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# SDS-PAGE Analysis of Storage Proteins of Cutivated Rice Collected in Tanzania, 1988

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## Introduction

Milled rice protein is one of the principal protein sources not only in the Japanese people but also in the Tanzanian 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 uesd 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 Tanzania during the period from June 30 to August 10 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 Tanzania. 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 Tanzania 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.

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## Materials and Methods

One hundred and twenty nine strains assorted according to the morphological observations from 106 seed samples collected in Tanzania[14], including 127 strains of *O. sativa* cultivars and two strains of *O. glaberrima*, 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), 7 $\mu$l 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 129 strains of 106 seed samples including 127 strains of *O. sativa* and two strains of *O. glaberrima* through the use of 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 Tanzania. The storage proteins in the starchy endosperm of the rice collected in Tanzania 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 polypeptydes, designated $\alpha$-1 $\alpha$-2 and $\alpha$-3. Based on the migrating distance or staining intensities of the individual polypeptide bands, these strains were classified into three types for 37-39 kDa polypeptides, tentatively named types A, B and C. Type A was distinguished from type B in the difference in migrating distance of $\alpha$-3 band. $\alpha$-3

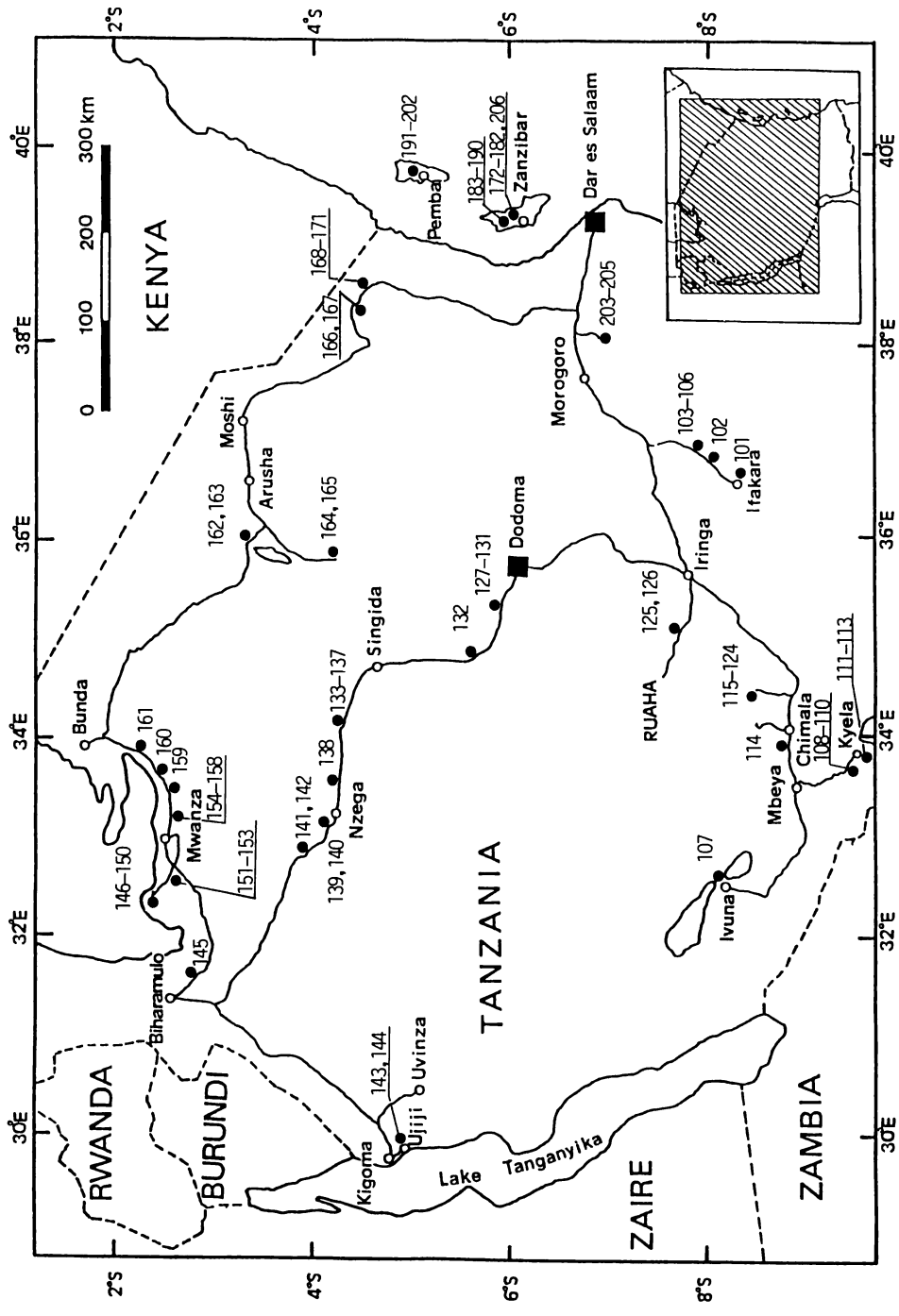


Fig. 1. Map showing several localities where the cultivated rices were collected in Tanzania. Solid line; route of observation, filled circles; collection areas, open circles; main towns. Code-numbers used in the figure are corresponding to the strain number used in the table.

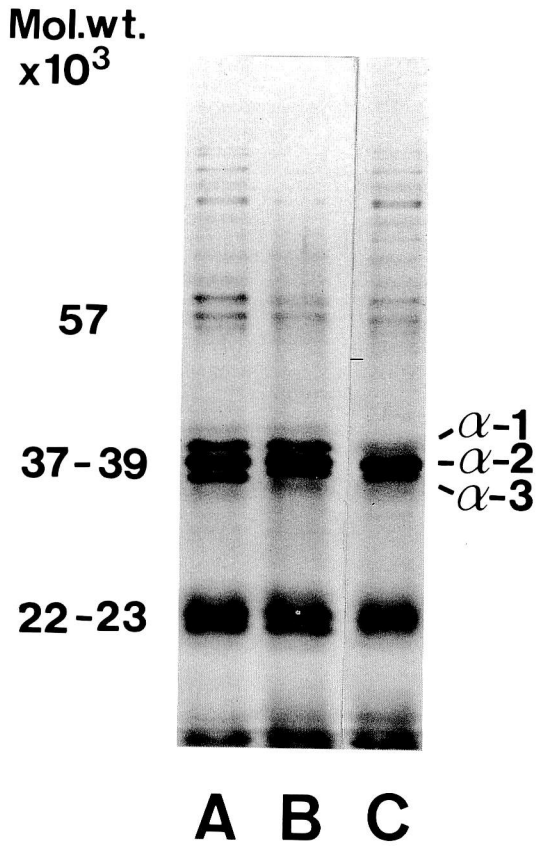


Fig. 2. Electrophoretic patterns of two types of variations for glutelin α subunits of endosperm storage proteins in rice collected in Tanzania.
A; Type A, B; Type B, C; Type C

band of type A migrated faster than that of type B (Fig. 2). Type C was distinguished apparently from the others in the absence of α-1 band.

Two major bands were identified in the 13 kDa polypeptides, designated 13a and 13b bands, by using a polyacrylamide gel containing a high concentration of BIS. Basing on the staining intensities or the migration distance of the individual polypeptide bands, these strains were classified into six types for 16, 13a, 13b and 10 kDa polypeptides, tentatively named types 1, 2, 3, 4, 5 and 6, respectively. 'Type 1' was characterized by the two bands of 13 kDa polypeptides with the same intensity, 'type 2' by a low intensity of 13b band, 'type 3' by the high intensity of 13a band without 13b band, 'type 4' by the high intensity of 13b band with a low intensity of 13a band and 'type 5' by the high intensity of 13b band without 13a band (Fig. 3). Type 6 was to be distinguished from the others according to the band pattern, in which another band was identified between 16 kDa and 13 kDa polypeptides.

The results were given in Table 1. Of 129 strains used in the SDS-PAGE analysis for gluterin α subunits, types A and B were observed in *O. sativa* cultivars, *i.e.*, 31

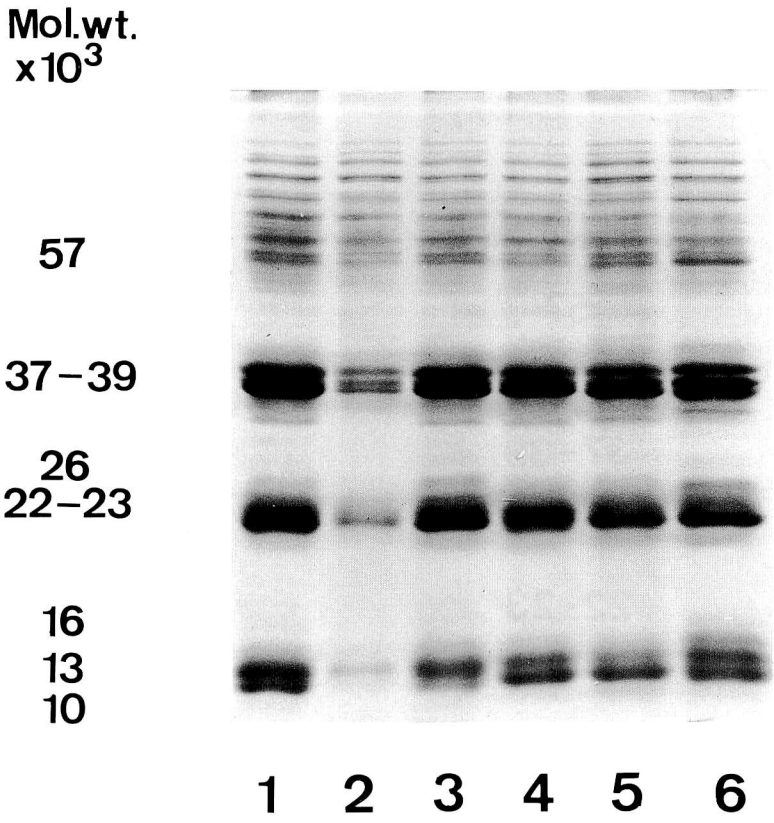


Fig. 3. Electrophoretic patterns of six types of variations for 13 kDa polypeptide bands of storage proteins in rice collected in Tanzania.
1; Type 1, 2; Type 2, 3; Type 3, 4; Type 4, 5; Type 5, 6; Type 6

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

| Strain No. | Local name | 37-39 kDa polypeptides | 13kDa polypeptides |
|---|---|---|---|
| 101 | India Rangi | B | 2 |
| 102 | Limoto | A | 2 |
| 103 | India | B | 2 |
| 104 | Kisaki | B | 2 |
| 105 | Arusha | A | 3 |
| 106 | Afaa | A | 2 |
| 107 | Kilombero | B | 1 |
| 108 | Mwangle | A | 1 |
| 109 | Mwasungo | B | 2 |
| 110 | Kilombero | B | 1 |
| 111 | Supa | B | 2 |
| 112 | Kilombero | B | 4 |
| 113 | Supa Mwasungo | B | 2 |

| | | | |
|---|---|---|---|
| 114 | Kilombero | B | 2 |
| 115 | Kibibi | B | 1 |
| 116 | Kilombero | B | 3 |
| 117 | Taiwan | B | 1 |
| 118 | Kihogo | B | 1 |
| 119 | Afaa Mwanza | A | 3 |
| 120 | Selemwa | B | 4 |
| 121 | Shindano | B | 2 |
| 122 | India | B | 2 |
| 123 | Kula Na Bwana | B | 2 |
| 124 | Cola (Unknown) | B | 4 |
| 125 | Supa | B | 2 |
| 126 | Ngohi | B | 2 |
| 127 | Supa-1 | B | 1 |
| 128 | Supa-2 | B | 1 |
| 129 | Supa-3 | B | 2 |
| 130 | Kihogo-1 | B | 2 |
| 131 | Kihogo-2 | B | 2 |
| 132 | -Unknown (Supa)- | B | 1 |
| 133 | -Unknown- | B | 1 |
| 134 | -Unknown- | B | 1 |
| 135 | -Unknown- | B | 3 |
| 136 | -Unknown- | B | 1 |
| 137 | -Unknown- | B | 1 |
| 138 | Supa | B | 3 |
| 139 | Supa | B | 4 |
| 140 | Kihogo | A | 1 |
| 141 | Supa | B | 1 |
| 142 | Kihogo | B | 1 |
| 143 | Supa | B | 2 |
| 144 | Supa | B | 2 |
| 145 | Horonadi | A | 5 |
| 146-1 | -Unknown (Supa) | B | 5 |
| 146-2 | -Unknown (Supa) | B | 5 |
| 147 | Moshi | B | 2 |
| 148-1 | Supa | B | 4 |
| 148-2 | Supa | B | 4 |
| 149 | Faya | A | 2 |
| 150 | Kihogo | B | 1 |
| 151 | Supa | B | 3 |
| 152 | Kihogo | A | 2 |
| 153 | Shindano | B | 3 |
| 154 | Supa | B | 3 |
| 155 | -Unknown (Mixture)- | B | 4 |
| 156 | Senga Senga | B | 2 |
| 157 | Moshi | A | 3 |
| 158 | -Unknown- | A | 2 |
| 159 | Lukata Kihogo | A | 5 |
| 160 | -Unknown- | A | 5 |
| 161 | -Mixture- | B | 2 |

| | | | |
|---|---|---|---|
| 162 | Supa | B | 2 |
| 163 | Moshi (Sigara) | A | 1 |
| 164 | Moshi (Sigara) | A | 2 |
| 165 | Supa | A | 4 |
| 166 | Supa | B | 2 |
| 167 | -Mixture- | B | 2 |
| 168-1 | Semanini | B | 3 |
| 168-2 | Semanini | B | 2 |
| 169 | Kihogo | B | 2 |
| 170 | Supa | B | 3 |
| 171 | Wahi Wahi | B | 3 |
| 172 | -Unknown- | A | 3 |
| 173 | Pinlot-330 | A | 3 |
| 174 | Colombia-5179 | A | 2 |
| 175-1 | Supa | B | 1 |
| 175-2 | Supa | B | 3 |
| 176 | -Unknown- | B | 3 |
| 177 | -Unknown- | A | 3 |
| 178 | Kijicho | B | 3 |
| 179-1 | Moshi | A | 3 |
| 179-2 | Moshi | A | 3 |
| 180-1 | Wamba | B | 3 |
| 180-2 | Wamba | B | 1 |
| 181 | -Unknown- | B | 2 |
| 182 | -Unknown- | B | 4 |
| 183-1 | -Mixture- | B | 3 |
| 183-2 | -Mixture- | B | 2 |
| 183-3 | -Mixture- | A | 2 |
| 184 | Supa | B | 3 |
| 185-1 | Gamti | B | 2 |
| 185-2 | Gamti | B | 1 |
| 186 | Mkia Wa Ngawa | B | 3 |
| 187 | Singapuri | B | 1 |
| 188 | Ringa | A | 1 |
| 189-1 | Tarabizuma | A | 3 |
| 189-2 | Tarabizuma | B | 4 |
| 190-1 | Ringa | A | 5 |
| 190-2 | Ringa | A | 4 |
| 191-1 | Ringa | A | 3 |
| 191-2 | Ringa | A | 4 |
| 191-3 | Ringa | A | 3 |
| 192 | Afaa | B | 1 |
| 193-1 | Kivuli | B | 2 |
| 193-2 | Kivuli | B | 2 |
| 194 | Riziki | B | 4 |
| 195 | Kibawa | B | 4 |
| 196 | Ausbin | B | 2 |
| 197-1 | Afaa | B | 4 |
| 197-2 | Afaa | B | 2 |
| 198 | Tiwani | B | 3 |

| | | | |
|---|---|---|---|
| 199-1 | Zira | B | 4 |
| 199-2 | Zira | B | 5 |
| 200-1 | Malbora | B | 3 |
| 200-2 | Malbora | B | 4 |
| 201 | Kivuli | B | 4 |
| 202 | Supa | B | 4 |
| 203-1 | Mukia Wa Nyumba | B | 2 |
| 203-2 | Mukia Wa Nyumba | B | 2 |
| 203-3 | Mukia Wa Nyumba | B | 4 |
| 203-4 | Mukia Wa Nyumba | B | 3 |
| 203-5 | Mukia Wa Nyumba | B | 2 |
| 204-1 | Supa | B | 3 |
| 204-2 | Supa | B | 3 |
| 205 | Kula Na Bwana | B | 4 |
| 206-1 | *O. glaberrima* | C | 6 |
| 206-2 | *O. glaberrima* | C | 6 |

Table 2. Geographical distribution of variants for the α-3 subunit of storage proteins of *O. sativa* cultivars rice collected in Tanzania

| Locality | Number of strains | | | | |
|---|---|---|---|---|---|
| | Type A | % | Type B | % | Total |
| Southwestern Area | 6 | (23) | 20 | (77) | 26 |
| Central and Western Areas | 2 | (11) | 17 | (89) | 19 |
| Northern Area | 6 | (33) | 12 | (67) | 18 |
| Eastern Area | 3 | (16) | 16 | (84) | 19 |
| Zanzibar and Pemba Islands | 14 | (31) | 31 | (69) | 45 |
| Total | 31 | (24) | 96 | (76) | 127 |

strains of *O. sativa* cultivars belonging to the type A and 96 strains of *O. sativa* cultivars belonging to the type B, respectively. Type C was observed in two strains of *O.glaberrima*, collected in Zanzibar Island. No *O. sativa* cultivar belonging to the type C was found, as far as the cultivars collected in Tanzania were concerned. But, when a polyacrylamide gel containing the high concentration of BIS was used, α-1 band could be observed in the gel electrophoresed two strains of *O. glaberrima* (Fig. 3). This suggests that α-1 band of *O. glaberrima* might qualitatively be different from that of *O. sativa*.

Geographical distributions of strains for the two types in *O. sativa* cultivars were also shown in Table 2. In Southwestern Area (strains No.101 to No.126), *i.e.*,

Ifakara, Mbeya, Ivuna, Kyela, Mbalari, Iringa districts, 23 % of the strains collected in these areas, namely 6 strains, belonged to type A; and the frequency of type B was found to be 77 %, 20 strains. In Central and Western Areas (strains No.127 to No.145), *i.e.*, Dodoma, Singida, Nzega and Ujiji districts, 2 strains belonged to the types A, and 1 strains belonged to the type B. The frequencies of the types A and B were found to be 11 % and 89 %, respectively. In Northern Area (strains No.146 to No.161), *i.e.*, Biharamuro, Mwanza and Bunda districts, 6 strains belonged to the type A and 12 strains belonged to the type B. The frequencies of the types A and B were observed to be 33 % and 67 %, respectively. In Eastern Area (strains No.162 to No.171 and No.203-1 to No.205), *i.e.*, Arusha, Moshi, Same and Ruvu districts, 3 strains belonged to the type A, and 16 strains belonged to the type B. The frequencies of the types A and B were found to be 16 % and 84 %, respectively. In Zanzibar and Pemba Islands (strains No.172 to No.202), 14 strains belonged to the type A, and 31 strains belonged to the type B. The frequencies of the types A and B were found to be 31 % and 69 %, respectively.

KAGAWA *et al.*[3] reported that some rice cultivars in Asian countries deleted the α-3 subunit and that they were found in *Indica* type of rice, not in the *Japonica* type. In this analysis for the glutelin subunits of *O. sativa* cultivars collected in Tanzania, 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-type of α-3 subunit was 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 patterns

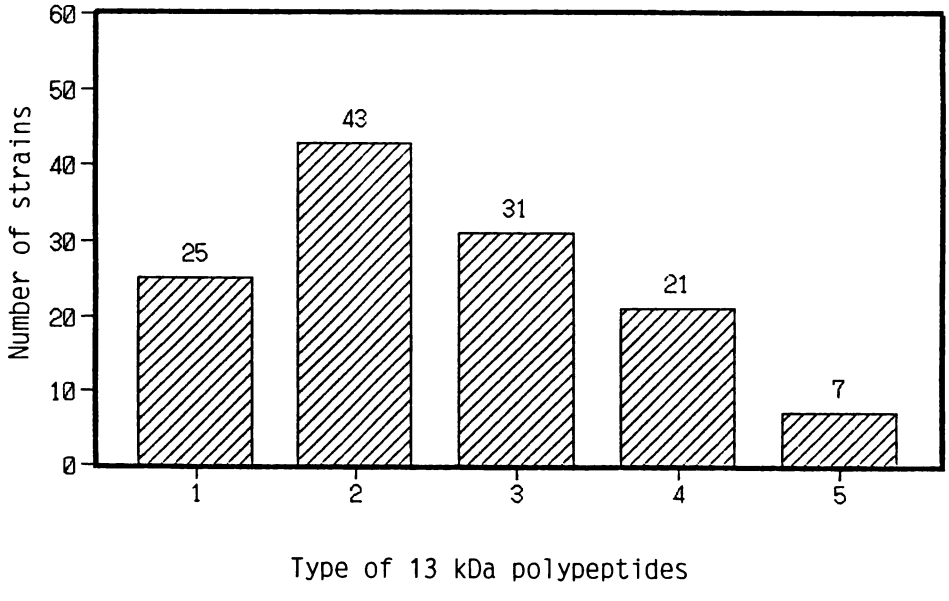


Fig. 4. Distribution of strains for the variation in 13 kDa polypeptide bands of endosperm storage proteins in *O. sativa* cultivars collected in Tanzania.

are seemingly quite similar. The majority of cultivated rices collected in Tanzania belonged to type B (76 %) (Table 2). Results obtained in this experiment might give some useful informations about the differentiation or distribution of the cultivated rice in Tanzania.

For 13 kDa polypeptides analysed by SDS-PAGE, types 1, 2, 3, 4 and 5 were found in *O. sativa* cultivars. Type 6 was observed in the two strains of *O. glaberrima* collected in Zanzibar Island.

Frequency distribution of variation for 13 kDa polypeptides of storage proteins of *O. sativa* cultivars collected in Tanzania was shown in Fig. 4. The highest frequencies was obtained in type 2 and the lowest was obtained in type 5. The frequencies of strains

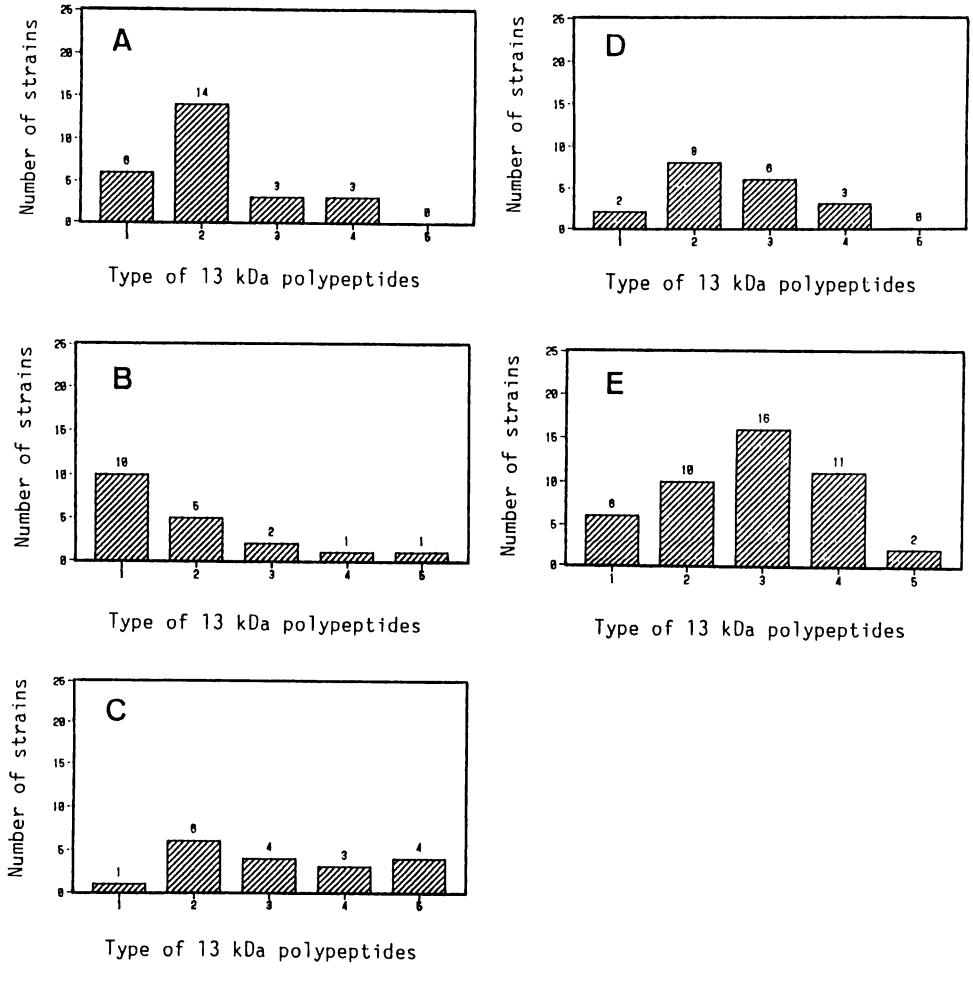


Fig. 5. Geographical distribution of cultivated rice for the variations in 13 kDa polypeptide bands of endosperm storage proteins in *O. sativa* cultivars collected in Tanzania.
A; Southwestern Area, B; Central and Western Areas,
C; Northern Area, D; Eastern Area,
E; Zanzibar and Pemba Islands

belonging to types 1, 2, 3, 4 and 5 were found to be 25 (20 %), 43 (34 %), 31 (24 %), 21 (16.5 %) and 7 (5.5 %), respectively. SATOH *et al.*[15] observed four types of variation for 13 kDa polypeptides in the cultivated rices collected in Madagascar. In this analysis, five types, *i.e.*, types 1, 2, 3, 4 and 5, were identified in the cultivated rices of *O. sativa* collected in Tanzania. Results obtained in this analysis might give some informations to the distribution or differentiation of the cultivated rice of *O. sativa* in Africa.

Geographical distribution of the five types was shown in Fig. 5A to Fig. 5E. The frequency of strains belonging to each type differed considerably among localities. In Southwestern Area (A), the frequency of 'type 3' was highest among them and no 'type 5' was observed. The frequencies of strains belonging to types 1, 2, 3, 4 and 5 were found to be 6 (23 %), 14 (54 %), 3 (11.5 %), 3 (11.5 %) and 0 (0 %), respectively. In Central and Western Areas (B), the frequency of 'type 1' was highest among them. The frequencies of strains belonging to types 1, 2, 3, 4 and 5 were found to be 10 (53 %), 5 (26 %), 2 (11 %), 1 (5 %) and 1 (5 %), respectively. In Northern Area (C), the highest frequency was observed in 'type 2', but the frequency was not so much different among them. The frequencies of strains belonging to types 1, 2, 3, 4 and 5 were found to be 1 (6 %), 6 (33 %), 4 (22 %), 3 (17 %) and 4 (22 %), respectively. In Eastern Area (D), the highest frequency was observed in 'type 2' and no 'type 5' was observed. The frequencies of strains belonging to types 1, 2, 3, 4 and 5 were found to be 2 (12 %), 8 (47 %), 6 (35 %), 3 (18 %) and 0 (0 %), respectively. In Zanzibar and Pemba Islands (E), the frequency of 'type 3' was highest among them. The frequencies of strains belonging to the types 1, 2, 3, 4 and 5 were found to be 6 (13 %), 10 (22 %), 16 (36 %), 11 (24 %) and 2 (4 %), respectively.

Wide variations were found in the polypeptide compositions of storage proteins in the cultivated rices collected in Tanzania. 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 usefulness of those mutants as the materials for breeding of qualitatively improved rice proteins and for biochemical and genetical studies on the regulation, biosysthesis 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 30 to August 10 in 1988, 105 seed samples of cultivated rice, *Oryza sativa* L., and a seed sample of *O. glaberrima* STEUD. were collected in Tanzania. Those were classified into 127 strains in *O. sativa* and 2 strains in *O. glaberrima*,

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 polypeptyde bands, strains were classified into three types for glutelin $\alpha$ subunits, tentatively named as types A, B and C (Fig. 2), and six types for 13 kDa polypeptides, tentatively named as types 1, 2, 3, 4, 5 and 6 (Fig. 3).

Of 127 strains of *O. sativa* cultivars, 31 strains (24 %) belonged to type A and 96 strains (76 %) belonged to type B (Table 1). Type C was observed in two strains of *O. glaberrima* collected in Zanzibar Island.

Geographical distribution of types A and B considerably differed among localities (Table 2). The frequencies of strains belonging to types A and B were 23 % and 77 % in Southwestern Area, 11 % and 89 % in Central and Western Areas, 33 % and 67 % in Northern Area, 16 % and 84 % in Eastern Area and 31 % and 69 % in Zanzibar and Pemba Islands, respectively.

For 13 kDa polypeptides analysed by SDS-PAGE, types 1, 2, 3, 4 and 5 were found in the strains of *O. sativa* cultivars. Type 6 was observed in two strains of *O. glaberrima* (Table 1). The frequencies of strains belonging to types 1, 2, 3, 4 and 5 were found to be 20 % (25 strains), 34 % (43 strains), 24 % (31 strains), 16.5 % (21 strains), and 5.5 % (7 strains), respectively.

Geographical distribution of strains belonging to each type also differed considerably among the localities (Figs. 4A to 4E). The frequencies of strains belonging to types 1, 2, 3, 4 and 5 were found to be 23 %, 54 %, 11.5 %, 11.5 % and 0 % in Southwestern Area, 53 %, 26 %, 11 %, 5 % and 5 % in Central and Western Areas, 6 %, 33 %, 22 %, 17 % and 22 % in Northern Area, 12 %, 47 %, 35 %, 18 % and 0 % in Eastern Area and 13 %, 22 %, 36 %, 24 % and 4 % in Zanzibar and Pemba Islands, respectively.

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