

Nonrandom association of genes and characters found in *indica* × *japonica* hybrids of rice

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Two subspecies of common rice, *indica* and *japonica*, are distinguishable by differences in alleles at a number of loci controlling biochemical and morphological traits. The nature of associations between twelve alleles and phenotypes was studied in both a sample of varieties and hybrid populations derived from a single *indica* × *japonica* cross. Many of the associations found in varieties were not found in the F₂ population. The nine sets of associations observed in F₂ are explained by linkage. However, seven nonrandom associations were recovered in an F₅ population, derived in such a way as to minimize the effect of zygotic selection, which were not found in F₂. The genes involved in these associations were not genetically linked and young zygotes showed no differences in fitness between parental and recombined genotypes. These results suggest that increasing parental association of genes and characters in a hybrid population is most probably caused by gametic selection. This gametic selection could be a causal factor promoting the *indica*-*japonica* differentiation in rice varieties.

INTRODUCTION

Among cultivars of common rice (*Oryza sativa* L.), genes and characters tend to be associated in a certain manner, causing the *indica*-*japonica* differentiation. Oka (1958) defined the Indica and Japonica types as two varietal groups having associations of contrasting states in phenol reaction (*Ph/ph*), apiculus hair length, KClO₃ susceptibility, and tolerance to cold and drought. Recently, Glaszmann (1987) reported that two major varietal groups represented by associations of alleles at 15 loci largely corresponded to the Indica and Japonica defined by Oka (1958).

The origin of the *indica*-*japonica* differentiation still remains as a puzzling question. The role of adaptation to different environmental conditions has been emphasized by many authors, while some internal factors have been suggested (Oka, 1958; Ikehashi and Araki, 1986).

In order to elucidate the factors causing non-random association of genes and characters which results in the *indica*-*japonica* differentiation among varieties, the pattern of their associations was observed in *indica* × *japonica* populations raised by a method designed to minimize distortion of genotypic frequencies by zygotic selection. Here

we described patterns of association in twelve genes and characters in F₂ and F₅ populations, and discuss the factors causing such nonrandom associations.

MATERIALS AND METHODS

Hybrid populations used in this study were derived from a cross Ac. 419 (*indica*) × Ac. 504 (*japonica*). Ac. 419 was released by pure line selection in India. Ac. 504 is a Taiwanese cultivar Taichung 65 (T65) which was raised from a cross between two Japanese native cultivars. Ac. 419 and T65 are typical *indica* and *japonica* respectively, and have contrasting sets of alleles at a number of loci and phenotypes of characters (table 1). The F₂ population consisted of 200 individuals. The F₃ and F₄ populations were raised by the single-seed-descent (SSD) method in which seed for the next generation was prepared as bulked single seed from each plant of the previous generation. The F₃ and F₄ populations consisted of 188 and 172 plants, respectively. The F₅ population was raised by bulk-ing two seeds from each F₄ individual, and about 300 plants were grown.

Table 1 Gene and character associations causing *indica-japonica* differentiation in varietal population

Character	Gene ¹	Phenotype or allele	
		<i>indica</i>	<i>japonica</i>
Phenol reaction	(<i>Ph/ph</i>) (II)	Positive (<i>Ph</i>)	Negative (<i>ph</i>)
KClO ₃ susceptibility	Unknown	Susceptible	Resistant
Apiculus hair length	(<i>Aph/aph</i>) (I)	Short (<i>aph</i>)	Long (<i>Aph</i>)
Purple apiculus	(<i>C</i>) (I)	Rare	Frequent
Red pericarp	(<i>Rc</i>) (IV)	Frequent	Rare
Black hull	(<i>Ph</i>) (II) (<i>Bh-a, Bh-b</i>) (I)	Sometimes	None
Awedness	Unknown	Rare	Sometimes
Isozymes			
Phosphoglucose isomerase-1	(<i>Pgi-2</i>) (I)	1, 2	1
Catalase-1	(<i>Cat-1</i>) (I)	1	2
Acid phosphatase-1	(<i>Acp-1</i>) (<i>d-33</i>)	-4	+9
Esterase-2	(<i>Est-2</i>) (I)	1, 2	<i>nul</i>
Aminopeptidase-2	(<i>Amp-2</i>) (<i>sug</i>)	2	1

¹ Linkage group is indicated in parenthesis.

All F₂ and F₅ plants were examined on a single plant basis for phenol reaction (*Ph/ph*, linkage group II), susceptibility to potassium chlorate (KClO₃) at the two or three leaf stage (quantitative traits), apiculus hair length (in mm, *Aph/aph*; see Sato, 1985), pericarp colour (*Rc/rc*, linkage group IV), apiculus colour (*C/c*, linkage group I) and hull colour (black or straw, complementary action of *Ph*, *Bh-a* and *Bh-b*), and presence or absence of awns (genes unknown). Methods for investigation were described by Sato *et al.* (1986). These plants were examined also for five enzyme-encoding loci segregating in the hybrid populations. The five loci assayed were *Est-2* (linkage group I), *Pgi-2* (linkage group I), *Amp-2* (linkage group *sug*), *Cat-1* (linkage group I, but independent from *Est-2* and *Pgi-2*) and *Acp-1* (linkage group *d-33*), by the method described by Ishikawa *et al.* (1987).

In addition, a sample of 200 native cultivars which were collected from various localities in Asia was also examined as a control population (varietal population). Records of the same characters and isozymes examined in hybrid populations were taken for each cultivar.

To check possible sources of selection operating in a breeding system, three characters representing adaptability *i.e.*, (1) seed fertility, (2) germination rate and (3) seedling survival, were measured at the young zygote stage, using seeds from each F₅ plant. Seed fertility was studied as a measurement of zygotic mortality before seed maturity. Twenty seeds were planted in a nursery bed, and non-germinating seeds and surviving seedlings were counted to estimate selection due

to seed dormancy and seedling survival, respectively.

RESULTS

Character and gene associations in the varietal sample

The pattern of association among 12 genes and quantitative characters, observed among varieties is illustrated in fig. 1. Solid and dotted lines indicate associations significant at 1 and 5 per cent levels, respectively, which are based on correlation

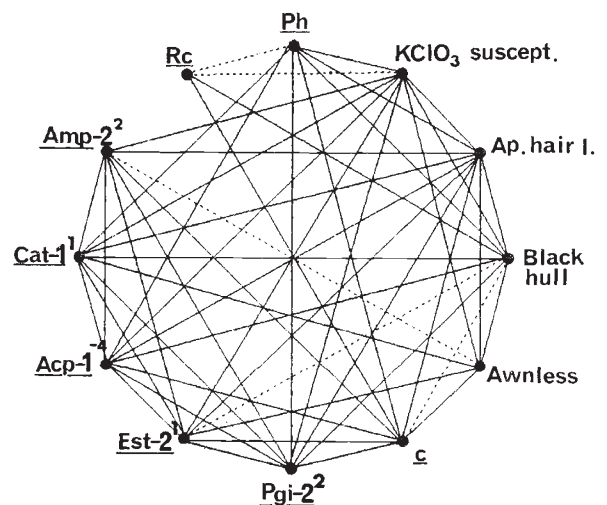


Figure 1 Pattern of association among genes and characters in varietal population.

coefficients (between quantitative characters), chi-square values (between qualitative characters), or *t* values (between qualitative and quantitative characters). Of the 66 possible combinations of genes and characters, 51 combinations (77 per cent) showed nonrandom association.

These associations give rise to two distinct groups of cultivars. The cultivars with the *Ph* allele tend to be susceptible to $KClO_3$, tend to have short apiculus hairs (the *aph* allele) and have *Cat-1*¹, *Acp-1*⁻⁴, and *Amp-2*² alleles. The cultivars with the *ph* allele, on the other hand, tend to have the opposite traits, i.e., resistance to $KClO_3$, long apiculus hairs (the *Aph* allele) and *Cat-1*², *Acp-1*⁺⁹, and *Amp-2*¹ alleles. These two groups of cultivars also showed different allelic constitutions at the *Pgi-2* and *Est-2* loci (Oka, 1958; Morishima and Gadrinab, 1987). The former and latter combination of alleles are *indica*- and *japonica*-specific, respectively (table 1). These associations were confirmed by the present analysis.

The pattern of association in hybrid populations

The pattern of associations between the 12 genes and characters in the F_2 and F_5 populations are shown in fig. 2. Out of 66 possible combinations, nine in F_2 and 15 in F_5 showed nonrandom associations. The associations found in the F_2 are likely to be due to linkage (e.g., *c*, *Est-2*¹ and *Pgi-2*¹, cf. RGN 1, p. 46). Recently, the *Aph* locus was also found to belong to this linkage group (Sato *et al.*, 1988). Black hull colour is controlled by three loci, *Ph*, *Bh-a* and *Bh-b*. Of these, *Ph* belongs to linkage group II. Either *Bh-a* or *Bh-b*, or both are linked to *Aph* (Sato, unpublished data).

In the F_5 population, seven sets of associations which did not appear in F_2 were recovered. These included *Ph* and *aph*, *aph* and $KClO_3$ susceptibility, black hull and *Rc* (red pericarp), *Ph* and black hull, *Ph* and *Rc*, *Rc* and *Amp-2*², and *Est-2*¹ and *Acp-1*⁻⁴. In all cases except the *Rc-Amp-2*² association, parental combinations of alleles found to be associated in *indica* and *japonica* (for instance, *Ph aph* and *ph Aph*, respectively) were more frequent than non-parental combinations (*Ph Aph* and *ph aph*).

Differences in mortality at the gametic and young zygotic stages

Differences in the three characters between parental and recombinant types in F_5 are shown in table 2. Parental genotypes showed no advantage to recombinant types in the three characters. Seed fertility reflects differential success in the development of female gametes and fertilized embryos. Differences in seed germinability and seedling surviving rate result from selection at young zygote stage. This result indicates that the associations found in F_5 were not due to zygotic selection between the time of fertilization to seed germination. The nonrandom associations may well be caused by gametic selection, however.

DISCUSSION

The distinction between *indica* and *japonica* is based on multi-locus association as indicated by many authors (Oka, 1958, Sato *et al.*, 1986; Glaszmann, 1987). They concluded that no single gene exists which is representative of *indica*-

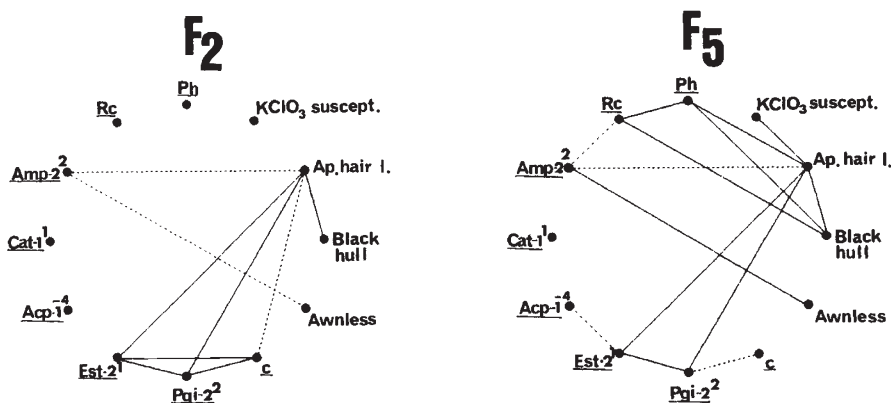


Figure 2 Associations among genes and characters in F_2 and F_5 populations.

Table 2 Differences in seed fertility (SF), seed germinability (SG) and seedling survival (SS) between parental and recombinant genotypes defined by two loci and characters associated nonrandomly in F₅

Associated loci and characters	SF (Percentage)			SG (Percentage)			SS (Percentage)		
	P†	R‡	D§	P†	R‡	D§	P†	R‡	D§
<i>Ph</i> -Black hull	85.2	84.0	1.2	95.0	96.8	-1.8	96.4	95.7	0.7
<i>Ph</i> - <i>Rc</i>	86.2	82.3	3.9	96.1	97.0	-0.9	98.2	95.2	3.0*
<i>Ph</i> - <i>Aph</i>	82.6	87.4	-4.8	96.2	96.4	-0.2	96.0	96.1	-0.1
KClO ₃ - <i>Aph</i>	83.2	86.7	-3.5	95.6	94.4	1.2	96.6	95.7	0.9
Black hull- <i>Rc</i>	85.7	82.5	3.2	95.4	94.1	0.3	97.0	96.3	0.7
<i>Awn</i> - <i>Amp</i> -2	85.1	84.5	0.6	97.0	95.1	1.9	97.2	96.0	1.2
<i>Est</i> -2- <i>Acp</i> -1	85.9	85.2	0.7	94.2	95.8	-1.6	95.7	96.8	-1.1

* Significant at 5 per cent level. No sign shows non-significant.

† Parental genotype

‡ Recombinant genotype

§ Difference (parental - recombinant)

japonica differentiation. Therefore, mechanisms responsible for multi-locus association among varieties should be examined to elucidate factors causing the *indica-japonica* differentiation.

Nonrandom association between alleles at two or more loci is caused by various evolutionary forces such as gametic selection, zygotic selection, random drift, linkage and nonrandom mating in higher plants (Hedrick *et al.*, 1978). Artificial selection also plays an important role in cultivated species.

In the *indica-japonica* hybrid populations, associations observed among the varietal sample largely disappeared in F₂. This indicates that the associations found among the varietal sample were partly caused by natural or artificial selections at the zygotic stage. Random drift may not be a causal factor because *indica-japonica* differentiation was also observed in African cultivars of *O. sativa* that have been established apart from Asian cultivars for a long time (de Kochko, 1987).

Associations found in the F₂ population are likely to be caused by linkage. Associations found in F₅ but absent in F₂, seven in total, could not, however, be explained by linkage. In fact, in five of the associations, the relevant loci are known to be carried on different chromosomes (*Ph* and *Rc*, *Acp*-1 and *Est*-2, *Rc* and *Amp*-2, *Ph* and *Bh*-a, *Bh*-b and *Rc* and *Bh*-a, *Bh*-b). Other association sets (KClO₃ susceptibility-apiculus hair length and *Ph*-apiculus hair length) appear also not to be explicable by linkage, although the linkage relationships of relevant genes are not fully known. In all cases except one, the nonrandom associations are brought about by an increase of parental genotypes relative to recombinants. No differences were observed in seed fertility, germination rate

and seedling survival between parental and recombinant genotypes (table 2), indicating that the non-random associations recovered in F₅ were not explained by selection due to differential fitness at the zygote stage.

In *indica* × *japonica* crosses, partial sterility of hybrids is frequently observed (*e.g.*, Oka, 1953, 1974; Oka and Doida, 1962; Yokoo, 1984; Ikehashi and Araki, 1986) and results in gametic selection. Competition between pollen grains during pollen tube elongation which is caused by particular genes also results in gametic selection. Many have argued that gametic selection and non-random fertilization, *e.g.*, competition among pollen grains and selective fertilization, play an important role in the evolution of higher plants (Ter Avanesian, 1978; Mulcahy, 1979; Hill and Load, 1986; Marshall and Ellstrand, 1986). It may be concluded that seven sets of associations found in F₅ were most probably due to gametic selection on the basis of the differential fertilizing abilities of gametes with different genotypes. It may be inferred that this trend for gametic selection acts as an internal mechanism for *indica-japonica* differentiation.

Acknowledgements The authors are grateful to Professors P. Hedrick, H. I. Oka, G. S. Khush and K. Yonezawa, for their encouragement and valuable advice.

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