

STUDY OF PROTEIN DISTRIBUTION PATTERNS IN SOME ACCESSIONS OF WILD AND CULTIVATED RICE, *ORYZA* Linn.

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ABSTRACT

The transverse sections of 52 accessions of rice comprising of 2 wild indigenous species, 1 cultivated indigenous species and 1 cultivated Asian species, including different land races and a commercial brand, were made. This was with a view to investigating the patterns of protein distribution in the rice species. The result showed a general protein distribution pattern of a dense, sometimes broad band of large protein bodies near the aleurone layer followed by tiny protein bodies which ramify into the endosperm, some reaching the core through the parenchyma covering of the amyloplasts. There is a considerable intra- and inter- specific variability which holds promise for exploitation for improvement. However, the accessions of *O. barthii* presented a poor protein profile while the land races of *O. sativa* had the best profile. The broad layer of deeply-diffused protein particles in some of the accessions, especially the land races, will survive milling even when the large protein bodies in the bran and aleurone layers are lost. This potential can be exploited for genetic improvement of rice.

Keywords: Protein Distribution, Wild Rices, Cultivated Land Races, Genetic Improvement

INTRODUCTION

Rice is one of the three major food crops of the world and forms the staple diet of about half of the world's population. Protein is available in a variety of dietary animal and plant sources. Hoffman and Falvo (2004) have shown that proteins from vegetables, grains, fruits and legumes, when combined to supply all the essential amino acids, provide an excellent source of protein without the fear of intake of saturated fat and cholesterol.

Besides carbohydrate, which form the bulk of food substances in the rice, the rice grains also contain proteins, fats, vitamins and minerals. Kido and Yanatori (1965) detected no protein in the pericarp, seed coat and aleurone layer. They reported that protein is distributed in the outer layer of the endosperm, richer in the ventral side than in the dorsal side and central part of the endosperm. According to Aadil *et al.* (2011) the protein is present in greater quantities in the germ and outer (bran) layers than in the starchy endosperm. Rice is subjected to a series of conditions and treatments from the time of its harvest to the time of consumption. The germ and bran layers are removed during the milling process and this leads to a great loss or depletion of the nutrients contained in the rice grains including niacin, iron and riboflavin. These nutritional contents removed from the rice grain

during milling make the rice bran protein of high nutritional quality. What is lost from the milled grain is gained by the by-product, the bran. Aadil *et al.* (2011) opined that milling is essential but faulty and that the loss of some of these nutrients are inevitable. They however concluded that milling processes should be designed to remove the pericarp but retain the other nutrients and as much of the aleurone layer as possible.

Recent rice researchers (Saunders, 1990; Helm and Burks, 1996; Wang *et al.*, 1999; Hye-Jung *et al.*, 2010; Aadil *et al.*, 2011) are contended with finding the quantity, quality and usefulness of protein and other nutrients lost to the bran during processing. In their various findings, rice bran contains a significant amount of protein (12-20%), thiamin (c 80%) and lysine (c 2-4%) which is higher than that of rice endosperm protein or proteins from other cereal bran or legumes. It has also been reported that the removal of the protecting pericarp facilitate the extraction of soluble substances from the aleurone layers during washing immediately before cooking the grains (Aadil *et al.*, 2011). Therefore, rice bran protein holds great promise as an alternative protein source for food improvement, evidenced by the work of Jiamyangyuen *et al.* (2005) which explored the rice bran protein concentrate for the improvement of bread quality.

Qualitative and quantitative improvement of protein content in rice is extremely important to human nutrition (Chu *et al.*, 1974). A number of rice breeders (Anon., 1967; Tong *et al.*, 1970; Chu *et al.*, 1973, 1974) have detected significant variation in protein content among varieties and mutant lines grown under the same environmental conditions which indicates that varieties with high protein content can be obtained by breeding. Kido and Yanatori (1965) and Tanaka and Tamura (1968) discussed the relationship between protein content of unpolished rice (brown rice) and polished rice. They suggested that the protein content of polished rice was highly correlated with that of brown rice but admitted that further information was not available. Based on the analysis of the F₃ population, workers in the International Rice Research Institute (IRRI) reported that the increase of protein in polished rice grains was associated with the increase in the bran of the seeds (Anon. 1969). Tanaka and Tamura (1968) reported that several mutant lines increased their protein content not only in the bran but also in the endosperm. Through their electron microscopic studies, they found that more protein bodies were located in the outer than in the inner layers of the grains. Grist (1959) reported the loss of about 29% of the total protein content in rice grains during milling and polishing due to the concentration of protein in the peripheral aleurone and sub-aleurone layers.

Chu *et al.* (1974), in their study, discovered that the variation in protein content of bran, aleurone and outer layer of the endosperm is significant. They reported that all the varieties studied showed more protein distribution in the outer than in the inner layer when subjected to the shortest polishing time of five seconds. Their result showed that the removed portion contained not only the seed coat and aleurone layer but also the ventral side of the endosperm which contains a large amount of protein. They noted that the bran and outer layers of rice grains are removed by grinding or milling to meet the market demand for polished rice and therefore submitted that any rice variety that contains protein only in the bran or outer layer of the grain would lose the protein to grinding/milling.

This experiment is designed to investigate the

patterns of protein distribution in rice grains among the accessions of the species present in Nigeria. This is with a view to exposing inter- and intra-variability in protein distribution that can be exploited for the genetic improvement of the crop.

METHODOLOGY

The method employed in this experiment is according to Kaul, Dhar and Swaminathan (1969) and Kaul, Dhar, Swaminathan and Ahnstrom (1969a). A total of 52 rice accessions consisting of 18 of *Oryza punctata*, 9 of *O. glaberrima*, 14 of *O. barthii*, 5 of *O. longistaminata*, and 6 of *O. sativa* (land races) were screened for patterns of protein distribution in the transverse section of the grains. A random sample of 20 F₂ seeds of AWGU DWARF x IJ86-W cross were also screened (Table 1.).

Rice grains of uniform sizes from all available accessions of both wild and cultivated species of rice, including a sample from commercially available varieties (AROSO), were fixed in Carnoy's fluid for 48 hours after which they were stored in 70% ethanol. Transverse sections (12 µm 15 µm) of the rice grains were cut using a sledge microtome and the sections were stored in 1% aqueous Bromophenol blue, dehydrated with ascending grades of alcohol/water (50%, 60%, 70%, 80%, 90% and absolute), then dehydrated in clove oil and finally in xylene. The sections were mounted in DPX and examined for protein distribution pattern following the categorization of Kaul *et al.* (1969, 1969a) and Annie *et al.* (1992). The major protein distribution patterns were documented in photomicrographs on a Leitz Dialux research microscope.

RESULTS

The protein profiles in the accessions are broadly described by species as follows:

Oryza punctata

A dense band consisting of 1 or 2 layers of large protein bodies next to the aleurone layer followed by a less dense, broader band of tiny protein particles ramifying into the endosperm along the parenchyma cells enclosing the amyloplasts. The dense layer of protein bodies is in isodiametric cells (1 cell - 2 cells thick) in the dorsal region as shown in two accessions, TOP 14097 and TOP 15114 (Figure 1A).

Table 1: Materials Used in the Study and their Sources

Accession Number	Collector/Source/Location	Chromosome Number	Description
Top ^a Ipetu	NWOKEOCHA: Ipetumodu, 7°30"N 4°45"E, Nigeria	2n = 48	Large population on marshy ground around pool of water. Close to human habitation. Location open.
Top Moro	NWOKEOCHA: Moro, 7°33"N 4°45"E, Nigeria	2n = 48	Few stands on the bed of a river, sandy soil. Location under shade of <i>Alchornea cordifolia</i>
Top Ikire	NWOKEOCHA: Ikire, 7°23"N 4°12"E, Nigeria	2n = 48	Few stands by pool of water on major road side. Location open.
Top Ede	NWOKEOCHA: Ede, 7°44"N 4°27"E, Nigeria	2n = 4	Few stands on river bed, road side. Location open
Top Sekona	NWOKEOCHA: Sekona, 7°33"N 4°38"E, Nigeria	2n = 48	Fairly large population on marshy ground around pool of water, close to major road. Location open.
Top Rd 7 ^f	FALUYI: O.A.U ^b Campus, Ile-Ife. 7°29"N 4°34"E, Nigeria	2n = 48	Along the valley of a river. Location, swampy, open in part, largely under shade.
TOP 15114	IITA ^c : 4°22"N 18°30"E, CAR	2n = 48	Wild on fallow land
TOP 15115	IITA: 4°23"N 18°30"E, CAR	2n = 48	Wild on fallow land
TOP 15116	IITA: 6°53"N 19°07"E, CAR	2n = 48	Wild on fallow land
TOP 15117	IITA: 6°55"N 19°06"E, CAR	2n = 48	Wild on fallow land
TOP 15118	IITA: 7°02"N 18°50"E, CAR	2n = 48	Wild on fallow land
TOP 15119	IITA: 4°20"N 18°30"E, CAR	2n = 48	Wild on fallow land
TOP 8222	IITA: 6°59"N 9°35"E, Nigeria	2n = 48	--
TOP 8221	IITA: 6°59"N 9°35"E, Nigeria	2n = 24	--
TOP 6788	IITA: 6°59"N 9°35"E, Nigeria	2n = 48	--
TOP 13596	IITA:-----Tanzania	2n = 48	--
TOP 13546	IITA: ----- Tanzania	2n = 24	--
TOP 14097	IITA:-----Tanzania	2n = 24	--
TOP 14460	IITA: ----- Nigeria	2n = 24	Wild on fallow land
TOP 5702	IITA: 7°22"N 7°50"E, Nigeria	2n = 24	Wild Vegetation
TOL ^e 5655	IITA:-----	2n = 24	--
TOL 7387	IITA:-----	2n = 24	--
TOB ^f 8218	IITA:-----	2n = 24	--
TOB 5660	IITA:-----	2n = 24	--
TOB 8226	IITA: 9°30"N 12°10"E, Nigeria	2n = 24	--
TOB 5646	IITA:-----	2n = 24	--
TOB 5658	IITA:-----	2n = 24	--
TOB 7337	IITA:-----	2n = 24	Wild
TOB 7306	IITA:-----	2n = 24	Wild
TOB 7311	IITA:-----	2n = 24	Wild
TOB 7307	IITA: 12°18"N 7°36"E, Mali	2n = 24	Cultivated
TOB 7382	IITA:-----	2n = 24	Wild
TOB 4645	IITA:-----	2n = 24	Wild
TOB 10838	IITA:-----	2n = 24	Wild
TOB KARI 83	OLORODE Nigeria	2n = 24	Wild
TOG ^g 12082	IITA:-----	2n = 24	Cultivated
TOG 12083	IITA:-----	2n = 24	"
TOG 10985	IITA:-----	2n = 24	"
TOG 12060	IITA:-----	2n = 24	"
TOG 5281	IITA:-----	2n = 24	"
TOG 5282	IITA:-----	2n = 24	"
TOG 5283	IITA:-----	2n = 24	"
TOG 5284	IITA:-----	2n = 24	"
TOG 5236	IITA:-----	2n = 24	"
TOG 3415	IITA:-----	2n = 24	"
TOG 16771	IITA:-----	2n = 24	"
TOG 7454	IITA:-----	2n = 24	"
TOS ^h 15223	IITA:-----	2n = 24	"

Table 1: contd.

Accession Number	Collector/Source/Location	Chromosome Number	Description
TOS purple	IITA: WARDA	2n = 24	"
TOS IJ86-W	FALUYI: Ijesa-Isu, 7°43"N 5°31"E, Nigeria	2n = 24	Upland rice from peasant farmers
TOS IJ86-B	FALUYI: Ijesa-Isu, 7°43"N 5°31"E, Nigeria	2n = 24	"
TOS AWGU DWARF-W	NWOKEOCHA: Awgu, 6°5"N 7°29"E, Nigeria.	2n = 24	"
TOS AWGU DWARF-B	NWOKEOCHA: Awgu, 6°5"N 7°29"E, Nigeria.	2n = 24	"
AROSO	NWOKEOCHA: Ile-Ife, Nigeria.	--	Processed rice collected from the open market

a = Tropical *Oryza punctata*; b = Obafemi Awolowo University; c = International Institute of Tropical Agriculture; d = Central African Republic

e = Tropical *Oryza longistaminata*; f = Tropical *Oryza barthii*; g = Tropical *Oryza glaberrima*; h = Tropical *Oryza sativa*.

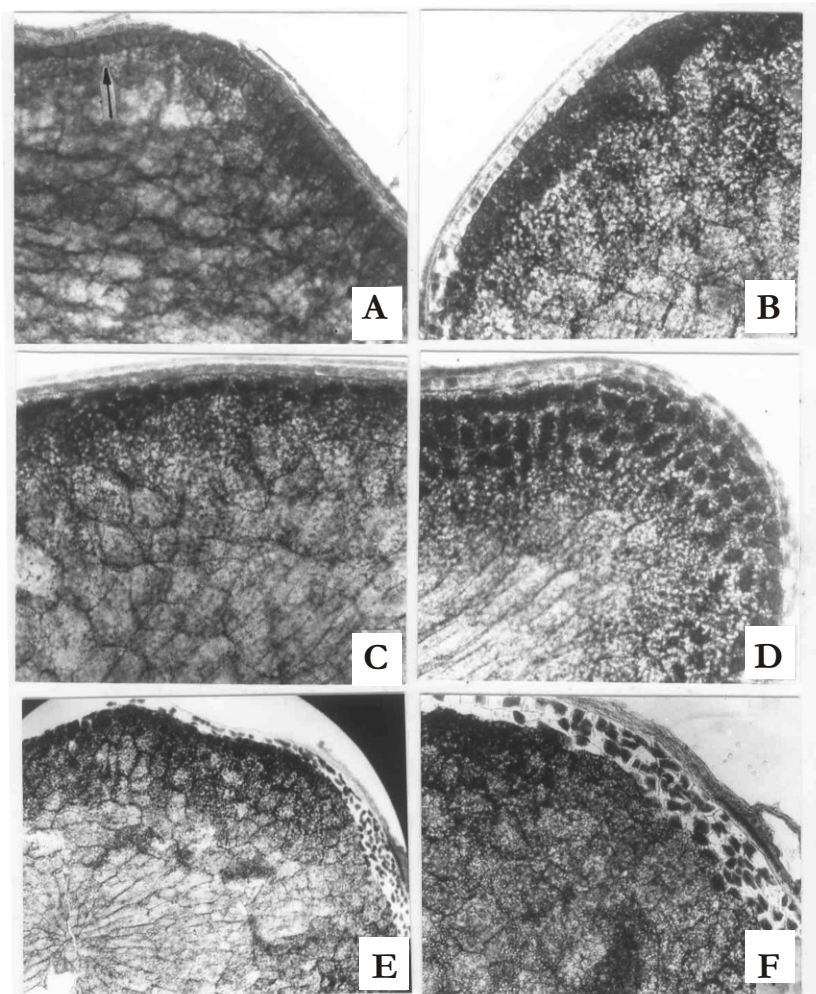


Plate 1: Major Distribution Patterns of Protein in the Rice Grains of the Accessions Studied.

- A. TOP 14097 (X10). Note the protein bodies in a row of isodiametric cells next to the aleurone.
- B. TOG 3415. Note the thick band of tiny protein bodies next to the aleurone and ramification of tiny particles into the endosperm.
- C. TOG 10985
- D-F. TOS IJ86-W (D=front. Note the infiltration of large protein bodies by tiny particles, E = aleurone end at scanning, F = aleurone X 40). Arrow shows a continuous row of protein bodies

Oryza glaberrima

A dense band of large protein bodies immediately after the aleurone layer followed by a less dense but broader band of tiny protein particles ramifying into the endosperm along the parenchyma cells enclosing the amyloplasts. This is typified in two accessions TOG 3415 and TOG 10985 (Figure 1B,C).

Oryza barthii

A band of large or small protein bodies next to the aleurone layer infiltrated by tiny protein particles which extend very minimally or about halfway into the endosperm through the parenchyma covering of the amyloplasts.

Oryza sativa

A wide band of large, distinct protein bodies massively infiltrated by tiny protein bodies which extend about halfway into the endosperm, some reaching the core through the parenchyma covering of the amyloplasts. IJ86-W and IJ86-B, both of which are land races, show additional protein bodies in the aleurone layer (Figure ID, E, F). The distribution pattern in the accession of *O. longistaminata* is similar to that in the *O. sativa* accessions.

DISCUSSION

The ideal protein distribution profile for rice is the deep, diffused network which ensures that most of the protein is preserved during milling (Kaul *et al.*, 1969). From the screening done in this study, it can be said that there is considerable inter- and intra- specific variability in protein distribution in the genus *Oryza*. This is consistent with the observation of Annie *et al.* (1992).

O. barthii accessions generally present a poor protein profile. The protein distribution pattern in the local accessions of *O. sativa* is the best. The broad layer of deeply-diffused protein particles will survive milling even if the large protein bodies in the aleurone layer are lost. Except for the absence of large protein bodies in the aleurone layer, the profile in the accessions of *O. longistaminata* and *O. glaberrima* are similar to that of *O. Sativa*.

This study has shown that there is considerable potential for good protein distribution pattern particularly in the grains of Nigerian land races. The interspecific variation observed is consistent

with the report of Annie *et al.* (1992). The screening of the F₂ seeds from a cross between two land races (IJ86-W x AWGU DWARF) shows that this potential can be exploited for genetic improvement.

REFERENCES

- Aadil, A., Shahzad, M., Faiza, A., Ayesha, K., Shakela, R. and Sumera, N. 2011. Effect of Processing on Nutritional value of Rice. *World Journal of Medical Sciences* 6(2), 68-73
- Adeyemi C.C. 2006. Breeding improved crop varieties for adaptation : Efforts and breakthroughs. In: *Climate Change and Food Security in Nigeria* . Adejuwon J.O. and Ogunkoya O.O. (eds.). Obafemi Awolowo University Press Ltd, Ile-Ife, Nigeria. Pp 217-228.
- Annie, P.T., Kumari, Sobha V.P. and Nair, G.P. 1992. Intra- and inter- specific variability in grain protein distribution pattern in cultivated and wild species of *Oryza*. *Rice Genetics Newsletter* 9, 40-42.
- Anonymous. 1967. *Screening for high protein*. A Report of International Rice Research Institute, 1967, 53-56.
- Anonymous, 1969. *Assessment of screening technique*. A Report of International Rice Research Institute 1969, 36-37
- Chu, Y.E., Wu, S.C. and Tsai, K.S. 1973. *Mutation breeding for high protein rice varieties*. Proceedings of Research meeting, Int. Atomic Energy Agency (Vienna, 1973), p 145-148.
- Chu, Y.E., Li, Y.S. and Tsai, K.S. 1974. Breeding for high protein and balanced Amino-acid rice varieties 4. The distribution of protein contents in a rice grain. *Euphytica* 23,153-158.
- Grist, D.H. 1959. *Rice*. Longman, London and New York, 472 pp.
- Helm, R.M. and Burks, A.W. 1996. Hypoallergenicity of rice bran protein. *Cereal Foods World* 41, 839-843.
- Hoffman, Jay R and Falvo. Michael J. 2004. Protein - Which is Best. *Journal of Sports Sciences and Medicine* 3, 118-130. <http://www.jssm.org>

- Hye-Jung, Y., Eun-Hye, L., Mi-Sun, H., Sang-Do, H. and Dong-Ho B. 2010. Production and Physicochemical properties of Rice Bran Protein Isolate prepared with Autoclaving and Enzymatic Hydrolysis. *J. Korean Soc. Appl. Biol. Chem.* 53(1), 62-70.
- Jiamyangyuen, S., Srijedaruk, V. and Harper, W.J. 2005. Extraction of rice bran protein concentrate and its application in bread. *Songlanakarin Journal of Science and Technology* 27(1), 55-64.
- Kaul, A.K., Dhar, R.D. and Swaminathan, M.S. 1969. Microscopic screening of rice for protein characteristics. *Current Science* 22, 529-531.
- Kaul, A.K., Dhar, R.D., Swaminathan, M.S. and Ahnstrom, G. 1969a. A rapid dye-binding method of screening single grains for protein characteristics. *Current Science* 14, 350-331.
- Kido, Mituro and Yanatori, Shozo 1965. Histochemical studies of protein accumulating process in rice grain. *Proc. Corp. Sci. Soc. Japan* 40, 204 - 209.
- Saunders, R.M. 1990. The properties of rice bran as a food stuff. *Cereal Food World* 35, 632-662.
- Tanaka, Sachihiko and Tamura, Sinpachiro 1968. A Short report on gamma-ray induced rice mutants having high protein content. *Japan Agric. Res.Q.* 3(3), 68.
- Tong, W.F., Chu, Y.E. and Li, H.W. 1970. Variation in protein and amino acid contents among genetic stock of Rice. *Bot. Bull. Acad. Sinica* 11, 55-60.
- Wang, M., Hettiarachchy, N.S., Qi, M., Burks, W., Siebenmorgen, T. 1999. Preparation and functional properties of rice bran protein isolate. *Journal of Agric. Food Chem.* 47, 411-416.