Research Journal of Agriculture and Biological Sciences 1(2): 184-189, 2005

Characterization of Proteins of Brown, Bran and Endosperm of Raw and Parboiled Rice

M.I.H. Reza, A.Q. Chowdhury and M.K. Pasha

Department of Botany, University of Chittagong , Chittagong-4231, Bangladesh.

Abstracts: SDS – PAGE analysis of total protein of brown, bran and endosperm of raw and parboiled rice *(Oryza sativa, Var. indica* cv pajam) showed clear and distinct protein patterns as revealed from both the number and distribution of peptide bands. The range of variations of bands were in between 14.5 kD and 240 kD. The number of peptide bands in raw and parboiled brown rice were 45 and 16 respectively. The major and minor bands and their distribution patterns in gel were also analysed. The brans of foot-pounding and milling of both raw and parboiled rice also showed much variations. The number of peptide bands were 15 and 7 in foot-pounding raw and parboiled brans, and 23 and 18 in milled raw and parboiled respectively. The range of MW of the peptide bands were 14.5 to 37 kD in the former and 14.5 to 45 kD in the latter. Many desimilar and less number of bands in parboiled rice than raw rice analysed with respect to their peptide groups. At least four bands were absent in endosperm of both milled and foot-pounded raw endosperm than the brown rice. On the other hand some new bands were appeared in both milled and foot-pounded parboiled endosperm.

Key words: SDS-PAGE, Brown, Bran, Endospern, Raw, Parboiled, Milled, Foot-punded

INTRODUCTION

Rice is one of the most important sources of protein. It has highest biological value among all the cereals but a low utilizable protein of rice is due to its poor protein content[5]. The mean storage protein content of rice is 9.9 ± 1.8 %[7]. Before taken as a food rice milling and boiling process is very important. Side by side variety of age old native foot-pounding and hand-pounding huller are also used for the process of rice and brown rice. But the modern taste in rice can only be machines that removes not only the husk but also the outer layers and germ of endosperm and thus polish the rice. Rice grain has a series of thin coats, about five, which are totally or partly removed as bran in process of pounding, milling and pearling.

Parboiling is an old process of conditioning the paddy. Nearly 80% of the rice consumed in Bangladesh is parboiled and the rest is used as raw. Many of the work on protein, by electrophoretic process, have been done on raw rice[2,8,9]. But there is no electrophoretic work on storage protein of parboiled rice. In the present investigation we have tried to determine the electrophoretic protein patterns of the storage proteins of brown, bran and endosperm of parboiled rich which were then compared with raw rice proteins.

MATERIALS AND METHODS

Plant material: Pajam paddy (*Oryza sativa* var. *indica* L.) were chosen for the study. It is the most popular rice of the eastern region of Bangladesh. Intact raw and parboiled pajam paddy were collected and processed, viz. i) dehulled by hand to obtain brown rice, ii) foot-pounded by mortar and pestle method, to obtain bran and endosperm, and finally iii) milled and polished by small holler mill to obtain bran and endosperm. The brans obtained from both milled and foot-pounded were separated by 60 mesh to remove the coarse husk material. Brown rice, brans and endosperms were than separated from the above processes. The samples thus collected were crushed to very fine powder by mortar and pestle in the laboratory.

Extraction of total proteins: One gram powder from each samples were crushed in five ml of total protein extraction buffer [5% SDS (w/v,) 2 – marcaptoethanal, 10% glycerol, 10 mM Tris HCI, pH 6.8] in a mortar and pestle for 30 min in room temperature. The extracts were centrifuged at 5,000 x g for 15 min. The supernatant was precipitated with ammonium sulphate at 80% saturation. The solution was taken and kept at 4°C for at least 12h. Soluble protein from the supernatant was found precipitated and separated by

Corresponding Author: M.I.H. Reza, Department of Botany, University of Chittagong , Chittagong – 4231, Bangladesh.

centrifugation at 5,000 x g for 10 minutes. The precipitant was then dissolved in the same extraction buffer, dialysed extensively against the same buffer for 24h in cold to remove ammonium sulphate and other ionic impurities. The dialysate was again centrifuged at 5,000 x g for ten minutes to remove undissolved substances.

The protein content in dialysates were estimated according to Lowry *et al*[11]. The extracted protein samples were separated by SDS – PAGE according to the method of Laemmli[10]. A 5ml of stacking gel (5%) was layered on 20 ml (10%) of separating gel (15 × 5 × 0.1 cm). The protein solutions, 200 µg/ ml, were mixed with equal volume of sample buffer and heated on a boiling water bath for three minutes. After cooling, the samples were applied to gel and electrophoresed with Tris-glycine buffer at 20 mA, constant current (120V) for stacking gel and 30 mA (200V) for the separating gel for about three hour. Bromophenol blue was used as marker dye. The gels were stained with coomassie brilliant blue in 50% methanol, 10% glacial acetic acid and 40% water for 12 h then destained with methanol, acetic acid, water (50:10:40). The Rf values and molecular weight (MW) for proteins were calculated and compared the bands with the standard proteins [" $_2$ Macroglobulin (180 kD), $-Galactosidase (116 kD), Fructose – 6 – phosphate kinase (84 kD), Pyruvate kinase (58 kD), Fumarase (48.5 kD), Lactate dehydrogenase (36.5 kD) and Triosephosphate isomerase (26.5 kD).

RESULTS AND DISCUSSIONS

The percentage of total protein in brown rice of both raw and parboiled rice was 7.8%. In case of raw rice the percentage of bran was 15% in milled and 17.5 % in foot pounded rice respectively. On the other hand, in parboiled rice, the percentage of bran was 11.54% and 7.7 % in milled and foot pounded rice respectively. The total protein in bran of raw rice was calculated to be 18% and 16% in case of milled and foot pounded rice respectively, and in the endosperm was 6.0% and 6.06% respectively. In parboiled rice the total protein in bran of foot-pounded and milled rice was calculated to be 21.05% and 11.95% in foot pounded rice respectively and that in the endosperm was 6.07% and 7.15 % respectively.

Protein electrophoretic pattern in different samples of rice SDS – PAGE showed very wide of MW of peptides in brown rice from raw and parboiled sample varied between 14.5 kD and 240 kD. Some of the peptide bands, when examined on light, showed most intense and deep in colour, and some of the bands were very low intense and diffuse in colour. The peptide patterns of raw rice were clear but the protein patterns parboiled rice showed poor extraction and separation as revealed from the intensity and number of peptide bands (figs. 1 and 2)

In the gel, the number of peptide bands were 45 in raw brown rice samples were examined. The same number of peptide band were also observed in raw milled endosperm as well as foot-pounded endosperm. Although the number of peptide bands were similar in all cases the relative MW of some peptide bands showed differences between brown rice and milled endosperm or foot-pounded endosperm. Out of 45 bands in brown rice at least four bands were identified to be absent both in the raw milled and foot-pounded endosperm and those bands were at 23.9 kD, 15.3 kD, 160 kD and 165 kD positions. Some deep and intense major peptide bands visible in brown rice were not found exactly similar in their intensities in milled and foot-pounded endosperm. In milled endosperm the peptides which were greatly reduced in their intensities were at the 14.5 kD, 25 kD, 25 kD, 46 kD and 70 kD positions. But in foot pounded endosperm these peptide bands were more intense than the milled rice, but less than the brown rice.

The total protein patterns in different sample of parboiled rice were quite different from that of raw rice. The number of bands were in between only 12 and 16. These peptide bands were also showed norrower range in between 14.5 kD and 63kD. Again most of these bands were of low MW position. The number of peptide bands in case of brown rice were minimum (12) than those of foot-pounded (16) and milled endosperm (16). Within the parboiled rice, the number of major bands were quite less in number in comparison to that of raw rice. A set of major bands around 52 kD positions were found more less almost in parboiled rice. The milled endosperm in parboiled rice showed some differences in the protein extractions. The major peptide groups around 19-21 kD and 29 – 35 kD were detected higher the amount in comparison to those observed in brown rice and foot-pounded rice. At the same time minor proteins were also showed less extraction when compared to other rice samples. These type of differences were not observed in raw rice (Fig. 1).

The peptide bands in bran of milled raw and parboiled rice was 23 and 18 respectively (Fig. 2). The range of these bands were in between 14.5 and 52 kD. Many of these bands between these two types of bran were detected in similar position. But some dissimilar peptide bands in raw rice bran were at 37.5 kD, 38 kD, 40 kD, 50 kD and 52 kD positions. The bran of raw and parboiled foot-pounded rice showed that the number of bands were 15 and 7 respectively. The range of these bands were in between 14.5 and 37 kD in raw bran and in between 14.5 and 21 kD in parboiled bran. These bands were also detected similar in raw rice also.

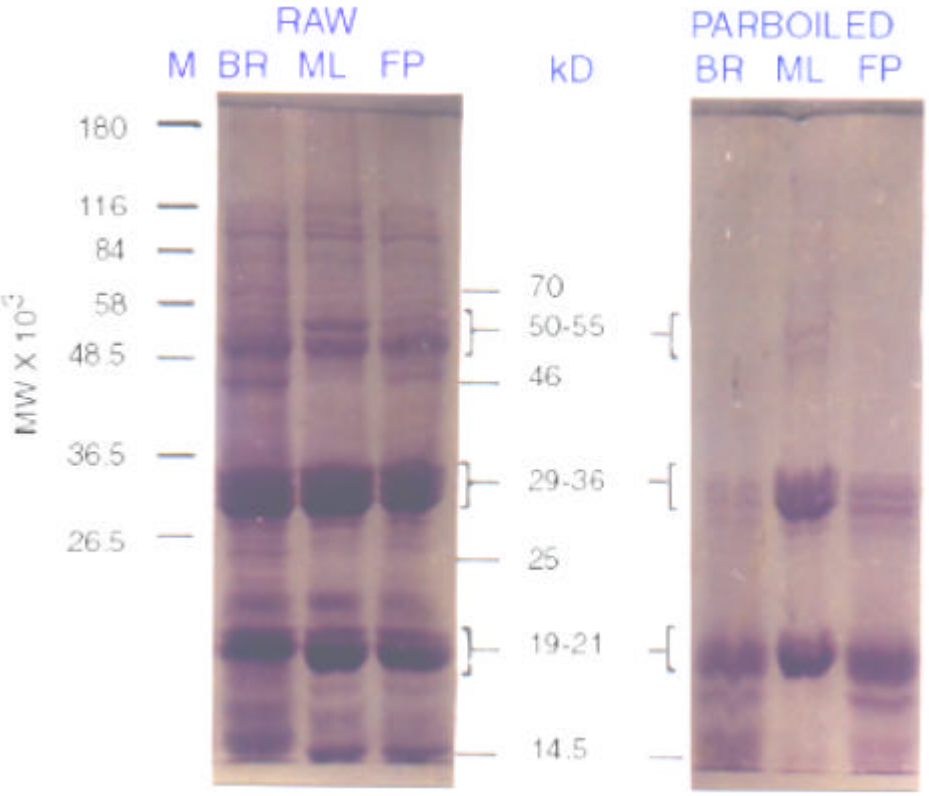

Fig. 1: Electrophoregram of total protein of brown rice and endosperm of raw and parboiled pajam rice samples obtained by 10% SDS – PAGE. Br, Brown rice ; ML, Milled endosperm; FP, Foot-pounded endosperm ; M, Marker.

Table 1: Distribution of peptide bands in brown, bran and endosperm of raw and parboiled rice proteins separated in 10% SDS – PAGE and grouped on the basis of MW.

Type		Total No. of band	Peptide Group on MW (kD)									
			P_1		P_2		P_3		P_4		P_5	
			10 – 25 kD		25.1 – 40 kD		40.1 – 55 kD		55.1 – 70 kD		70.1 kD +	
			No. of	Major	No. of	Major	No. of	Major	No. of	Major	No. of	Major
Raw	Brown	45	12	6	13	4	8	3	2	1	10	1
	Milled bran	23	10	5	9	2	4	1	-	-	-	-
	Milled endosperm	45	12	5	11	4	8	2	2	-	12	1
	Foot-pounded endosperm	45	12	5	11	4	8	3	2	-	12	1
	Foot- pounded bran	15	10	5	5	-	-	-	-	-	-	-
Parboiled	Brown	12	7	4	5	4	-	-	-	-	-	-
	Milled bran	18	10	5	6	2	2	1	-	-	-	-
	Milled endosperm	16	8	4	5	4	3	-	-	-	-	-
	Foot-pounded endosperm	16	8	4	6	4	2	-	-	-	-	-
	Foot- pounded bran	7	7	3	-	-	-	-	-	-	-	-

For convenience of analysis of separated proteins all these band were divided, on the basis of gel patterns, into five peptide groups. These groups were arranged in the series of 10 – 25 kD (P_1), 25.1 – 40 kD (P_2), 40.1 – 55 kD

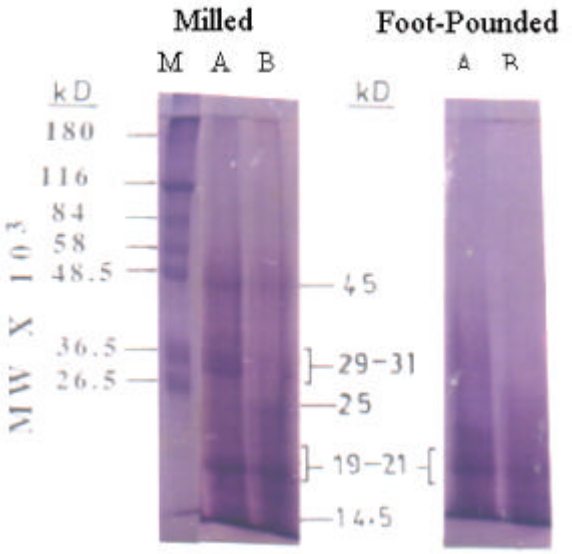

Fig. 2: Electrophoregram of total protein of bran of raw and parboiled pajam rice sample. A, Raw ; B, parboiled ; M, Marker.

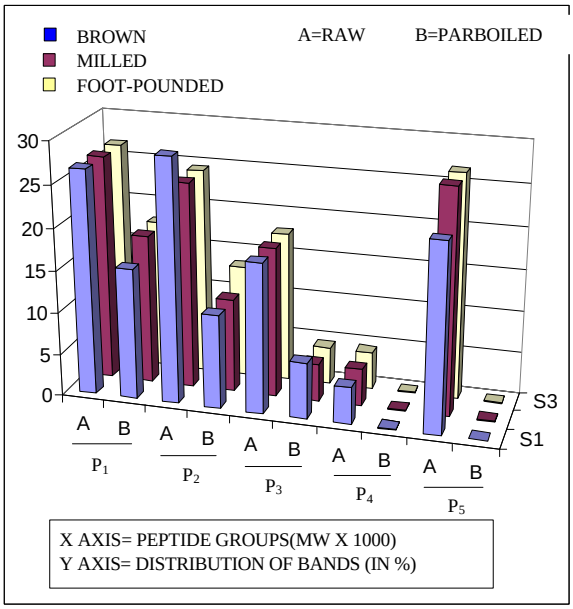

Fig. 3: The relative average distribution of five groups of proteins of brown and foot-pounded endosperm or raw parboiled rice on the basis of MWs.

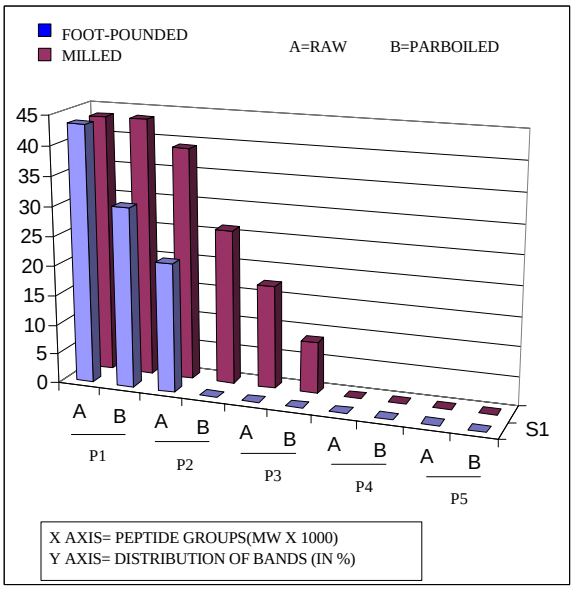

Fig. 4: The relative average distribution of five peptide groups of proteins of brans of raw and parboiled rice on the basis of Mws.

(P_3), 55.1 – 70 kD (P_4) and 70.1 kD to above (P_5) (Table 1). In different samples of raw rice the number of bands were 10 – 12, 5 – 13, 4 – 8, 2 and 10 – 12, in different peptide groups respectively. The number of major bands in raw bran rice were 6, 4, 3, 1 and 1 in the P_1, P_2, P_3, P_4 and P_5 peptide groups respectively. In raw milled bran the number of major bands were 5, 2, and 1 in P_1, P_2 and P_3. In foot-pounded bran the 5 major bands were detected only in P_1. But in raw milled and foot pounded endosperm the number of major band were 5, 4, 2 and 1 in the P_1, P_2, P_3 and P_5 peptide groups respectively. No such major band was distributed in the P_4 peptide group.

In parboiled rice the number of bands were 7 – 10, 5 – 6, 2 – 3 distributed in the P_1, P_2 and P_3 peptide group respectively. There was no such detectable band distributed in P_4 and P_5 peptide groups. Similarly the number of major bands in all the parboiled rice samples were 3 – 4, 2 – 4 and 1 each in P_1, P_2 and P_3 peptide group respectively. The percent distribution of these five peptide groups in raw and parboiled rice are presented in histogram (Fig. 3 and 4). These showed that the highest number of bands appeared in P_1 and P_2. Then the number gradually decreased in other peptide groups. It indicated that more than 50 percent bands were appeared in between 14 kD and 40 kD. Unusually, more than 20 percent bands appeared in case of raw rice, above 70 kD were totally absent in parboiled rice.

The wide range of MW peptides and also their number indicated that rice storage proteins are of diverse in nature. The protein extracted from the brown rice, from the milled bran, foot-pounded bran and endosperm of raw rice, showed no such major differences in the peptide patterns in gels. But some minor differences were observed in these rice samples. During the processing of rice the peptides which lost mostly in milled rice, were 14kD, 25kD, 46 kD and 70 klD peptide region. These peptides were partially removed during the foot-pounding

process. It can be predicted that some of the proteins were totally lost due to milling but partially lost due to foot-pounding process. This was confirmed when the milled and foot-pounded bran extracted protein analysed. It was also observed that some major bands at the position 14.5 kD, 19 – 21 kD, 25 kD, 29 – 36 kD, and 45 kD region, were less intense in milled and foot – pounded endosperm.

There was a great variation in the peptide patterns of the different samples of parboiled brown, bran and endosperm. The number of peptide band were quite less than raw rice was only 12 –16. This clearly indicated that the storage proteins of parboiled rice might be reduced its solubility characters during the parboiling process of rice. All the proteins appear during the developing rice are deposited either in PB – I, PB – II or in PB – III protein bodies[6,1]. These protein bodies seemed to be drastically changed during the parboiling process of rice. It was indicated that with the improvement of grain translucency and hardness, the disruption of protein bodies and the gelatinization of the starch granules were found accompanied parboiling. They also observed that the protein fraction were less efficiently extracted from parboiled rice. Therefore, the parboiling affected the physical properties more than the chemical properties of the grain. But there is no such previous reports about the electrophoretic patterns of parboiled rice. Our findings in this experiment clearly indicated that the solubility properties of the rice protein greatly modified, which was indicated by the poor number of peptides rice, most of the high MW peptides totally lost their soluble properties and thus no such detectable bands could be observed in the present study.

The analysis of different peptide groups in raw rice showed that most of the peptides had their position within the MW of 14 – 50 kD. A good number of peptides were also distributed within 70 kD to 150 kD positions. On the other hand, most of the peptides in parboiled brown rice and endosperm were distributed between the range of 14 kD and 35 kD. This finding clearly indicated that only the low MW peptides were soluble, but most of the high MW peptides were observed insoluble and thus not undetected due to parboiling. Therefore, the parboiling process or rice had a great impact on the soluble properties of proteins, specially, on the high MWs group.

In parboiled rice, it was able to record some of the effects of milling and foot-pounding process. The peptides around 14 – 16 kD and one of the major protein at 18kD positions were removed in milled rice but were able to detect in foot-pounded rice. From the above analysis, it was possible to find out that there are some differences also in the protein retaining capacity of the endosperm. As because some of the notable proteins were removed during the milling process, but not in foot-pounding process, higher protein retaining capacity in the foot-pounded rice than the milled rice is again supported here. The proteins which were greatly retained in foot-pounded rice, both in raw and parboiled, pointed it's advantages with respect to the protein quantity, may be also quality, than milled endosperm.

The number of peptide bands were always in parboiled foot-pounded and milled bran than the raw bran. In raw rice bran most of the proteins were of low MWs. In foot-pounded raw bran, the presence of some major peptide bands indicated greater loss of protein than the foot-pounded parboiled rice. When compared with the foot-pounded bran, milled bran showed greater number of peptides both in raw and parboiled. Here again, many of the peptides found in raw were absent in parboiled rice. Most of the additional bands appeared in milled bran were of medium weight polypeptides. These differences in the number of peptides showed difference not only the processing of boiling but were also in the process of polishing. In both the cases raw rice bran removed rice indicated the lesser number of protein removed from endosperm. In that respect parboiling process of rice is very important, with respect to the yield in quality and quantity of proteins.

Cagampang et al[2] polished three varieties of raw rice. In all the cases they observed glutelin to be the dominant fraction in the whole as well as in the milled grain. They recorded that albumin and globulin formed bulk of the bran and polished endosperm proteins. Prolamins showed evenly distributed in all the polished fraction of milled rice. They also observed that albulin and also the prolamin contents were gradually lower from periphery to the centre of endosperm which was quite opposite in case of glutelin. But they did not analysed those proteins electrophoretically.

In our experiment, we could identify, from their MWs, some of the major bands. In foot-pounded rice only the albumins, globulins and also the $- glutelin (partially) were removed. In foot-pounded raw bran, a minor amount of "- glutelin also was removed. But these proteins were mostly retain in parboiled rice. In case of milled rice, many of the albumins, globulins and also gluetelins were removed as bran during milling process. As because most of the layers are affected and removed during milling process, the number of bands appeared higher in the foot-pounded rice. It is clearly indicated that, as because a lesser number of peptides in foot-pounded bran, and thus the quality of endosperm is better in foot-pounding than the milling processes.

REFERENCES

1. Bechtet, D.B. and. B.O. Juliano, 1980. Formation of protein bodies in the starchy endosperm of rice (*Oryza stiva* L.) : a reinvestigation. Ann. Bot. 45: 503 – 509.
2. Cagampang, G.B., L.J. Cruz, S.G. Espiritu, R.G. Santiag and B.O. Juliano, 1966. Studies on the extraction and composition of rice protein. Cereal Chem., 43: 145- 155.
3. Chen, S.C.G. and M.C. Cheng, 1986. Characterization of storage proteins of *indica* rice. Bot. Bull. Academia Sinica (Taipei), 27: 147-162.
4. Chen, L.F.O., M.C. Cheng and S.C.G. Chen, 1987. Similarity and diversity of seed proteins in rice varieties. Bot. Bull. Academia Sinica. (Taipei), 28: 169-183.
5. Eggum, B.O., 1979. Proc. Workshop, Chemical Aspects of Rice Grain Quality. IRRI, the Philippines, p. 91.
6. Harris, N. and B.O. Juliano, 1977. Ultrastructure of endosperm protein bodies in developing rice grains differing in protein content. Ann. Bot., 41: 1-5.
7. IRRI., 1967. Annual Report for 1967, Los Benos, the Philippines.
8. Juliano, B.O. and D. Boulter, 1976. Extraction and composition of rice endosperm glutelin. Phytochem., 15: 1601-1606.
9. Kaul, A.K., Khan, M.R.I. and K.M. Munir, 1982. Rice quality–A survey of Bangladesh Germplasm. Bangladesh Rice Research Institute, Joydebpur, Dhaka.
10. Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature, 227 : 680 – 685.
11. Lowry, O.H., N.J. Risebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., 193 : 265 – 275.
12. Padhye, V.M. and D.K. Saluakhe, 1979. Extraction and characterization of rice proteins Cereal Chem., 56: 389-393.
13. Pasha, M.K., Nazma Begum and Q.A. Baset, 1995. Electrophoretic characterization of albumin and globulin proteins or rice grains. Bang. J. Bot., 24 (2): 115-120.
14. Yupsanis, T., M. Moustakas, and S. Karakoli, 1992. Seed protein electrophoresis for varietal identification in rice (*Oryza sativa* L.) J. Agro Crop. Sci., 168 : 95-99.