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Gametic selection in anther culture of rice (*Oryza sativa* L.)*

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Summary. The segregation and recombination of heterozygous isozyme markers have been monitored in anther culture derivatives (i.e., six nonmorphogenic microspore-derived callus [NMC] populations and two anther culture plant [ACP] populations) and F_2 plants generated from six F_1 hybrids of rice, including five japonica upland/improved indica tropical hybrids. The alleles in excess at some loci displaying skewed segregations in the F_2s were consistently overrepresented in the NMC populations. These alleles were also generally found to be overabundant in the two ACP populations except for certain loci that contrastingly segregated in a 1:1 ratio. Additional distortions were found to be specific to AC derivatives indicating the existence of in vitro gametic selection. Overall, however, the gametic selection in the ACP materials was neutral with regard to the indica and japonica differentiation. Estimates of linkages between markers borne by chromosome 6 using AC-derivative data were consistent with those noted in the F_2s and with current knowledge of the isozyme locus linkage map. Given the average neutrality of gametic selection and the consistency of linkage relationships in the ACPs, their further use as rice molecular mapping and gene tagging populations can be investigated with confidence.

Key words: Rice – Japonica/indica – Anther culture – Isozyme markers segregation – Linkage

Introduction

During the last decade the technique of anther culture (AC) has been widely integrated into breeding programs of major crops in order to speed up the fixation of true homozygous lines developed from hybrid materials. In addition, sets of inbred lines rapidly generated through AC have been considered to be relevant and convenient materials in the identification of genes that control traits of interest by classical genetic analysis (Chen et al. 1983) or molecular mapping (Rivard et al. 1989). It is thus important for both plant breeders and geneticists to know whether the AC-derived plants (ACP) represent a random array of the microspore population.

A sensitive test of gametic selection involves comparing segregations of heterozygous morphological or biochemical markers among the AC derivatives and the backcross or F_2 progenies of F_1 hybrids. Mutant loci controlling gross phenotypical changes were the first to be employed. In general, segregations fitting the expected 1:1 ratios among the AC plants were reported from such genetic analyses, notably in rice (Chen et al. 1983; Siva Reddy et al. 1989) and petunia (Raquin 1982). In barley, however, studies on the segregation of monogenic traits in AC-derived lines provided contrasting evidence for gametic selection (Kao et al. 1983; Foroughi-Wehr and Friedt 1984; Powell et al. 1986).

Heterozygous isozyme markers in turn have been used to test for the existence of segregational skewings in the microspore derivatives. The natural occurrence of these markers (which sometimes exist in large numbers in the hybrids commonly used in breeding program is a big advantage. Strong segregation distortions of such markers have been reported in AC-derived plants of pearl millet (Bui Dang Ha and Pernès 1982) and perennial raygrass (Hayward et al. 1990) and in AC-derived embryos of broccoli (Orton and Browers 1985). Hybrids between the two main varietal groups of rice (*Oryza sativa* L.) japonica and indica, display specific traits that are useful in assessing the impact of gametic selection using isozyme markers: (a) the interparental allelic polymorphism permits the survey of 6–16 isozyme markers in young shoots versus 0–14 and 0–5 for indica/indica and

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japonica/japonica hybrids, respectively (J. C. Glaszmann, unpublished); (b) japonica varieties are well known to be more amenable to AC than indica varieties (Chen 1986) and thus offer an interesting system for studying putative in vitro selection and possibly a means of identifying loci tightly linked with genes responsible for the AC culture response. Along this line, an obvious overrepresentation of the japonica plant type among AC derivatives of japonica/indica hybrids has been reported (Shen et al. 1978); (c) F_2 progenies of indica/japonica hybrids are commonly prone to segregational skewings due to the complex action of F_1 gametophyte or sporophyte genes (Nakagahra 1986; Ikehashi and Araki 1986; Oka 1988), making concurrent analysis of these segregations in the in vitro derivatives of particular interest. Another advantage in using isozyme genes as markers for detecting in vitro gametic selection is their wide expression in the microspore-derived callus that can be reliably scored up to 3 weeks after callus transfer onto the regeneration medium when plant regeneration has occurred (Guiderdoni et al. 1988). This permits concurrent assessment of gametic selection in nonmorphogenic and morphogenic androgenetic products.

We recently reported the segregation of 13 isozyme genes among nonmorphogenic, microspore-derived calli (NMC) and doubled haploid lines derived from ACs of a japonica/indica hybrid. There were consistent deviations at two loci in both the F_2 plants and AC derivatives and distortions specific to AC-derived materials at two other loci (Guiderdoni et al. 1989a, b). However, neither in vivo or in vitro gametic selection favored a single parental allelic type, and the distortions were found to be balanced between the indica and japonica alleles when averaged over all 13 loci. The recombination rates among four isozyme loci located on chromosome 12 estimated from F_2 and doubled haploid data were very consistent.

In the experiments reported in this paper we carried out AC of various rice hybrids, most of which involved an indica and a japonica parent, a high level of interparental allozyme variation, and the presence of heterozygous markers on chromosome 6 (known to be a "hot spot" for F_2 segregational skewings and pseudolinkages [Nakaghara 1986; Ikehashi and Araki 1986; Pham et al. 1990]). We obtained a sufficient number of calli and plants to make the isozyme marker segregation analysis meaningful in six and two progenies respectively.

Materials and methods

Parentals, F_1, and F_2 progenies

The origin, seed source and classification of the parental varieties are listed in Table 1. 'IRAT216' (also known as IDSA6) and 'Azucena' are improved and traditional tropical upland japonica rice varieties. 'IRAM31-8-6', 'Eloni', 'UPlRi-7', and 'IR64' are improved irrigated indica varieties. The elite rainfed lowland line 'IR21567-9-2-2-3-1-3' also belongs the indica group. 'Ramjaewen', a traditionnal lowland variety from Burma is atypical; it is usually considered an indica, but falls under isozyme group 5 (Glaszmann 1987) and is distinct from typical indica varieties in this respect. F_1 seeds of 'IR21567-9-2-2-3-1-3'/'Ramjaewen', 'IRAT216'/'Eloni', 'IRAT216'/'IRAM31-8-6', 'Azucena'/'UPlRi-7', 'IR64'/'Azucena', and 'IR64'/'IRAT216' were germinated and grown in the IRRI phytotron under natural light conditions, a day and night temperature of 29° and 21 °C, respectively, and 70% relative humidity during the 1988 and 1989 dry seasons. The hybrid legitimacy of all F_1 plants was tested by means of isozyme analyses of their leaf tissue and further comparison with the parental isozyme phenotypes. Tillers for AC were collected from the plants at the booting stage. For pollen fertility evaluation, three groups of three spikelets each were collected at various levels of the main tiller panicle from five heading F_1 plants. At least one anther per group (i.e., 15 anthers per cross) was dissected and its pollen stained using Alexander's technique (Alexander 1969). Bagged panicles of these five plants were used for F_2 seed collection. The mean values of pollen fertilities of the six hybrids ranged from 71.4% to 92.3% (Table 2).

Production of AC calli and plants

The panicles were extracted from the flag leaf sheath of surface-sterilized boots, plated onto imbibed Whatman no. 4 paper fil-

Table 1. Origin, isozyme profile, and classification of the parentals of the six rice crosses

Parent[a]	Origin	Isozyme group[b]	Classification	Isozyme locus											
				Icd-1 (1)[d]	Amp-3 (6)	Est-2 (6)	Pgi-2 (6)	Cat-1 (6)	Pgi-1 (3)	Sdh-1 (12)	Acp-1 (12)	Est-9 (7)	Amp-2 (8)	Pgd-1 (11)	Mal-1 (?)
IRAT 216	Ivory Coast	VI	Japonica	2	2	1	1	2	2	2	2	2	1	3	1
Azucena	Philippines	VI	Japonica	1	2	1	1	2	2	2	2	2	1	1	1
Eloni	Suriname	I	Indica	2	2	1	1	1	1	1	1	1	2	3	2
IRAM 31-8-6	Malagasy	I	Indica	1	1	2	1	2	1	2	1	1	2	1	2
UPLRi-7	Philippines	I	Indica	1	2	1	1	1	1	2	1	1	2	3	2
IR 21567-9-2 -2-3-1-3	Philippines	I	Indica	1	1	2	1	1	1	1	1	2	2	1	2
IR 64	Philippines	I	Indica	1	1	2	2	1	1	4	1	1	2	1	2
Ramjaewen	Burma	V	Atypical	1	4	0	4	1	2	2	2	2	1	1	2

[a] Seeds of 'IR 21567-9-2-2-3-1-3' and 'Ramjaewen' and of their F_1 hybrid were kindly supplied by Dr. D.J. Mackill, IRRI
[b] Isozyme group and variety classification based on isozymes (Glaszmann 1987)
[c] Gene and allele designations according to Glaszmann et al. (1988)
[d] The chromosomal locations are given in the synthesis by Wu et al. (1988) and follow the revised chromosome numbering system (Rice Genetics Cooperative, 1990)

Table 2. Mean values of pollen fertility and anther culturability of the six rice crosses, and respective sizes of the isozymed F_2 plant and anther culture derivative populations

Hybrid	Isozyme groups involved[a]	Hybrid pollen fertility[b]	Anther cultures				Isozyme analyses		
			Anthers plated	Percentage of callusing anthers	Green plant regenerating calli per 1000 anthers cultured	Heterozygous isozyme loci surveyed	Non morphogenic calli (NMC)	Anther culture plants[c] (ACP)	F_2 plants (F_2)
IR 21567/Ramjaewen	I/V	79.1	8,500	8.6	2.3	6	284	–	205
IRAT 216/Eloni	VI/I	89.7	12,500	11.9	8.1	7	399	–	336
IRAT 216/IRAM 31-8-6	VI/I	71.4	8,500	17.0	2.8	9	394	–	420
Azucena/UPLRi-7	VI/I	91.9	9,500	10.7	12.6	7	256	–	377
IR 64/Azucena	I/VI	83.2	23,850	27.3	19.7	10	516	405	364
IR 64/IRAT 216	I/VI	92.3	28,400	22.3	10.1	12	508	271	232

[a] Classification of the parentals based on their isozyme profiles (Glaszmann 1987)
[b] Estimated with the Alexander staining technique (Alexander 1969)
[c] Isozyme analysis has been carried out only when the ACP number is equal to or is higher than 100

ters in 100-mm-diameter glass petri dishes, and then cold-treated for 8 days at 8 °C. The anthers were dissected from the spikelets, plated into 60-mm petri dishes containing 10 ml of semi-solid (solidified with 0.5% w/v agarose) N6AK medium containing N6 salts, iron source, and vitamins (Chu 1978), and supplemented with 2 mg/l α-naphthalene acetic acid (NAA), 0.5 mg/l kinetin and 6% sucrose, and incubated in the dark at 26 °C. Calli of lengths between 1 and 2 mm that emerged from the anthers between the 3rd and the 8th week after plating were transferred for 3 more weeks into 100-mm petri dishes containing 25 ml semi-solid R6 medium (N6 basal medium supplemented with 1 mg/l kinetin, 0.1 mg/l NAA, and 4% sucrose) under light. The calli regenerating green plantlets were then transferred into test tubes onto semi-solid P medium (MS salts, iron source, and vitamins as per Murashige and Skoog (1962) supplemented with 8% sucrose), whereas calli that remained nonmorphogenic were collected for isozyme analysis. After 4 weeks of growth in the test tubes, plants with vigorous roots and canopy were transferred to a nutrient solution and, 2 weeks later, to pots in the greenhouse. Anther culturability of the six hybrids is given in Table 2.

Isozyme analyses

Tissues of NMC, coleoptiles of 5-day-old F_2 seedlings, and of nonchlorophyllous and green portions of expanding leaves of young ACP, 3 weeks after transfer of the plants into pots, were used for isozyme analyses. Enzyme extraction was performed by simple manual grinding of the leaf or callus tissue in a few drops of cold distilled water. Electrophoresis was performed in starch gels using methods described by Second (1982) (pH 8, system I) and Glaszmann et al. (1988) (pH 8.7, system II). Nine enzymes were revealed: catalase (CAT), isocitrate dehydrogenase (ICD), phosphoglucose isomerase (PGI), shikimate dehydrogenase (SDH), acid phosphatase (ACP), esterase (EST), aminopeptidases specific to either alanyl-β-naphthylamide or L-leucyl-β-naphthylamide (AMP), phosphogluconate dehydrogenase (PGD), and malic enzyme (MAL). The isozyme loci resolved and the isozyme genotypes of the parentals are given in Table 1.

To avoid artifacts in zymogram scoring, only the genes presenting two expressed alleles in the hybrids were considered for segregation analyses. The number of heterozygous isozyme loci surveyed ranged from 6 to 12, and the respective sizes of the NMC, ACP, and F_2 populations tested are listed for the six hybrids in Table 2. In 'IR64'/'Azucena' and 'IR64'/'IRAT216', the number of regenerated ACP was sufficient to make statistical analysis and comparison with NMC and F_2 populations meaningful.

Results

Origin of the AC derivatives

Isozyme analyses surveyed at least six isozyme loci per cross and covered over 2000 NMC and 700 ACP samples generated from the six hybrids. During this survey, neither a fully heterozygous isozyme phenotype (which would have resulted from analysis of somatic tissue-derived NMC or ACP) nor an isozyme profile presenting both parental bands at some isozymes (which would have resulted from analysis of clumps of several coalescent microspore calli) were observed. Duplicated NMC and ACP isozyme phenotypes were detected in 3% of the samples. Given the high number of heterozygous markers surveyed, the random occurrence of two identical isozyme sequences from two different meiosis products is rather unlikely. Duplicated isozyme phenotypes may result from the fragmentation of a microspore-derived callus during its growth. Only one record was kept for further statistical analysis in such a case. All the other samples were original nonheterozygous recombination products of the parental phenotypes and were, therefore, of single microspore origin.

Segregation of isozyme loci among F_2 plants, NMC and ACPs

1. *'IR21567'-/'Ramjaewen'*. Among the six segregations monitored in the F_2, three deviated from the expected 1:2:1 ratio due to an overabundance of the IR line al-

Table 3. Segregation of heterozygous isozyme markers among anther culture derivatives (NMC, nonmorphogenic microspore-derived calli; ACP, anther culture plants) and F_2 progenies generated from six rice crosses. Segregation of the isozyme phenotypes follow the order JJ:JI:II for the F_2 progeny and J:I for the NMC and ACP populations (J and I are the alleles contributed by the japonica- or atypical- parental and by the indica parental, respectively). Segregations given on *black* and *screened background* deviate significantly (at least 5%) from the expected 1:2:1 (F_2 progeny) or 1:1 (AC derivatives) ratio due to an overrepresentation of the J and I parental allele, respectively. *Asterisk* (*) indicates significance at the 5% level of Chi-square values for a panmictic segregation indicating a nonrandom assortment of parental alleles

Cross	Progeny	Isozyme locus											
		Icd-1	Amp-3	Est-2	Pgi-2	Cat-1	Pgi-1	Sdh-1	Acp-1	Est-9	Amp-2	Pgd-1	Mal-1
IR21567 × Ramjaewen	NMC	–	100:178	–	147:135	–	134:148	95:189	69:172	–	145:122	–	–
	F_2	–	48:67:83*	–	57:94:51	–	36:102:63	17:66:121	24:77:79	–	61:103:37	–	–
IRAT216 × Eloni	NMC	–	–	–	–	190:199	218:155	249:146	180:186	189:184	243:152	–	139:132
	F_2	–	–	–	–	27:68:48	53:90:73	126:122:80*	76:127:67	43:84:54	84:134:67	–	32:74:60
IRAT216 × IRAM31-8-6	NMC	185:210	217:175	216:177	–	–	246:145	–	60:122	240:153	193:146	228:164	124:45
	F_2	87:133:100	77:145:62	67:145:77	–	–	40:99:51	–	61:82:35	87:120:42	41:80:64	38:83:58	51:26:22*
Azucena × UPLRi-7	NMC	–	–	–	–	103:151	143:113	–	147:45	161:93	85:135	167:89	105:130
	F_2	–	–	–	–	–	74:115:60	–	137:64:82	123:136:54	50:93:69	85:53:55*	61:66:55*
IR64 × Azucena	NMC	–	285:199	300:211	266:230	184:288	232:279	273:243	190:162	269:168	112:207	–	174:238
	ACP	–	233:172	222:174	214:191	168:225	162:243	178:207	88:106	195:210	143:259	–	151:210
	F_2	–	90:145:96	78:126:92	101:157:106*	46:37:77*	95:186:101	67:122:72	58:97:61	79:140:67	47:146:67	–	52:100:109*
IR64 × IRAT216	NMC	313:177	294:189	292:186	287:191	182:279	264:244	239:245	240:160	298:171	143:249	243:246	242:216
	ACP	142:129	141:130	141:130	151:120	123:148	124:147	137:134	43:33	166:105	112:155	124:147	129:128
	F_2	133:64:34*	83:98:51*	83:96:53*	88:84:50*	46:90:95	62:80:90*	54:31:49*	35:52:44	96:76:60*	74:71:87*	45:121:44*	54:40:49*

leles. The alleles, overrepresented in the F_2, were also found in excess in the NMC population, whereas the other three loci fit the expected 1:1 ratio. There were no significant differences between the allelic frequencies evaluated from F_2 and NMC data at the three distorted loci (Table 3).

2. *'IRAT216'/'Eloni'*. Among the seven gene segregations monitored in the F_2, only one deviated significantly from Mendelian expectations, and this was partly due to an excess of the allele contributed by the japonica parental. That allele was consistently overrepresented in the NMC population. Moreover, additional deviations in favor of the japonica allele at two loci were specific to AC-derived material. At these loci, allelic frequencies differed significantly from those recorded in the F_2.

3. *'IRAT216'/'IRAM31-8-6'*. Among the nine segregations monitored in the F_2 progeny, Chi-square values for a fixed ratio hypothesis were found to be significant at two isozyme loci due to an excess of the alleles contributed by the japonica parental. These alleles were also overrepresented in the NMC population at corresponding loci, while their frequencies did not differ significantly from those in the F_2s. Moreover, additional deviations at four other loci, all in favor of the japonica allele, were specific to the NMC. At these four loci the allelic frequencies differed significantly from those evaluated from F_2 data.

4. *'Azucena'/'UPlRi-7'*. Three out of the five segregations monitored in the F_2 population significantly departed from the expected 1:2:1 ratio due to excess japonica alleles. These alleles were consistently overrepresented in the NMC, whereas allelic frequencies calculated from F_2 and NMC data did not differ significantly at these genes. An additional locus segregation departed from the expected 1:1 ratio in favor of the indica parental, specifically in the NMC. The allelic frequencies were significantly different from those in the F_2s at that locus.

5. *'IR64'/'Azucena'*. The segregation of ten isozyme genes were monitored in the F_2, NMC and ACP populations generated from that hybrid. Significant departures from the expected 1:2:1 ratio due to an excess of alleles contributed by the japonica parental were observed at two loci in the F_2. The same alleles were overabundant in the NMC and ACP populations. There were no significant differences among the allele frequencies calculated from NMC, ACP, and F_2 data at these loci. Four additional deviations were specific to both ACP and NMC. The alleles contributed by the indica and the japonica parental were each favored at two loci. At these loci the allelic frequencies calculated from ACP and NMC data did not differ, but they did differ significantly from F_2s. A seventh segregation distortion was found to be specific to NMC. Calculation of the allelic frequencies showed an excess of the indica allele at this locus in NMC and significant differences from those calculated using F_2 and ACP data.

6. *'IR64'/'IRAT216'*. Six out of the twelve gene segregations monitored in the F_2 progeny deviated from the expected Mendelian ratio due to an overrepresentation of the allele contributed by the japonica and indica parental at one locus and five loci, respectively. The alleles in excess in the F_2 were also found to be overabundant in the NMC population, and their allelic frequencies did not differ significantly from those in the F_2s at the six loci. One of these loci also showed dissimilar frequencies of the parental alleles in the ACP. However, segregations at the other five loci that deviated in the F_2 and NMC populations did fit the expected 1:1 ratio in the ACP. The allelic frequencies calculated from F_2 and NMC data differed significantly from those of ACP at these loci borne by chromosomes 1 and 6. Additional deviations favoring the japonica allele at one locus and the indica allele at another were found to be specific to NMC and to NMC and ACP, respectively. For these two loci the allelic frequencies calculated from ACP and NMC did not differ significantly, but did differ from those found in the F_2.

Overall deviation

When averaged over all the loci surveyed, the mean japonica and indica allelic frequencies fitted a 1:1 ratio in the two ACP populations, but deviated in favor of the indica and japonica allele in one and four, respectively, of the six NMC populations analyzed.

Recombination among isozyme markers in F_2 and AC derivatives

In examining the recombination data obtained from the F_2 progenies and anther culture derivatives generated from 'IRAT216'/'IRAM31-8-6', 'IR64'/'Azucena', and 'IRAT216', Chi-square values for independence were found to be nonsignificant for all combinations of pairs of loci except Est-2 = Amp-3, Amp-3 = Pgi-2, and Est-2 = Pgi-2. Consistent linkage values were calculated from the percentage of recombinant AC derivatives in NMC and ACP populations and through the maximum likelihood method in the F_2 progenies (Table 4).

Cosegregation analyses also identified linkages among the three loci borne by chromosome 6 (i.e., Est-2, Amp-3, and Pgi-2) and Sdh-1 and Pgi-1 known to be located on chromosomes (2 and 3), respectively, specifically in the F_2 progeny of 'IR64'/'IRAT216'. The linkage values among the three markers of chromosome 6 and Sdh-1 and Pgi-1 calculated through the maximum likelihood method appeared rather loose, ranging from 33% to 35%.

Table 4. Linkage values between isozyme loci borne by chromosome 6 estimated from NMC, ACP, and F_2 populations generated from 'IRAT216'/IRAM 31-8-6', 'IR64'/'Azucena', and 'IR64'/'IRAT216'

Cross	Progeny	No. of pair of loci surveyed	Est-2/Amp-3	Amp-3/Pgi-2	Est-2/Pgi-2
IRAT216/IRAM 31-8-6	NMC	392	3.8±1.0	–	–
	F_2	187	10.5±1.7	–	–
IR64/Azucena	NMC	484	1.9±0.6	27.9±2.0	29.3±2.0
	ACP	396	3.3±0.9	17.5±1.9	17.4±1.9
	F_2	364	11.7±1.4	23.8±1.9	24.4±2.1
IR64/IRAT216	NMC	478	0.8±0.4	25.2±1.9	25.1±1.9
	ACP	271	0.0	16.2±2.2	16.2±2.2
	F_2	232	1.3±0.5	20.6±2.1	21.1±2.2
Current isozyme locus linkage map (Pham et al. 1990)	F_2 or trisomics	–	0–7	7–17	6–23

Recombination rates in anther culture derivatives and in F_2s were deduced from the percentage of recombinant associations and through the maximum likelihood method, respectively

Discussion

Almost 100% of the 3,000 AC derivatives analyzed for isozymes in this study were found to be of single microspore origin. This result confirms the conclusions drawn from comparable genetic analyses carried out earlier in rice (Chen et al. 1983, Guiderdoni et al. 1988). The experiments also confirm the existence of callus fragmentation during microspore callus growth that results in duplicated isozyme phenotypes.

Segregation distortions were detected in a variable proportion of the loci under study in the F_2 progenies of the five japonica/indica hybrids. Segregation bias is a common feature in the progeny of partially sterile intersubspecific hybrids in rice. These aberrant segregations, evidenced in the use of morphological (Nakagahra 1986), isozyme (Pham et al. 1990), and RFLP (McCouch et al. 1988) markers, have been tentatively explained by several genetic mechanisms involving the action of gametophytic genes, sporophytic genes, or sporogametophytic interactions (Oka 1988). However, other causes may be responsible for gametic selection in our study since the F_1 fertilities of the japonica/indica hybrids were rather high. The influence of the environment on the randomness of F_1 hybrid gametes has been recently reported in rice (Sato et al. 1986). These authors suggest that selection at the gametic phase is a more general phenomenon that the previously admitted intersubspecific nature of segregation distortions. In our study segregational skewings were also noted among the F_2 population of 'IR21567'/'Ramjaewen', which confirms the results of a survey of isozyme segregations in 24 intra- and intersubspecific F_2 populations in which deviations in some japonica/japonica and indica/indica hybrid progenies were noted (Pham et al. 1990). These deviations suggest either that hybrid sterility genes are present in intrasubspecific crosses or that another distorting mechanism exists.

The alleles overrepresented in the F_2s were always found in excess at the corresponding loci in the six NMC populations. These alleles were also generally overabundant in the ACP population. In these cases there are generally no significant differences among the allelic frequencies calculated from the F_2 and the NMC and ACP populations, which would suggest that mechanisms of microspore selection during gametogenesis, which are responsible for segregational skewings in the F_2 progeny, interfere indiscriminately with the morphogenic and nonmorphogenic pathways of androgenesis. Alternately, the selection may take place in the microsporogenesis before the mid-uninucleated stage that is suitable for triggering rice androgenesis.

However, the absence of segregational skewings in ACP of 'IR64'/'IRAT216' at certain isozyme loci borne by chromosomes 1 and 6, which were found distorted in the NMC and F_2 populations, appears to be a noteworthy exception to this rule. This discrepancy can be explained if one assumes that the microspore selection that caused distortion at these loci operated after the mid-uninucleated stage. In rice AC it has been shown that the regenerability of the callus initiated from microspores decreases from the mid-uninucleated stage until the first pollen mitosis (Genovesi and Magill 1979). Microspores at the mid-uninucleated stage may divide symmetrically and form embryoids that may regenerate green plants, whereas microspores at a later stage tend to divide asymmetrically and form albino plants and nonmorphogenic calli (Chen 1977). Therefore, one can tentatively explain the absence of distortions in the ACP by the fact that they are derived from early-stage microspores that avoid the selection by means of a divergent division pattern. On the other hand, NMC are most frequently derived from late-uninucleated microspores that divide asymmetrically and, therefore, sustain sterility gene action.

Our results clearly indicate that ACP populations do not represent a truly random F_1 gametic array. Either distortions likely related to in vitro gametic selection are found at some additional loci in the ACP populations or some of the loci found distorted in the F_2s segregate normally in the ACP. The existence of in vitro gametic selection contradicts previous reports on the segregations of morphological markers fitting the expected ratios in ACP and F_2 plants generated from japonica/japonica (Chen et al. 1983) and indica/indica (Siva Reddy et al. 1989) rice hybrids. This discrepancy in these studies could be explained by the use of hybrids from closely related varieties and of few markers.

As to the use of microspore-derived plants in rice genetics and breeding, the partial and slight gametic selection noticed in our ACP materials does not appear to be a major drawback since the pooled allelic frequencies do not deviate from the expected 1 : 1 ratio. This indicates that the observed gametic selection is neutral with regard to the indica and japonica differentiation.

The consistency of the linkage values estimated from AC derivative and F_2 data also suggests that ACP populations can be reliably integrated into genetic studies. It is also important to note that the loose but significant pseudolinkages as well as the segregation distortions observed at certain loci borne by chromosome 6 in the F_2 progeny of 'IR64'/'IRAT216' were not found in the ACP population. This would suggest that the morphogenic androgenetic pathway may, in some instance, bypass problems related to hybrid sterility existing in the microsporogenesis of distant rice hybrids.

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