Kagoshima Univ. Res. Center S. Pac., Occasional Papers, No. 18, 101-113, 1990

SDS-PAGE Analysis of Storage Proteins of Cultivated Rice Collected in Madagascar, 1988

Hikaru SATOH*, RATOKONJANAHARY Xavier Roland** and Tadao C. KATAYAMA***

(*Faculty of Agriculture, Kyushu University, JAPAN, **Ministere de la Recherches Scientifique et Technologique pour le developpement, REPOBLIKA DEMOKRATIKA MALAGASY, ***Faculty of Agriculture, Kagoshima University, JAPAN)

Introduction

Milled rice protein is one of the principal protein sources not only in the Japanese people but also in the Madagascar people. Therefore, it is apparent that there is a considerable potentiality for the qualitative and quantitative improvements of rice proteins. For these purposes, it must be necessary to search and collect the genetic resources of seed storage protein in rice. As to the genetical research and improvement of the seed storage protein, a lot of works have been done, and mutants for seed storage protein were reported to have occourred in maize [8,10], barley[9, 16], wheat[12], soybean[5], oat[13] ; and those have been used for materials of genetic studies and breedings. In rice, the nutritional improvements have been emphasized in the increase of protein contents in the endosperm owing to the fact that rices have relatively better storage proteins compared with other cereals. For example some mutants of increased protein contents were reported[1, 2, 4, 11] as well as a mutant for lysine contents[17]. Recently KUMAMARU *et al.* [6] found some mutants for rice storage proteins and discussed on the possibility of qualitative improvement in rice storage protein.

From the view-point of searching any new genetic resources for rice storage proteins, the writers took a trip to Madagascar during the period from June 1 to June 28 in 1988 for collecting the wild and cultivated rices under the project, "Studies on the Distribution and Ecotypic Differentiation of Wild and Cultivated Rice Species in Africa", supported by a Grant from the Ministry of Education, Science and Culture of the Japanese Government. In this trip, various types of cultivated rice, distributed and under cultivation, were collected in Madagascar. Those seed samples were investigated to fix the seed storage proteins for the purposes of making some nutritional improvement in rice.

In this report, only the SDS-PAGE analysis on storage proteins of the cultivated rice collected in Madagascar was described. Based on the data obtained in the further

analyses of seed storage proteins, more detailed characteristics are going to be informed in the following papers.

The writers are most grateful to the Government Officials in Madagascar. Thanks are also due to the following persons; Mon. Zafera Abtoine RABESA, Mrs. Yvonne RABENATOANDRO, Prof. Zefaniasy BEMANANJARA, Ambassade de Madagascar au Japon, Embassy of Japan in Antananarivo.

Materials and Methods

One hundred and thirty strains assorted according to the morphological observations from 99 seed samples collected in Madagascar[14] were used for SDS-PAGE analysis of storage proteins in the starchy endosperm.

Extracted storage proteins of the collected samples were electrophoresed using the discontinuous buffer system of LAEMMLI[7] on a slab gel containing an acrylamide/BIS concentration of 32:0.8 and 30:0.135 (SDS-PAGE). Proteins were extracted from one grain for each line. Each grain was crashed by pliers, suspended in 0.5 ml extraction buffer (50 mM KH_2PO_4-NaOH, pH 6.8, containing 4M urea, 4 % SDS, 20 % glycerin and 5 % mercaptoethanol) and sonicated for several minutes. After centrifugation (15,000 rpm, for 10 min), 7μl of the supernatant was used for SDS-PAGE. After electrophoresis, the proteins were stained with Coomassie brilliant blue R 250.

Results and Discussion

Geographical distribution and habitats of the seed samples used in this experiment were briefly illustrated in Fig. 1, in which the trip route and collection site were given, too.

After electrophoresing 130 strains of 99 seed samples by using the two systems of electrophoresis, it was found that there was a wide variation on the electrophoretic pattern of seed storage proteins in the cultivated rice collected in Madagascar. The storage proteins in the starchy endosperm of the rice collected in Madagascar were dissociated and separated by SDS-PAGE; and they were grouped, for an apparent molecular mass, into seven groups of 57, 37-39, 26, 22-23, 16, 13 and 10 kDa. By using a polyacrylamide gel containing a low concentration of BIS in SDS-PAGE, three bands were identified in 37-39 kDa polypeptides, designated α-1, α-2, and α-3. On the basis of migrating distance of the individual polypeptide bands, these strains were classified into two types for 37-39 kDa polypeptides, tentatively named types A and B. Type A was distinguished from type B in the difference in migrating distance of α-3 band. α-3 band of type A migrated faster than that of type B (Fig. 2).

Two major bands were identified in the 13 kDa polypeptides, designated 13a and

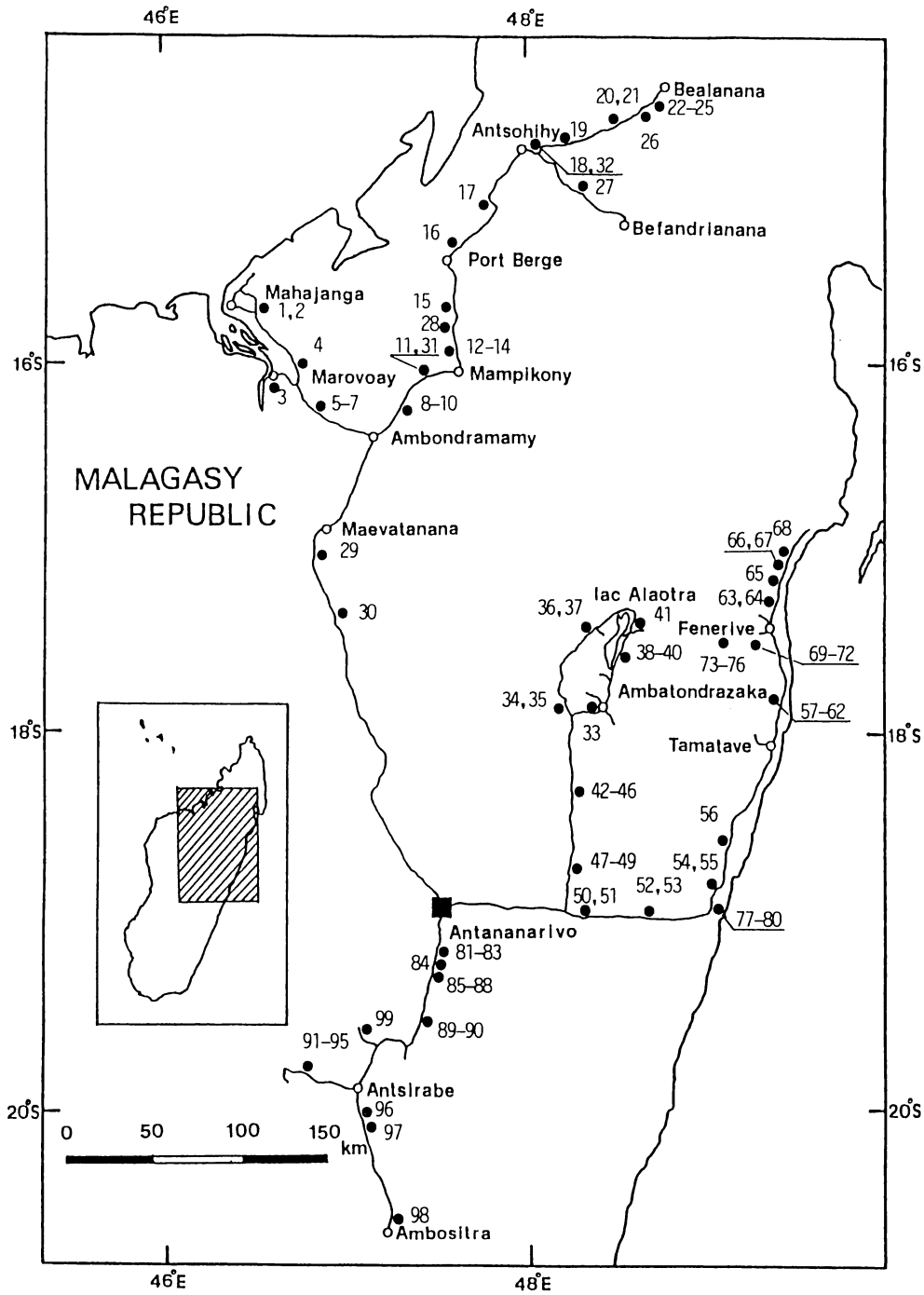

Fig. 1. Map showing several localities where the cultivated rices were collected in Madagascar. Solid line; route of observations, filled circles; collection areas; open circles; main towns. Code-numbers used in the figure are corresponding to the strain number used in the table.

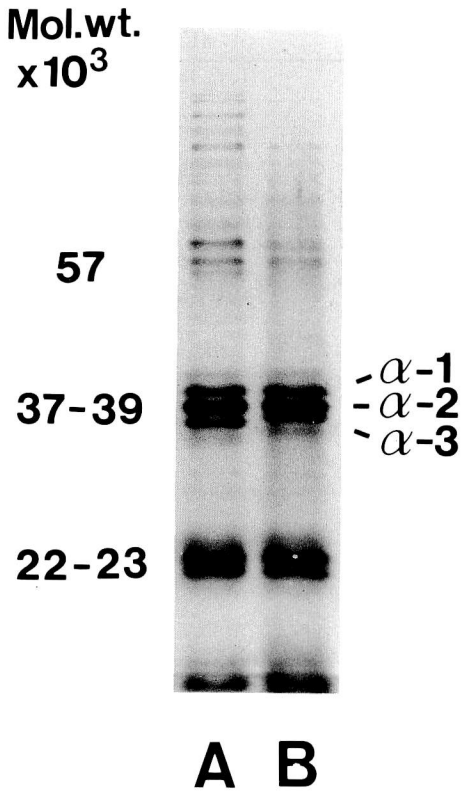

Fig. 2. Electrophoretic patterns of two types of variations for glutelin α-3 subunit of endosperm storage proteins in rice collected in Madagascar.
A; Type A, B; Type B

13b bands, by using a polyacrylamide gel containing a high concentration of BIS. Basing on the staining intensities or the migration-distance of the 16, 13a, 13b and 10 kDa polypeptide bands, these strains were classified into four types, tentatively named types 1, 2, 3 and 4. 'Type 1' was characterized by the two bands of 13 KDa polypeptides with the same intensity, 'type 2' by the low intensity of 13b band, 'type 3' by the high intensity of 13a band without 13b band and 'type 4' by the high intensity of 13b band with low intensity of 13a band (Fig. 3).

The results were given in Table 1. Of 130 strains used in the SDS-PAGE analysis for gluterin α-3 subunit, 21 strains belonged to the type A and 109 strains belonged to the type B (Table 2). The frequencies of types A and B were observed to be 16 % and 84 %, respectively. 113 strains of lowland rice and 17 strains of upland rice were collected in Madagascar. On the lowland rices, 16 strains belonged to the type A and 97 strains belonged to the type B. The frequencies of types A and B were found to be 14 % and 86 %, respectively. On the upland rices, 5 strains belonged to the type A and 12 strains belonged to the type B (Table 1). The frequencies of types A and B were

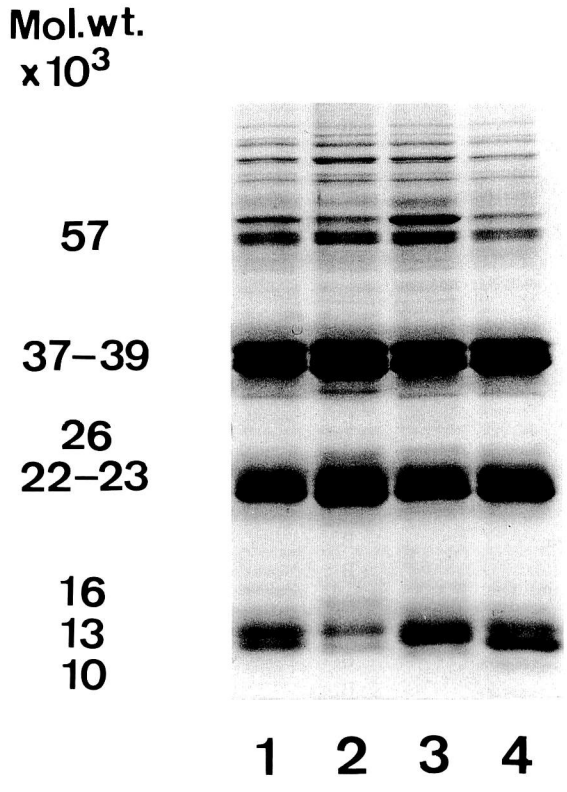

Fig. 3. Electrophoretic patterns of four types of variations for 13 kDa polypeptide bands of storage proteins in rice collected in Madagascar.
1; Type 1, 2; Type 2, 3; Type 3, 4; Type 4

Table 1. SDS-PAGE analysis of seed storage proteins of cultivated rice collected in Madagascar in 1988

Strain No.	Local name	37-39 kDa polypeptides	13 kDa polypeptides
1	Avia Mizaha	B	4
2	Sanabody	A	3
3-1	Andramonta	B	2
3-2	Andramonta	B	2
3-3	Andramonta	B	3
4-1	Tsipala	B	2
4-2	Tsipala	B	3
5	Tsipala	B	2
6	Andramonta	B	3
7	-Unknown-	B	3
8	-Unknown-	B	3
9	Tsipala	B	4
10	Masokibobo	B	3
11	Vary Vatosoa	A	3

12	Vary Vato	A	3
13	Tsipala	B	2
14-1	Naoromidina	B	3
14-2	Naoromidina	B	2
15	Tsipala	B	3
16	Makalioka	B	2
17	Bemarijy & Andramonta	B	4
18	Mamoriake	A	1
19-1	Tsitaitra	B	3
19-2	Tsitaitra	B	4
20	Tsivimbina	B	3
21	Tahosy	A	2
22-1	Makalioka	B	4
22-2	Makalioka	B	3
23	Rakaraka	B	1
24-1	Komojy	B	2
24-2	Komojy	B	3
25	Rojo	A	4
26*	Tsra Voa Banga	B	3
27	Makalioka	B	3
28-1	Vary Patsa	B	3
28-2	Vary Patsa	B	3
28-3	Vary Patsa	B	3
28-4	Vary Patsa	B	3
29	Bekimondro	B	3
30-1	-Unknown-	B	3
30-2	-Unknown-	B	3
31	-Unknown-	B	3
32	-Unknown-	B	4
33	Makalioka	B	2
34-1	Makalioka	B	3
34-2	Makalioka	B	2
35	-Unknown-	B	3
36-1	Makalioka	B	2
36-2	Makalioka	B	4
37	Vary Malady	B	2
38	Vary Malady	B	3
39	-Unknown-	B	2
40	Rojomena Rojofotsy	B	4
41-1	Makalioka	B	3
41-2	Makalioka	B	3
42	Vonjy	B	3
43	Bestileo	B	4
44-1	Makalioka	B	3
44-2	Makalioka	B	3
45	Rojhofotsy	B	3
46	Vary Be	B	3
47	Makalioka	B	3
48	Telovolana	A	4
49	Rojofotsy	B	2
50-1*	Langakafotsy	B	4
50-2*	Langakafotsy	B	1

50-3*	Langakafotsy	B	2
51*	Somotra	B	4
52*	Somotra	B	4
53-1	Vanjakohnandiana	A	2
53-2	Vanjakohnandiana	B	2
54*	Mena Vazana	A	2
55*	Mena Vazana	A	1
56	Ramaditra	B	3
57*	Vimboahangy	B	2
58*	Mintimalady	A	3
59*	Telovorana	B	3
60	Diara	B	3
61	Makalioka	B	2
62-1*	Vary Be	A	2
62-2*	Vary Be	A	1
63-1	Vary Gonibe	B	3
63-2	Vary Gonibe	B	3
64*	Vary Be Malady	B	1
65-1	Marotia	B	4
65-2	Marotia	B	3
66	Kirimy	B	3
67	Lohambitro	A	1
68-1	Lohambitro (Menamongo)	A	1
68-2	Lohambitro (Menamongo)	A	1
69*	Bemahasoa	A	3
70*	Lohambitrobe	B	2
71*	Rambompiso	B	1
72-1	Tsipala	B	3
72-2	Tsipala	B	4
73-1	Vary Gony	B	3
73-2	Vary Gony	B	3
74*	Telovorana	B	3
75	Bary Botrika	A	3
76	Vary Kitrana	B	3
77	Vary Kitrana	B	3
78*	Vary Somotra	B	3
79	Kirotsaka	B	2
80	Ramilona	B	2
81	Rojomena	B	2
82-1	Botry-Tsindrilahy	B	3
82-2	Botry-Tsindrilahy	B	3
83-1	Botry	B	3
83-2	Botry	B	4
84	Ambolavava	B	3
85	Botry	B	2
86-1	Rojo	B	2
86-2	Rojo	B	2
87	Japone (Unknown)	A	1
88	Botry	A	2
89	Telorirana	B	2
90-1	Rojomena	B	3

90-2	Rojomena	B	3
91	Rijakely	A	1
92	Rijakely	B	2
93	Mangakely	B	3
94	Tsipala	B	1
95-1	Mavokely	B	2
95-2	Mavokely	B	2
96-1	Manfe	B	2
96-2	Manfe	B	2
97-1	Mangatovo	B	1
97-2	Mangatovo	B	2
98	Kalafohindrazaha	B	2
99	Latsika	B	2

* Upland rice.

Table 2. Geographical distribution of strains for the variation of α-3 subunit of storage proteins of cultivated rice collected in Madagascar

Locality	Number of strains				
	Type A	%	Type B	%	Total
Northern Area	6	(14)	37	(86)	43
Central Area	2	(7)	26	(93)	28
Eastern Area	10	(30)	23	(70)	33
South Mountain Area	3	(12)	23	(88)	26
Total	21	(16)	109	(84)	130

found to be 29 % and 71 %, respectively.

Geographical distributions of two types were also shown in Table 2. In Northern Area (strains No.1 to No.32), *i.e.*, Mahajanga, Marovoay, Maevatanana, Mampikony, Antsohihy and Bealanana districts, 86 % of the strains collected in these areas, namely 37 strains, belonged to type B; and the frequency of type A was found to be 14 %, 6 strains. In Central Area (strains No.33 to No.53), *i.e.*, Moramanga and Lac Alaotra districts, 2 strains belonged to the type A, and 26 strains belonged to the type B. The frequencies of the types A and B were found to be 7 % and 93 %, respectively. In Eastern Area (strains No.54 to No.80), *i.e.*, Brickaville, Anosibe and Fenerive districts, 10 strains belonged to the type A, and 23 strains belonged to the type B. The frequencies of the types A and B were observed to be 30 % and 70 %, respectively. In South Mountain Area (strains No.81 to No.99), *i.e.*, Ansirabe and Ambositra districts, 3 strains belonged to the type A, and 23 strains belonged to the type B. The frequencies of the types A and B were found to be 12 % and 88 %, respectively.

Most of upland rices were collected in the Eastern Area. It seems that one of the reasons for the high frequency of type A in this area is caused by the high frequency of

type A in upland rices.

KAGAWA *et al.*[3] reported that some rice cultivars in Asian countries deleted the α-3 subunits and that they were found in *Indica* type of rice, not in the *Japonica* type. In this analysis for the glutelin subunits of cultivated rices collected in Madagascar, both the faster migrated α-3 subunit, or type A, and the slower migrated α-3 subunit, or type B, were observed (Fig. 2). The deletion-types of α-3 subunit were not found in these strains. Although there remains a genetical question whether or not the slower-migrated-type is the same as the deletion-type of α-3 subunit, both of the electrophoretic pattern is seemingly quite similar. The majority of cultivated rices collected in Madagascar belonged to type B (84 %) (Table 2). Results obtained in this experiment might give some useful informations about the differentiation or distribution of the cultivated rice in Madagascar.

Frequency distribution of variation for 13 kDa polypeptides of storage proteins of cultivated rice collected in Madagascar was shown in Fig. 4. The frequencies of strains belonging types 1, 2, 3 and 4 were found to be 14 (11 %), 39 (30 %), 57 (44 %) and 20 (15 %), respectively. On the lowland rices, the frequencies of strains belonging to types 1, 2, 3 and 4 were found to be 10 (9 %), 34 (30 %), 51 (45 %) and 18 (16 %), respectively. On the upland rices, the frequencies of strains belonging to types 1, 2, 3 and 4 were found to be 4 (24 %), 5 (29 %), 6 (35 %) and 2 (12 %), respectively. SATOH *et al.*[15] identified the five types of variation for 13 kDa polypeptides in the cultivated rices collected from Tanzania. In this analysis, four types, namely types 1, 2, 3 and 4, were identified in the cultivated rices collected in Madagascar, but 'type 5' which lacks the 13a band was not found.

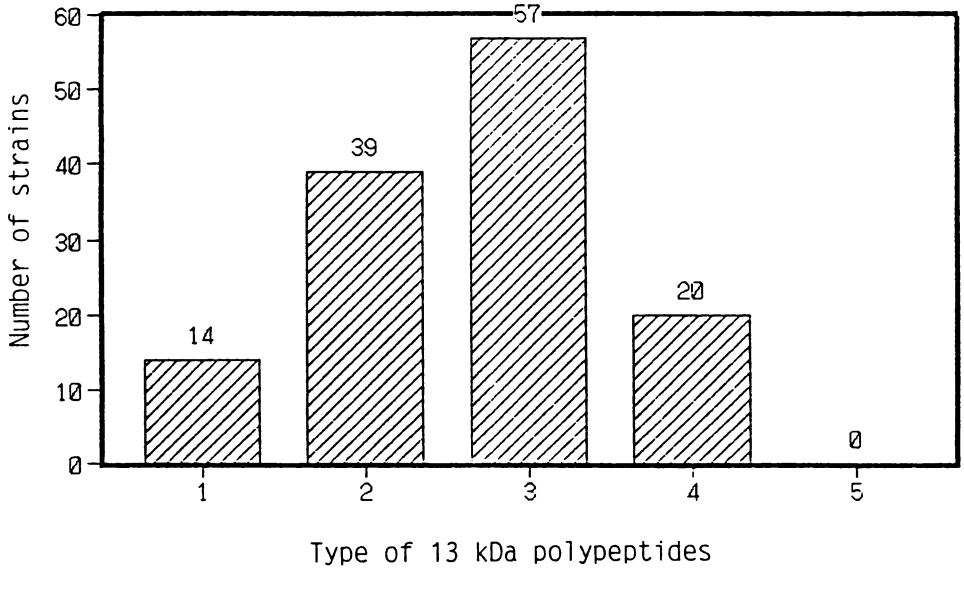

Fig. 4. Distribution of strains for the variations in 13 kDa polypeptides bands of endosperm storage proteins of cultivated rice collected in Madagascar.

Geographical distribution of the four types was shown in Fig. 5A to Fig. 5D. The frequencies of strains belonging to each type differed considerably among localities. In Northern Area (A), the highest frequency was observed in type 3 and the lowest one was found in 'type 1'. The frequencies of strains belonging to types 1, 2, 3 and 4 were found to be 2 (5 %), 9 (21 %), 22 (51 %) and 10 (23 %), respectively. In Central Area (B), the frequency of 'type 1' was considerably lower than other 3 types. The frequencies of strains belonging to types 1, 2, 3 and 4 were found to be 1 (4 %), 9 (32 %), 11 (39 %) and 7 (25 %), respectively. In Eastern Area (C), the highest frequency was observed in 'type 3' and the lowest was observed in 'type 4'. The frequencies of strains belonging to types 1, 2, 3 and 4 were found to be 7 (21 %), 7 (21 %), 17 (52 %) and 2 (6 %), respectively. In South Mountain Area (D), the highest frequency was observed in 'type 2' and the lowest was observed in 'type 4'. The frequencies of strains belonging to types 1, 2, 3 and 4 were found to be 4 (15 %), 14 (54 %), 7 (27 %) and 1 (4 %), respectively.

Wide variations were found in the polypeptide compositions of storage protein in the cultivated rices collected in Madagascar. Emphasis should be placed on the selection and the characterization of genetic materials for the qualitative and quantitative improvements of storage proteins of rice. KUMAMARU *et al.*[6] reported four types of mutants for storage proteins in starchy endosperm of rice, and discussed of the useful-

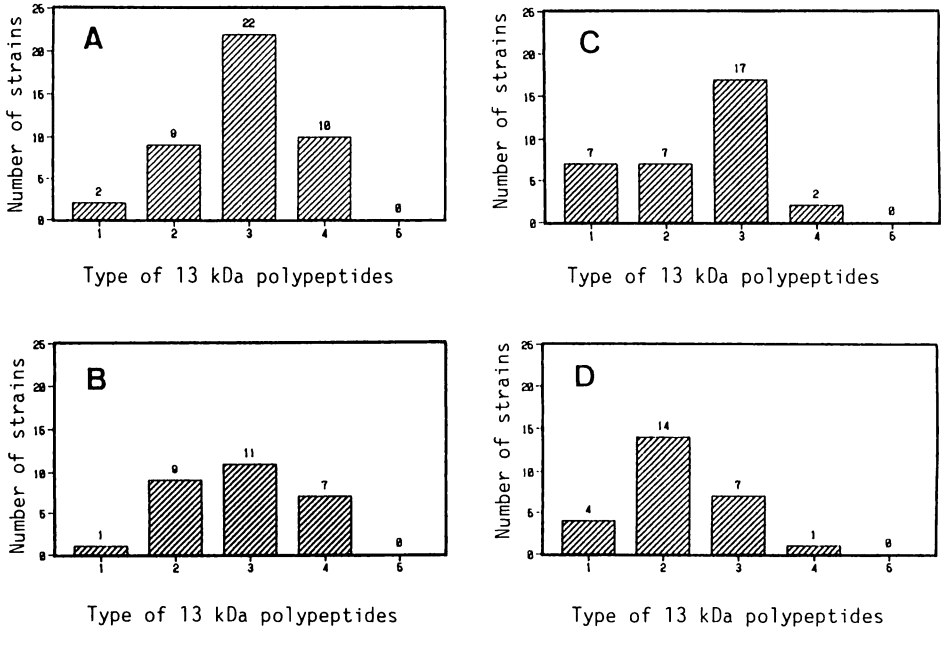

Fig. 5. Geographical distribution of strains for the variations in 13 kDa polypeptide bands of endosperm storage proteins of cultivated rice collected in Madagascar.
A; Northern Area, B; Central Area, C; Eastern Area,
D; South Mountain Area

ness of those mutants as the materials for breeding of qualitatively improved rice proteins and for biochemical and genetical studies on the regulation, biosynthesis and accumulation mechanisms of rice storage protein. The variations obtained in this analysis may also be promising and useful genetic materials for breeding and for genetical and biochemical studies of seed storage proteins of rice, though the detailed characteristics are under examination.

Summary

During the trip from June 1 to June 28 in 1988, 99 seed samples of cultivated rice, *Oryza sativa* L., were collected. Those were classified into 130 strains according to the morphological observations. Seed storage proteins extracted from one rice seed of each strain were subjected to SDS-PAGE. The SDS-PAGE analysis of seed storage proteins was reported.

Basing on the migration mode or staining intensity of the individual polypeptide bands, strains were classified into two types for glutelin α -3 subunit, tentatively named as types A and B (Fig. 2), and four types for 13 kDa polypeptides, tentatively named types 1, 2, 3 and 4 (Fig. 3).

Of 130 strains, 21 strains (16 % of the whole) belonged to type A and 109 strains (84 %) belonged to type B (Table 1). The number of strains belonging to types A and B were 16 (14 %) and 97 (86 %) in the lowland rice, and 5 (29 %) and 12 (71 %) in the upland rice, respectively.

Geographical distribution of types A and B considerably differed among localities (Table 2). The frequencies of strains belonging to types A and B were 14 % and 86 % in Northern Area, 7 % and 93 % in Central Area, 30 % and 70 % in Eastern Area and 12 % and 88 % in South Mountain Area, respectively. It seems that one of the reasons for the high frequencies of type A in Eastern Area is caused by the high frequency of type A in upland rices.

The frequencies of strains belonging to types 1, 2, 3 and 4 were found to be 11 % (14 strains), 30 % (39 strains), 44 % (57 strains) and 15 % (20 strains), respectively. The frequencies of types 1, 2, 3 and 4 were found 9 %, 30 %, 45 % and 16 % in the lowland rice, and 24 %, 29 %, 35 % and 12 % in the upland rice, respectively.

Geographical distribution of strains belonging to respective types also differed considerably among localities (Figs. 4A to 4D). The frequencies of strains belonging to types 1, 2, 3 and 4 were found to be 5 %, 21 %, 51 % and 23 % in Northern Area, 4 %, 32 %, 39 % and 25 % in Central Area, 21 %, 21 %, 52 % and 6 % in Eastern Area, and 15 %, 54 %, 27 % and 4 % in South Mountain Area, respectively.

References

1) BEACHELL, H. M., G. S. KHUSH and B. O. JULIANO: Breeding for high protein content in rice. *In* : Rice Breeding. IRRI, Los Banõs, Philippines, pp. 419-428 (1972)
2) HIGASI, T., K. KUSHIBUCHI and R. ITO: Studies on breeding for high protein rice. I. Protein content of different rice varieties and their relations with some agronomic traits including yield. Jpn. J. Breed., **24**:88-96 (1974)
3) KAGAWA, H., H. HIRANO and F. KIKUCHI: Variation of glutelin seed storage protein in rice (*Oryza sativa* L.). Jpn. J. Breed., **38**:327-332 (1988)
4) KAMBAYASHI, M., I. TSURUMI and T. SASAHARA: Genetic studies on improvement of protein content in rice grain. Jpn. J. Breed., **34**:356-363 (1984)
5) KITAMURA, K. and N. KAIZUMA: Mutant strains with low level of subunits of 7S globulin in soybean (*Glycine max* Merr.) seed. Jpn. J. Breed., **31**:353-359 (1981)
6) KUMAMARU, T., H. SATOH, N. IWATA, T. OMURA, M. OGAWA and K. TANAKA: Mutants for rice storage proteins. 1. Screening of mutants for rice storage proteins of protein bodies in the starchy endosperm. Theor. Appl. Genet., **76**:11-16 (1988)
7) LAEMMLI, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, **227**:680-685 (1970)
8) MERTZ, E. T., L. S. BATES and O. E. NELSON: Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science, **145**:279-280 (1964)
9) MUNCK, L., K. E. KARLSSON, A. HAGBERG and B. O. EGGUM: Gene for improved nutritional value in barley seed protein. Science,**168**:985-987 (1970)
10) NELSON, O. E., E. T. MERTZ and L. S. BATES: Second mutant gene affecting the amino acid pattern of maize endosperm proteins. Science, **150**:1469-1470 (1965)
11) OSONE, K. and T. TAKAGI: Studies on breeding for high protein content and quality in rice. I. Estimation of seed-protein content using the Dye-binding methodJpn. J. Breed., **20**:301-304 (1970)
12) PAYNEP, I., L. M. HOLT and C. N. LAW: Structual and genetical studies on the high-molecular-weight subunits of wheat glutenin. Part 1. Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum*). Theor. Appl. Genet., **60**:229-236 (1981)
13) ROBERT, L. S., C. NOZZOLILLO and C. ALTOSAAR I: Molecular weight and charge heterogeneity of prolamines (Avenins) from nine oat (*Avena sativa* L.) cultivars of different protein content and from developing seeds. Cereal Chem., **60**:438-442 (1983)
14) SATOH, H., X. RAKOTONJANAHARY and T. C. KATAYAMA: On distribution and grain morphology of cultivated rice collected in Madagascar, 1988. Kagoshima Univ. Res. Center S. Pac., Occ. Papers, **18**:63-72 (1990)

15) SATOH, H., H. M. CHING'ANG'A, D. ILAILA and T. C. KATAYAMA: SDS-PAGE analysis of storage proteins of cultivated rice collected in Tanzania, 1988. Kagoshima Univ. Res. Center S. Pac., Occ. Papers, **18**:114-126 (1990)

16) SHEWRY, P. R., J. R. S. ELLIS, H. M. PRATT and B. J. MIFLIN: A comparison of methods for the extraction and separation of hordein fractions from 29 varieties. J. Sci. Food Agric., **29**:433-441 (1978)

17) SHIN, Y. B., S. TANAKA and T. KATAYAMA: Studies on the quantitative and qualitative improvement of rice. Application of DBC as a screening technique and assessment of Japanese local rice varieties for high protein and lysine. Sci. Bull. Fac. Agric., Kyushu Univ., **31**:145-150 (1977)