Theor Appl Genet (1998) 96: 526–538

R. H. Li · C. G. Xue · L. P. Yuan · Y. Q. He
C. Q. Sun · S. B. Yu · X. H. Li · X. K. Wang

Differentiation and classification of parental lines and favorable genic interactions affecting F_1 fertility in distant crosses of rice (*Oryza sativa* L.)

Received: 5 June 1997 / Accepted: 10 September 1997

Abstract This study was intended to investigate the extent of genetic differentiation in parental lines of rice hybrids and to analyze the genetic basis underlying the fertility phenomenon in distant crosses. Two subsets of rice material (111 entries in total) were used, including 81 doubled-haploid (DH) lines and 30 Indica and Japonica rice varieties or lines (as a control). The DH lines was derived from a heterotic Indica/Japonica cross (Gui630/02428) by anther culture. The materials in the control represent a broad spectrum of the Asian cultivated rice gene pool including landraces, primitive cultivars, historically important cultivars, modern elite cultivars, super rice and parents of superior hybrids. In accordance with the NC II design, 57 out of the DH lines were test-crossed to two important wide compatibility lines: photoperiod-sensitive genetic male sterile (PGMS) line N422s and thermo-sensitive genetic male sterile (TGMS) line Peiai64s. The F_1s and their parents, 182 entries in total, were examined for the performance of seven traits in a replicated field trial. All the rice materials was surveyed for polymorphisms using 92 RFLP markers selected from two published molecular marker linkage maps. Genotypes of the F_1 hybrids at the molecular-marker loci were deduced from the parental genotypes. The analysis showed that there were two types of genetic differentiation in the two subsets of rice material; that is, qualitative differentiation in the control and quantitative differentiation in the DH lines. In addition, favorable genic interactions (both intra- or inter-locus) contributed to better increase the fertility in hybrids of distant crosses through incorporation of a wide-compatibility line as the female parent. Favorable genic interactions can be applied in hybrid rice breeding programs by selecting parents with an appropriate extent of genetic differentiation.

Key words *Oryza sativa* L. · Genetic differentiation · Classification of parental lines · Restriction fragment length polymorphism (RFLP) · Genic interaction

Communicated by G. Kush

R. H. Li · C. Q. Sun · X. K. Wang (✉)
Department of Plant Genetics and Breeding, China Agricultural University, Haidian, Beijing 10094, China
Fax: 086-010-62891055
E-mail: rhlksy@ihw.com.cn

C. G. Xue · Y. Q. He · S. B. Yu · X. H. Li
State Key Laboratory in Genetic Improvements of Crops, Huazhong Agricultural University, Wuhan 430070, China

L. P. Yuan
National Hybrid Rice Research and Development Center, Changsha 410125, China

Introduction

The differentiation of Indica and Japonica subspecies is of major importance for both the genetic and morphological differentiation of Asian cultivated rice (*Oryza sativa* L.) (Cheng and Wang 1987; Oka 1988; Wang and Tanksley 1989). Indica/Japonica hybrid rice promises to be of greater yield potential compared with intra-subspecific rice hybrids because two parents of the former are more distantly related (Yuan 1987). Scientists in China, therefore, have been pursuing exploration of such heterosis for almost a decade, although a series of problems have been encountered. Among the frequently occurring problems, the partial sterility of Indica/Japonica hybrid F_1s looms large. A recent prevailing explanation for this phenomenon is allelic interaction at several loci in the rice genome (Nakagahra 1972; Ikehashi and Araki 1986, 1988; Sano 1990, 1993; Lin and Ikehashi 1991; Lin et al. 1992; Yanagihara et al. 1992; Zhang and Lu 1993; Zhang et al. 1994 a).

Heterosis in rice is a complex biological phenomenon which has just started to be characterized (Zhang et al. 1994 b, 1996; Xiao et al. 1995). Two major hypotheses have been promulgated to explain the genetic basis of heterosis (Crow 1952). The dominant hypothesis, proposed by Davenpot (1908), Bruce (1910) and Keebl and Pellew (1910), supposes that heterosis is due to the suppression of deleterious recessives from one parent by dominant alleles from the other parent in the heterozygous F_1. The over-dominant hypothesis, proposed by Shull (1908) and East (1936), on the other hand, assumes that a heterozygous combination of alleles at a single locus is superior to either of the homozygous combinations of alleles at that locus. Evidence for these two hypotheses has been recently observed in many plants through a molecular genetic approach (Stuber et al. 1992; Bradshaw and Stettler 1995; Mitchell-Olds 1995; Xiao et al. 1995). However, heterosis can also be due to non-allelic interactions between different genic loci. Jinks and Jones (1958) first included epistasis into their quantitative genetic models dealing with heterosis. By means of a two-locus diallelic model, Minvielle (1987) later showed that heterosis might stem from multiplicative epistatic interaction rather than dominance effects.

Epistasis is the phenotypic effect of interaction among alleles at multiple loci. A belief in the importance of genetic interaction lies at the core of Wright's ideas concerning the genetic basis of evolution (Wright 1932, 1980; Provine 1986; Wade 1992) and plays a central role in founder-effect models of speciation (Templeton 1979, 1980; Carson and Templeton 1984). Allard (1996) illustrated marker-assisted analysis of the genetic basis of adaptedness with a sample of allozyme data from three species groups, two heavily selfing groups (two wild *Avena* species and barley) and one outcrossing species (corn, maize), and then drew the conclusion that the single most important genetic mechanism in all three species groups was the assembly of favorable epistatic combinations of different loci by means of recurring cycles of selection, intercrossing superior selections, and inbreeding to near homozygosity leading to stable superior multilocus genotypes adapted to specific habitats.

In the present study, we have used RFLP markers and a series of DH lines derived from a heterotic Indica/Japonica cross to study the effects of genic interactions on fertility in two test-cross F_1 populations. We were able to identify two kinds of favorable epistasis (interaction); namely, favorable homologous epistasis which results from allelic- or non-allelic interactions with genes from the same subspecies genome (i.e. Indica or Japonica genome), and favorable heterologous epistasis, which results from allelic- or non allelic interactions with genes from the two different genomes.

Materials and methods

Materials and mating design

A total of 111 entries of rice lines were composed of two subsets. The first subset was a doubled-haploid (DH) population consisting of 81 lines derived from a heterotic Indica/Japonica cross (Gui 630/02428), kindly provided by Dr. Ping Li, Sichuan Agricultural University. The second subset included 30 Indica and Japonica varieties or lines (used as a control) which represent a broad spectrum of the cultivated rice gene pool including landraces, primitive cultivars, historically important cultivars, modern elite cultivars and parents of superior hybrids (Table 1).

In 1996, the DH lines were test-crossed to two important rice lines, wide-compatibility Peiai64s (TGMS) and N422s (PGMS) respectively, following the NC II genetic mating design. The Indica line Peiai64s was developed by Prof. Xiao-he Luo and the first author in the Hunan Hybrid Rice Research Center (HHRRC). The morphologically Japonica-cline line, N422s, was developed by the same author and Prof. Xiang-kun Wang. Two test-cross F_1 populations were established including 56 F_1s for TCP1 (test-cross population 1 in which Peiai64s was used as a tester line) and 57 F_1s for TCP2 (test-cross population 2 in which N422s was used as the tester line).

Table 1 Lines used in the present study, their varietal type and origin

Line	Type[a]	Origin	Line	Type	Origin
G01-G81	DH	China			
IR64446-7-10-5	New plant type, J	IRRI	Padi-1	I	Indonesia
IR66160-14-5-3-2	New plant type, J	IRRI	Padi-3	I	Indonesia
IR65600-21-2-2-3	New plant type, J	IRRI	padi-4	I	Indonesia
IR65598-112-2	New plant type, J	IRRI	Peiai 64	I	China
Shennung	New plant type, J	IRRI	6078	I	China
Akihikari	J	Japan	Minghui 63	I	China
Nongken58s	J	China	Gui 630	I	Guiana
Wuyukeng 3	J	China	IR8	I	IRRI
02428	J	China	Fu 1312	I	China
C8420	J	China	Zhong 413	I	China
Liao 198	J	China	Teqing	I	China
C418	J	China	Nanjing 11	I	China
N422s	Intermediate	China	ZS97A	I	China
Lunhui 422	Intermediate	China	Peiai64s-1	I	China
NP190	Intermediate	China	Peiai64s-2	I	China

[a] I – Indica; J – Japonica

Field experiment and data collection

In the late spring of 1996, Seeds of F_1s and corresponding parents (182 entries in total) were sown in a paddy nursery in the Rice Experiment Farm of Huazhong Agricultural University, Wuchang. At the 4–5 leaf stage, seedlings were transplanted at a 16.7×26.7-cm spacing following a completely randomized block design with three replications. Ten seedlings for each plot were planted, with purple rice used as guard lines. Field management followed essentially the practice under normal rice cultivation.

Six-to-eight plants in the middle of each plot were examined for seven quantitative traits: (1) heading date, scored as number of days starting from May 15, the seeding date, (2) plant height, as the length of the tallest tiller for each plant, (3) panicle length, as the average length of all the panicles of each plant, (4) tillers per plant, as the number of seed-setting tillers of a plant, (5) seeds per panicle, as the total number of seeds threshed from each plant divided by the number of tillers per plant, (6) yield per plant, measured as the weight of all filled seeds, and (7) grain weight, which is the total seed weight divided by the total number of seeds per plant.

Molecular-marker assay

Tissue for each of the materials was collected *en mass* from about 20 seedlings planted in the nursery. DNA was extracted following the protocol proposed by Saghai Maroof et al. (1984).

The co-dominant markers, restriction fragment length polymorphisms (RFLPs), were used for surveying the DNA polymorphism of all the lines and varieties. About 2.5 μg of total cellular DNA for each sample was digested with each of the five restriction endonucleases, *Eco*RI, *Bam*HI, *Hin*dIII, *Eco*RV, and *Dra*I, and probed with 92 polymorphic cloned markers selected from two published RFLP linkage maps (Causse et al. 1994; Kurata et al. 1994). Electrophoresis, blotting, probe-labelling and hybridization followed the methods described by Zhang et al. (1994).

Measurement of genetic differentiation in DH lines

Morphological character-index methods as proposed by Cheng and Wang (1987) proved to be effective in differentiating Indica (*O. sativa* L. subsp. *indica*) and Japonica (*O. sativa* L. subsp. *japonica*). The comprehensive index was based on scoring the following six diagnostic traits: apiculus hair, leaf pubescence, length-to-width ratio of grains, phenol reaction, length of 1st and 2nd rachis internode and glume color at anthesis. According to Cheng's scoring system, a rice line with a total scoring value (index) of 0–8 belongs to Indica, 9–13 belongs to Indica-cline, 14–17 belongs to Japonica-cline and 18–24 belongs to Japonica.

The 111 entries of lines and varieties were assigned to either of the subspecies, i.e. Hsien (Indica) or Keng (Japonica), according to Cheng's morphological trait index. The gene frequency of each allele detected by RFLP analysis was calculated following a two-way classification of the Indica and Japonica groups. If the Indica and/or Japonica gene frequency at a certain locus was over 80% then we could say that it was a specific marker locus associated with Indica and/or Japonica differentiation. Two major alleles at the locus are specific alleles assigned for Indica and/or Japonica.

Data processing and statistical analysis

The RFLP data were scored and processed using the following method. The marker genotype of a F_1 hybrid was inferred from its parental genotypes. For the F_1s within a certain test-cross population the polymorphism of alleles at a locus from paternal DH lines reflected the polymorphism of each test-cross population, because the tester lines (Peiai64s or N422s) contain identical alleles at that locus. Thus it was convenient to conduct analysis of variance by grouping alleles from the male parents. For most single-copy co-dominant markers used in this study, a score of 1 was assigned to those that possessed an identical band, like Gui 630, an Indica, and a score of 2 to those that possessed an identical band, like 02428, a Japonica. For some dominant markers, 1 was given to the band identical to that of Gui 630 with presence, and 0 to that like 02428 with absence. In contrast, 2 was assigned to the band identical to 02428 with presence, and 0 to that like Gui 630 with absence.

To conduct cluster analysis, the score of RFLPs was transferred to data of type 0 and 1, that is, 1 was assigned to the presence of a phenotype and 0 to the absence of a phenotype. Genetic distance was calculated following Nei (1987). The 92 polymorphic loci were subjected to cluster analysis using the UPGMA (unpaired group-mean arithmetic) method.

The effect of a chromosomal region, as marked by a molecular marker, on a trait (as represented by heterosis over a male parent) was assayed with a one-way analysis of variance using markers (allele types or bands) as groups and entries within marker types as the error term. Markers that detected significant effects on a trait at the 0.01 (P value) probability level were referred to as positive markers for that trait. The proportion of the total phenotypic variation explained by each marker associated with a putative QTL was calculated as an R^2 value (R^2 stands for the ratio of the sum of squares explained by the marker locus to the total sum of squares).The effects of genic epistasis (interaction) at two loci on a trait was examined with two-way analysis of variance, using the combinations of marker types from two loci as groups and entries within marker-type combinations as the error term. All the analyses were run on a Macintosh 7200 using the SAS program (SAS institute 1988).

Results

Detection of putative marker loci associated with Indica and Japonica differentiation

Among the 92 RFLP markers assayed, 42 RFLP markers were detected as specific markers which were associated with Indica and Japonica differentiation (Table 2). They were distributed on all 12 chromosomes of rice, suggesting that multi-loci were involved in the differentiation of Indica and Japonica. Among the 111 entries of rice lines, 98 kinds of genotypes were grouped based on 41 specific marker loci, which implies that the differentiation of *O. sativa* is a complicated evolutionary process.

Genetic differentiation and classification of all entries

The frequency distribution of Cheng's index in the DH lines and control is shown in Fig. 1. In DH lines the histogram showed a continuous variation with an almost normal distribution, while a bimodal distribution was present in the control, suggesting two different types of morphological differentiation persisted within *O. sativa.*

Considering the specific marker loci associated with Indica and Japonica differentiation, two types of

Table 2 Diagnostic marker loci and their chromosomal distribution

Agreement (%)	Marker (location)					
82.1	RG64(6)[a] R2677(7)[a]	RG101(3)[ab]	RG256(2)[ab]	RZ993(3)[ab]	C217(3)[b]	C1087(4)[n]
85.7	RG118(11)[n] C25(3)[ab]	RG167A(11)[a] C445(4)[ab]	RG462(1)[a] C1232(9)[b]	RG553(9)[b] C1336(12)[n]	RG667(9)[ab] R2071(6)[ab]	RG869(12)[n]
89.3	RG104(3)[ab] C595(3)[ab]	RG375(4)[ab] C621B(?)[ab]	RG470(5)[b] C1057(7)[ab]	RG598(8)[ab]	C153C(?)[n]	C454(12)[ab]
92.9	RG134(10)*	RG324(2)[ab]	RG482(3)[n]	RG536(1)[ab]	RG811(1)[ab]	R2635(1)[ab]
96.4	RG303(11)[n]	C39B(7)[ab]	C560(2)[b]	R1927(3)[b]		
100	RG351(7)[ab]	C621A(2)[ab]	C728(7)[ab]	C1236(2)[ab]	G44(11)[ab]	

[a] Positive markers for grain yield and/or yield components in TCP1
[b] Positive markers for grain yield and/or yield components in TCP2
[a b] Positive markers for grain yield and/or yield components in both TCP1 and TCP2
[n] Not positive markers for either TCP1 or TCP2
* Not included in statistics because of the vague hybridization band in the X-ray sheet of DH lines

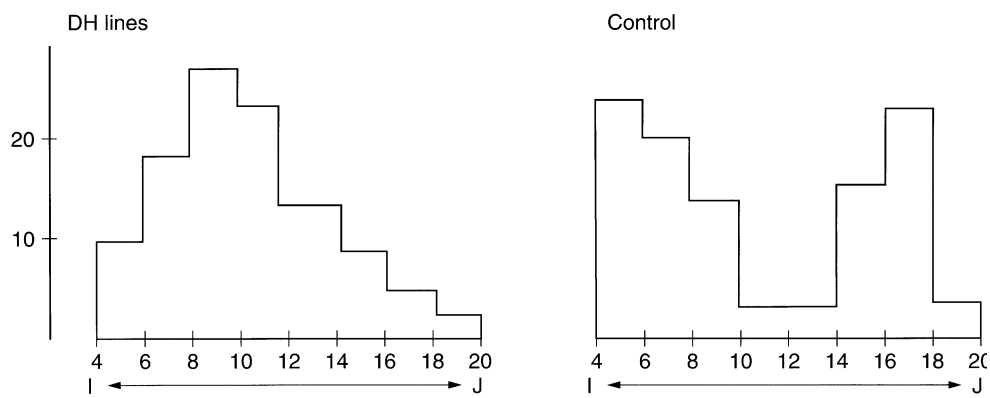


Fig. 1 Frequency distribution of Cheng's six diagnostic character indices based on 81 DH lines and their parents, including another set of lines used as a control

genetic differentiation were present in the two subsets of rice lines. The first subset of DH lines was characterized by on average 51.5% (30.8–69.2) alleles differentiating toward Indica and 48.5% (30.8–69.2) alleles differentiating toward Japonica (Table 3), suggesting that quantitative differentiation (or accumulative differentiation) was overdominant. Qualitative differentiation, however, was observed in most varieties in the control (accounting for 22.5% of the total entries), characterized by on average 94.4% (85.4–100) alleles differentiated well into Indica or 94.1% (90–100) alleles differentiated well into Japonica (Table 4).

The 111 entries of rice lines were subjected to cluster analysis using the genetic distance following Nei (1987). The dendrogram is shown in Fig. 2. At a distance of 0.3, three groups could be classified; namely, Indica, Japonica, and intermediate groups. The Indica group included all the morphological Indica varieties or lines in the control and 17 DH lines. The Japonica group consisted of all the morphological Japonica varieties or lines in the control and only one DH line. The third group, intermediate between Indica and Japonica, was composed of 63 DH lines which accounted for 77.8% of the total DH lines. The classification was almost consistent with that based on Cheng's morphological trait index, regarding the Hsien-cline and Keng-cline types as intermediate.

One-way analysis of variance

In TCP1, one-way ANOVA was conducted to test the significance of phenotypic effects (as measured by the heterosis of yield and yield components as well as growth duration and plant height). As a result, 59 positive markers were detected at the probability level of 0.01 in TCP1 and 57 positive markers in TCP2 (Table 2). Eighty one per cent of the markers detected were duplicative between the two test-cross populations. Two major putative QTLs associated with grain yield (GY) were detected. One, linked to the marker C223 on chromosome 10, could explain total variance by 18.2% in TCP1 and 20.4% in TCP2. The other putative QTL, found located between two closely linked markers on chromosome 1, R1928 and RG811, explained total variance by 7.6% vs 13.6% in TCP1

Table 3 Accumulative differentiation in the genetics of DH lines

Line	No. Indica loci (I)	No. Japonica loci (J)	Percent I (%)	Percent J (%)	Line	No. Indica loci (I)	No. Japonica loci (J)	Percent I (%)	Percent J (%)
G02	23	18	56.1	43.9	G42	20	21	48.8	51.2
G03	24	16	60.0	40.0	G43	25	15	62.5	37.5
G04	24	17	58.5	41.5	G44	18	23	43.9	56.1
G05	24	17	58.5	41.5	G45	28	13	68.3	31.7
G06	23	18	56.1	43.9	G46	28	13	68.3	31.7
G07	21	20	51.2	48.8	G47	17	24	41.5	58.5
G08	19	22	46.3	53.7	G49	18	22	45.0	55.0
G09	21	20	51.2	48.8	G50	19	21	47.5	52.5
G10	19	13	59.4	40.6	G51	18	22	45.0	55.0
G11	17	23	42.5	57.5	G55	24	17	58.5	41.5
G12	24	15	61.5	38.5	G56	20	21	48.8	51.2
G13	27	12	69.2	30.8	G57	22	18	55.0	45.0
G14	25	16	61.0	39.0	G58	20	21	48.8	51.2
G15	25	16	61.0	39.0	G59	24	16	60.0	40.0
G16	27	14	65.9	34.1	G61	19	19	50.0	50.0
G17	25	16	61.0	39.0	G62	14	27	34.1	65.9
G18	12	27	30.8	69.2	G63	28	13	68.3	31.7
G20	17	23	42.5	57.5	G64	18	23	43.9	56.1
G22	19	21	47.5	52.5	G65	15	23	39.5	60.5
G23	20	21	48.8	51.2	G66	19	22	46.3	53.7
G24	19	22	46.3	53.7	G67	16	20	44.4	55.6
G25	25	16	61.0	39.0	G68	23	17	57.5	42.5
G26	21	20	51.2	48.8	G69	17	19	47.2	52.8
G27	19	22	46.3	53.7	G70	18	22	45.0	55.0
G28	20	21	48.8	51.2	G71	15	16	48.4	51.6
G29	19	19	50.0	50.0	G73	18	23	43.9	56.1
G30	19	22	46.3	53.7	G74	18	21	46.2	53.8
G31	24	17	58.5	41.5	G75	18	21	46.2	53.8
G32	20	21	48.8	51.2	G76	25	14	64.1	35.9
G33	20	19	51.3	48.7	G77	19	22	46.3	53.7
G34	18	23	43.9	56.1	G78	19	22	46.3	53.7
G35	23	18	56.1	43.9	G79	19	22	46.3	53.7
G36	21	20	51.2	48.8	G80	13	28	31.7	68.3
G37	22	17	56.4	43.6	G81	25	16	61.0	39.0
G38	20	19	51.3	48.7	Ave			51.5	48.5
G39	23	18	56.1	43.9	Min			30.8	30.8
G40	20	21	48.8	51.2	Max			69.2	69.2
G41	19	22	46.3	53.7	Stdev			8.5	8.5

and 15.1% vs 14.0% in TCP2. These three important markers were also found associated with the heterosis of yield components, suggesting the presence of pleiotropic effects of the major QTLs.

More interesting, among the 41 specific markers associated with Indica and Japonica differentiation, 28 (68.3%) were also positive markers associated with heterosis of grain yield and yield components in TCP1, and 30 were positive markers in TCP2 (Table 2). Twenty four such markers (accounting for 58.5%) were shared by the two populations. Thirty six (87.8%) markers were found to be positive in at least one population, with the exception of only five markers (12.2%).

Effects of genic interaction on seedset and other traits in TCP1

Favorable genes are those that best adapt to a specific environment after long-term human and natural selection. Favorable genic epistasis is the type of genic interaction that produces significant positive phenotypic effects to best adapt to a specific environment. For example, augmented fertility can increase productivity and adaptability. We detected two types of fovourable inter-locus interactions, and representative examples are provided in Table 5.

The results of two-way ANOVA in TCP1 are shown in Table 6. A total of 344 favorable genic interactions at the probability level of 0.05 were detected based on 28 specific markers and four other important positive markers (C223, R1928, C181, RG776). A total of 118 out of the 344 genic interactions (accounting for 34.6%) were on seedset (SS) and the remaining two-thirds on the other five traits including grain yield (GD), grain weight per panicle (GW/PNL), 1000-grain weight (1000GW), spikelets per panicle (SN/PNL) and panicle number (PN). With regard to the yield components like SS, 1000GW, SN/PNL and PN, as large a proportion as 57.8% of the genic interactions persisted on SS.

Table 4 Qualitative genetic differentiation in the subset of most lines of the control

Line	No. Indica loci (I)	No. Japonica loci (J)	Percent I (%)	Percent J (%)
Akihikari	0	41		100
02428	0	41		100
Nongken58s	1	40		97.6
Wuyujing 3	2	39		95.1
Shennung	2	38		95.0
C8420	3	38		92.7
Liao198	4	37		90.2
IR66160-14-5-13-2	4	37		90.2
IR65598-112-2	4	37		90.2
IR65600-21-2-2-3	4	36		90.0
Peiai64s-1	35	6	85.4	
Peiai64s-2	35	6	85.4	
Padi-4	35	4	89.7	
ZS97A	36	4	90.0	
Nanjing11	36	4	90.0	
Padi-1	39	2	95.1	
Padi-3	39	2	95.1	
Peiai64	39	2	95.1	
Zhong413	40	1	97.6	
FU1312	40	1	97.6	
Teqing	40	1	97.6	
IR8	40	1	97.6	
6078	41	0	100	
Minghui63	41	0	100	
Gui630	41	0	100	
Ave			94.4	94.1
Max			100	100
Min			85.4	90.0
Stdev			5.1	4.0

Digenic interaction, therefore, can to a larger extent explain the fertility problem in distantly crossing F_1 hybrids.

From Table 6 we can idenifty the proportion of epistasis types I, II and III for the different traits. With the exception of SS, all other traits displayed the order of III or II > I, suggesting the relative importance of inter-locus interactions. For SS the order was reversed, indicating the relative importance of intra-locus allelic interactions. As a result of both inter- and intra-locus interactions for each yield component and higher order interactions among yield components, grain yield is affected by complicated high-order epistasis. Hence, digenic interactions could hardly explain it.

Further, 86 (73%) of the 118 favorable genic interactions acting on SS belonged to type-I epistasis (Table 6), i.e. involving significant intra-locus interactions for both loci and significant inter-locus interactions. Type-II epistasis (significant intra-locus interaction for either locus and significant inter-locus interactions) and type-III epistasis (non-significant intra-locus interaction for neither locus but significant inter-locus interactions) accounted for only 19% and 7.6%, respectively (Table 7). Moreover, for type-I, 55 (64%) genic interactions were significant at the probability level of 0.01 with respect to the three *P* values (two for intra-locus and one for inter-locus), suggesting the importance of both intra- and inter-locus genic interactions for SS.

The classification of inter-locus interactions is shown in Table 7. In terminology, we defined two types of favorable inter-locus genic interactions; namely, heterologous interaction and homologous interaction. The former is referred to as favorable epistasis between allele 1 from the Indica genome (Gui 630) at one locus and allele 2 from the Japonica genome (02428) at the other locus (12E and/or 21E). The latter is the result of favorable non-allelic epistasis between two loci from the single Indica (Gui 630) or Japonica (02428) genome (11E and/or 22E). In type-I epistasis for SS, 68% (59) were attributed to favorable heterologous interactions and 32% (27) to favorable homologous interactions. Favorable heterologous interactions were also overdominant in type-II and type-III epistasis (see Table 7). It can be inferred that favorable heterologous interactions have a great bearing on SS in the F_1s from crosses of distantly related parents. As far as F_1 genotypes were concerned, there was another pair of inter-locus genic interactions for each locus-pair originating from a single maternal line. Such kinds of favorable genic interaction were also inherent products of long-term human and natural selection in elite lines.

Further analysis of intra-locus epistasis is shown in Table 8 with regard to type-I epistasis for SS in TCP1. There were also two kinds of favorable intra-locus epistasis, namely, homologous (A type) and heterologous (H type). Such intra-locus allelic interaction was parallel to the notion of overdominance. We drew two conclusions from the data. First, the direction of interaction for a favorable gene with any other one was not changed, i.e. the positive or negative effects of homozygous or heterozygous combinations of alleles on SS were invariable, although locus-pairs were varied. Second, dominant genes were favorable for higher values of SS and recessive genes were less favorable or unfavorable, which was displayed by all the dominant markers.

Effects of genic interaction on fertility and other traits in TCP2

To compare the reliability of results, the same method was adopted to analyze TCP2. A total of 366 favorable genic interactions were detected using 30 specific markers associated with Indica and Japonica differentiation plus three important positive markers (C223, R1928, C181) (Table 9). Favorable genic interactions of 116 pairs (31.7%) acted on SS, which was similar to that in TCP1. The trend of proportions among epistasis types I, II and III was in agreement with that in TCP1. For SS the proportion of favorable interactions was 56% for type I, 23.3% for II and 20.7% for III. Considering the yield components (PN, SN/PNL, SS

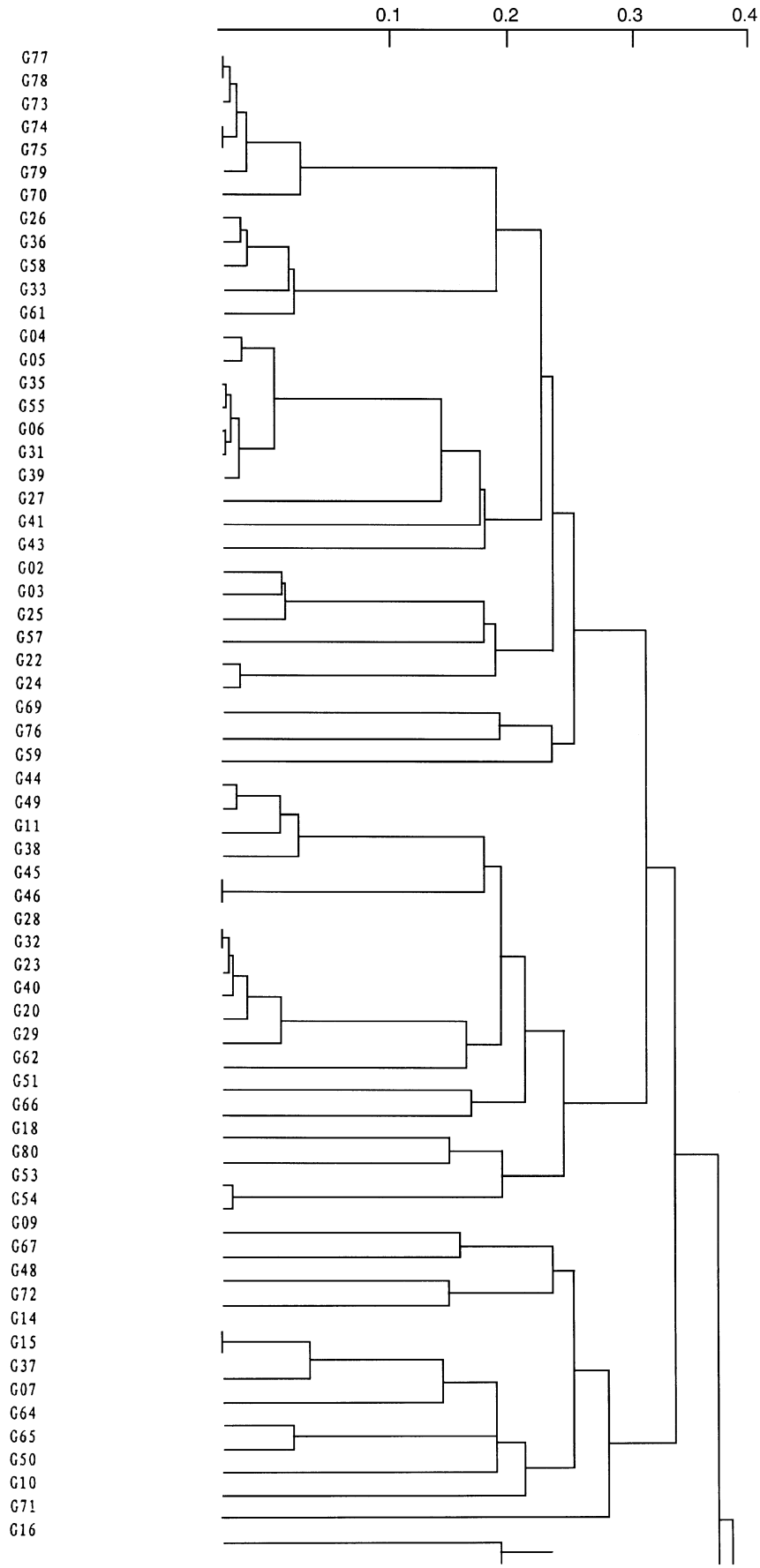
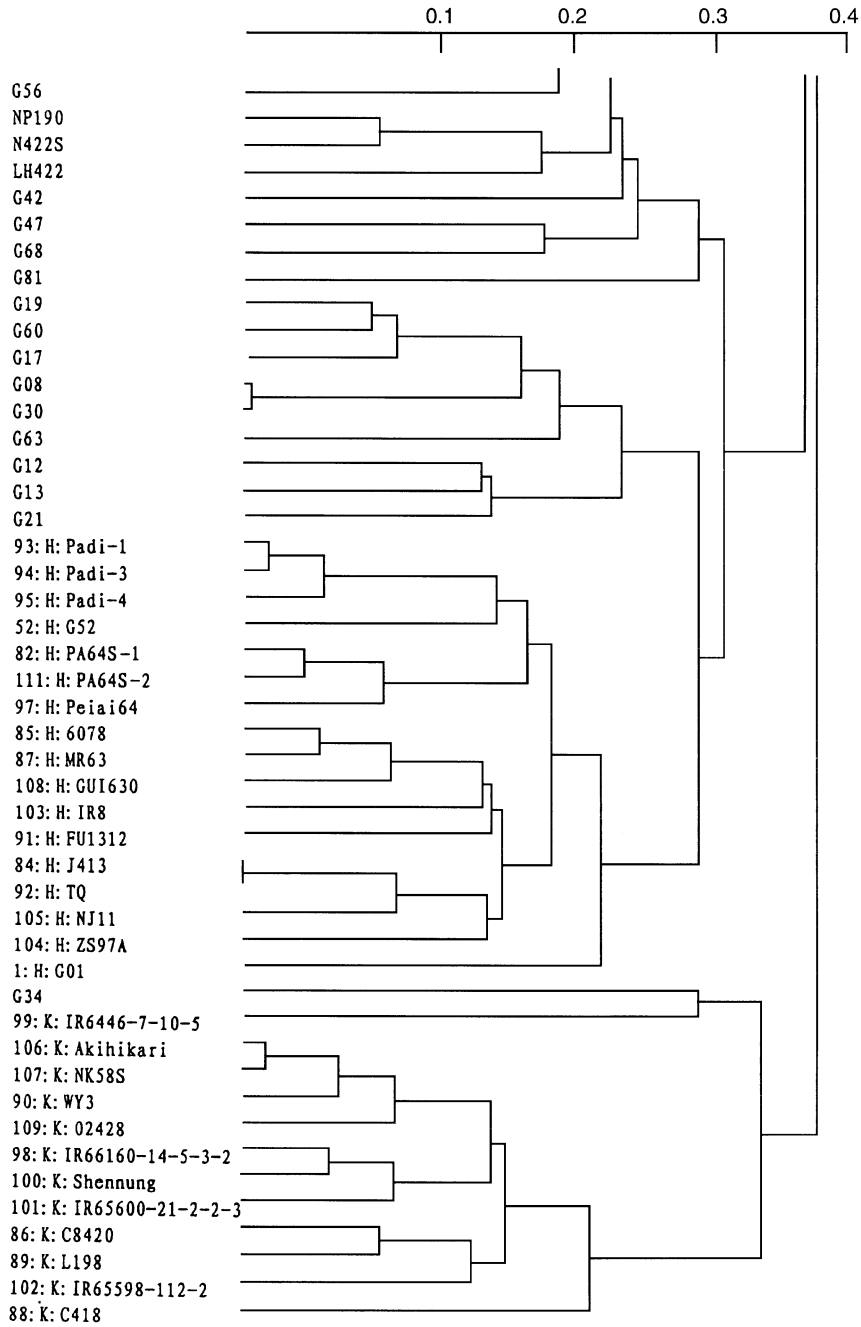


Fig. 2 Dendrogram of 111 rice lines based on 185 phenotypes detected by RFLP analysis



and 1000GW) only, the proportion was 62.7% for SS. Hence, the view was supported that digenic interactions (both inter- and intra-locus) can reasonably explain the genetic basis of the fertility problem in distant crosses of rice.

Discussion

The advent of DNA markers such as RFLPs allows hundreds of markers to be surveyed in a single population. Segments of DNA, and therefore the genes carried on them, from both parents can be compared pair-wise to examine the genetic basis of heterosis. Evidence has indicates that the complexity of the genetic basis of hybrid performance and heterosis has only just started to be characterized (Zhang et al. 1994, 1996; Xiao et al. 1995). Epistasis is an important genetic basis for complex traits such as yield and yield components like grain number per panicle (GN) and grain weight per panicle (GWP) (Li et al. 1997; Yu et al. 1997).

Table 5 Examples of favorable inter-locus homologous and heterologous epistasis on SS

12E:

RG536:	Allele 1	Allele 2	Mean phenotype
C621A: Allele 1	0.09[a] (25)	1.2366 (7)	0.3408
Allele 2	0.2931 (9)	0.1727 (11)	0.2269
Mean phenotype	0.1438	0.5864	0.297

21E:

C1057:	Allele 1	Allele 2	Mean phenotype
R2635: Allele 1	0.19 (13)	0.0734 (20)	0.1199
Allele 2	1.4347 (6)	0.2113 (17)	0.5305
Mean phenotype	0.583	0.1368	0.2882

11E:

RZ993:	Allele 1	Allele 2	Mean phenotype
RG375: Allele 1	0.9355 (10)	0.3421 (8)	0.6717
Allele 2	0.082 (24)	0.1399 (11)	0.1002
Mean phenotype	0.333	0.225	0.2943

22E:

R2635:	Allele 1	Allele 2	Mean phenotype
R2071: Allele 1	0.1402 (21)	0.2388 (11)	0.1741
Allele 2	0.0727 (11)	0.9166 (10)	0.4746
Mean phenotype	0.117	0.5616	0.2932

[a] Average phenotypic value based on the number of hybrid combinations in parentheses

A total of 42 marker loci, whose allele frequency was over 80% in Indica and/or Japonica, was detected after surveying 92 RFLP markers which were deliberately selected based on published data. Our parallel study indicated that SS was the most sensitive and important factor which has a close relationship with the extent of parental differentiation characterized by the relative gene frequency of Japonica [Dj = Fj/(Fi + Fj), where Fj is the gene frequency of Japonica at a locus, Fi is the gene frequency of Indica at the same locus]. Other yield components like PN and 1000GW were less influenced by changes in the extent of parental differentiation (data not shown). Further, 87.8% of the specific marker loci associated with Indica and Japonica differentiation were involved in significant effects on heterosis of yield and yield components in at least one of the test-cross populations. Alleles present at these loci are the result of the evolution of adaptedness after long-term human and natural selection. A non-random association of alleles at the 41 specific marker loci has been established for well-differentiated Indica and Japonica rice lines (accounting for 22.8%) which can adapt to specific environments as is evident in the distribution of different rice ecotypes. But the situation is quite different in DH lines derived from a cross between Indica and Japonica. Quantitative differentiation was dominant in the population of such lines due to the fact that recombination of genes occurred only once in the process of its development and the lack of rigorous selection under a specific environment.

The hybrid fertility of rice is a complicated phenomenon which has been documented and studied for more than 70 years. To determine the gene system for F_1 sterility, different hypothetical models have been set up and tested for fitness to the data (Oka 1974, 1988). Two influential hypothetical models have been proposed and adopted to explain the genetic basis of F_1 sterility in distant rice crosses. The first model refers to the duplicate gametic lethal hypothesis proposed by Oka (1974). Two independent loci are involved in this model. Gametes carrying s_1s_2 are aborted, while the genes affect both male and female gemetes or male gametes only. The second model,

Table 6 Two-way ANOVA using 28 diagnostic marker loci, plus another four important positive markers, to test for favourable genic interactions in TCP1

Type of epistasis[a]	GY	GW/PNL	1000GW	SS	SN/PNL	PN	Total
I	18 (0.22)	14 (0.24)	2 0.04)	86 (0.73)	1 (0.06)	2 (0.08)	123
II	35 (0.42)	16 (0.27)	29 (0.64)	23 (0.19)	4 (0.2)	6 (0.25)	113
III	28 (0.34)	29 (0.49)	13 (0.29)	9 (0.076)	13 (0.72)	16 (0.67)	108
Total	81 (0.24)	59 (0.17)	44 (0.13)	118 (0.346)	18 (0.05)	24 (0.07)	344

[a] I – significant intra-locus interaction for both loci and significant inter-locus interaction
II – significant intra-locus interaction for either locus and significant inter-locus interaction
III– non-significant intra-locus interaction for neither locus but sigficant inter-locus interaction

Table 7 Inter-locus epistasis analysis for SS in TCP1

Type I				Type II				Type III			
12E[a]	21E[b]	11E[c]	22E[d]	12E	21E	11E	22E	12E	21E	11E	22E
40 (0.46) 59(0.68)	19 (0.22)	9 (0.10) 27(0.31)	18 (0.21)	8 (0.35) 15(0.65)	7 (0.30)	4 (0.17) 8(0.34)	4 (0.17)	5 (0.55) 7(0.77)	2 (0.22)	1 (0.11) 2(0.22)	1 (0.11)

[a,b] 12E vs 21E: favorable non-allelic interactions between allele 1 from the Indica genome (Gui 630) at one locus and allele 2 from the Japonica genome (02428) at the other locus
[c,d] 11E vs 22E: favorable non-allelic interaction between two loci from the single Indica (Gui 630) or Japonica genome (02428)

Table 8 Favorable intra-locus allelic interaction on SS (type I) in TCP1

Epistasic locus pair	Inter-locus		First locus		Second locus	
	Type	*P*	Type[a]	*P*	Type	*P*
C1057-C181	12E	0.0001	H > A	0.0001	H > A	0.0001
C1057-C445	12E	0.0004	H > A	0.0001	H > A	0.0001
C1057-RG324	12E	0.0079	H > A	0.0379	H > A	0.0364
C1057-RG375	11E	0.0038	H > A	0.0011	A > H	0.0001
C1057-RG536	12E	0.0001	H > A	0.0001	A > H	0.0001
C1057-RG598	12E	0.0001	H > A	0.0001	H > A	0.0015
C1057-RG667	12E	0.0011	H > A	0.0009	H > A	0.0047
C1057-RG776	12E	0.0001	H > A	0.0001	H > A	0.0001
C1057-RG811	12E	0.0015	H > A	0.0016	H > A	0.0002
C1236-C181	12E	0.0048	A > H	0.0182	H > A	0.013
C1236-RG375	11E	0.0453	A > H	0.0288	A > H	0.0011
C1236-RG536	12E	0.01	A > H	0.0283	A > H	0.0288
C1236-RG598	12E	0.0001	A > H	0.0001	H > A	0.0001
C1236-RG667	12E	0.0001	A > H	0.0002	H > A	0.0003
C1236-RG811	12E	0.0001	A > H	0.0001	H > A	0.0001
C153B-C1236	11E	0.0469	01 > 00	0.005	A > H	0.0289
C153B-C181	12E	0.007	01 > 00	0.0015	H > A	0.0065
C153B-C223	12E	0.0219	01 > 00	0.0052	A > H	0.0087
C153B-C223	12E	0.0219	01 > 00	0.0052	A > H	0.0087
C153B-RG536	12E	0.0161	01 > 00	0.0019	A > H	0.0069
C181-RG375	21E	0.0015	H > A	0.002	A > H	0.0002
C181-RG811	22E	0.0034	H > A	0.0026	H > A	0.0002
C223-C1236	21E	0.0311	A > H	0.0082	A > H	0.0339
C223-C598	22E	0.0045	A > H	0.0015	H > A	0.036
C223-C621A	21E	0.0002	A > H	0.0007	A > H	0.0069
C223-R1928	22E	0.0141	A > H	0.0019	H > A	0.0003
C223-R2635	22E	0.0329	A > H	0.0021	H > A	0.0019
C223-RG101	21E	0.0001	A > H	0.0001	H > A	0.0009
C223-RG811	22E	0.0022	A > H	0.0009	H > A	0.0002
C39B-C1057	21E	0.0007	02 > 00	0.0019	H > A	0.0009
C454-RG536	12E	0.0001	A > H	0.0009	A > H	0.0003
C595-C1057	21E	0.0303	H > A	0.0113	H > A	0.0283
C621A-RG536	12E	0.0002	A > H	0.0088	A > H	0.0021
C621A-RG811	12E	0.0049	A > H	0.0378	H > A	0.001
C621B-C1057	11E	0.0008	11 > 10	0.0001	H > A	0.0001
C621B-C1236	11E	0.0276	11 > 10	0.0085	A > H	0.0227
C621B-RG536	12E	0.0438	11 > 10	0.0096	A > H	0.0154
C621B-RG598	12E	0.0076	11 > 10	0.0028	H > A	0.0288
C728-C445	12E	0.0482	A > H	0.1468	H > A	0.0415
C728-R1928	12E	0.0073	A > H	0.0201	H > A	0.0004
C728-RG536	12E	0.0008	A > H	0.0088	A > H	0.0021
C728-RG811	12E	0.0078	A > H	0.072	H > A	0.0042
R1928-C1236	21E	0.0287	H > A	0.0007	A > H	0.021
R1928-C181	22E	0.0017	H > A	0.0006	H > A	0.0033
R1928-C445	22E	0.0099	H > A	0.0006	H > A	0.0057
R1928-C536	22E	0.0003	H > A	0.0001	A > H	0.0001
R1928-RG101	21E	0.0001	H > A	0.0001	A > H	0.0009

Table 8 Continued

Epistasic locus pair	Inter-locus		First locus		Second locus	
	Type	*P*	Type[a]	*P*	Type	*P*
R1928-RG351	21E	0.0073	H > A	0.0003	A > H	0.0155
R1928-RG64	22E	0.0001	H > A	0.0001	H > A	0.0001
R1928-RG667	22E	0.002	H > A	0.0001	H > A	0.0058
R2071-C1057	21E	0.0001	H > A	0.001	H > A	0.0001
R2071-C1236	21E	0.0001	H > A	0.0002	A > H	0.0001
R2071-C621B	21E	0.025	H > A	0.0823	11 > 10	0.0123
R2071-R1928	22E	0.0055	H > A	0.0093	H > A	0.0001
R2071-RG536	22E	0.0015	H > A	0.0095	A > H	0.003
R2071-RG811	22E	0.0063	H > A	0.0238	H > A	0.0003
R2635-C1057	21E	0.0002	H > A	0.0001	H > A	0.0001
R2635-C1236	21E	0.0406	H > A	0.006	A > H	0.0307
R2635-C181	22E	0.0197	H > A	0.0029	H > A	0.0096
R2635-RG101	21E	0.0015	H > A	0.0006	A > H	0.0081
R2635-RG536	22E	0.0026	H > A	0.0004	A > H	0.0016
R2635-RG598	22E	0.0005	H > A	0.0001	H > A	0.0021
R2677-C1057	21E	0.0343	A > H	0.0362	H > A	0.0447
RG101-C445	12E	0.0143	A > H	0.0449	H > A	0.0114
RG101-RG536	12E	0.0001	A > H	0.0001	A > H	0.0001
RG101-RG811	12E	0.0001	A > H	0.0015	H > A	0.0001
RG2635-RG64	22E	0.0095	H > A	0.0034	H > A	0.0159
RG375-C445	12E	0.0291	A > H	0.0022	H > A	0.0211
RG375-RG101	11E	0.0004	A > H	0.0001	A > H	0.0043
RG375-RG351	11E	0.0162	A > H	0.0006	A > H	0.0272
RG375-RG462	11E	0.0026	A > H	0.0001	A > H	0.007
RG375-RG536	12E	0.007	A > H	0.0002	A > H	0.0033
RG375-RG598	12E	0.0018	A > H	0.0001	H > A	0.004
RG375-RG64	12E	0.0001	A > H	0.0001	H > A	0.0001
RG375-RG667	12E	0.0106	A > H	0.001	H > A	0.0363
RG462-RG811	12E	0.0012	A > H	0.008	H > A	0.0001
RG536-RG351	21E	0.0009	A > H	0.0012	A > H	0.0051
RG536-RG351	21E	0.0009	A > H	0.0012	A > H	0.0051
RG536-RG462	21E	0.0001	A > H	0.0001	A > H	0.0001
RG536-RG598	22E	0.0001	A > H	0.0001	H > A	0.0001
RG536-RG811	22E	0.0012	A > H	0.0002	H > A	0.0001
RG598-C445	22E	0.0001	H > A	0.0001	H > A	0.0001
RG598-RG811	22E	0.0001	H > A	0.0001	H > A	0.0004
RG811-C445	22E	0.0261	H > A	0.0003	H > A	0.003
RG811-RG64	22E	0.0027	H > A	0.0002	H > A	0.0026
RZ993-RG811	12E	0.0001	H > A	0.0006	H > A	0.0001

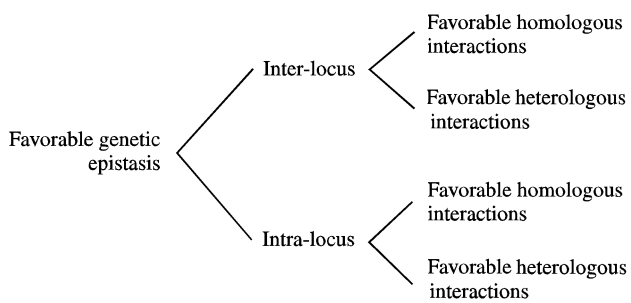
[a] A and H indicate homozygous and heterozygous combinations of alleles at a locus, respectively; 1 and 2 represent dominant genes from the Indica or Japonica genome, respectively; 0 stands for recessive genes

Table 9 Two-way ANOVA using 30 diagnostic marker loci plus three important positive markers to test favorable genic interactions in TCP2

Type of epistasis[a]	GY	GW/PNL	1000GW	SS	SN/PNL	PN	Totals
I	10 (0.103)	9 (0.107)	3 (0.111)	65 (0.560)	1 (0.053)	6 (0.261)	94
II	30 (0.309)	18 (0.214)	8 (0.296)	27 (0.233)	10 (0.526)	7 (0.304)	100
III	57 (0.588)	57 (0.679)	16 (0.593)	24 (0.207)	8 (0.421)	10 (0.435)	172
(Total)	97 (0.265)	84 (0.229)	27 (0.073)	116 (0.317)	19 (0.052)	23 (0.063)	366

[a] I – significant intra-locus interaction for both loci and significant inter-locus interaction
II – significant intra-locus interaction for either locus and significant inter-locus interaction
III – non-significant intra-locus interaction for either locus but significant inter-locus interaction

one-locus sporo-gametophytic interaction, was adopted by Kitamura (1962) and by Ikehashi and Araki (1986). More recently, a multiple-allele locus (S-5) on chromosome 6 was found to be responsible for hybrid sterility through allelic interaction (Ikehashi and Araki 1986, 1988). Female gametes were aborted in the genotype of $S\text{-}5^{i}$/S-$5^{j,}$ whereas the neutral allele $S\text{-}5^{n}$ did not cause abortion in the heterozygotes of $S\text{-}5^{i}/S\text{-}5^{n}$ and $S\text{-}5^{j}/S\text{-}5^{n}$. Since then, the $S\text{-}5^{n}$ allele has been used to overcome hybrid sterility in Indica/Japonica hybrids (Araki et al. 1990; Yuan 1992), and the donor of $S\text{-}5^{n}$ is called a wide-compatibility gene (WCV). In our experiment, both maternal parents of the two test-cross population are WCVs as reported by our previous studies (Luo et al. 1992; Li et al. 1996). All F_1s in the two test-cross populations showed heterosis over male parents with respect to SS but the degree of heterosis differed markedly. Based on the results of a two-way ANOVA, a digenic interaction model acting on the heterosis of SS was summed up as follows:



Our parallel report showed that a moderate extent of genetic differentiation (average Dj 0.50–0.60) in parental lines could best increase hybrid fertility which resulted in the highest grain-yield heterosis (data not shown). In breeding programs, favourable heterologous interactions of genes both between two loci and within a single locus can be employed by selecting parental lines with an optimum extent of genetic differentiation to produce orchestrated F_1 hybrid genome combinations. Additional theoretical and applied studies on this aspect deserve attention.

Acknowledgements The authors thank Dr. S. D. Tanksley' s group and Dr. N. Kurata's group for kindly providing the rice RFLP clones. This research, supported by China National High-Tech Research and Development Program, was conducted in the State Key Laboratory in Genetic Improvement of Crops in Huazhong Agricultural University and under the guidance of Dr. Q. F. Zhang.

References

Allard RW (1996) Genetic basis of the evolution of adaptedness in plants. Euphytica 92:1–11

Araki H, Ikehashi H, Toya K, Matsumoto S (1990) Development of wide compatibility rice, 'Norin PL 9'. JAQR 24:78–81

Bradshaw HD, Stettler RF (1995) Molecular genetics of growth and development in *Populus*. IV. Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. Genetics 139:963–973

Bruce AB (1910) The Mendelian theory of heredity and the augment of vigor. Science 32:627–628

Carson HL, Templeton AR (1984) Genetic revolutions in relation to speciation phenomena: the founding of new populations. Annu Rev Ecol Syst 15:97–131

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu KS, Xiao JH, Yu ZH, Ronald PC, Harrington SE, Second G, McCouch S, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics:1251–1274

Cheng KS, Wang XK (1987) Comprehensive study and application of rice germplasmic resources in Yunan' IV. Classification of Hsien and Keng in Asian cultivated rice (in chinese). Genetic Res Crops 1:1–5

Crow JF (1952) Dominance and overdominance. In: Gowen JW (ed) Heterosis. Iowa State College Press. Ames. pp 282–297

Davenpot CB (1908) Degeneration, albinism, and inbreeding. Science 28:454–455

East EM (1936) Heterosis. Genetics 21:375–397

Ikehashi H, Araki H (1986) Genetics of F_1 sterility in remote crosses of rice. In: Rice genetics, IRRI, P.O. Box 933, Manila, Philippines, pp 119–130

Ikehashi H, Araki H (1988) Multiple alleles controlling F_1 sterility in remote crosses of rice. Jpn J Breed 38:283–291

Jinks JL, Jones RM (1958) Estimation of the components of heterosis. Genetics 97:639–666

Keeble F, Pellew C (1910) The mode of inheritance of stature and of time of flowering in peas (*Pisum sativum*). J Genet 1:47–56

Kitamura E (1962) Genetic studies on sterility observed in hybrids between distantly related varieties of rice, *Oryza sativa* L. (Japanese/English) Bull Chugoku Agr Exp Station, A8: 141–205

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin SY, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y et al. (1994) A 300-kilobase interval genetic map of rice including 883 expressed sequences. Nat Genet 8:365–372

Li RH, Wang XK, Yang ZY (1996) Classification of a wide compatibility rice N422s, a photoperiod-sensitive genic male-sterile line. Acta Crop Sinica 22:495–499

Li ZK, Pinson SRM, Park WD, Paterson AH, Stansel JW (1997) Epistasis for three grain yield components in rice (*Oryza sativa* L.) Genetics 145:453–465

Lin SY, Ikehashi H (1991) A gamate eliminator induced by Est-9 in chromosome 7 of rice (*Oryza sativa* L.). Jpn J Breed 41:350–351

Lin SY, Ikehashi H, Yanagihara S, Kawashima A (1992) Segregation distorted via male gametes in hybrids between Indica and Japonica or wide-compatibility varieties of rice (O*ryza sativa* L.) Theor Appl Genet 84:812–818

Luo XH, Yuan LP (1992) Development of a thermosensitive-genic male sterile rice line – Peiai 64s. Hybrid Rice 1:27–30 (in Chinese)

Minvielle F (1987) Doninance is not necessary for heterosis: a two-locus model. Genet Res 49:245–247

Mitchell-Olds T (1995) Interval mapping of viability loci causing heterosis in *Arabidoposis*. Genetics 140:1105–1109

Nakagahra M (1972) Genetic mechanism of the distorted segrer-ation of marker genes belonging to the eleventh linkage group in cultivated rice. Jpn J Breed 22:232–238

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Oka HI (1974) Analysis of genes controlling F_1 sterility in rice by the use of isogenic lines. Genetics 77:521–534

Oka HI (1988) Origin of cultivated rice . Jpn Sci Soc Press, Amsterdam New York

Provine WB (1986) Sewall Wright and Evolutionary Biology. University of Chicago Press, Chicago

Saghai Maroof MA, Soliman KM, Jorgensen RA, Allard R W (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81 : 8014–8018

Sano Y (1990) Genetic analysis of an alien chromosome segment from Oryza sativa. Jpn J Breed 40 [suppl 2] : 306–307

Sano Y (1993) Is an egg-killer present in rice? Theor Appl Genet 86 : 1038–1042

SAS Institute Inc (1988) SAS users guide: statistic. SAS Institute, Cary, North Carolina

Shull GH (1908) The composition of a field of maize. Am Breed Assoc 4 : 296–301

Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132 : 823–839

Templeton AR (1979) The unit of selection in *Drosophila mercatorum*. II. Genetic revolution and the origin of coadapted genomes on parthenogenetic strains. Genetics 92 : 1265–1282

Templeton AR (1980) The theory of speciation via the founder principle. Genetics 94 : 1011–1038

Wade MJ (1992) Sewall Wright: gene interaction and the shifting balance theory. Oxf Surv Evol Biol 8 : 33–62

Wang ZY, Tanksley SD (1989) Restriction fragment length polymorphism in *Oryza sativa* L. Genome 32 : 1113–1118

Wright S (1932) The role of mutation, inbreeding, crossbreeding, and selection in evolution. Proc 6th Int Congr Genet 1 : 356–366

Wright S (1980) Genetic and organismic selection. Evolution 34 : 825–843

Xiao J, Li J, Yuan L, Tanksley SD (1995) Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics 140 : 745–754

Yanagihara S, Kato H, Ikehashi H (1992) A new locus for multiple alleles causing hybrid sterility between an Aus variety and Javanica varieties in rice (*Oryza sativa* L.). Jpn J Breed 42 : 793–801

Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang QF, Saghai Maroof MA (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci 19 : 9226–9231

Yuan LP (1987) Strategic hypotheses of breeding for hybrid rice (in chinese). Hybrid Rice 1 : 1–3

Yuan LP (1992) The strategy of the development of hybrid rice breeding. In: Yuan LP (ed) 'Current status of two-line hybrid rice research' Agricultural Publishing Ltd, Beijing, pp 1–5

Zhang G , Lu Y (1993) Genetic studies of the hybrid sterility in cultivated rice (*Oryza sativa* L.). II. A genic model for F_1 pollen sterility. Acta Genet Sinica 20 : 222–228

Zhang G, Lu Y, Zhang H, Yang G (1994 a) Genetic studies of the hybrid sterility in cultivated rice (*Oryza sativa* L.). IV. Genotypes for F_1 pollen sterility. Acta Genet Sinica 21 : 34–41

Zhang QF, Gao YJ, Yang SH, Ragab R, Saghai Maroof MA, Li ZB (1994 b) A diallel analysis of heterosis in elite hybrid rice based on RFLPs and microsatellites. Theor Appl Genet 89 : 185–192

Zhang QF, Zhou ZQ, Yang GP, Liu KD, Saghai Maroof MA (1996) Molecular marker heterozygosity and hybrid performance in indica and japonica rice. Theor Appl Genet 93 : 1218–1224