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Genetic characterization of weedy rice (*Oryza sativa* L.) based on morpho-physiology, isozymes and RAPD markers

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Abstract Weedy rice (*Oryza sativa* L.) is an important resource for breeding and for studying the evolution of rice. The present study was carried out to identify the genetic basis of the weedy rices distributed in various countries of the world. One hundred and fifty two strains of weedy rice collected from Bangladesh, Brazil, Bhutan, China, India, Japan, Korea, Nepal, Thailand and the USA were tested for variations in six morpho-physiological characteristics and in 14 isozyme loci. Twenty six weedy strains selected from the above materials were assayed for the *Est-10* locus, six RAPD loci of the nuclear genome, and one chloroplast locus. From the results of multivariate analysis based on the morpho-physiological characteristics and the isozymes, weedy rice strains were classified into *indica* and *japonica* types, and each type was further divided into forms resembling cultivated and wild rice. Thus, four groups designated as I, II, III and IV were identified. Weedy strains of group I (*indica*-type similar to cultivars) were distributed mostly in temperate countries, group II (*indica*-type similar to wild rice) in tropical countries, group III (*japonica*-type similar to cultivars) in Bhutan and Korea, group IV (*japonica*-type similar to wild rice) in China and Korea. In group I, classified as *indica*, several strains showed *japonica*-specific RAPD markers, while some others had *japonica* cytoplasm with *indica*-specific RAPD markers in a heterozygous state at several loci. One weedy strain belonging to group II showed a wild rice-specific allele at the *Est-10* locus. However, in groups III and IV, no variation was ound either for the markers on *Est-10* or for the RAPD loci tested. Judging from this study, weedy rice of group I might have originated at least partly from gene flow between *indica* and *japonica*, whereas that of group II most probably originated from gene flow between wild and cultivated *indica* rice. Weedy rice of group III is thought to have originated from old rice cultivars which had reverted to a weedy form, and that of group IV from gene flow between *japonica* cultivars and wild rice having *japonica* backgrounds.

Key words Weedy rice · *Oryza sativa* · Classification · Origin of weedy rice · Isozyme · Randomly amplified polymorphic DNA

Introduction

Crops are often associated with respective weedy forms which are genetically related (de Wett and Harlan 1975). Weedy rice is distributed over a wide area in rice-growing regions, and seems to possess a wide variation in characteristics. It is not intentionally cultivated, but grows naturally in and around rice fields. Some weedy rice strains are similar to wild rice in that they have a red pericarp, black hull, light seed weight, high degree of seed dormancy and high degree of seed shattering, but other strains are similar to cultivars (Arashi 1974; Heu 1988; Oka 1988; Tang and Morishima 1988; Suh et al. 1992). A high degree of cold tolerance at the seedling stage and a high degree of shoot-emergence ability in deep water were observed in some weedy strains (Suh and Morishima, unpublished). A wide range of tolerance to various adverse conditions is expected in weedy rice.

Weedy rice strains reproduce themselves by dispersed seeds, or by being sown together with cultivar seed by man, and are classified into two categories, one

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occurring together with common wild rice and the other distributed in regions where no wild rice occurs (Oka 1988).

We have tried to classify 152 weedy rice strains selected randomly from more than 1000 strains collected in Korea and nine other countries. We used multivariate analysis based on six morpho-physiological characteristics and 14 isozyme loci. We hope to trace the origin of these weedy rice strains by assaying variations in an isozyme marker specific to wild rice and DNA markers of the nuclear genome and the chloroplast.

Materials and methods

One hundred and fifty two strains of weedy rice (9 from Bangladesh, 16 from Bhutan, 2 from Brazil, 4 from China, 3 from India, 3 from Japan, 101 from Korea, 10 from Nepal, 2 from Thailand and 2 from the USA) were examined, together with two *indica* cultivars (IR36 and Peh-kuh) and two *japonica* cultivars (Taichung 65 and Nakdong). Six morpho-physiological characters were measured: three *indica/japonica* diagnostic characteristics (resistance to $KClO_3$, glume hair length, phenol reaction) and three wild/cultivated characteristics (100-grain weight, degree of seed dormancy and degree of seed shattering). The degree of seed dormancy was represented by the number of days after harvest when at least 95% of the seeds germinated. The degree of seed shattering was measured using the instrument devised by Ichikawa et al. (1990); the smaller number represents easy shattering and the large number represents hard shattering. Other characters were measured by the methods described by Sato (1990). Fourteen isozyme loci (*Acp-1*, *Amp-1*,*Amp-2*, *Amp-3*, *Cat-1*, *Est-2*, *Est-5*, *Pgd-1*, *Pgd-2*, *Pgi-1*, *Pgi-2*, *Pgi-3*, *Pox-2*, and *Sdh-1*) were assayed by the starch-gel method (IRRI 1990). Enzyme extracts were prepared from 4-day-old shoots of etiolated seedlings. After electrophoresis and staining, bands were read by the system suggested by Morishima and Glaszmann (1990). Allozyme variation in 14 isozyme loci from 152 strains (including four control cultivars) was assayed by principal component analysis (PCA). Factor analysis was applied to assay the variations in the six morpho-physiological characteristics tested along with the score of the first component of PCA from isozyme data, in order to classify weedy rice strains. Twenty six weedy strains selected from the 152 strains tested, and two *indica* and two *japonica* cultivars, were assayed for the isozyme marker at the *Est-10* locus, six RAPD markers of the nuclear genome and one chloroplast DNA locus. The polyacrylamide-gel system was applied to analyze the *Est-10* locus. Bands 1, 2 and 4, which were specific to *japonica*, *indica* and wild rice, respectively, were measured by the method described by Cai and Wang (1994). Six genomic RAPD markers (OPN-16, OPO-15, OPQ-05, OPR-13, OPR-15 and CMNA-32) which showed specific bands in *indica* and *japonica* cultivars, and deletion at locus ORF-100 of the chloroplast, were assayed by the polymerase chain reaction. Primers for the amplification of genomic DNA fragments were:

5′-AAGCGACCTG-3′(OPN-16), 5′-TGGCGTCCTT-3′(OPO-15), 5′-CCGCGTCTTG-3′(OPQ-05), 5′-GGACGACAAG-3′(OPR-13), 5′-GGACAACGAG-3′(OPR-15) and 5′-CTTGTCATGTGT-3′ (CMNA-32).

Two oligonucleotides, 5′-AGTCCACTCAGCCATCTCTC-3′ and 5′-GGCCATCATTTTCTTCTTTAG-3′, were used as primers for detecting deletion at the ORF-100 chloroplast locus. DNA extraction and the PCR process were followed by the method described by Nakamura and Sato (1991).

Results

Classification of weedy rice

The 14 isozyme loci examined were all polymorphic in the weedy strains tested. The weedy strains were scattered in the plane defined by the first and second components of PCA based on allozyme data (Fig. 1). They were clearly divided into two distinct groups by the first component, which included 48.4% of the total variation. One group containing the *japonica* cultivars Taichung 65 (T65) and Nakdong, which scattered on the right side of the figure, seemed to represent the *japonica* type. The other group containing the *indica* cultivars IR36 and Peh-kuh, which scattered on the left side, seemed to represent the *indica* type. The strains from Bhutan, China and Korea contained both *indica* and *japonica*, while those from other countries were all *indica*. As shown in Fig. 1, variation in the *japonica* group was narrow and simple, but the *indica* types were much more variable. In the *indica* group, the strains from tropical countries tended to scatter in lower part of the figure, with those from temperate countries in the upper part.

The scatter diagram of the first and second vectors of the factor analysis from all 152 weedy strains, based on six morpho-physiological characteristics, and the score of the first component of PCA from 14 isozyme loci, is shown in Fig. 2. All the weedy strains tested were classified into four groups designated as groups I, II, III and IV. Judging from the mean values of resistance to $KClO_3$ and glume hair length (Table 1), groups I and II are of the *indica* type characterized by susceptibility to $KClO_3$ and short glume hair, groups III and IV are of the *japonica* type having the opposite characteristics. When mean values of 100-grain weight, degree of seed shattering and seed dormancy were compared (Table 1) in the four groups, I and III appeared to be similar to cultivars, while II and IV were similar to wild rice. Thus, weedy rice strains were classified into an *indica*-type similar to cultivars (group I), an *indica*-type similar to wild rice (group II), a *japonica*-type similar to cultivars (group III) and a *japonica*-type similar to wild rice (group IV). Fifty four weedy strains from temperate countries were identified as group I, 24 strains from tropical countries as group II, 63 strains from Bhutan and Korea as group III, and 11 strains from China and Korea as group IV.

Est-10 and RAPD analysis

The band patterns of *Est-10*, RAPD markers at the genomic loci OPN-16, OPO-15, OPQ-05, OPR-13, OPR-15 and CMNA-32, and the DNA marker at ORF-100 locus of the chloroplast from 26 weedy strains and four cultivars are given in Table 2. For the

Fig. 1 Scatter diagram of the first (*I*) and second (*II*) vectors of principal component analysis based on the allele composition of 14 isozyme loci in 152 weedy rice strains (*Ba* Bangladesh, *Bh* Bhutan, *Br* Brazil, *Ch* China, *In* India, *Ja* Japan, *Kl* Korean long grain, *Ks* Korean short grain, *Ne* Nepal, *Th* Thailand, *US* USA weedy rice, *Peh-kuh indica* cultivar, *T65 japonica* cultivar)

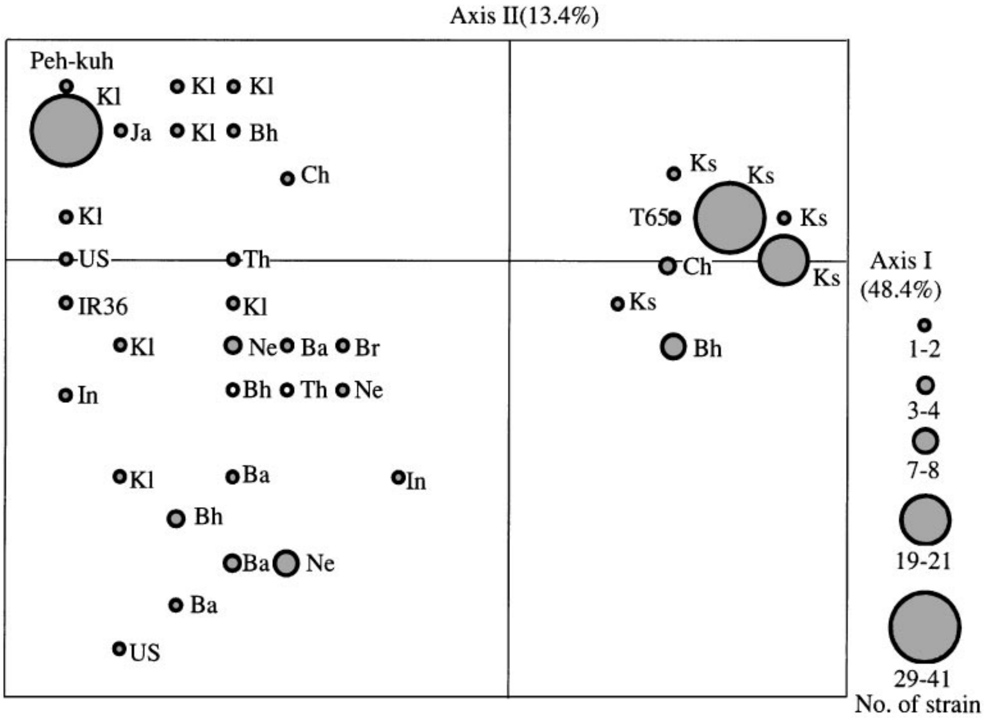

Fig. 2 Scatter diagram of 152 strains of weedy rice in planes (*I*, *II*) of factor analysis based on resistance of $KClO_3$, glume hair length, phenol reaction, 100-grain weight, degree of seed dormancy, degree of seed shattering and PCA score of 14 isozyme loci. (● with *Ba* Bangladesh, *Bh* Bhutan, *Br* Brazil, *Ch* China, *In* India, *Ja* Japan, *Ne* Nepal, *Th* Thailand, *US* USA weedy rice, ● Korean long-grain, ○: Korean short-grain weedy rice, *IR36* and *Peh-kuh indica*, *T65* and *Nakdong japonica* cultivar)

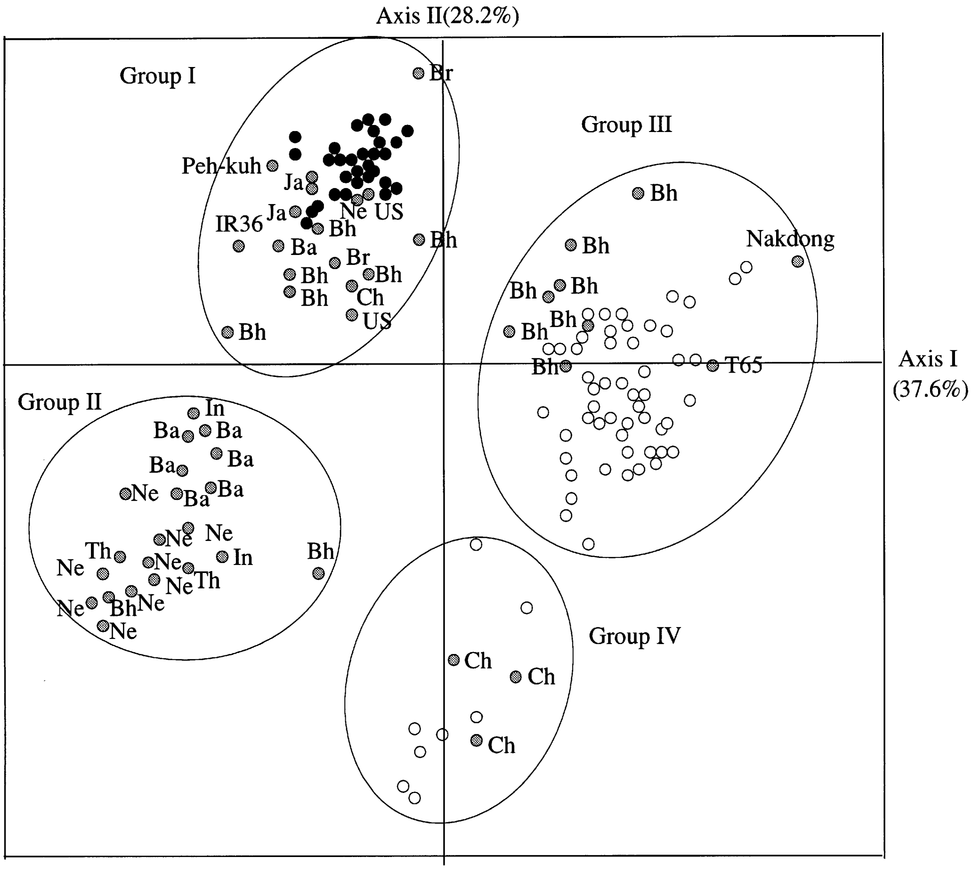

Table 1 Comparison of resistance to $KClO_3$, glume hair length, 100-grain weight, seed shattering and seed dormancy among four groups of weedy rice strains grouped by multivariate analysis

Characteristics	Group			
	I	II	III	IV
Resistance to $KClO_3$ (0–2)	1.80a[a]	1.62b	0.39c	0.10d
Glume hair length (mm)	0.50c	0.49c	0.76b	0.89a
100-grain weight (g)	2.08c	1.75b	2.03a	1.77b
Seed shattering (gf)	124b	12d	150a	39c
Seed dormancy (days)	29b	161a	31b	161a

[a] Mean values within a row followed by different letters are significantly diferent at the 0.05 probability level, according to Duncan's Multiple Range Test

Est-10 locus, 19 weedy strains showed allele 1, six strains allele 2 and the strain W2064 from Bangladesh allele 4, which were known to be specific to *indica*, *japonica* and wild rice respectively (Cai and Wang 1994).

For RAPD markers, all the *japonica*-type weedy strains defined by isozymes showed *japonica*-specific RAPD bands at six loci of the nuclear genome and a non-deletion type at the chloroplast ORF-100 locus. In *indica*-type weedy strains, however, polymorphism was found at five of the the six genomic RAPD loci tested and at the chloroplast DNA locus (Table 2). The *indica* weedy strain W1713 from Brazil appeared to have hybrid bands at loci OPQ-05 and OPR-15. Strains W1714 from Brazil, as well as US 1 and US 2 from the USA, appeared to have a hybrid band at locus OPR-15. Among the 20 weedy rice strains classified as *indica*-type by isozymes, five strains (W1713 and W1714 from Brazil, US 1 from the USA, S514 from China and C9520 from Nepal) had a non-deletion chloroplast at the ORF-100 locus, which was known to be specific to *japonica* (Chen et al. 1993). Among the *indica*-type, weedy strains carrying *indica* isozymes and chloroplast, S401 from Korea showed the *japonica*-specific band at genomic loci OPO-15 and four others (CMNA-32, and S416 from Korea, C9574 and C9575

Table 2 Characteristics of weedy rice strains for six RAPD markers in the nuclear genome (OPN-16, OPO-15, OPQ-05, OPR-13, OPR-15 and CMNA-32), one chloroplast DNA locus (ORF-100) and one isozyme locus *Est-10*

Type	Strain	Origin	Isozyme group[a]	RAPD markers[b]							
				OPN-16	OPO-15	OPQ-05	OPR-13	OPR-15	CMNA-32	ORF-100	*Est-10*[c]
indica	IR36	Philippines	I	a	a	a	a	a	a	D	1
indica	Peh-kuh	Taiwan	I	a	a	a	a	a	a	D	1
Weedy	S401	Korea	I	a	b	a	a	a	b	D	1
Weedy	S416	Korea	I	a	a	a	a	a	b	D	1
Weedy	C9574	Japan	I	a	a	a	a	a	b	D	1
Weedy	C9575	Japan	I	a	a	a	a	a	b	D	1
Weedy	W1713	Brazil	I	a	a	a/b	a	a/b	a	N	1
Weedy	W1714	Brazil	I	a	a	a	a	a/b	a	N	1
Weedy	US1	USA	I	a	a	a	a	a/b	a	N	1
Weedy	US2	USA	I	a	a	a	a	a/b	a	D	1
Weedy	S514	China	I	a	a	a	a	a	a	N	1
Weedy	85-27	China	I	a	a	a	a	a	a	D	1
Weedy	C9520	Nepal	I	a	a	a	a	a	a	N	1
Weedy	C9496	Nepal	I	a	a	a	a	a	a	D	1
Weedy	BT5d	Bhutan	I	a	a	a	a	a	a	D	1
Weedy	BT5f	Bhutan	I	a	a	a	a	a	a	D	1
Weedy	W150	India	I	a	a	a	a	a	a	D	1
Weedy	W1670	India	I	a	a	a	a	a	a	D	1
Weedy	SS18-2	Thailand	I	a	a	a	a	a	a	D	1
Weedy	SS21-1	Thailand	I	a	a	a	a	a	a	D	1
Weedy	W2063	Bangladesh	I	a	a	a	a	a	a	D	1
Weedy	W2064	Bangladesh	I	a	a	a	a	a	a	D	4
Weedy	S434	Korea	J	b	b	b	b	b	b	N	2
Weedy	S436	Korea	J	b	b	b	b	b	b	N	2
Weedy	Ch79	China	J	b	b	b	b	b	b	N	2
Weedy	Ch80	China	J	b	b	b	b	b	b	N	2
Weedy	BT1Ac	Bhutan	J	b	b	b	b	b	b	N	2
Weedy	BT2Cb	Bhutan	J	b	b	b	b	b	b	N	2
japonica	Taichung 65	Taiwan	J	b	b	b	b	b	b	N	2
japonica	Nakdong	Korea	J	b	b	b	b	b	b	N	2

[a] I: *indica*, J: *japonica*, classified by PCA score of 14 isozyme

[b] a,b: nuclear DNA specific to *indica*(a) and *japonica*(b); D,N: chloroplast DNA of deletion(D) and non-deletion(N) type

[c] 1: *japonica*, 2: *indica*, 4: wild-rice specific

from Japan) showed the *japonica*-specific band at locus CMNA-32. This result indicated that some *indica*-type weedy rices from Brazil, China, Korea, Japan, Nepal and the USA might have originated from gene flow between *indica* and *japonica* rice, whether they were cultivated or wild-type.

Discussion

The present study reveals two distinct groups of weedy rice strains corresponding to *indica* and *japonica* cultivars, respectively. Each group was further classified into forms resembling cultivars and wild rice, respectively. The origin of each group will be discussed below based on their genetic nature and geographical distribution.

Some *indica* weedy strains in group I from Brazil, Korea, Japan and the USA showed *japonica*-specific RAPD genes in one or two of the six loci tested. A further, five *indica* weedy strains of group I from Brazil, China, Nepal and the USA were found to have *japonica* cytoplasm, and some of them carried *japonica* nuclear markers in a heterozygous state at one or two loci. Following RFLP analysis, Cho et al. (1995) reported that the long-grain Korean weedy rices, which belong to group I of this report, had 49–57% genetic similarity with the *indica* cultivar IR26, 5–11% with the *japonica* cultivar Gihobyeo, and 24–37% were specific weedy rice compositions. Judging from these results, we cannot rule out the possibility that at least some of the weedy rices belonging to group I might have originated from hybridization between the *indica* and *japonica*, sub-species, whether in a cultivated or a wild form, with *japonica* as the female. Tang and Morishima (1995, in preparation) pointed out that this type of weedy rice was supposed to represent old rice cultivars which had reverted to a weedy form.

Among the *indica* weedy rices of group II distributed in tropical Asian countries, strain C9520 from Nepal appeared to have *japonica* cytoplasm with an *indica* genome, and strain W2064 from Bangladesh showed the wild rice-specific allele at the *Est-10* locus. This observations suggested that weedy rice strains of group II may have originated from hybridization between wild and cultivated rice. Oka and Chang (1961) observed wide variation from wild to cultivated types in the rice population of tropical regions. Gene flow from cultivated to wild species was earlier proposed by several researchers (Oka and Chang 1961; Baker 1974; de Wet and Harlan 1975; Oka 1988). The present study provides clear evidence of gene flow between wild and cultivated rice.

The *japonica* weedy strains of group III distributed in Bhutan and Korea showed only minor variation in the isozyme and DNA characteristics tested. These strains were assumed to be old rice cultivars which had reverted to a weedy form. Baker (1974) and de Wet and Harlan (1975) have discussed three principal processes in the evolution of weeds: (1) from wild colonizing plants through selection toward adaptation to continuous habitat disturbance, along with the evolution of domestication, (2) as derivatives of hybrids between wild and cultivated races, (3) from abandoned domesticates through selection toward a less intimate association with man. The origin of the weedy strains in group III seems to agree with the third of these processes.

The *japonica* strains of group IV found in China and Korea showed only minor variation in the isozyme and DNA characters tested. This group, however, was quite different from group III in diagnostic characteristics for wild vs cultivated types, with easy shattering, long dormancy and light grain weight (Table 1). The wildness characteristics found in Chinese weedy strains of this group seem to have originated from gene flow between the *japonica* cultivar and the *japonica* wild rice which was distributed in China (Morishima and Gadrinab 1987; Dally and Second 1990; Chen et al. 1993). The group IV weedy rices in Korea were found at the west side of the Korean peninsula, directly opposite the Yangtze river across the Yellow Sea. Thus, this may have been introduced from China along with cultivated rice in ancient times, because no wild rice is native to the Korean peninsula.

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