphic products were used to calculate the genetic similarity coefficients. These coefficients were calculated using Nei and Li's distance measure (Nei and Li, 1979) in the NTSYS–PC Numerical Taxonomy and Multivariate Analysis System (Rohlf, 1990). The lines were clustered on the basis of similarity coefficients using the unweighted pair group method- arithmetic average (UPGMA) clustering algorithm.

RESULTS AND DISCUSSION

Polymorphism of microsatellite markers

Many researchers have reported the genetic basis of the salinity trait, which was governed by 1 recessive allele located in chromosome 1. Therefore, molecular markers linked tightly to the target salinity gene is considered a powerful tool to support breeding efforts to develop salt-tolerant rice varieties rapidly. The results indicate that phenotypic analysis was affected strongly by environmental factors. To overcome this, an assessment of genetic diversity of initial material sources is necessary.

PCR amplification was performed with DNA samples extracted from 100 traditional rice varieties. Several representative DNA samples were used as template in the PCR amplification reaction using SSR markers as 105 primers, but

only 55 primers were polymorphic. Amplified PCR products were electrophoresed on 3% agarose gel with 1X TBE buffer solution, stained with ethidium bromide, then observed under UV-transilluminator.

In the amplification of genomic DNA of the 100 rice genotypes using 135 primers, 55 were found to be polymorphic. The number of amplified fragments ranged from 2 to 4. All of the primer pairs used in this study generated polymorphic bands among the genotypes. A total of 25 loci were assigned to the 55 microsatellite primer pairs. A total of 163 alleles were detected among the 100 rice genotypes with an average of 1.46 alleles per locus (Table 3). The number of alleles per locus ranged from 2 to 5 (in RM11125). The total alleles identified in the 100 genotypes were classified into 4 categories:

The PIC values for the microsatellite loci ranged from 0.4 3 to 0.79 with an average of 0.67 (Table 2). The low PIC values were observed among the primers of RM148 (0.43) RM243, and RM10649(0.45); the PIC value high such as primers RM11125 (0.79), RM 21, and RM5629 (0.78).

A dendrogram based on cluster analysis using UPGMA with the module of SAHN in the NTSYS-pc package was created. Cluster analysis showed significant genetic variation among the landrace rice varieties studied, with genetic distance ranging from 0 to 0.74 (Figure 1). With a genetic distance of 0.60, the cluster revealed 4major groups, A, B, C, and D, in the VietNam rice varieties. Group A was divided into sub-clusters A1 and A2 (46%); Group B and Group C (39%); and Group D consisted of 6 traditional varieties (6%) such as Trang Luu, Nanh Chon, Mbarla, Lun Rang, Lua Thong, and HTA 88086.

Table 2. Primers and Chromosome, PIC values for survival 100 varieties from Vietnam.

No.	Primer	Chromosome	No. of allele	Size (bp)	PIC values
1	RM105	9	2	210-215	0.46
2	RM10115	1	2	240-250	0.49
3	RM243	1	2	190-210	0.45
4	RM10649	1	2	180-210	0.45
5	RM24	1	3	200-205	0.63
6	RM7643	1	3	205-220	0.66
7	RM472	1	3	210-242	0.64
8	RM11125	1	5	160-200	0.79
9	RM10843	1	4	180-200	0.73
10	RM3412b	1	3	190-200	0.64
11	RM10793	1	3	210-220	0.63
12	Salt 1	1	4	200-220	0.74
13	Salt 2	1	2	210-220	0.45
14	RM 152	8	3	175-200	0.63
15	RM5806	10	3	210-230	0.66
16	RM5806	10	3	230-250	0.64
17	RM211	2	3		0.65
18	RM17	12	5	160-190	0.79
19	RM310	8	4	200-210	0.72
20	RM27877	12	3	215-240	0.63
21	RM221	2	3	220-230	0.66

22	RM28746	12	3	200-210	0.63
23	RM5436	7	4	200-210	0.73
24	RM3867	3	4	210-230	0.74
25	RM6329	3	3	220-230	0.64
26	RM249	5	3	210-230	0.64
27	RM5626	3	5	200-210	0.78
28	RM18	7	3	190-200	0.64
29	RM21	11	5	210-220	0.78
30	RM163	5	2	255-260	0.45
31	S11049	11	4	200-210	0.74
32	RM140	1	3	190-200	0.61
33	RM169	5	4	240-250	0.73
34	RM9	1	2	230-240	0.49
35	RM10852	1	3	220-230	0.64
36	RM10890	1	3	205-210	0.66
37	RM10927	1	2	240-245	0.40
38	RM154	2	2	160-180	0.45
39	RM231	3	3	200-210	0.67
40	RM21539	7	2	205-210	0.45
41	RM122	5	3	205-230	0.64
42	RM510	6	2	220-230	0.42
43	RM547	8	2	200-210	0.49
44	RM23662	9	3	210-220	0.64
45	RM219	9	3	200-215	0.65
46	RM24013	9	2	215-220	0.42
47	RM3	6	2	220-225	0.50
48	RM223	8	2	200-210	0.46
49	RM315	1	2	210-230	0.49
50	RM13	5	3	190-210	0.63
51	RM166	2	3	190-200	0.65
52	RM140	1	3	200-210	0.63
53	RM220	1	3	210-220	0.64
54	RM227	3	3	200-220	0.65
55	RM148	3	2	190-210	0.43

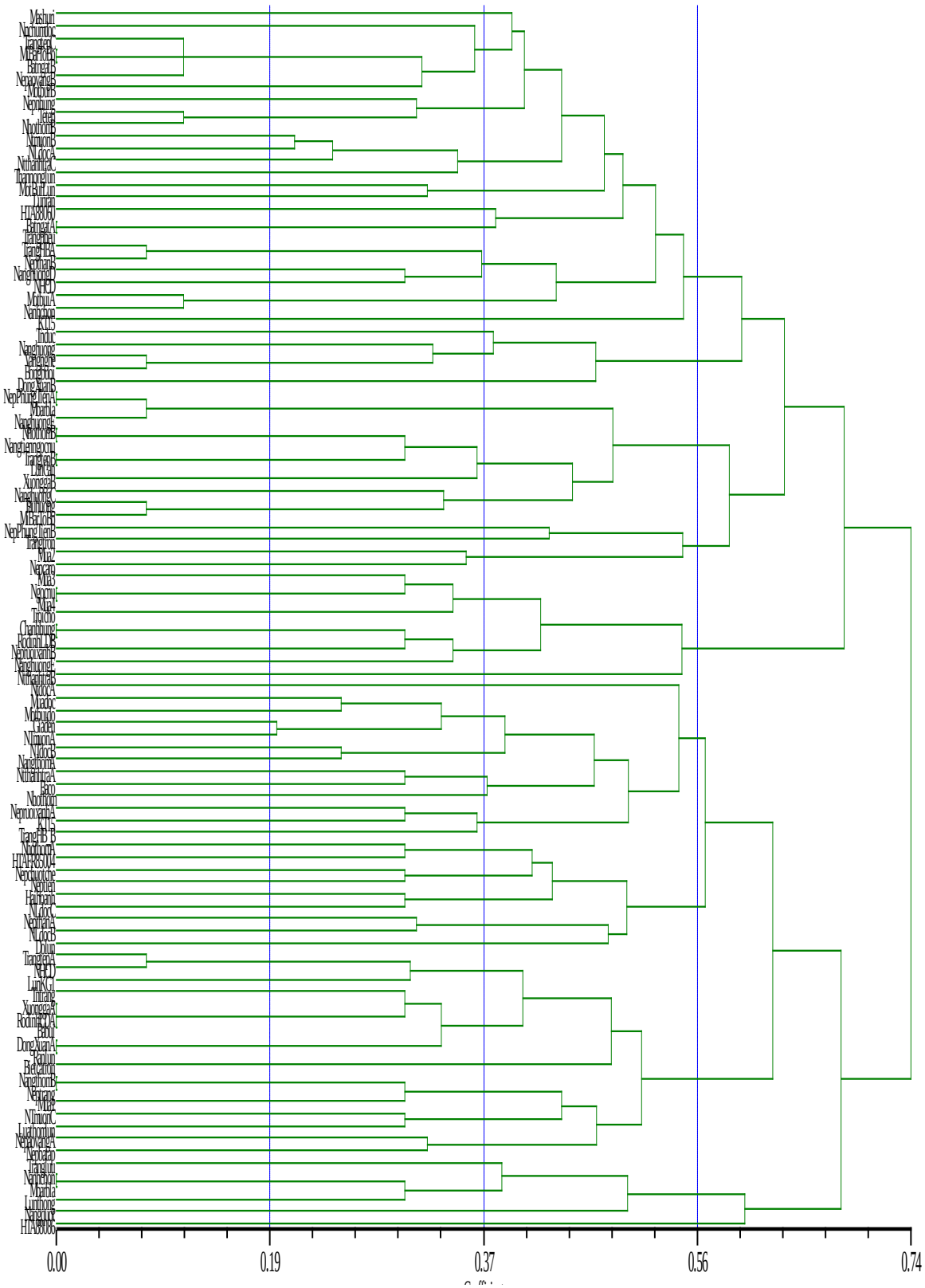

Figure 1. Classification of rice varieties based on genetic distance calculated from 55 microsatellite markers of 100 rice varieties

Table 3. Mean number of alleles on different rice chromosomes based on microsatellite markers.

Group	Sub group	Mean of allele number. per SSR marker												Mean
		Chromosome												
		1	2	3	4	5	6	7	8	9	10	11	12	
A	1	1.30	1.26	1.44	0.00	1.32	0.92	1.35	1.17	1.24	1.53	1.82	1.48	1.24
	2	1.33	1.12	1.49	0.00	1.39	0.83	1.60	1.03	1.30	1.83	1.83	1.62	1.28
	Mean	*1.32*	*1.19*	*1.47*	*0.00*	*1.36*	*0.88*	*1.48*	*1.10*	*1.27*	*1.68*	*1.83*	*1.55*	*1.26*
B	1	1.41	1.31	1.65	0.00	1.76	1.11	1,63	1.58	1.33	1.39	2.50	1.74	1.43
	Mean	1.41	1.31	1.65	0.00	1.76	1.11	1,63	1.58	1.33	1.39	2.5	1.74	*1.43*
C	1	1.40	1.63	1.44	0.00	1.70	1.00	1.78	1.46	1.50	1.00	2.92	1.78	1.47
	2	1.24	1.41	1.53	0.00	1.28	0.97	1.08	1.17	1.14	1.28	1.91	1.69	1.23
	3	1.47	1.18	1.55	0.00	1.52	0.59	1.55	1.32	1.25	1.79	2.32	1.65	1.35
	Mean	*1.37*	*1.41*	*1.51*	*0.00*	*1.50*	*0.85*	*1.47*	*1.32*	*1.30*	*1.36*	*2.38*	*1.71*	*1.35*
D		1.09	1.04	1.08	0.00	1.23	0.58	1.22	1.00	0.79	1.08	1.83	0.83	0.98
	Mean	*1.09*	*1.04*	*1.08*	*0.00*	*1.23*	*0.58*	*1.22*	*1.00*	*0.79*	*1.08*	*1.83*	*0.83*	*0.98*
Mean		1.30	1.24	1.43	0.00	1.46	0.85	1.39	1.25	1.17	1.38	2.13	1.46	1.26

Table 4. Descriptive statistics of quantitative traits among 100 landraces.

Trait	Max	Min	Mean	CV	P	h^2
Plant height (cm)	139.75	78.75	113.91	0.73	<0.01	0.99
Panicles/ hill (no.)	31.22	8.69	18.89	4.07	<0.01	0.98
Panicle length (cm)	29.06	19.17	23.86	3.07	<0.01	0.87
Fertile grains (%)	94.38	39.94	80.38	1.07	<0.01	0.99
Unfertile grains (%)	60.06	5.62	19.62	4.19	<0.01	0.99
1000-grain weight (g)	32.72	24.47	26.70	3.00	<0.01	0.84
Duration (days)	174.00	120.00	155.11	0.51	<0.01	0.99
Biomass (g)	180.00	16.00	62.32	1.26	<0.01	0.99
Yield (g/hill)	105.16	3.10	41.92	1.90	<0.01	0.99
Harvest index (%)	0.45	0.10	0.41	3.91	<0.01	0.99
Salt stress(days)	30.00	21.00	25.59	3.26	<0.01	0.86

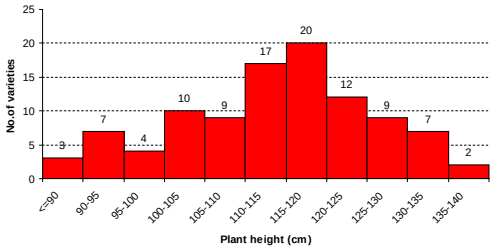

Figure 2a

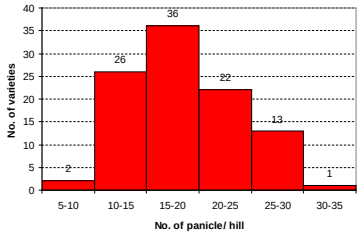

Figure 2b

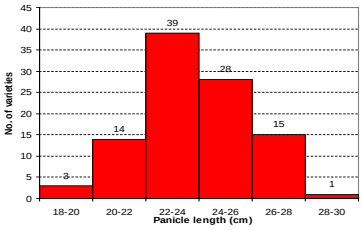

Figure 2c

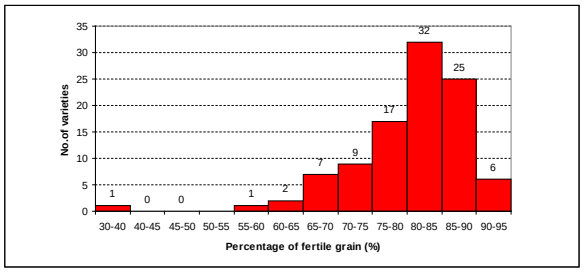

Figure 2d

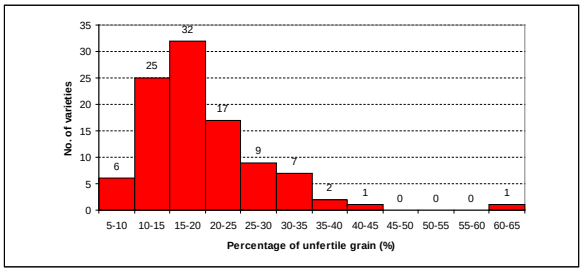

Figure 2e

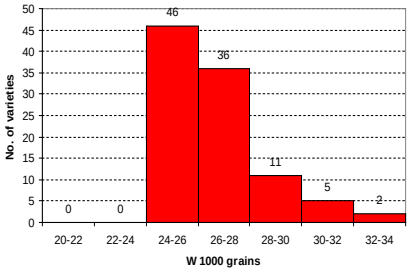

Figure 2f

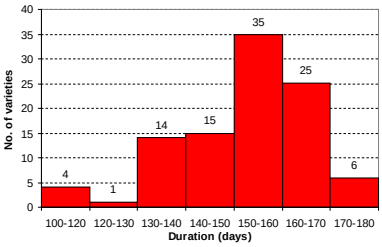

Figure 2g

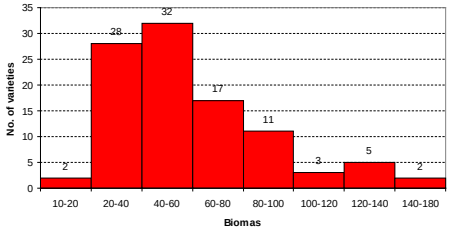

Figure 2h

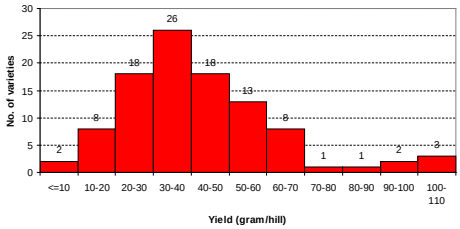

Figure g

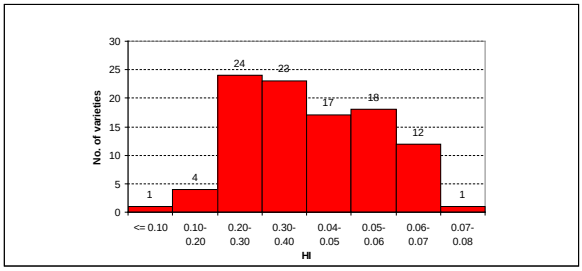

Figure f

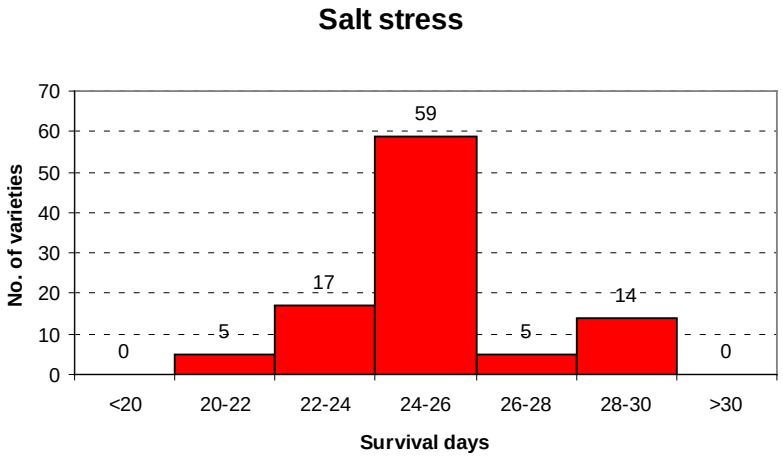

Figure h

Figure 2. Frequency distribution of the varieties with respect to maturity, high plant duration , panicles per plant, , number of filled grains, number of unfilled grains, 1000-g weight, yield , biomass , Harvest Index and survival days showed the diversity of landrace varieties.

The first subgroup, A, contained 1 upland and 2 lowland rice varieties. The second group, B, which was the largest, contained 126 (90.2 %) rice varieties. Most varieties in this group were lowland rice varieties. The second group was divided into 2 sub-groups, 1 and 2. The first subgroup, B1, contained 122 rice varieties consisting of 26 upland rice varieties andB2 included 4 rice varieties.

Allele number per locus and per chromosome was much lower than 1.46 (Table 3). The mean allele number per locus in group A was 1.26.That in group B was 1.43; C had 1.35 and D, 0.98.

Agro-morphological characters

Analysis of variance. For each of the 11 quantitative traits, the mean, range (maximum and minimum), standard deviation, coefficient of variation (CV), mean standard error, and F values were calculated (Table 4). Highly significant differences in various traits of the 100 traditional varieties were obtained—e.g., number of unfilled grains, 1,000-grain weight, harvest index, yield, and biomass. Results show that most of the quantitative traits were highly variable. With respect to maturity, the earliest maturing genotype matured in 120 days; the latest maturing one took 175 days. Maximum yield (236.46 g/hill) was seen in Nep Nho, whereas Nang Huong had 151.0 g/hill. Some varieties had very low yields: 2.617 g from Nep Phu and 9.228 g from Nep Phung Tien. Panicles of some varieties were long- LunRang's panicle length was found to be 29.66 cm. Some varieties had high grain weight- in Mashuri, it was 32.72 g. However, other varieties were very light; the Nang Co varieties had low grain weight (16.06 g) and KT 15 was observed to have 94.38 filled grains (≈39.94.8%). Both showed high fertility, which means that they are good breeding materials.

Highly significant differences in number of unfilled grains and 1000 grain weight were seen in the 100 traditional varieties studied.

The frequency distribution of varieties with respect to maturity, panicles per plant, number of filled grains, number of unfilled grains, 1000-grain weight, yield, biomass, harvest index, and days of survival after salt stress showed the diversity of traditional varieties. These quantitative characters were found to be significant at 1% and all measurements were normally distributed (Figures 2a to h).

Plant height showed normal distribution (Figure 2a). Distribution of varieties in terms of number of filled grains was slightly skewed to the right, with only a few varieties found near the maximum value (Figure 2d). As to number of unfilled grains, the distribution was slightly skewed to the left, with only a few varieties near the maximum value (Figure 2e). For traits such as 1000-grain weight, yield, and panicles per plant, unimodal distribution was observed with most varieties skewed to the left of the curve. Such distribution is favorable, particularly with respect to number of unfilled grains, because the lower number of unfilled grains would mean higher yield. This is an important objective for most plant breeders, improving present-day varieties.

Yield showed near normal distribution-slightly skewed to the right with only a few varieties nearing the maximum value (Figure 2g). With regard to maturity, almost half of the varieties investigated exhibited long growth duration. The analysis of variance showed high variability among the varieties in terms of number of unfilled grains, yield, and number of filled grains.

Considering 1000-grain weight, only 2 varieties had weights greater than 32 g; most varieties had weights less than 24-26 g (Figure 2g). Since this trait is one of the most important yield components, the landraces identified can be important starting materials for the development of varieties with higher grain weight. This study also found that most varieties were tall, height range being 120-130 cm. Only 2 varieties (Nang huong and Huyet tuong) had heights greater than 140 cm (Figure 2a). The semi-dwarf stature contributed most to production gains during the green revolution due to associated improvements in harvest index and lodging under heavy fertilizer doses (Hargrove *et al*., 1980). As to maturity, some varieties such as NepTrang mature in 174-180 days. The challenge still exists for breeders to develop varieties with shorter duration without sacrificing yield.

Morphological characterization showed that most traditional varieties generally are taller with broader leaves and had more filled grains, less unfilled grains, late maturity, , higher 1000-g weight. The variation in agro-morphological characters discussed above can be explained by the genetic variation among the varieties examined. This variability can be used to find raw materials that plant breeders can use to develop rice with better plant type, better grain quality, and higher photosynthetic efficiency.

For salt stress tolerance, Trang Tep, Mot Bui Do, Nho Thom, Mot Bui Lun, and Do Lun hold promise as good donors of this important trait.

Correlation among agro-morphological traits

The correlation coefficients of the traits measured in the study are shown in Table 5. Panicle length was significantly correlated with plant height (r = 0.625) and harvest index was significantly correlated with yield, (r = 0.688**), confirming the findings that varieties with high harvest index also have higher yield (Lang *et al.*, 2009). Significant negative correlations were also found between harvest index and biomass (r = -0.603), which can be explained by the principle of morphogenic compatibility in rice plant architecture with landrace varieties. Other traits were found to be poorly correlated with other agro-morphological traits. There was negative correlation between yield and filling grain (r =0.093), panicles per plant (r = -0.093), and panicle length (r= -0.042). Some late-maturing varieties had a negative correlation with yield (r = -0.043), again supporting the results of other studies (Lang *et al*., 2009).

Table 6 presents the Shannon-Weaver diversity indices (H') of the 11 quantitative agro-morphological traits. The H' values ranged from 0.68 to 0.95 with a mean of 0.79. The highest diversity indices were observed in 1000grain weight (H' = 0.95), yield (H' = 0.82), harvest index (H' = 0.94), and number of filled grains (H' = 0.92). The lowest diversity index was 0.68, for salt stress (survival days).

The 100 landrace varieties held in the Cuu Long genebank exhibited high diversity in the 11 quantitative agro-morphological characters evaluated. The collection can be a valuable resource for developing rice varieties in Vietnam. The information will also help germplasm managers' plan for future acquisitions.

Cluster Analysis

The 100 landrace varieties were classified based on agro-morphological markers using UPGMA and SAHN clustering methods (Figure 5). At a similarity coefficient of 22.50, the dendrogram generated 3 clusters: A, B, and C. Characters that were distinct in the formation of the 3 clusters included origin of the varieties and 11 agro-morphological features. The clusters are as follows:

Cluster A- 24 varieties; this group was subdivided into subclusters A 1 and A 2.

Cluster B- 61 varieties; there were 3 sub-clusters, B1, B2, and B3. B1 includes 39 varieties collected from different places: Southeast Vietnam (7 varieties), Songhau, Western Vietnam, and Cambodia (Mibartobo), Camau Peninsula Vietnam (3 varieties), and Kien Giang, Plain of Reeds, Longan, Longxuyen of Vietnam and Thailand (one variety each). These show that, although these varieties are from different places, they are grouped together because of close similarities in quantitative traits. They may also have descended from related parents.

Cluster B2 only had 19 traditional varieties collected from the Mekong Delta and Cluster B3 had 3 varieties from Kien Giang and Cambodia.

Cluster C consisted of 8 varieties (8%): Do Lun and KT 5 were collected from Kien Giang, Mibartobo was collected from Cambodia, and the remaining 5 varieties were collected from Tien Giang and Long An, Vietnam.

Cluster D only had 1 variety, HTA88060, which was collected at fromThailand (deepwater rice). Cluter E's 5 varieties (5%) were collected from Long An; and Cluter F only had 1 Nep ao vang B obtained from central Vietnam (Quang Tri).

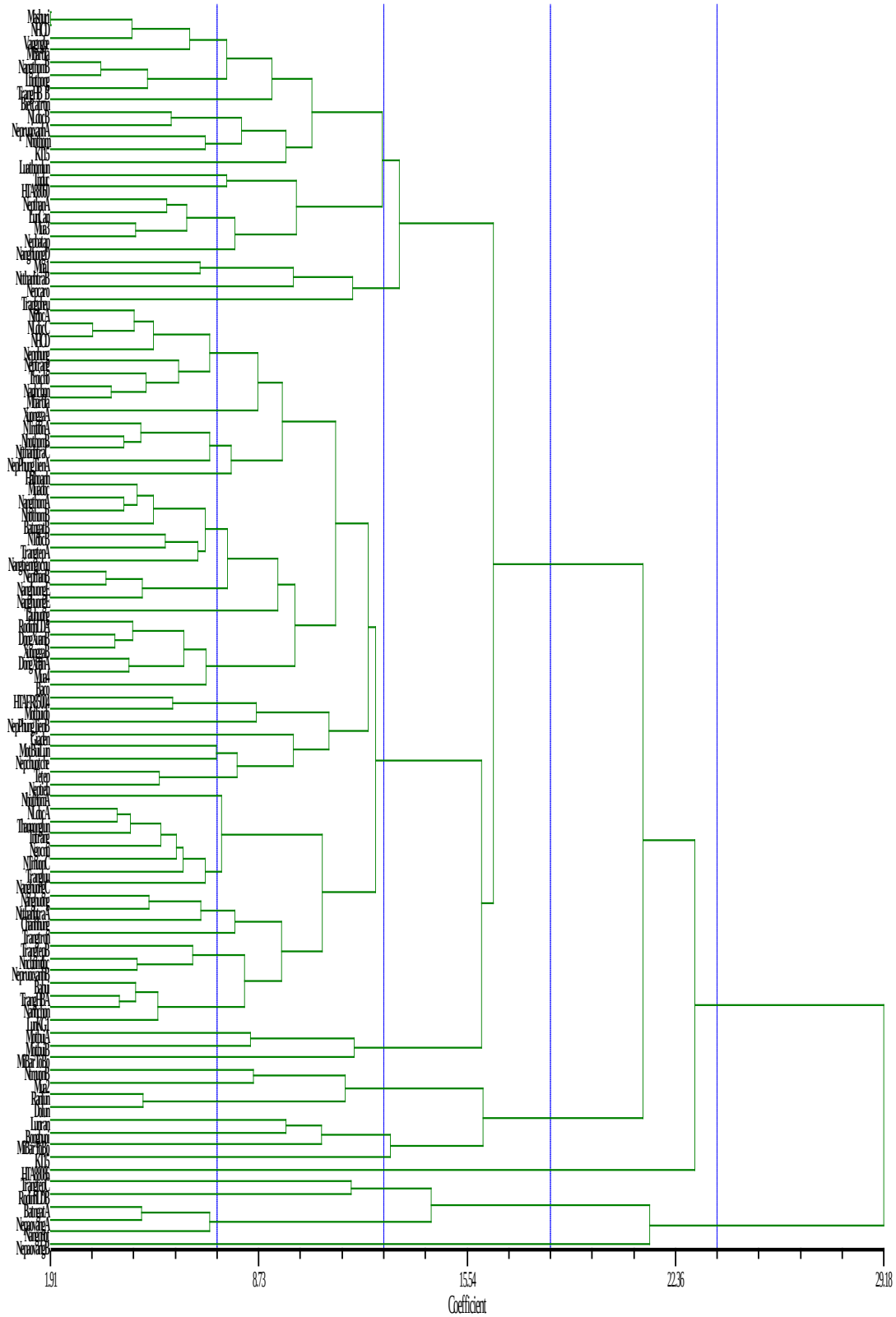

Figure 3. Dendrogram of 100 traditional varieties based on Euclidean distance coefficients estimated from the 11 agro-morphological traits.

Table 5. Correlation coefficients among 11 agro-morphological traits of 100 landrace rice varieties.

	Plant height (cm)	Panicles/hill (no.)	Panicle length (cm)	Fertile grains (%)	Unfertile grains (%)	1000-grain weight (g)	Duration (days)	Biomass (g)	Yield (gram/hill)	*HI*	*Salt stress*
Plant height (cm)	-										
Panicles/hill (no.)	-0.010ns	-									
Panicle length (cm)	0.625**	0.133ns	-								
Fertile grains (%)	-0.005ns	0.081ns	0.006ns	-							
Unfertile grains (%)	0.005ns	-0.081ns	-0.006ns	-1.000**	-						
1000-grain weight (g)	0.262ns	-0.110ns	0.161ns	0.035ns	-0.035ns	-					
Duration (days)	-0.171ns	0.004ns	-0.087ns	0.024ns	-0.024ns	-0.153ns	-				
Biomass (g)	-0.072ns	-0.073ns	-0.028ns	-0.016ns	0.016ns	-0.169ns	-0.040ns	-			
Yield (g/hill)	-0.043ns	-0.105ns	-0.042ns	0.093ns	-0.093ns	0.145ns	-0.009ns	0.034ns	-		
Harvest index (%)	0.085ns	-0.051ns	0.046ns	0.114ns	-0.114ns	0.246ns	-0.004ns	-0.603**	0.688**	-	
Salt stress(days)	-0.037ns	-0.001ns	-0.035ns	-0.156ns	0.156ns	0.232ns	-0.113ns	-0.079ns	-0.155ns	-0.093ns	-

Shannon-Weaver diversity indices

Table 6. Shannon-Weaver diversity indices for quantitative traits of 96 traditional varieties.

Traits	H'
Plant height (cm)	0.92
Panicles/hill (no.)	0.92
Panicle length (cm)	0.90
Fertile grains (%)	0.92
Unfertile grains (%)	0.88
1000-grain weight (g)	0.95
Duration (days)	0.85
Biomass (g)	0.68
Yield (g/hill)	0.82
Harvest index (%)	0.94
Salt stress (days)	0.68
Mean diversity index	0.79

CONCLUSIONS AND RECOMMENDATIONS

Agro-morphological characters and PCR-based markers have provided valuable information about genetic diversity in the rice collection of CLRRI. Results of molecular-based analysis showed that SSR markers were very useful and effective in characterizing and estimating the extent and distribution of genetic variation in the 100 rice landraces considered in the study. Clustering of varieties based on genetic distance (0.60) allowed the grouping of the 100 varieties into 4 clusters.

In general, both morphological and SSR markers were able to group the varieties into ecotypes, rainfed and landraces.

The quantitative agro-morphological characters and molecular markers of 100 accessions were analyzed using clustering, correlation coefficient, principal component analysis, and ANOVA. Diversity of the collection was analyzed using the Shannon-Weaver diversity index. The objective of the study was to determine the extent of diversity using agro-morphological and molecular markers (SSRs).

Using quantitative agro-morphological characters, ANOVA showed highly significant differences among the traits of the 100 rice landraces, except panicles per plant and yield. Correlation coefficients showed that all the traits were significantly correlated with each other, except yield, which was only slightly correlated with other traits. The diversity indices for quantitative descriptors were high, ranging from 0.68 to 0.95. Mean diversity index for all traits among the 100 traditional varieties was high (H' = 0.88). Cluster analysis using UPGMA grouped the 100 landraces into clusters A, B, C, D, E and F at a similarity coefficient of 15.45. The 6 clusters were distinct in terms of number of filled grains, panicle length, panicles per plant, harvest index, yield, and biomass. Varieties collected from the same longitude and latitude were grouped together in the same cluster. Almost all varieties were collected from Mekong Province.

On the basis of these results, the following recommendations are presented:

1. Diversity analysis based on agro-morphological traits of rice landraces should be continued to further confirm relationships among them.

2. Extensive molecular marker analysis may be conducted by considering more primers for relevant application and efficient attainment of breeding objectives.

3. Analysis of the rest of the accessions in the CLRRI genebank may be continued to identify novel resistance genes that would be used in developing salt-tolerant rice varieties.

ACKNOWLEDGEMENTS

This paper presents findings from the 'Climate Change Affecting Land Use in the Mekong Delta: Adaptation of Rice-based Cropping Systems (CLUES)"Project. We thank ACIAR for supporting this project and we also acknowledge the support of CLRRI, IRRI, Can Tho

University, IAS, and the gene bank of the Plant Breeding and Genetic Division.

REFERENCES

Bar-Hen A, Charcosset A, Bourgoin M and Cuiard J (1995). Relationships between genetic markers and morphological traits in a maize inbred line collection. *Euphytica* 84: 145–154.

Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet* 32: 314–331.

Bernardo R (1993). Estimation of coefficient of coancestry using molecular markers in maize. *Theor. Appl. Genet.* 85: 1055–1062.

Gottlieb D (1984). Genetics and morphological evolution of plants. *Am. Nat.* 123: 681–709.

Hamrick JL and Godt MJW (1997). Allozyme diversity in cultivated crops. *Crop Sci.* 37: 2630.

Lang NT (2002). Protocol for basics of biotechnology. Agricultural Publishing House, Ho Chi Minh, Vietnam.

Lang (NT), Pham Thi Be Tu, Nguyen Chi Thanh, Bui Chi Buu and Ismail A (2009). Genetic diversity of salt-tolerant rice landraces in Vietnam. *J. Plant Breed. Crop Sci.* 1(5): 230-243.

Lang NT, Buu CB (2007). Rice breeding and inheritance of herbicide resistance in Clearfield rice (*Oryza sativa*). Omonrice 15: 36-45.

McCouch SR (1988). Molecular mapping of rice chromosomes. *Theor. Appl. Genet.* 76: 815-829.

Ni J, Colowit PM and MacKill DJ (2002). Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Sci.* 42: 601-607.

Nei M and Wen-Hsiung LI (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci.* USA76 (10): 5269-5273.

Nei M (1973). Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci.* USA. 70: 395-401.

Rohlf FJ (1990). NTSYS-pc. Numerical taxonomy and multivariate analysis system. Applied Biostatistics Inc., New York. 175 p.

Newbury HJ and Ford Lloyd BV (1993). The use of RAPD in accessing variation in plants. *Plant Growth Reg.* 12: 45-51.

SAS Institute (1999). SAS/STAT: user's guide: version 8.SAS Institute, Cary.

Sambrook J, Fritsch EF and Maniatis T (1989). Molecular cloning: a laboratory manual. Vol. I. 2nd ed. Cold Spring Harbor Laboratory Press.

Sneath PA and Sokal RR (1973). Numerical taxonomy. W.H. Freeman Co, San Francisco, USA.

Smith OS and Smith JSC (1992). Measurement of genetic diversity among maize hybrids: a comparison of isozyme, RFLP, pedigree, and heterosis data. *Maydica* 37: 53–60.

Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T and McCouch SR (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 100: 697-712.

Tran D (2000). *Oryza sativa*. Food and Agriculture and Organization of the United Nations, Rome, Italy. 17 p.

Thormann EC, Ferreira ME, Camargo LEA, Tivang JG and Osborn TC (1994). Comparison of RFLP and RAPD markers to estimate genetic relationships within and among cruciferous species. *Theor. Appl. Genet.* 88: 976-980.

Van Hintum THJL and Haalman D (1994). Pedigree analysis for composing a core collection of modern cultivars, with examples from barley (*Hordeum vulgare*). *Theor. Appl. Genet.* 88: 70–74.

Yoshida S, Forno DA, Cork JH, Gomez KA (1976). Laboratory manual for physiological studies of rice (3rd Ed.). International Rice Research Institute. Los Banos, Philippines. 83 p.