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Genetic diversity of rice germplasm used in Taiwan breeding programs

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ABSTRACT. Rice is one of the most important cereal crops in the world and is the major crop in Taiwan. Assessment of genetic diversity of rice germplasm is imperative for conservation and breeding. We used 114 simple sequence repeat (SSR) and 5 sequence tagged site (STS) markers with 80 common rice varieties, including 52 *japonica* and 28 *indica* varieties used in Taiwan breeding programs, and detected 395 alleles. The mean number of alleles per marker was 3.5, range 2 to 7. The mean polymorphism information content (PIC) for each marker was 0.43 (range 0.04-0.76). The *japonica* and *indica* varieties were obviously separated into clusters by the distance-based unweighted pair-group method with averages (UPGMA) and principal coordinate analysis (PCoA). Taiwan and Japanese *japonica* varieties aggregated in the same clusters because of their highly similar genetic background. The genetic diversity was greater for *indica* than *japonica* varieties. The genetic diversity of Taiwan cultivars was relatively narrow, specifically in *japonica* varieties, and introduced exotic elite cultivars exhibited enriched alleles. The differentiation of domestic and introduced *indica* cultivars was moderate (F_{ST} =0.25), which was the same revealed *japonica* cultivars (F_{ST} =0.15). However, there was no significant differentiation between Taiwan and Japanese *japonica* cultivars as supported by the small F_{ST} (0.05); thus, introducing exotic germplasm other than from Japan should enlarge the gene pool of Taiwan cultivars. This DNA polymorphism analysis revealed genomic relationships in Taiwan rice germplasm, and the database on 'The Resource of Rice Genetic Markers in Taiwan' is useful for cultivar identification, local germplasm conservation and breeding programs.

Keywords: Genetic diversity; Rice (*Oryza sativa*); SSR markers; Taiwan rice cultivar.

INTRODUCTION

Rice is one of the most important cereal crops in the world and provides more than 50% of the calories consumed by humans in Asia (Khush, 1997). The two cultivated rice species are *Oryza sativa,* widely grown in Asia and other countries, and *O. glaberrima*, grown in Africa only. *O. sativa* evolved from perennial or annual types of *O. rufipogon* and diversified into two subspecies, *indica* and *japonica* (Oka, 1974; Chang, 1985). Ancient *indica* and *japonica* diversified at estimated 200,000~440,000 and 86,000~200,000 years ago according to the nuclear genome and chloroplast DNA sequence, respectively (Ma and Bennetzen, 2004; Vitte et al., 2004). Two possible centers of domestication are proposed: *japonica* arising in the Yangtze and Yellow River Basin of China, and *indica* arising in South Asia (Khush, 1997). On the basis of the distinct morphologic features of *indica* and *japonica* rice, they were recorded as Hsien and Keng, respectively, during the Han dynasty in China (Chou, 1948). Later, five separate groups were classified, and the evolutionary relationship was found closely between the *indica* and *aus* groups, and among tropical *japonica*, temperate *japonica*, and *aromatic* groups by analyses of genetic diversity and population structure (Garris et al., 2005).

Many rice genotypes have evolved to adapt to various environments, including irrigated, rain-fed lowland, and upland ecosystems between 55 N° and 36 S° latitude (Khush, 1997). More than 780,000 varieties have been collected worldwide; 109,136 varieties are deposited in the International Rice Genebank of the International Rice Research Institute (IRRI), and more than 80,000 and 70,000 varieties have been collected in India and China (FAO, 2009). The genetic diversity of rice germplasm was assessed by use of molecular markers, such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), and Simple Se-

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quence Repeats (SSRs). SSRs have been frequently used in genetic and breeding research because of relatively high allelic polymorphism and easy genotyping by PCR. Moreover, approximately 20,000 SSR markers were mined from the genome sequence of *japonica* cv. Nipponbare and are publicly accessible (IRGSP, 2005). Thus, SSR markers have been extensively used in evaluating the genetic diversity of wild relatives, landraces, and cultivars of rice (Ram et al., 2007; Pusadee et al., 2009; Thomson et al., 2007, 2009). For example, SSRs were used to cluster rice landraces collected from Yunnan into 7 subgroups corresponding to subspecies, soil-watery species, and seasonal ecotype (Zhang et al., 2007). As well, the genetic diversity of rice was not decreased when comparing the major rice varieties planted in the 1950s and the past decade in China with use of SSRs (Yuan et al., 2007). SSRs were used in identifying varieties and authenticating polished rice sold in markets in Taiwan (Chuang et al., 2011).

During domestication, some key agronomy traits, such as grain shattering, grain dormancy, and grain size, were strongly selected, which led to greatly diminished genetic diversity in rice. The rice genome encountered a severe early domestication bottleneck; thus, landraces represent only small proportion of the genetic variation of wild rice (Kovach and McCouch, 2008). Moreover, modern breeding programs continuously select desirable characters under highly controlled conditions to achieve an ideotype, which exacerbates the reduction in gene pool of cultivars (McCouch, 2004). The genetic diversity of *japonica* and *indica* cultivars is only 10% and 20% of wild rice; *japonica* encountered greater bottleneck stringency than did *indica* (Caicedo et al., 2007). In Taiwan, the genetic diversity of *japonica* varieties was relative narrow, which was revealed by two varieties had great genetic contribution to the varieties released between 1940 and 1987 and by the field uniformity of *japonica* rice (Lin, 1991a, b).

To breed new varieties for facing the effects of global climate change, the gene pool of cultivars must be broadened by introducing wild species, landraces, and exotic germplasm into breeding programs. Wild species, resistant to biotic and abiotic stresses, are an important genetic resource (Khush, 1997). However, the incompatibility of wild species with cultivars delimits the introgression of wild species' genes to cultivars (Brar and Khush, 1997). Landraces whose seeds are maintained by farmers still contain useful genes (Jackson, 1997; Thomson et al., 2007; Pusadee et al., 2009). Many genes conferring resistance to abiotic and biotic stresses, such as salinity, rice stripe virus, and rice blast, are preserved and are used in modern breeding programs (Shi et al., 2010). Nevertheless, inferior genes might be introgressed along with favorable genes because of linkage drag, which impedes breeding efficiency to obtain elite varieties. To avoid the shortage of introgressing genes from wild rice and landraces, the introduction of exotic elite cultivars is common to increase the gene pool of domestic cultivars, which has been routinely used in rice breeding programs in Taiwan.

By pedigree analysis, 99 varieties released before 1988 were bred by using germplam introduced largely from Japan, and 10 major parental varieties, except one Taiwan landrace Oloan-chu, were from Japan (Lin, 1991a). Improving grain quality has been a high priority; therefore, elite Japanese cultivars with good grain appearance and eating quality have been used in modern rice-breeding programs. Because 85% of Taiwan varieties descended from Japanese varieties, the genetic diversity of modern cultivars was expected to be very narrow (Wu and Lin, 2008). Nevertheless, germplasm introduced from IRRI the Philippines and other countries has been incorporated into rice breeding programs to increase the gene pool of cultivars and to breed new varieties with resistance to biotic and abiotic stresses later on.

In this study, we employed molecular markers rather than pedigree analysis to evaluate the genetic diversity of modern elite cultivars and common germplasm used in rice breeding programs in Taiwan. We used 80 varieties genotyped with 119 PCR-based markers to reveal the genetic relationship based on phylogenetic tree analysis, genetic diversity, and principal coordinate analysis (PCoA). This study provides substantial information to maintain and use rice genetic resources in breeding and in researches of genetic diversity and linkage analysis.

MATERIALS AND METHODS

Plant materials

We used 80 rice varieties (*Oryza sativa*), encompassing modern elite cultivars, and domestic and imported germplasm in Taiwan, to evaluate genetic diversity: 52 *japonica* varieties, including 24 from Taiwan and 28 from abroad; and 28 *indica* varieties, including 10 from Taiwan and 18 from abroad (Table 1). To achieve a breeding goal of grain quality, most of the varieties selected have good taste or aroma; examples are Taikeng 2, Taikeng 8, Kaohsiung 139, Kaohsiung 145, Taitung 30, and Tainung 71. The other Taiwan varieties have resistance to brown plant hopper and rice blast (Table 1). The introduction of exotic germplasm and elite cultivars has been routine work by introgressing useful genes and enlarging genetic diversity in rice breeding recently. To improve grain quality, several varieties were introduced from Japan, including the leading varieties, Koshihikari, and Kinuhikari. To improve insect and disease resistance, several varieties were introduced from IRRI. Some exotic varieties possess abiotic resistant genes or high yield (Table 1). In addition, we included *japonica* cv. Nipponbare and *indica* 93-11, whose genomes have been sequenced for basic scientific research (IRGSP, 2005; Yu et al., 2002).

Assessment of molecular marker genotypes

The extraction of rice nuclear genomic DNA was as described (Lin et al., 2011). We used 119 molecular markers, including 114 SSRs with the prefix RM and 5 STSs with the prefixes C, E, S, and STS to evaluate genetic diversity.

Table 1. The origins and major important traits of 80 rice varieties.

Variety[a]	Subspecies	Origin	Major important trait
Chianung 242 (CN 242)	*japonica*	Taiwan	Rice blast resistance
Hsinchu 64 (HC 64)	*japonica*	Taiwan	Brown planthopper resistance
Hualien 19 (HL 19)	*japonica*	Taiwan	Rice blast resistance
Kaohsiung 139 (KH 139)	*japonica*	Taiwan	Rice blast resistance
Kaohsiung 143 (KH 143)	*japonica*	Taiwan	Good taste quality
Kwangfu 1 (KF 1)	*japonica*	Taiwan	Rice blast resistance
Taichung 65 (TC 65)	*japonica*	Taiwan	Photoperiod insensitivity
Taikeng 2 (TK 2)	*japonica*	Taiwan	Brown planthopper resistance
Taikeng 8 (TK 8)	*japonica*	Taiwan	Good taste quality
Taikeng 14 (TK 14)	*japonica*	Taiwan	High yield
Taikeng 16 (TK 16)	*japonica*	Taiwan	Brown planthopper resistance
Taikeng 17 (TK 17)	*japonica*	Taiwan	Good taste quality
Taikeng Glutinous1 (TKG 1)	*japonica*	Taiwan	Good taste quality
Tainan 5 (TN 5)	*japonica*	Taiwan	Good taste quality
Tainung 67 (TNG 67)	*japonica*	Taiwan	High yield
Tainung 69 (TNG 69)	*japonica*	Taiwan	Rice blast resistance
Tainung 70 (TNG 70)	*japonica*	Taiwan	Rice blast resistance
Tainung 71 (TNG 71)	*japonica*	Taiwan	Taro aroma
Tainung 72 (TNG 72)	*japonica*	Taiwan	Good taste quality
Taitung 29 (TT 29)	*japonica*	Taiwan	Rice blast resistance
Taitung 30 (TT 30)	*japonica*	Taiwan	Brown planthopper resistance
Taoyuan 1 (TY 1)	*japonica*	Taiwan	Good taste quality
Taoyuan Glutinous 2 (TYG 2)	*japonica*	Taiwan	Cold tolerance
Tung Lu 1 (TL 1)	*japonica*	Taiwan	Rice blast resistance
Akitakomachi (ATM)	*japonica*	Japan	Good taste quality
Chiyonisiki (CNK)	*japonica*	Japan	Lodging resistance
Fukunikari (FNR)	*japonica*	Japan	Good taste quality
Hoshiyutaka (HYK)	*japonica*	Japan	High amylose content
Kinuhikari (KHR)	*japonica*	Japan	Good rice appearance
Koshihikari (KHR)	*japonica*	Japan	Good taste quality
Nipponbare (NB)	*japonica*	Japan	Lodging resistance
Todorokiwase (TRW)	*japonica*	Japan	Cold tolerance
Toyonishiki (TNK)	*japonica*	Japan	Early maturity
Tsukinohikari (TNK)	*japonica*	Japan	Good taste quality
Milagrosa	*japonica*	Philippines	Aroma
Milfor	*japonica*	Philippines	High yield
Della	*japonica*	U.S.A	Aroma
L202	*japonica*	U.S.A	Lodging resistance
M103	*japonica*	U.S.A	Salt tolerance
M202	*japonica*	U.S.A	Early maturity
M401	*japonica*	U.S.A	High yield

Table 1. (Continued)

Variety[a]	Subspecies	Origin	Major important trait
Nortai	*japonica*	U.S.A	Low protein content
S301	*japonica*	U.S.A	High protein content
Giza 4120-205	*japonica*	Egypt	Rice blast resistance
Gz 5379	*japonica*	Egypt	Good taste quality
MGG	*japonica*	Haiti	
Start Bomet	*japonica*	Haiti	
Basmati 370	*japonica*	India	Aroma
Basmati T3	*japonica*	India	Aroma
Parkistam Basmati	*japonica*	Pakistan	Aroma
Khao-kueng	*japonica*	Thailand	
Ku79-2	*japonica*		
Chianung Sen 11 (CNS 11)	*indica*	Taiwan	Rice blast resistance
Kaohsiung Sen 7 (KHS 7)	*indica*	Taiwan	Sheath blight resistance
Tai Sen 2 (TS 2)	*indica*	Taiwan	Rice blast resistance
Tai Sen Glutinous 2 (TSG 2)	*indica*	Taiwan	Cold tolerance
Taichung Sen 3 (TCS 3)	*indica*	Taiwan	Rice blast resistance
Taichung Sen 10 (TCS 10)	*indica*	Taiwan	Sheath rot resistance
Taichung Sen 17 (TCS 17)	*indica*	Taiwan	Lodging resistance
Tainung Sen 14 (TNS 14)	*indica*	Taiwan	Rice blast resistance
Tainung Sen 19 (TNS 19)	*indica*	Taiwan	Rice blast resistance
Tainung Sen 20 (TNS 20)	*indica*	Taiwan	Rice blast resistance
Khosakau	*indica*	Japan	
PsBRc4	*indica*	Philippines	Green rice leafhopper resistance
PsBRc10	*indica*	Philippines	Green rice leafhopper resistance
PsBRc18	*indica*	Philippines	Green rice leafhopper resistance
PsBRc20	*indica*	Philippines	High yield
IR29	*indica*	IRRI	Bacterial leaf blight resistance
IR30	*indica*	IRRI	Bacterial leaf blight resistance
IR36	*indica*	IRRI	Brown planthopper resistance
IR64	*indica*	IRRI	Brown planthopper resistance
IR72	*indica*	IRRI	Brown planthopper resistance
IR1545-339	*indica*	IRRI	Bacterial leaf blight resistance
IR1552	*indica*	IRRI	Manganese resistance
IR2105	*indica*	IRRI	High yield
93-11	*indica*	China	Brown planthopper resistance
Huakeng 74 (HK 74)	*indica*	China	Bacterial leaf blight resistance
FKR19	*indica*	Burkina Faso	High yield
ASD16	*indica*	India	Brown planthopper resistance
Pokhareli	*indica*	Nepal	Low amylose content

[a]The abbreviation of each variety is indicated in parentheses.

All primer sequences and other information for SSRs can be accessed from Gramene (http://www.gramene.org). Four of the 5 STSs with prefixes C, E, and S were obtained from the Rice Genome Research Project (http://rgp.dna.affrc.go.jp, Inoue et al., 1994). The last STS marker, STS208 (F: 5'-CAAAGGTATGATGAGGATAAGG-3', R: 5'-TAGATTCGTCTCGCAGTTTAC-3') was designed as described (Wu et al., 2010). All markers displayed primary polymorphism between *indica* and *japonica* (Wu et al., 2010; Lin et al., 2011). PCR reactions and amplification were as described (Lin et al., 2011). The PCR products underwent electrophoresis on 2.5% of Super Fine Resolution (SFR) agarose (Amresco, Solon, Ohio, USA) by Rapid Agarose Electrophoresis (RAGE, Cascade Biologics, Portland, Oregon, USA) in 1× TAE at 250 V for 20 min. The resolution of the gel system was approximately 5 bp.

Data analysis

Because cultivated rice is inbred, one homozygous allele is revealed by one DNA band after gel electrophoresis, and one marker represents one locus for the 119 markers used in this study. Presence or absence of a specific allele was indicated as 1 or 0, respectively, and the matrix of 1 and 0 data underwent analysis by polymorphic information content (PIC), genetic similarity and *Fst.* The PIC was estimated as follows:

$$\widehat{PIC_l} = 1 - \sum_{u=1}^{k} \tilde{P}_{lu}{}^2 - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\,\tilde{P}_{lu}^2\,\tilde{P}_{lv}^2$$

where l is locus; k is the number of alleles of locus l; P_{lu} is the frequency of allele u; P_{lv} is the frequency of allele v (Botstein et al., 1980). The PIC value for each marker was used to justify the polymorphic information, and the mean PIC value for a group of individuals implies the genetic diversity within the group. Both the PIC for each marker and mean PIC for each group were determined by use of PowerMarker (Liu and Muse, 2005). Genetic similarity and dissimilarity were evaluated by modified Rogers' distance (Goodman and Stuber, 1983). We used sequential agglomerative hierarchic non-overlapping (SAHN) clustering and then unweighted pair-group method with arithmetic mean (UPGMA) dendrogram to infer genetic relationships and construct a phylogenetic tree. Decnter and Eigene modules were used to transform and calculate two dimension of principle coordinate analysis (PCoA, Gower, 1966). UPGMA and PCoA analysis involved use of NTSYS-pc v.2.21 (Rohlf, 2005).

RESULTS

Polymorphic levels of molecular markers

Using 114 SSR and 5 STS markers to analyze the genetic diversity of 80 modern cultivars and germplasm used in Taiwan rice-breeding programs, we found 395 alleles with a mean of 3.5 alleles (Table 2). The allele number per locus ranged from 2 to 7, and most markers, 43 (36.13%), revealed 3 alleles. The PIC value for each marker was used to assess the polymorphic level. The mean PIC value for markers was 0.43 with the range of 0.04 (RM6407) to 0.76 (RM481). According to the definition of informative level (Botstein et al. 1980), 35 (29.41%) and 76 (63.87%) markers were highly and reasonably informative, respectively; only 8 (6.72%) were slightly informative. Overall, 119 markers provided sufficient informative polymorphism to evaluate genetic diversity of these 80 varieties.

Analysis of genetic distance

The Dice coefficient was used to assess genetic similarity between two varieties (Dice, 1945), which was consequently subjected to clustering analysis based on UPGMA to construct a dendrogram (Figure 1). The *japonica* and *indica* varieties were distinguishably separated into two major different groups with a similarity coefficient of 0.23. Subgroups of *japonica* and *indica* varieties further identified by high genetic similarity were classified into 3 clusters (clusters J1 to J3) and 2 clusters (clusters I1 and I2) with a similarity coefficient of 0.78 and 0.63, respectively. However, 12 *japonica* varieties and 4 *indica* varieties originated from several countries share great genetic dissimilarity, which were miscellaneous and not easily classified to suitable clusters. According to the Dice coefficient, most *japonica* varieties, clusters J1 to J3, had greater genetic similarity than did *indica* clusters (Figure 1).

All Taiwan and Japanese *japonica* varieties were grouped into 3 clusters, clusters J1 to J3, and each cluster contained varieties from these two countries. Koshihikari, a leading Japanese variety well known to possess good grain quality, is closely related to Kaohsiung 139 from Taiwan, also with good grain quality. Several varieties released recently, Taikeng 8, Taikeng 16, Taitung 30, Tainung 69, Tainung 72, and Tainan 5, showed high genetic similarity and were grouped together in cluster J2 with Tainung 67, an elite germplasm that has undergone modern rice breeding (Figure 1). Tung Lu 1, an old upland variety known as tropical *japonica*, showed little genetic similarity to the other temperate *japonica* varieties by UPGMA analysis and was not grouped into the other three major *japonica* clusters. Varieties introduced from the United States, such as M103, M202, M401, and S301, share a genetic background with a similarity coefficient of 0.91 and were clustered together in cluster J1 (Figure 1). Most varieties introduced from the other countries exhibited larger genetic distance than those introduced from Japan and the United States, which were classified as in *japonica* group but not easily separated as distinct clusters (Figure 1). Nevertheless, three aromatic varieties, Pakistan Basmati, Basmati T3, and Basmati 370, were in the same cluster, which was distinguished from the other *japonica* varieties.

All 28 *indica* varieties formed the *indica* group with 2 distinct clusters, clusters I1 and I2 (Figure 1). All Taiwan *indica* varieties shared high genetic similarity with the oth-

Table 2. Polymorphic levels of 119 markers for genotyping 80 rice varieties.

Chr.	Marker[a]	PIC	Chr.	Marker	PIC	Chr.	Marker	PIC	Chr.	Marker	PIC
1	RM3252 (6)	0.42	3	RM251 (5)	0.49	6	RM225 (3)	0.49	8	RM447 (3)	0.46
1	RM001 (4)	0.45	3	RM282 (2)	0.36	6	RM6734 (4)	0.49	9	S12569 (3)	0.59
1	RM243 (5)	0.34	3	RM016 (2)	0.36	6	RM253 (3)	0.52	9	RM3912 (2)	0.37
1	RM580 (6)	0.51	3	RM5626 (2)	0.15	6	RM276 (7)	0.67	9	RM257 (5)	0.60
1	RM009 (6)	0.50	3	RM135 (5)	0.54	6	RM527 (3)	0.41	9	RM278 (3)	0.39
1	RM246 (3)	0.43	3	RM168 (3)	0.31	6	RM3330 (3)	0.39	9	RM201 (4)	0.43
1	RM3411 (3)	0.39	3	RM186 (3)	0.28	6	RM541 (3)	0.39	9	RM3744 (2)	0.36
1	RM302 (4)	0.38	3	RM143 (5)	0.59	6	RM003 (4)	0.48	9	RM205 (3)	0.37
1	RM212 (2)	0.37	3	RM085 (3)	0.35	6	RM162 (4)	0.54	10	RM222 (3)	0.37
1	RM1387 (5)	0.59	4	RM551 (4)	0.35	6	RM528 (5)	0.53	10	C51124 (2)	0.37
1	RM104 (2)	0.37	4	RM518 (3)	0.55	6	RM030 (2)	0.34	10	RM3311 (2)	0.23
1	RM6407 (2)	0.04	4	RM5687 (3)	0.45	6	RM3138 (4)	0.47	10	RM1375 (7)	0.72
2	RM154 (4)	0.39	4	RM1359 (5)	0.50	6	RM340 (5)	0.62	10	RM258 (4)	0.45
2	RM211 (4)	0.35	4	RM252 (5)	0.47	7	RM481 (7)	0.76	10	RM6673 (5)	0.52
2	RM5780 (4)	0.52	4	RM470 (3)	0.39	7	RM125 (5)	0.39	10	RM333 (6)	0.72
2	RM145 (4)	0.44	4	RM6089 (2)	0.37	7	RM214 (3)	0.49	10	RM496 (4)	0.42
2	RM5356 (3)	0.42	4	RM567 (3)	0.18	7	RM6767 (3)	0.52	11	RM286 (3)	0.52
2	RM324 (4)	0.49	5	RM1024 (3)	0.38	7	RM011 (3)	0.42	11	RM167 (2)	0.36
2	RM341 (4)	0.40	5	RM267 (2)	0.37	7	RM010 (3)	0.51	11	RM536 (3)	0.50
2	RM475 (3)	0.57	5	E3528 (3)	0.37	7	RM3826 (3)	0.45	11	RM287 (2)	0.30
2	RM263 (3)	0.40	5	RM289 (3)	0.36	7	RM234 (4)	0.41	11	RM021 (4)	0.59
2	RM1367 (4)	0.45	5	RM3838 (3)	0.15	7	RM420 (2)	0.38	11	RM206 (7)	0.61
2	RM240 (3)	0.38	5	RM430 (4)	0.51	8	RM152 (4)	0.54	11	RM224 (5)	0.57
2	RM250 (2)	0.37	5	RM161 (2)	0.35	8	RM1376 (2)	0.26	12	RM019 (3)	0.42
2	RM207 (7)	0.64	5	STS208 (2)	0.36	8	RM025 (2)	0.15	12	RM101 (2)	0.37
3	RM022 (2)	0.36	5	RM480 (3)	0.47	8	RM072 (4)	0.61	12	RM247 (4)	0.47
3	RM6038 (3)	0.38	5	E1113 (2)	0.34	8	RM331 (2)	0.36	12	RM270 (2)	0.38
3	RM545 (5)	0.52	6	RM508 (3)	0.52	8	RM515 (3)	0.45	12	RM235 (5)	0.51
3	RM517 (2)	0.17	6	RM190 (3)	0.53	8	RM223 (3)	0.38	12	RM017 (3)	0.34
3	RM218 (5)	0.63	6	RM584 (3)	0.43	8	RM256 (2)	0.11			

[a]The number in parentheses indicates the number of alleles of markers detected in the 80 varieties.

ers introduced from abroad, which led to their grouping in one cluster by UPGMA analysis. Taichung Sen 3 and Taichung Sen 17, with a similarity coefficient of 0.86, were more related to each other than to the others in cluster I1. All varieties introduced through IRRI and the Philippines, except for IR1545-339 with rice blast resistance, were in the same cluster, which revealed high genetic similarity with a similarity coefficient of 0.73. Two varieties, 93-11 from China and Khosakau from Japan, showed great dissimilarity to the other *indica* varieties and clustered each other (Figure 1). One unexpected clustering result was Huakeng 74, a Chinese *japonica* variety, related to Taiwan *indica* varieties.

The genetic relationship among these 80 varieties was assessed by PCoA based on the modified Roger's distance (Goodman and Stuber, 1983). The first and second

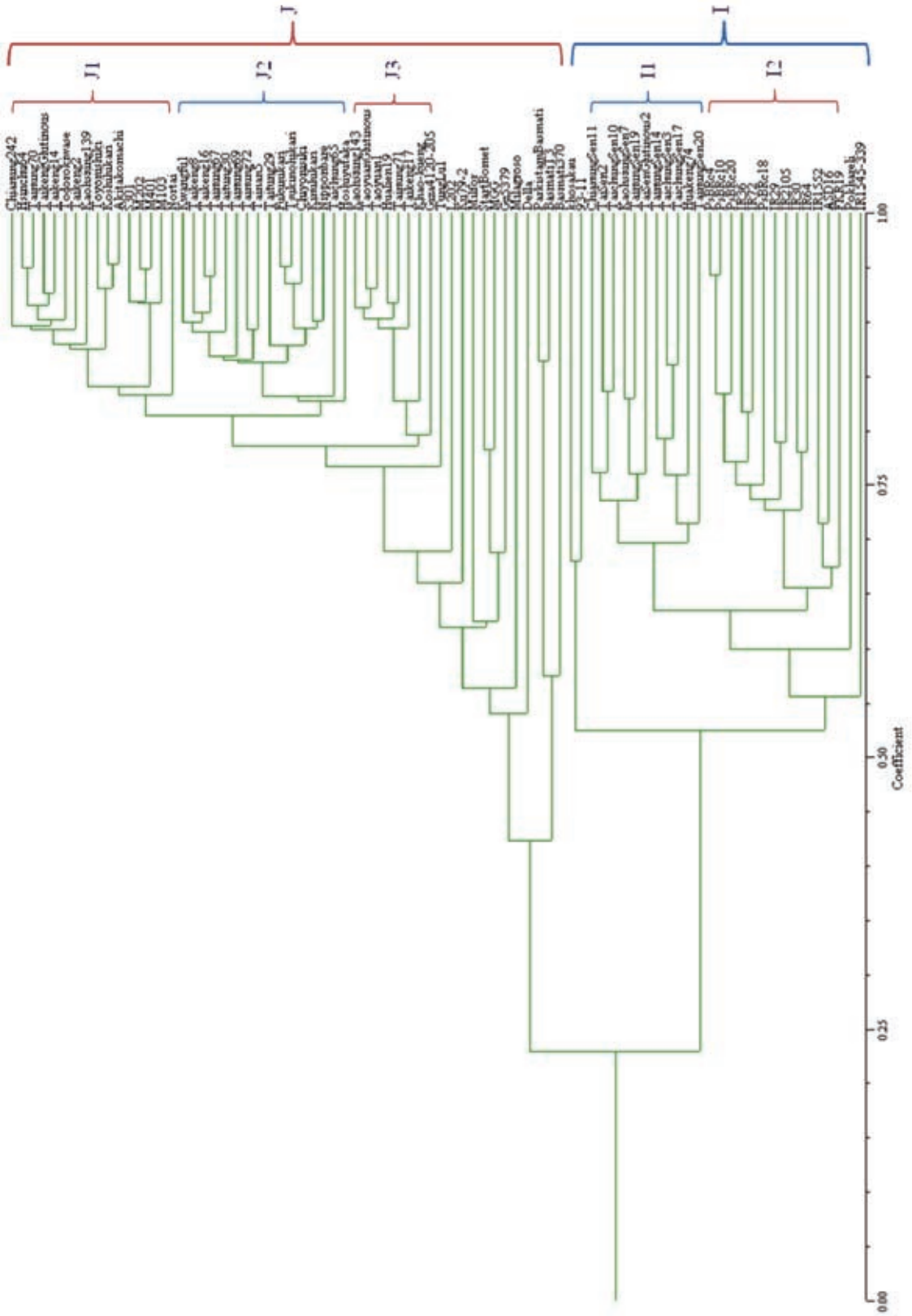

Figure 1. Dendrogram of 80 rice varieties in Taiwan based on unweighted pair-group method with averages. Two major groups corresponding to *japonica* and *indica* rice were indicated as J and I, respectively. The three distinct clusters of *japonica* group were assigned as cluster J1, J2, and J3; the two distinct clusters of *indica* group were assigned as cluster I1 and I2. The X axis indicates the Dice similarity coefficient.

Table 3. The clusters formed by principal coordinate analysis.

Cluster	Variety
A	Akitakomachi, Chianung 242, Chiyonisiki, Fukunikari, Hoshiyutaka, Hsinchu 64, Kaohsiung 139, Khosakau, Kinuhikari, Koshihikari, Kwangfu 1, M103, M202, M401, Nipponbare, Nortai, S301, Taichung 65, Taikeng 14, Taikeng 16, Taikeng 2, Taikeng 8, Taikeng Glutinous 1, Tainan 5, Tainung 67, Tainung 69, Tainung 70, Tainung 72, Taitung 29, Taitung 30, Todorokiwase, Toyonishiki, Tsukinohikari, Tung Lu 1
B	Giza 4120-205, Hualien 19, Kaohsiung 143, Khao-kueng, Taikeng 17, Tainung 71, Taoyuan 1, Taoyuan Glutinous 2
C	Della, Gz5379, Ku79-2, L202, MGG, Milagroso, Milfor, Start Bomet
D	Basamati 370, Basmati T3, Pakistam Basmati
E	Chianung Sen 11, Huakeng 74, Tai Sen 2, Tai Sen Glutinous 2, Taichung Sen 10, Taichung Sen 17, Taichung Sen 3, Tainung Sen 14, Tainung Sen 19, Tainung Sen 20
F	93-11, ASD16, FKR19, IR1545-339, IR1552, IR2105, IR29, IR30, IR36, IR64, IR72, Khosakau, Pokhareli, PsBRc10, PsBRc18, PsBRc20, PsBRc4

dimensions of PCoA explained 18.03% and 1.87% of the genetic diversity, respectively. In total, 52 *japonica* varieties showed close distribution and were classified into four clusters (Table 3; Figure 2). All *japonica* varieties introduced from Japan were aggregated in cluster A, and most Taiwan *japonica* varieties were also in cluster A; 6 varieties were separate from the others and formed another cluster, cluster B. Four American varieties shared genetic similarity and were in cluster A. These 2 clusters, clusters A and B, were corresponded to the 3 clusters, clusters J1, J2, and J3, of UPGMA dendrogram. The other *japonica* varieties introduced from other countries were scattered on the PCoA plot, which indicated relative genetic diversification among them. Nevertheless, the three aromatic varieties still were located nearby (Table 3; Figure 2). Both the PCoA plot and UPGMA dendrogram revealed approximately the same genetic relationship among these *japonica* varieties.

The *indica* varieties were grouped into two clusters by PCoA. All Taiwan *indica* varieties were grouped in one cluster, cluster E, and all varieties introduced from IRRI and the Philippines were grouped in cluster F (Table 3; Figure 2). Two varieties, 93-11 and Khosakau, were separated into a unique cluster by UPGMA with a similarity coefficient of 0.68 and dispersed in cluster F with some genetic distance by PCoA (Table 3; Figure 2).

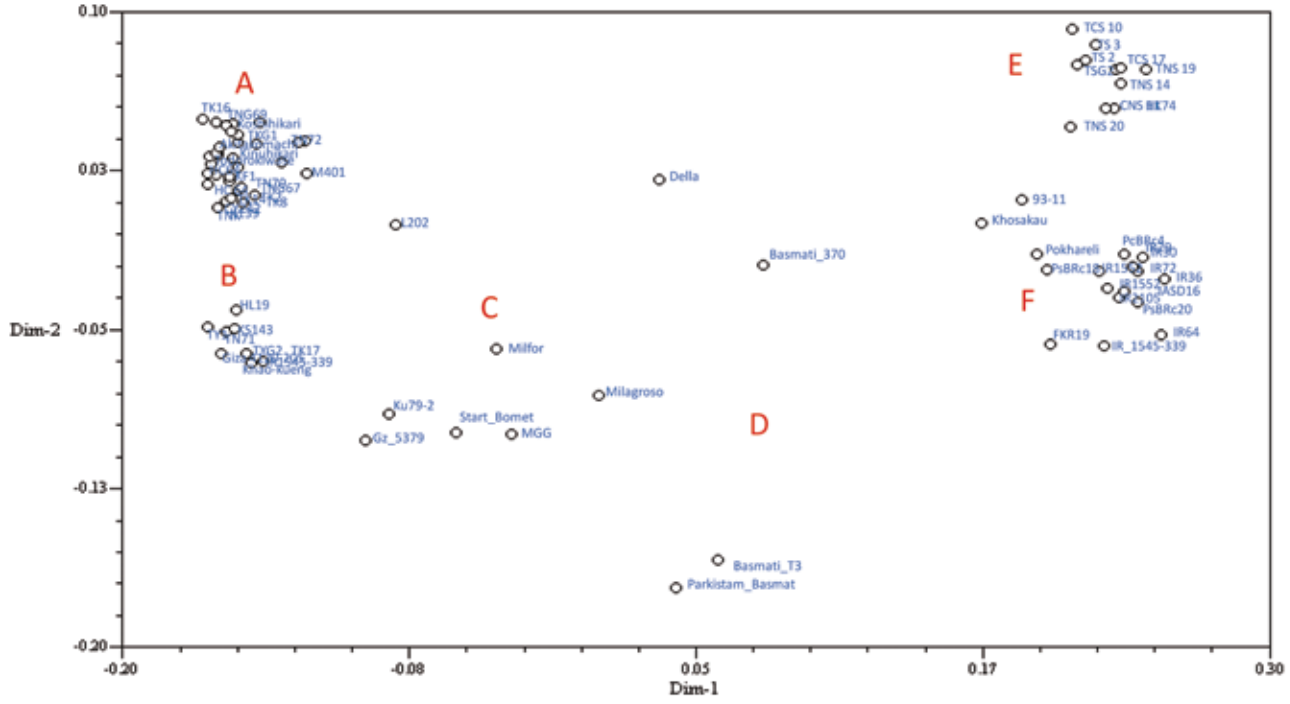

Figure 2. Two-dimension plot of principal coordinate analysis for all 80 rice varieties in Taiwan. The first and second dimensions explained 18.03% and 1.87%, respectively, of the genetic diversity.

Genetic diversity and genetic differentiation within and between subgroups

We divided the germplasm into several subgroups to evaluate genetic diversity by mean allele number per locus, major allele frequency, mean gene diversity, and mean PIC value, and genetic differentiation between two subgroups by F_{ST} (Table 4). The genetic diversity for the *indica* group, including all 28 varieties, was greater than that for the *japonica* group, of 52 varieties, as revealed by smaller major allele frequency and larger mean gene diversity and mean PIC value (Table 4). The same phenomenon was observed in comparing Taiwan *indica* and *japonica* groups, too. Taiwan *indica* varieties exhibited slightly less genetic diversity as compared with introduced *indica* varieties. In contrast, the mean gene diversity and mean PIC value were lower for Taiwan *japonica* varieties than the 28 introduced *japonica* varieties but did not differ from those of the 10 Japanese *japonica* varieties (Table 4). Thus, the genetic diversity of Taiwan and Japanese *japonica* cultivars was narrow.

Overall, the differentiation between the *indica* groups and *japonica* groups was high, with F_{ST} values of 0.58 and 0.50 for all 80 varieties and 34 Taiwan varieties, respectively (Table 4). Genetic differentiation was moderate between Taiwan domestic varieties and introduced varieties for both *indica* and *japonica* groups, with F_{ST} values of 0.25 and 0.15, respectively. However, Taiwan and Japanese *japonica* varieties showed very low genetic differentiation (F_{ST} =0.05, Table 4).

DISCUSSION

Assessment of the polymorphic levels of SSR markers uncovered in this study

SSR markers have been commonly used in evaluating genetic diversity and phylogenetic analysis because of their abundance in genomes and high allelic polymorphism, co-dominance, and easy manipulation by PCR. Gel electrophoresis with agarose, polycrylamide, and capillary gel can be used to observe allelic variation of SSR markers caused by various repeat numbers of one type of SSR or SSR with at least two types of SSRs even with embedded single nucleotide polymorphism (SNP) and/or indels. We used 2.5% SFR agarose, which could resolve the DNA fragments with a difference of 5 bp, and revealed 395 alleles by using 119 markers with 80 rice varieties; the mean allele number per locus was 3.5 (Table 2, 4). The mean allele number we found was lower than that for SSRs labeled with fluorescence and separated by capillary gel electrophoresis, which is expensive (Jain et al., 2004; Thomson et al., 2007). One reason for the difference may be the lower resolution in separating SSR alleles in agarose gel than in capillary gel. As well, we used cultivars rather than landraces and wild relatives because of less allelic variation in cultivars in general (Ram et al., 2007; Thomson et al., 2007; Yuan et al., 2007; Pusadee et al., 2009). However, our result was comparable to the mean of 4.5 alleles per locus found in 35 Asian cultivars and 2.0–5.5 alleles per locus found with different kinds of

Table 4. Genetic diversity and differentiation within and between subgroups of 80 rice varieties.

Subgroups	Sample size	Mean no. alleles/locus	Major allele frequency	Mean gene diversity	Mean PIC value	F*st*
All 80 varieties	80	3.50	0.61	0.50	0.43	0.58
indica	28	2.91	0.74	0.35	0.31	
japonica	52	2.96	0.80	0.29	0.26	
Taiwan varieties	34	2.72	0.70	0.41	0.35	0.50
indica	10	1.89	0.80	0.25	0.21	
japonica	24	1.79	0.89	0.15	0.13	
indica varieties	28	2.91	0.74	0.35	0.31	0.25
Taiwan	10	1.89	0.80	0.25	0.21	
introduced	18	2.91	0.71	0.38	0.34	
japonica varieties	52	2.96	0.80	0.29	0.26	0.15
Taiwan	24	1.79	0.89	0.15	0.13	
introduced	28	2.82	0.75	0.35	0.31	
japonica varieties	34	1.84	0.89	0.15	0.13	0.05
Taiwan	24	1.79	0.89	0.15	0.13	
Japan	10	1.30	0.85	0.17	0.16	

microsatellites (Cho et al., 2000; Pervaiz et al., 2009).

PIC values, which could avoid the bias estimation with major alleles, are also a good indicator of the polymorphic levels of molecular markers. The PIC values were high and varied, with a mean of 0.43 and range 0.04–0.76 (Table 2). The PIC values we estimated were comparable to those from previous studies of SSR markers used with rice cultivars (Lu et al., 2005; Pervaiz et al., 2009) but were lower than those with rice landraces (Thomson et al., 2007; Pervaiz et al., 2010).

Only 8 of 119 markers (6.72%), with PIC values < 0.25, were considered slightly informative; the other 111 markers were reasonably to highly informative (Table 2). The 5 STS markers we used were reasonably and highly informative, with PIC values 0.34 (E1113) to 0.59 (S12569), which could offer allelic variation for analysis of genetic diversity. Nevertheless, informative polymorphism was greater for SSRs than indels in general; for example, 4 SSRs revealed 7 alleles with PIC value up to 0.76 (Table 2). The allelic information for the 119 markers with the 80 varieties and the polymorphic markers between any 2 varieties can be accessed from the website for 'The Resource of Rice Genetic Markers in Taiwan' (http://rice.sinica.edu.tw, Lin et al., 2008). The polymorphic markers we uncovered could be applied to genetic linkage analysis, quantitative trait locus (QTL) mapping, and marker assisted selection (MAS) to improve rice breeding efficiency. Moreover, some variety-specific alleles can be exploited in DNA fingerprinting to variety identification (Chuang et al., 2011).

Clusters of Taiwan rice-breeding germplasm based on genetic distance

We identified two major groups corresponding to ssp. *japonica* and *indica* by both cluster analyses, UPGMA and PCoA, on the basis of genetic distance of the Dice similarity coefficient among the 80 varieties used in rice breeding programs (Figures 1, 2). By the classification of Garris et al. (2005), the subspecies *japonica* could be distinguished as *tropical japonica*, *temperate japonica*, and *aromatic* groups, and ssp. *indica* could be distinguished as *indica* and *aus* groups. Because most popular rice varieties in Taiwan were *temperate japonica*, the common *japonica* germplasm used in breeding belonged to *temperate japonica*, except for Tung Lu 1, which is a domestic *tropical japonica* and showed little similarity to the other *japonica* varieties and had some genetic distances to the other *temperate japonica* by UPGMA but still was grouped in cluster A by PCoA. We included only 3 Basmati varieties belonging to the *aromatic* group; therefore, they could not form a distinct group and also exhibited genetic dissimilarity to the imported *japonica* varieties other than those introduced from Japan (Table 3; Figures 1, 2). The *aus*, closely related to *indica*, was considered to be ssp. *indica* and is habituated along the Himalayan hills. Pokhareli introduced from India might contain the genetic background of *aus*, which displayed large genetic distance to the other *indica* varieties (Table 3; Figures 1, 2). Overall, clustering analyses of these 80 rice germplasm used in Taiwan were consistent with the new classification of sub-groups of Asian rice, *O. sativa*.

According to the pedigree analysis of 99 Taiwan *japonica* varieties released between 1940 and 1987, 85% of the parentages of the varieties were introduced from Japan. Especially, Shinriki and Kameji, the two major parents of Taichung 65 which was the first reported photoperiod-insensitive *temperate japonica* variety, showed great genetic contribution to genomes, 21.3% and 16.7%, respectively (Lin, 1991a). As a consequence, 24 Taiwan and 10 Japanese varieties shared highly similar genetic background, which was supported by both UPGMA and PCoA (Table 3; Figures 1, 2). Since the prime rice-breeding goal of yield shifted to rice grain quality, several elite Japanese cultivars such as Koshihikari and Kinuhikari were introduced and are being extensively used in regular rice-breeding programs. These cultivars, with good grain appearance and eating quality, were grouped in clusters J1 and J2 by UPGMA or cluster A by PCoA (Tables 1, 3; Figures 1, 2).

All Taiwan *indica* varieties share a similar genetic background and formed one cluster and exhibited higher genetic distance than the newly introduced *indica* varieties (Table 3; Figures 1, 2). Many *indica* landraces, brought and cultivated by Chinese people when they migrated from Guangdong and Fujian provinces of mainland China about 300 years ago, might have been incorporated in early modern *indica* rice breeding. The *indica* varieties from the IRRI and the Philippines were closely related because of selection under similar environments for specific breeding aims. Nevertheless, the other *indica* varieties originating from other than the Philippines showed relative genetics distance as well.

Genetic diversity and differentiation among subgroups of germplasm for the 80 varieties

Japonica and *indica* rice are easy to distinguish by obvious distinct morphologic and physiologic characters (Oka and Morishima, 1982). The F_1 seeds of inter-subspecific crosses always exhibit low fertility and hybrid breakdown in successive selfed generations because of the accumulation of genetic differentiation; these two subspecies had undergone divergence and adaptation to various environments and domestication. The genetic divergence could also be detected by DNA sequences of nuclear DNA and chloroplast genes (Vitte et al., 2004; Londo et al., 2006). With our analysis of F_{ST}, an index of divergent level between two subpopulations, the *indica* varieties greatly differentiated from *japonica* varieties. As compared with other studies of worldwide collections (F_{ST} =0.43) and Indonesian germplasm (F_{ST} =0.38) and landraces (F_{ST} =0.59) (Garris et al., 2005; Thomson et al., 2007, 2009), we observed a certain degree of differentiation for all *indica* varieties vs. *japonica* varieties (F_{ST} =0.58) herein (Table 4). Even different germplasm collections might account for the different outcomes; the high F_{ST} value for cultivated

indica and *japonica* varieties might be associated with strongly diversified selection for different breeding goals during breeding processes. Furthermore, this phenomenon was supported by the high differentiation in Taiwan *indica* and *japonica* cultivars (F_{ST} =0.50; Table 4) under intensive selection for specific breeding goals, high yield for *indica* cultivars and grain quality for *japonica* cultivars.

Asian rice, *O. sativa* L., encountered a severe early domestication bottleneck, which led to narrower genetic diversity than its perennial ancestor, *O. rufipogon*, and annual ancestor, *O. nivara* (Kovach and McCouch, 2008). Because of different geographic distribution and selection, ssp. *japonica* rice experienced more severe bottleneck than ssp. *indica* rice did as indicated by bottleneck stringency K values (Zhu et al., 2007). The genetic diversity and gene richness was lower for *japonica* rice than *indica* rice collected from many countries and worldwide in general, as revealed by STS or SSR markers (Yang et al., 1994; Ni et al., 2002; Garris et al., 2005; Caicedo et al., 2007). From the major allele frequency and high mean gene diversity and mean PIC value in *indica* rice from all 28 varieties and 10 Taiwan varieties (Table 4), our results also reflected the evolutionary processes and agreed with previous findings. Furthermore, the genetic diversity was lower for Taiwan cultivars than introduced cultivars, not only *indica* but also *japonica* rice (Table 4), because of limited genetic bases.

In Taiwan, *indica* rice was the major staple food before the 21st century and is commonly used in making rice noodles, rice cake, and other derived products. The breeding goals for *indica* rice differ slightly from those for *japonica* rice: high yield and resistance to biotic and abiotic stresses. Because yield and resistance are affected by many factors, the 10 Taiwan *indica* varieties became genetically diverse but could still maintain the considerable level as the varieties introduced from abroad, as indicated by the lower major allele frequency, mean gene diversity, and mean PIC value (Table 4). Furthermore, Taiwan domestic and introduced *indica* varieties showed moderate differentiation (F_{ST} =0.25; Table 4).

The current Taiwan *japonica* cultivars have a very narrow gene pool because of relatively little germplasm used in breeding programs and field uniformity (Lin, 1991a, b; Wu and Lin, 2008). The mean genetic diversity and PIC value were lower for Taiwan *japonica* than introduced *japonica* varieties and even lower than for introduced Japanese *japonica* varieties (Table 4). Taiwan and Japanese *japonica* varieties did not differ greatly in genetic diversity (F_{ST} =0.05). In addition, clustering analyses based on genetic distances revealed Taiwan modern *japonica* cultivars highly related to introduced Japanese *japonica* cultivars, which was consistent with results by pedigree analysis (Lin, 1991a; Wu and Lin, 2008). The breeding goal has been grain quality for better selling price since 1980; the tradeoff has been a great reduction in genetic diversity of cultivated rice.

The assessment of genetic diversity of germplasm is essential for breeding programs to characterize the genetic bases of commercial cultivars. The genetic diversity of rice cultivars in Taiwan is very narrow, as determined by pedigree analysis or molecular marker assay, because of the high selection pressure for good grain quality and repeated use of a few germplasm in breeding programs. To broaden the gene pool of current cultivars, exotic elite cultivars were recently introduced from various countries besides Japan (Table 1). Those introduced cultivars provide beneficial genes for Taiwan cultivars, such as those for biotic and abiotic resistance, aroma, and high yield (Table 1). The genomes of introduced exotic germplasm broadened the gene pool of Taiwan cultivars, as revealed by increased mean number alleles per locus, mean gene diversity, and mean PIC value, and lower major allele frequency (Table 4). Nevertheless, the genetic diversity of cultivars is greatly reduced during systematicly extensive selection for a few target traits. Enlarging the gene pool of current cultivars for new challenges of unknown biotic and abiotic stresses due to global warming is a crucial task in modern breeding programs. Investigating untapped natural diversity in landraces or wild relative rice, *O. rufipogon* and *O. nivara*, is ongoing (Thomson et al., 2007, 2009; Kovach and McCouch, 2008; Pusadee et al., 2009). Previous assessment of genetic diversity of Taiwan landraces and cultivars of rice found landraces with greater variation in allele richness by SSR markers (Y.-R. Lin, Y.-P. Wu, and Su-Huang Chang, unpublished data). How to eliminate inferior alleles from landraces and wild relative rice while introgressing useful genes in cultivars is critical. Marker-assisted selection (MAS) for precise selection of favorable genotypes will accelerate current breeding and increase gene pyramiding in rice modern breeding.

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水稻是世界最重要的禾穀類作物之一，在臺灣更是主要的栽培作物，因此水稻種原遺傳歧異度的評估，對於種原之保存及育種上之利用均扮演著重要的角色，故利用 114 個簡單序列重複分子標幟 (simple sequence repeat, SSR) 及 5 個序列標籤位點 (sequence tagged site, STS) 分子標幟，針對 52 個粳稻和 28 個秈稻等 80 個栽培稻於臺灣育種過程常用之種原進行探討遺傳結構。總共偵測出 395 個對偶基因，每個分子標幟可偵測 2 至 7 個對偶基因，平均為 3.5 個；而每個分子標幟之多型性訊息量 (polymorphism information content，PIC) 介於 0.04-0.76，平均為 0.43。以遺傳距離為導向之不加權平均重法 (UPGMA) 及主座標分析法 (PCoA) 探討 80 個水稻品種之結構，可將粳稻及秈稻明顯區分在不同群集，其中臺灣及日本之粳稻分布於相同的群集中，顯示具有高度相似的遺傳背景。秈稻品種之遺傳歧異度大於粳稻，而臺灣栽培稻之遺傳歧異度相對較小，尤其是臺灣粳稻，但國外引進之栽培稻種原則呈現較豐富遺傳變異。檢視遺傳分化指數 (genetic differentiation index, F_{ST}) 分析，發現國內外之秈稻和粳稻的分化指數僅屬於中等 (分別為 0.25 和 0.15)，而由臺灣及日本來源之粳稻間之遺傳分化指數相當小 (F_{ST} =0.05)，顯示兩地區來源之水稻粳稻品種未呈現明顯分化，另從日本以外之地區引進臺灣之種原會有現較大的分化指數，顯示其有擴大臺灣栽培稻基因池之效益。由 DNA 分子標幟多型性的分析解析了臺灣水稻種原在基因體上之關係，更建立了臺灣栽培稻之遺傳標幟資料庫，可供未來在不同品種鑑別、種原保存和育種上進行相關利用。

關鍵詞：遺傳歧異；水稻；簡單序列重複分子標幟；臺灣栽培稻。