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Variation of Vascular Bundle System Corresponds to *indica*, Tropical－and Temperate－*japonica* Differentiation of Asian Rice(*Oryza sativa* L.)

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Summary

Variation of the vascular bundle system in 83 Asian rice cultivars and its usefulness in the distinction of the 3 types, *indica*, tropical－*japonica* and temperate－*japonica* were investigated. The 83 cultivars were classified into 46 *indica* and 37 *japonica* cultivars by the discriminant function, D_1, based on the data of phenol reaction, sensitivity to $KClO_3$ and apiculus hair length. Next, the 37 *japonica* cultivars were subdivided into 8 tropical－ and 29 temperate－*japonica* using the score of D_2 which was computed from the ratio of length to width of the kernel, alkali digestibility and mesocotyl length. The number of vascular bundles(Vb) in the peduncle and the ratio of Vb to rachis branches(Rb)(V/R ratio) differed among the 3 types; the *indica* and tropical－*japonica* cultivars had more Vbs and showed a higher V/R ratio than the temperate－*japonica* cultivars. Then, the new discriminant function(D_3) for identification of *indica* and *japonica* and D_4 for that of tropical－ and temperate－*japonica* were calculated based on Vb, Rb and V/R ratio. D_3 and D_4 improved the precision of distinguishing *indica* from *japonica*, and tropical－ from temperate－*japonica*; the probability of misclassification decreased from 1.4%(D_1) to 0.7%(D_3), and from 4.8%(D_2) to 0.4%(D_4), respectively. In conclusion, the variation of vascular bundle system was a highly useful character for identification of Asian rice cultivars.

Key Words : *Oryza sativa*, *indica*, *japonica*, classification, vascular bundle.

Introduction

Asian rice cultivars, *Oryza sativa* L., have been classified into two groups, *indica* and *japonica*, after the pioneering work of Kato *et al.*(1928). Oka(1958) demonstrated that the *japonica* type, which was formerly called insular type, could be subdivided into tropical and temperate types based on the pattern of many characters in combination, and he also emphasized that there was no single character or gene by which *indica* and *japonica* types could be distinguished with certainty. Classification into varietal groups has always been a matter of concern for rice geneticists and breeders. Hitherto, much information has accumulated for the identification of Asian rice cultivars using morphological, physiological and ecological traits(Oka 1958, Matsuo 1952), isozymes(Second 1982, Glaszmann 1987), and molecular markers including RFLPs and RAPDs of nuclear or cytoplasmic genomes(Wang and Tanksley 1989, Tanaka *et al.* 1989, Fukuoka *et al.* 1992, Mackill 1995, Sano and Sano 1990, Kadowaki *et al.* 1988, Dally and Second 1990). However, since isozymes and molecular markers are neutral to selection, polymorphisms in these markers between varietal types seem to have resulted from linkage with adaptive genes(hitchhiking effect) and/or linkage with the genes for reproductive isolation. To elucidate more precisely the adaptive differentiation in Asian rice, we need to search for the characters linked to the neutral markers mentioned above.

The vascular bundle system in plants is important for transport of photosynthetic products, water and nutrients, and it is highly possible that this character intimately relates with the selection. Huang(1988) and Fukuyama and Takayama(1995) indicated the possibility that *indica* and *japonica* could be identified by the relation of the number of large vascular bundles in the peduncle(first internode from the top) and primary rachis branches; i.e., *indica* bore more vascular bundles than *japonica*, although the number of rachis branches was nearly equal. However, only 10 *indica* and 9 *japonica* cultivars were examined(Huang 1988), or there was no evidence for classification of *indica* and *japonica* using diagnostic characters such as sensitivity to $KClO_3$ and apiculus hair length other than phenol reaction(Fukuyama and Takayama 1995).

The present investigation was carried out to examine whether the variation of the vascular bundle system corresponds to the *indica*/*japonica* classification in Asian rice.

Materials and Methods

A total of 83 Asian rice cultivars were used. Their origins were: 17 from Japan, 15 from China, 6 from Nepal, 19 from Indonesia, 17 from Bangladesh and 9 from India. Japanese cultivars consisted of land races or improved cultivars, and the other Asian cultivars were land races introduced in 1986 and 1989 to National Institute of Agrobiological Resources, Tsukuba. Twenty

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plants of each cultivar were grown in the field of Hokuriku National Agricultural Experiment Station, Joetsu, in 1992. After heading, the panicle of the longest tiller of 5 plants randomly selected from each cultivar was sampled, and the number of large vascular bundles(Vb) in the peduncle 1cm below the neck of panicle was investigated under a dissecting microscope. The number of primary rachis branches(Rb) of the same panicle was recorded, and the ratio of Vb to Rb(V/R ratio) was calculated in each cultivar.

The cultivars were classified into 3 types, *indica*, tropical- and temperate-*japonica*, following Sato (1991); the discriminant function, D_1=Ph+1.313K − 0.82H − 1.251, where Ph, K and H standing for the phenol reaction of the hull, sensitivity to $KClO_3$ and apiculus hair length, respectively, was used for identification of *indica* and *japonica* types. Next, for the cultivars classified into *japonica* type by the D_1 score, another discriminant function, D_2=R+0.093M -0.044E -2.01, where R, M and E referring to the ratio of length to width of the spikelet, the length of mesocotyl and the alkali digestibility of grain, respectively, was applied for identification of tropical- and temperate-*japonica* types. Briefly, the cultivars were tested for each diagnostic character as follows. For the phenol reaction of the hull, 3 grains were examined after 3 days' soaking in a 1.5% phenol solution. To determine the sensitivity to $KClO_3$, 10 seedlings at the 2- to 3-leaf stage were treated with a 1.5% solution of $KClO_3$ at 30 ℃ for 3 days, and evaluated by the injury reading(0-2). For apiculus hair length, the lengths of 2 longest apiculus hairs in each of the 10 kernels were measured. To obtain the ratio of length to width of the kernel, 10 kernels were evaluated. To determine mesocotyl elongation, 15 grains were grown for 10 days at 30 ℃ in the dark, and mesocotyl length was measured. Alkali digestibility was evaluated by cutting each of 5 grains into two, soaking in a 2% KOH solution for 24 hours at 30 ℃, and calculating the percentage of the weight of digested endosperm to the control.

Results

In Fig. 1, the 83 cultivars are classified according to the score given by the discriminant function, D_1. They formed two groups at the boundary point of − 0.5. The 37 cultivars with the score lower than − 0.5, which consisted of 17 from Japan, 9 from China and 11 from Indonesia, were assumed to be of the *japonica* type. The remaining 46 cultivars with a score higher than -0.5, which consisted of those from Nepal, Bangladesh and India, 6 cultivars from China and 8 cultivars from Indonesia, were considered to be of the *indica* type.

Fig. 2 shows the distribution of D_2 scores among the 37 cultivars classified into *japonica* type by the D_1 score. Although no clear discreteness was found in the distribution, the cultivars could be divided into two groups at the D_2 score of 0.5. All 8 cultivars with a score higher than 0.5 were from Indonesia. Then, it was assumed that these cultivars were of the tropical-*japonica* type, and the remaining 29 cultivars were of the temperate-*japonica* type originating from Japan, China and Indonesia. The cultivars showing a boundary score of 0 consisted of 3 from Indonesia and 1 from Japan.

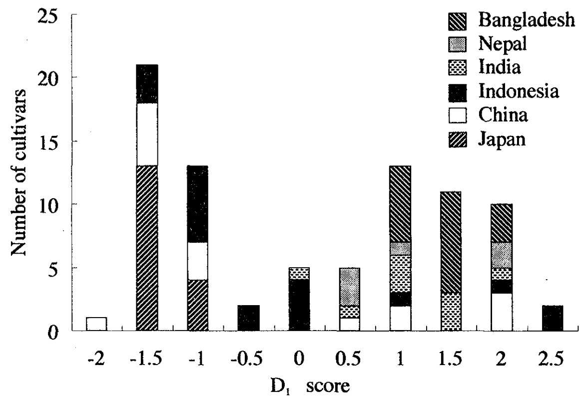

Fig. 1. Frequency distribution of the D_1 scores among 83 Asian cultivars.

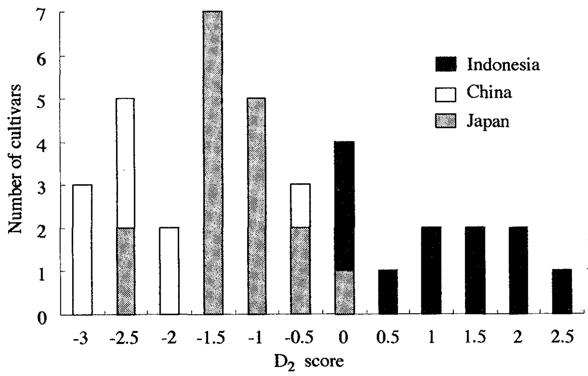

Fig. 2. Frequency distribution of the D_2 scores among 37 *japonica* cultivars.

The agreement between the classification of the cultivars into *indica*, tropical-*japonica* and temperate-*japonica* and the Vb, Rb and V/R ratio within each group was examined(Table 1). The Vbs in the peduncle ranged from 14 to 24 in the *indica* type, from 14 to 22 in the tropical-*japonica* type, and from 8 to 18 in the temperate-*japonica* type, showing a slight distinction of the character between the temperate-*japonica* type and the other two types. No differences were recognized in the number of Rb among the 3 types. On the other hand, the V/R ratios showed a more obvious distinction among the 3 types; *indica* had the highest V/R ratio, ranging from 1.31 to 2.00, followed by tropical-*japonica*(1.37-1.66) and temperate-*japonica*(0.84-1.38).

Since we ascertained that Vb and the V/R ratio could be useful to distinguish between *indica* and *japonica* types, the new discriminant function, D_3, for the *indica*-*japonica* classification was recomputed between 46 *indica* and 37 *japonica* cultivars using the characters of Vb, Rb, V/R ratio, phenol reaction(Ph), sensitivity to $KClO_3$(K) and apiculus hair length(H). The obtained formula was D_3 =7.83Ph+8.53K− 2.08H− 0.22Vb+0.46

Table 1. Distribution of the number of vascular bundle(a), primary rachis branch number(b), and the ratio of vascular bundles to rachis branches(c) in *indica*, tropical- and temperate-*japonica* cultivars

a) Number of vascular bundles

Type	No. of cvs	8	10	12	14	16	18	20	22	24
indica	46				5	9	16	8	3	5
Tr-*japonica*	8				1	5		1	1	
Tm-*japonica*	29	1	7	9	7	3	2			

b) Number of rachis branches

Type	No. of cvs	7	8	9	10	11	12	13	14	15
indica	46		4	11	11	5	6	2	6	1
Tr-*japonica*	8			1	1	2	2	1		1
Tm-*japonica*	29	1	2	1	4	5	7	5	3	1

c) Ratio of vascular bundles to rachis branches

Type	No. of cvs	1.0	1.2	1.4	1.6	1.8	2.0
indica	46			3	9	20	14
Tr-*japonica*	8			2	5	1	
Tm-*japonica*	29	10	11	8			

Rb+15.53V/R − 32.78. To compare the degree of contribution of six characters in D_3, standardization was made by dividing each coefficient by the standard deviation of each character. The highest standardized value was obtained in V/R ratio(48.2), followed by Ph(15.7), K(12.7) and H(9.8), while the values were very low in Rb(0.2) and Vb(0.06). Fig. 3 shows the distribution of D_3 scores. Except a cultivar, Rakka Rayak from Indonesia, 82 cultivars were classified into *indica* and *japonica* with the plus or minus D_3 score, respectively. Likewise, the new D_4 scores were computed using Vb, Rb, V/R ratio, the ratio of length to width of the kernel(R), mesocotyl elongation(M), and alkali digestibility(E) between 8 tropical-*japonica* and 29 temperate-*japonica* cultivars classified by D_2. The obtained formula was $D_4 = -14.80R - 0.06M + 0.41E + 5.43Vb - 7.98Rb - 84.07 V/R + 131.41$.The standardized coefficient of each character in D_4 was highest in V/R(415.6), followed by R(41.7), and Rb(4.5), while those of Vb, E and M were less than 1.8. As shown in Fig. 4, the distribution of the D_4 scores was discontinuous between tropical- and temperate-*japonica*.

To compare the discriminant power among the characters investigated, we estimated the probabilities of misclassification for *indica* and *japonica* types and those for tropical-*japonica* and temperate-*japonica* types following Snedecor and Cochran(1967). The obtained results are shown in Table 2, where Ph was excluded because it was controlled by a single gene. The *indica* and *japonica* types could be best distinguished by the sensitivity to $KClO_3$, the probability of misclassification being 5.0%, followed by V/R ratio(6.7%), the number of Vb(20.9%), and the apiculus hair length(31.2%). The number of Rb was useless for the identification of *indica* and *japonica*. Such order of discriminating power was almost coincident with that of the standardized coefficient described above. Indeed, the scores given by the

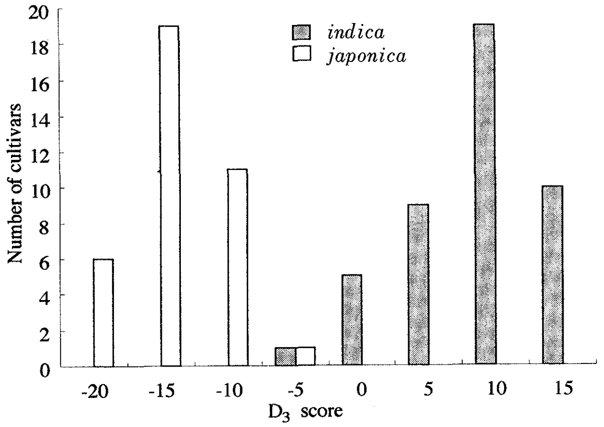

Fig. 3. Frequency distribution of the D_3 scores among 83 Asian cultivars.

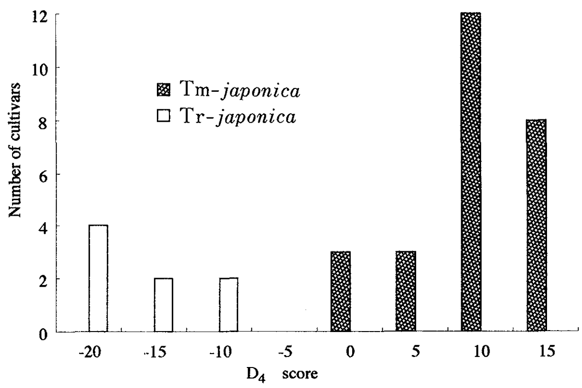

Fig. 4. Frequency distribution of the D_4 scores among 37 *japonica* cultivars.

Table 2. Probabilities of misclassification between *indica* and *japonica* types and between tropical- and temperate-*japonica* types by different characters

Character	Mean ± SD		d	d/2SD	P(%)
	indica (n=46)	*japonica* (n=37)			
Vascular bundle (Vb)	17.6 ± 2.7	13.0 ± 3.0	4.6	0.82	20.9
Rachis branch (Rb)	10.4 ± 1.9	11.2 ± 1.8	− 0.8	− 0.22	41.7
V/R ratio	1.7 ± 0.2	1.2 ± 0.2	0.5	1.50	6.7
Apiculus hair length (H)	0.5 ± 0.1	0.7 ± 0.3	− 0.2	− 0.49	31.2
Tolerance to $KClO_3$ (K)	1.4 ± 0.4	0.2 ± 0.2	1.2	1.65	5.0
D_1 [1)]	0.96 ± 0.70	− 1.52 ± 0.35	2.48	2.19	1.4
D_3 [1)]	11.79 ± 5.68	− 11.79 ± 3.56	23.58	2.44	0.7
	Tr-*japonica*(n=8)	Tm-*japonica*(n=29)			
Vb	16.5 ± 2.6	12.0 ± 2.3	4.5	0.97	16.6
Rb	11.4 ± 1.9	11.1 ± 1.8	0.3	0.08	46.8
V/R ratio	1.5 ± 0.1	1.1 ± 0.1	0.4	1.82	3.4
L/W (R) [2)]	2.7 ± 0.3	2.0 ± 0.2	0.7	1.60	5.5
Mesocotyl length (M)	29.4 ± 5.8	13.2 ± 7.2	16.2	1.18	11.9
Alkali digestibility (E)	49.7 ± 6.1	66.5 ± 12.1	− 16.8	− 0.76	22.4
D_2 [1)]	1.22 ± 0.58	− 1.71 ± 0.95	2.93	1.67	4.8
D_4 [1)]	− 13.35 ± 4.12	13.35 ± 5.40	− 26.70	− 2.62	0.4

[1)]: D_1 =Ph (phenol reaction) +1.313K − 0.82H − 1.251 (Sato 1991).
D_2 =R+0.093M − 0.044E − 2.01 (Sato 1991).
D_3 =7.83Ph+8.53K − 2.08H − 0.22Vb+0.46Rb+15.53V/R − 32.78 (see text).
D_4 = − 14.80R − 0.06M+0.41E+5.43Vb − 7.98Rb − 84.07V/R+131.41 (see text).
[2)]: Ratio of length to width of the spikelet.

discriminant function, D_1 and D_3, were most powerful tools for the classification as compared with single character measurements, the misclassification probability of D_1 and D_3 being 1.4% and 0.7%, respectively. It should be noted that through the use of V/R ratio for the identification characters, the discriminant power of D_3 became about two times as compared with that of D_1. For the tropical- and temperate-*japonica* types, the V/R ratio showed the lowest misclassification probability(3.4%), which was lower than that of the D_2 score(4.8%), followed by the ratio of length to width of the kernel(5.5%), mesocotyl elongation(11.9%), the number of Vb(16.6%), and alkali digestibility(22.4%). The highest discriminant power(0.4%) was obtained by the D_4 score calculated from six characters, R, M, E, Vb, Rb and V/R ratio.

Discussion

The present investigation clearly demonstrates that the *indica* and *japonica* types can be classified according to the number of large vascular bundles in the peduncle(Vb) and the ratio of Vb to primary rachis branches. Likewise, it is emphasized that these two diagnostic characters are highly effective for identification of the two types; the probability of misclassification became quite low(0.7%) by the use of Vb, Rb and V/R in addition to already established traits such as phenol reaction, sensitivity to $KClO_3$ and apiculus hair length. Moreover, the difficulty of classification into tropical- and temperate-*japonica* was decreased when Vb, Rb and V/R were used as the identification characters. Ishikawa *et al.*(1991) indicated that the isozyme alleles, *Amp3*-2 and *Pgd1-2*, were unique to the tropical-*japonica* cultivars, suggesting that these genes were useful for the distinction of the two types of *japonica*, although their materials were restricted to those of Japanese lowland cultivars. Glaszmann(1987) classified a number of Asian rice cultivars into 6 groups, I-VI, based on the variation of 15 isozyme loci, where both tropical- and temperate-*japonica* were included in a single group VI. Then, he successfully subdivided group VI into two types of *japonica*, the so-called "Japonica" and "Javanica" in the sense of Matsuo(1952), by adding the data for 46 quantitative characters estimated by Jacquot and Arnaud(1979). Therefore, it may be hard to identify tropical- and temperate-*japonica* through only isozyme variations. In this study, the discriminant power was markedly improved by adding the variation of Vb, Rb and V/R ratio to the ratio of length to width of the spikelets, the length of mesocotyl and the alkali digestibility of grain. Thus, we propose here that the variations for vascular bundle system are useful not only for distinction between *indica* and *japonica* but also between tropical- and temperate-*japonica*.

The variation of vascular bundle system reported here might be adaptive because the Vbs are primarily requisite to the transportation of photosynthetic products from source to sink and play an important role for the growth. Studies on the differentiation in Vb system between *indica* and *japonica* types are underway using the isogenic lines with high and low V/R ratios derived from the cross of *indica* and *japonica* cultivars.

Literature Cited

Dally, A.M. and G. Second (1990) Chloroplast DNA diversity in wild and cultivated species of rice(Genus *Oryza*, section *Oryza*). Cladistic-mutation and genetic-distance analysis. Theor. Appl. Genet. 80: 209-222.

Fukuoka, S., K. Hosokawa and O. Kamijima (1992) Use of random amplified polymorphic DNAs(RAPDs) for identification of rice accessions. Jpn. J. Genet. 67: 243-252.

Fukuyama, T. and T. Takayama (1995) Variation of the vascular bundle system in Asian rice cultivars. Euphytica 86: 227-231.

Glaszmann, J. C. (1987) Isozymes and classification of Asian rice varieties. Theor. Appl. Genet. 74: 21-30.

Huang, H. (1988) *Japonica* and *indica* differences in large vascular bundles in culm. Intn. Rice Res. Newsl. 13:1.

Ishikawa, R., K. Maeda, T. Harada, M. Niizeki and K. Saito(1991) Classification of Japanese rice varieties into *indica* and *japonica* types by using isozyme genotypes. Japan. J. Breed. 41: 605-622.

Jacquot, M. and M. Arnaud (1979) Classification numerique de varieties de riz. Agron. Trop. 33:157-173.

Kadowaki, K., K. Yazaki, T. Osumi, K. Harada, M. Katsuta and M. Nakagahra (1988) Distribution of mitochondrial plasmid-like DNA in cultivated rice(*Oryza sativa* L.) and its relationship with varietal groups. Theor. Appl. Genet. 76: 809-814.

Kato, S., H. Kosaka and S. Hara (1928) On the affinity of rice varieties as shown by fertility of hybrid plant. Bull. Sci., Fac. Agric. Kyushu Univ. 3: 132-147.

Mackill, D. J. (1995) Classifying *japonica* rice cultivars with RAPD markers.Crop Sci. 35: 889-894.

Matsuo, T. (1952) Genecological studies on cultivated rice. Bull. Natl. Inst. Agr. Sci. Jpn. D3: 1-111.

Oka, H. I. (1958) Intervarietal variation and classification of cultivated rice. Indian J. Genet. Plant Breed. 18: 79-89.

Sano, Y. and R. Sano (1990) Variation of the intergenic spacer region of ribosomal DNA in cultivated and wild species. Genome 33: 209-218.

Sato, Y. I. (1991) Variation in spikelet shape of the *indica* and *japonica* rice cultivars in Asian origin. Japan. J. Breed. 41: 121-134.

Second, G. (1982) Origin of the genic diversity of cultivated rice(*Oryza* spp.): Study of the polymorphism scored at 40 isozyme loci. Jpn. J. Genet. 57: 25-57.

Snedecor, G.W. and W.G. Cochran (1967) Statistical Methods(6th ed.) Iowa State University Press, Ames, 414-415.

Tanaka, T., M. Kawase, A. Saito, M. Yano, N. Kishimoto, K. Saito, A. Yoshimura, H. Takeda, T. Nagamine and M. Nakagahra (1989) Intraspecific variation on restriction fragment length polymorphism of nuclear DNA in rice, *Oryza sativa* L. In Breeding Research: The key to the survival of the earth. Org. Commit. 6th Intl. Congr. SABRAO, Tokyo, 599-602.

Wang, Z. Y. and S. D. Tanksley (1989) Restriction fragment length polymorphism in *Oryza sativa* L. Genome 32: 1113-1118.