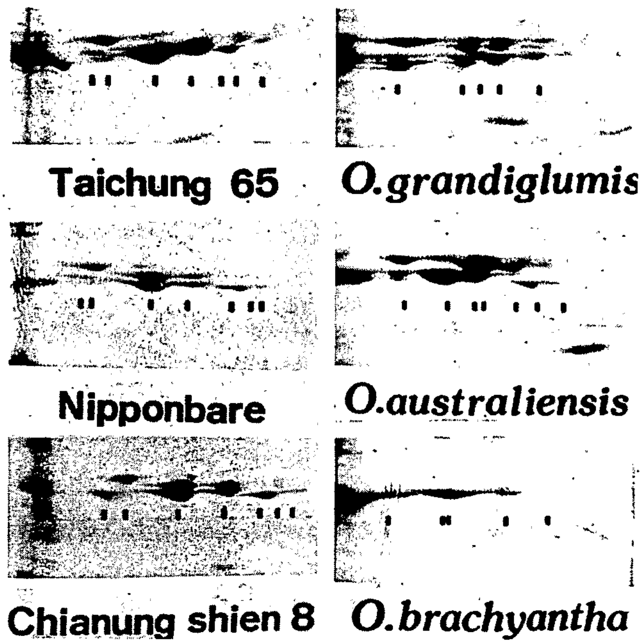

Fig. 1. Two-dimensional separation of glutelin α subunit. Vertical axis represents molecular weight (*e.g.*, 32.5, 31.0, 30.0 and 29.0 KD from upper to lower point for Taichung 65). Dots along the horizontal axis indicates pI value (*e.g.*, 7.60, 7.50, 7.30, 7.10, 6.80, 6.70 and 6.50 from left to right for Taichung 65).

16. Analysis of total chloroplast DNA RFLPs in cultivated and wild species of rice

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We have improved the non-aqueous method for the isolation of chloroplasts from freeze-dried leaves. The changes include the mechanization of grinding, the use of less toxic solvents, and differential centrifugation in the presence of Triton to remove contaminant DNA. Total chloroplast DNA was digested by restriction enzymes and restriction patterns from single plants of various species of the *Eu-Oryza* section of Genus *Oryza* were obtained.

Chloroplast DNA from 320 plants were digested by EcoR1 and Ava I restriction enzymes. Thirty-two distinguishable patterns ("plastotypes") were observed and further characterized by digestion with Bam H1, Hin dIII, Sma I, Pst I, Bst EII and Sal I restriction enzymes.

A careful determination of apparent molecular weights of restriction bands allowed for the distinction of 112 mutations. Forty of these were recognized as

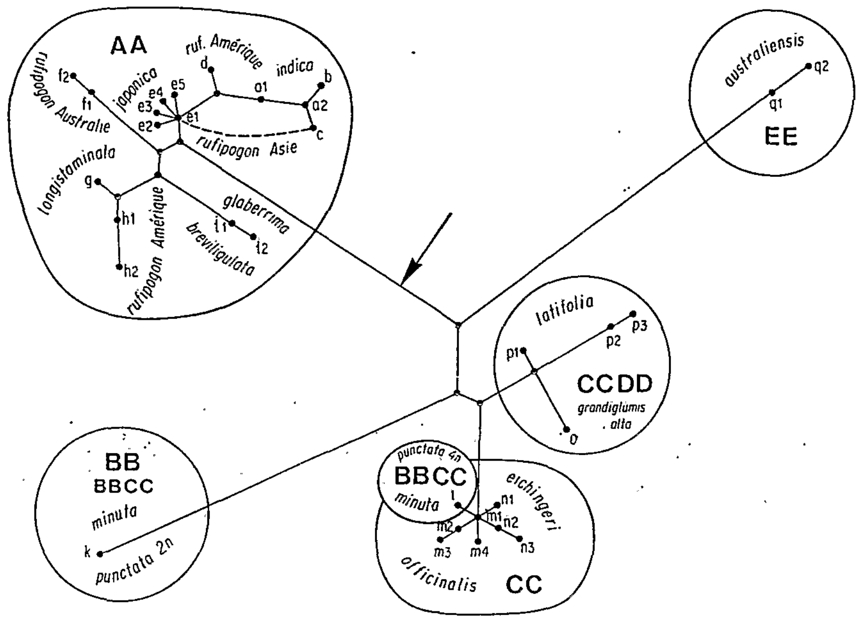

Fig. 1. A cladogram showing interrelationships among 32 plastotypes distinguished. Capital letters stand for the nuclear genomes and small letters for the plastotypes. The length of a branch is approximately proportional to the number of mutations specific to that branch. The arrow indicates the "root".

restriction site mutations for 220 restriction sites surveyed representing 1300 base pairs of DNA sequences.

A cladistic analysis of the 112 mutations allowed us to produce a cladogram of the 32 plastotypes. Its "root" was deduced from a preliminary study of the Oryzeae tribe with digestion by enzyme Bsp x1 (Fig. 1).

This cladogram agreed completely with the cytological distinction of 5 basic genomes, and the recognition of the *sativa* and *latifolia* groups of species in the section *Eu-Oryza*. It also largely agreed with the finer genetic structure as revealed by isozyme electrophoresis patterns. Additional information on the maternal parents of allotetraploids was obtained. Several strong discrepancies between the relationships at nuclear and chloroplastic marker levels suggested some past nucleo-cytoplasmic substitution events.

Observations of cultivated rices

Ten plastotypes were distinguished in cultivated rice. One corresponded to *O. glaberrima*, and 9 others were found in *O. sativa*. They clustered in the cladistic analysis in two groups which, as shown in Table 1, corresponded largely to the *indica-japonica* differentiation with one dominant type each. Intermediate *indica-japonica* varieties shared the *japonica* plastotype more often than the *indica* plasto-

Table 1. Different plastotypes observed in *O. sativa*

Plastotype	No. of varieties	Glaszmann's isozyme grouy	Remarks
indica			
a1	19	I, II	Most *indica* varieties and some GMS lines
a1	9	I	Most GMS lines analyzed
b	1	I	An African cultivar
c	1	I	A Thai upland cultivar
japonica			
e1	38	VI, I, II, III, IV, V	All *japonica* varieties, most intermediate *indica-japonica* and some *indica* varieties
e2	1	VI	The most cold resistant cv from Madagascar
e3	2	II	Two varieties from Iran (Gherdeh and Gharib)
e4	1	IV	A Thai cultivar (Howm Om)
e5	1	/	A land race of Western India with affinity to *O. glaberrima**

* Reported by Lolo and Second (this volume)

Table 2. Plastotypes observed in CMS plants

Acc. No.	Line	Source of cytoplasm	Plastotype
103435	Zhen-shan 97 A	WA	a1
103806	V20 A	WA	a1+a2*
103919	V41 A	WA	a1+a2
103804	Er-chiu-nan A	WA	a2
104054	Er-jiu-ai A	WA	a2
—	IR 46829 A	WA	a2
—	IR 46830 A	WA	a2
—	IR 54752 A	WA	a2
104052	Lian-tang zao A	—	a1
103871	Gang-yi-ya-ai-zao	Gambiaca	a2

* Differing according to plants

type. The rare plastotypes corresponded 1) to certain cytoplasms which induced male sterility (see below) and 2) to unique varieties with particular features.

Some observations of cytoplasmic male-sterile plants

Thirteen cytoplasmic male sterile (CMS) *O. sativa* plants were analyzed. They represented 10 different lines related to at least two different sources of CMS: Wild Abortive (WA) and Gambiaca.

Two different plastotypes were found: a1, the most common plastotype found in *indica* varieties, and a2, not found in fertile cultivated varieties but observed in various lines of *O. rufipogon*. They differed by a restriction site substitution detectable in Sma I and Ava I digestion patterns (Table 2).

WA source of CMS, with 11 plants studied appeared to be heterogeneous with different plants presenting one or the other plastotype, sometimes in the same line. One plant even showed a half and half mixture of the two restriction patterns a1 and a2.

This result suggested possible biparental inheritance of chloroplasts in rice. Because a few controlled hybrids so far studied always showed maternal inheritance of chloroplasts, biparental inheritance must not be frequent. Its detection could be favored under the breeding scheme used to maintain CMS lines, *i.e.* pollination with a fertile variety. Further studies are necessary to elucidate the possible relation of this finding with the origin and instability of CMS

References

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

17. "Alien pollen primacy" detected in rice

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In rice species, partial self-incompatibility has been found in *O. longistaminata* (Chu et al. 1969), and other mechanisms favoring outcrossing also have been known. The author has found a trend towards selective fertilization in which the stigma prefers alien to its own pollen in a mixed pollination experiment conducted in the summer of 1987.

Four kinds of crosses between two varieties, Taichung 65 (T65, Japonica) and IR36 (Indica) were made as shown in Table 1, where, for instance, T65× (T65+IR36) means that T65's pollen was set on the stigma first, and IR36's pollen was deposited five minutes later on the same stigma. Care was taken to deposit

Table 1. Successive pollination of pollen grains of T65 and IR36 in different orders resulting in differential rates of fertilization according to the maternal parent

Cross	Plants obtained from fertilization by pollen		
	T65*lg*	IR36	Total
T65*lg*×(T65*lg*+IR36)	39	42	81
T65*lg*×(IR36+T65*lg*)	18	58	76
IR36×(T65*lg*+IR36)	38	19	57
IR36×(IR36×T65*lg*)	29	32	61

T65*lg*: An isogenic line of T65 with gene *lg*.