

## Relationship of Grain Protein Content to Other Grain Quality Traits in Interspecific *Oryza sativa* L. X *Oryza glaberrima* Steud. Progenies

<sup>1</sup>K. Futakuchi, <sup>2</sup>H. Watanabe and <sup>3</sup>M.P. Jones

<sup>1</sup>Africa Rice Center (WARDA), 01 B.P. 2031 Cotonou, Benin

<sup>2</sup>RECS International Inc. Tokyo, Japan

<sup>3</sup>Forum for Agricultural Research in Africa (FARA), PMB CT 173 Accra, Ghana

**Abstract:** High protein rice will improve the nutritional status of West African people. Promising varieties require high quality as well as good agronomic performance in West Africa. Fifteen interspecific *Oryza sativa* × *Oryza glaberrima* progenies showed a wide range of milled rice protein contents though all were derived from a single cross. These interspecifics were tested in terms of the relationship between protein content and other grain quality traits, i.e., husking yield, milling yield, head rice ratio, thousand grain weight of paddy, milled grain dimensions, milled grain appearance, viscosity parameters and milled rice amylose content. The trial was repeated for three seasons. No grain quality traits consistently showed significant correlation with protein content in all three seasons. Only thousand grain weight had a significant negative correlation in 2 seasons. Between the ten highest and ten lowest interspecific progenies for protein content, significant difference was observed only in thousand grain weight (in two seasons), husking yield, milling yield and minimum viscosity (one season each). Protein content was remotely associated with other grain quality traits in the interspecific progenies in general. Compared to protein content, amylose content strongly influenced grain dimensions and viscosity parameters. Selection of high protein interspecific rice could be made independently of the grain quality traits evaluated.

**Key words:** Grain quality, interspecific rice, *Oryza glaberrima* Steud., *Oryza sativa* L., protein content, West Africa

### INTRODUCTION

There are 2 cultivated rice species: *Oryza sativa* L. and *Oryza glaberrima* Steud. The former is of Asian origin and is now cultivated worldwide. The latter was domesticated in West Africa more than 3500 years ago (Jones *et al.*, 1997b) and is still cultivated in this sub-region (Singh *et al.*, 1997). However, growing area of *O. glaberrima* has been decreasing because its yield level is generally low compared to *O. sativa* due to grain shattering and poor resistance to lodging, which enhances grain shattering (Dingkuhn *et al.*, 1998). On the other hand, *O. glaberrima* lines are known to have resistance to a wide variety of rice cultivation constraints in West Africa (Jones *et al.*, 1997b; Johnson *et al.*, 1998; Jones and Singh, 1999; Futakuchi *et al.*, 2001) and are important as genetic resources to develop tailor-made rice varieties for resource-poor farmers in West Africa, who suffer from low yield caused by various biotic and abiotic stresses. To exploit resistance of *O. glaberrima* to these

major constraints and to overcome of its inherent low yield, the Africa Rice Center (WARDA) began interspecific breeding in 1992 to combine characteristics of *O. sativa* favorable to yield generation and the resistance of *O. glaberrima* (Jones *et al.*, 1997a). The first fixed fertile interspecific progenies were obtained in 1994 (Jones *et al.*, 1997a). Out of the wide crosses made in 1992, a number of promising lines, termed the WAB450 series, were obtained from a one cross combination: WAB56-104 as an *O. sativa* parent and CG 14 as an *O. glaberrima* parent.

Grain quality inclusive of protein content was not included in the selection criteria at the beginning of the interspecific breeding by WARDA. During the evaluation of fixed interspecific progenies, however, transgressive segregation in relation to protein content of milled rice was observed in the WAB450 series and several high protein content interspecific lines were identified (Watanabe *et al.*, 2006). Low yielding is likely to be associated with high grain protein content (Kataoka, 1978)

because of the physiological regulation that the accumulation of starch in grains is more disrupted than that of protein since amino acids are firstly translocated to grains and later to sucrose (Higashi *et al.*, 1974). However, no negative correlation was observed between yield and protein content in the interspecifics and it was suggested that the high protein content of some interspecifics was considered to have some mechanism to concentrate protein in the grains (Watanabe *et al.*, 2006).

Tagwireyi and Greiner (1994) have reported that 70% of the total protein for human nutrition in African regions comes from cereals. Protein content of milled rice is as low as 8.1% on a dry matter basis, whereas it is 9.5% in maize grits and 10.5% in first-grade medium-wheat flour (Resources Council/Science and Technology Agency, 1982). However, protein quality of rice is high compared to that of wheat or maize: the amino acid score is 65, 41 and 32 for milled rice, wheat flour and maize grits, respectively (Ohtsubo, 1995; Resources Council/Science and Technology Agency, 1982). Furthermore, rice consumption in West Africa has been increasing rapidly, thereby compensating the reduction of the consumption of other cereals such as millet with the result rice has now become an important protein source (Watanabe, 1998). The development of high protein rice can therefore contribute to improving the level of human nutrition in West Africa.

Since 1996, WARDA has adopted participatory approaches for varietal development (Farmers' Participatory Varietal Selection), where farmers' own selection criteria were collected and became feedback to the breeding program. Grain quality and taste are often listed as important criteria as well as agronomic characteristics such as short growth duration and high yield, etc. Sakurai *et al.* (2006) have conducted a survey in Ghana and showed that a market price for milled rice in an urban area reflects grain quality, i.e., rice with a higher whole grain content and shorter cooking time was sold at a higher price. Promising varieties therefore should possess not only high agronomic performance but also high quality inclusive of good taste. Protein content has an influence on the water absorption, swelling of cooked rice and gelatinization properties (Taira, 1998) and eventually on the taste of rice (Ishima *et al.*, 1974; Matsue *et al.*, 1994; Wada *et al.*, 2006). The relationship between protein content and some grain traits such as grain size has also been reported in *O. sativa* (Higashi *et al.*, 1974; Hillerislambert *et al.*, 1973; Kataoka, 1978; Tanaka *et al.*, 1970; Tsuzuki and Furusho, 1986). However, such data have not been available in interspecific rice developed

from the cross of *O. sativa* and *O. glaberrima*. Thus, in this study existing interspecific progenies in the WAB450 series were tested for relationships between protein content and several other grain quality traits in order to examine if a particular requirement for any of those grain quality traits could become an obstacle for the development of high protein content interspecific rice varieties. *O. glaberrima* lines possessing much higher protein content than existing high protein interspecific progenies have already been identified (Watanabe *et al.*, 2004) so that the development of high protein rice will be one of the objectives of the interspecific breeding for the next generation.

## MATERIALS AND METHODS

**Plant materials:** Before the beginning of the wide hybridization between *O. sativa* and *O. glaberrima*, 1130 *O. glaberrima* lines were evaluated in relation to early maturity, high tillering ability and rapid seedling growth in 1991 and 1992, after which eight lines were nominated for interspecific crosses. These 8 *O. glaberrima* lines were expected to compete well with weeds and escape from the drought which comes at the end of the wet season. Grain quality traits other than grain dimension were not evaluated during that screening. Five high yielding, elite upland *O. sativa* lines developed by WARDA were also nominated to use for the wide cross. Crossing was carried out in 1992. A few fertile grains were collected from seven crosses and those F<sub>1</sub> progenies were successively backcrossed to the respective *O. sativa* parents twice. Individuals from the BC<sub>2</sub>F<sub>1</sub> were subjected to pedigree selection in upland using selection criteria such as high tillering, rapid seedling growth, early maturity, lodging resistance and panicle type. The increment of fertility at each generation was observed only in progenies derived from 2 crosses, WAB449 and WAB450. The selection continued for an additional 6 generations until varietal traits were fixed. This is how promising interspecific progenies were selected from the WAB450 series, the parents of which are WAB56-104/CG 14//2\*WAB56-104. WAB56-104 is a japonica-type, improved upland *O. sativa* variety officially released in Côte d'Ivoire in 1998 (MINAGRA, 1998). CG 14 was an *O. glaberrima* line collected from a farmer's upland field in Casamance, Senegal.

Fifty lines of the interspecific progenies (WAB450 series) were used for the trial. The experiment was conducted and repeated in three consecutive years, the wet seasons of 1997 and 1998 and the dry season of 1999,

in the irrigated lowland field at WARDA headquarters at M'bé in Côte d'Ivoire (7°52'N, 5°6'W and 300 m of altitude). Seeds were sown in a semi-irrigated nursery bed on 14 July 1997, 28 July 1998 and 10 February 1999. The seedlings were transplanted 21 days after seeding at a rate of two seedlings per hill with a spacing of 0.25×0.25 m in clean-weeded plots, based on a randomized block design with three replications. Each plot measured 5×5 m (19 hills ×19 rows) and received a basal application of compound fertilizer (10% N, 18% P<sub>2</sub>O<sub>5</sub> and 18% K<sub>2</sub>O) at a rate of 10 g m<sup>-2</sup> and top-dressing of urea (46% N) at a rate of 8.70 g m<sup>-2</sup> at the maximum tillering and panicle initiation stages. The plots were irrigated and weeded by hand as necessary to keep the plots clean. Sixteen hills were sampled from each plot at maturity. At harvesting, panicles from a single hill were put in a cotton sack and the whole aboveground part was collected. The collected samples were dried in sacks outside on paved ground. Cotton sacks were used to avoid possible grain loss by shattering and over-fast drying, which causes grain breakage. When the moisture content of paddy reaches about 14%, paddy was threshed manually. Threshed paddy was dried at about 55°C until the moisture content of all samples was around 11% and the dried paddy could be used for the grain quality analysis. Moisture content was monitored by moisture meter (KETT C600). Three lines were eliminated from the quality analysis because of suspected contamination of the paddy sample by grains from a different line in one of the 3 experimental years. Forty-seven lines were therefore tested for protein content and other grain traits.

This experiment was part of a larger trial to evaluate the interspecific progenies in relation to leaf area expansion and other important characteristics associated with weed competitiveness.

**Thousand grain weight of paddy:** Paddy samples collected from the three plots were mixed together and winnowed. Two hundred paddy grains were randomly selected and weighed. Thousand Grain Weight of paddy (TGW) was calculated and expressed on a 14% moisture basis.

**Milling characteristics:** Husking Yield (HY), Milling Yield (MY) and Head Rice Ratio (HRR) -the percentage ratios of, respectively, brown rice to paddy, milled rice to brown rice and head rice to milled rice on a weight basis-were determined. For each interspecific line, the 250 g of winnowed paddy was hulled with a Satake Testing Husker (THU 35H). Brown rice was weighed and HY was determined. The 150 g of brown rice was milled with a

milling machine for laboratory use (Yamamoto Test Rice Whitener VP-31T). After milling, rice bran was removed with a 1.7 mm sieve. A cleaned sample of milled rice was weighed and MY was determined. From the 20 g of cleaned milled rice, all head rice was taken and weighed. HRR was then calculated. Milled rice grains with a length greater than three-quarters that of complete grains were considered as head rice. Colored and damaged grains were also removed from the category of head rice.

**Grain appearance of milled rice:** For each line, 10 whole grains were randomly selected from the cleaned milled rice and processed for the determination of the milling characteristics after grain Length (L), Width (W) and Thickness (T) had been measured by micrometer. The Length/Width ratio (L/W) and Thickness/Width ratio (T/W) were also calculated. Regarding the determination of chalkiness, 200 whole grains were selected from the cleaned milled rice and a score of 0, 1, 5 or 9 was given to each sample by eye measurement according to the standard evaluation systems of the International Rice Research Institute (IRRI, 1996). Whiteness and translucency of milled rice were determined using the cleaned milled rice, excluding damaged and colored grains and a Satake Milling Meter (MM-1B).

**Determination of amylose content:** The cleaned milled rice was ground and powdered by an Udy Cyclone Mill with a 1.0 mm mesh screen. Amylose content was determined by auto-analyzer based on the iodine-colorimetric method (Julliano, 1971).

**Determination of viscosity characteristics:** The powdered samples mentioned above were passed through a mesh (No. 40). For each interspecific line, the powdered sample-the amount of which was equivalent to 50 g at 13.5% moisture content-was suspended in 450 mL of distilled water. The following viscosity parameters were determined with a Brabender viscogram by using the rapid method (Watanabe and Futakuchi, 2000): Peak Viscosity (PV), Minimum Viscosity (MIN), Breakdown viscosity (BD), Final Viscosity (FV) and Setback viscosity (SB).

**Determination of protein content:** The powdered samples prepared for the determination of amylose contents were used. Total nitrogen content was determined by near Infrared Reflectance (NIR) analyzer. Protein content was calculated by multiplying total nitrogen content by 5.95-the constant to convert nitrogen content to protein content in rice-and expressed on a dry matter basis of milled rice.

## RESULTS

**Protein content and other grain quality traits:** Although all interspecific progenies tested were derived from the same cross (WAB56-101/CG 14//2\*WAB56-104), protein

Table 1: Protein content of the interspecific progenies and their parents: CG 14 (*Oryza glaberrima*) and WAB56-104 (*Oryza sativa*)

	Protein content (%)		
	1997	1998	1999
Mean	8.3	9.0	9.9
se	0.5	0.5	0.8
Max	9.4	10.2	11.8
Min	7.4	7.8	8.5
CG 14	7.9	8.3	8.9
WAB56-104	8.1	8.9	10.3

Table 2: Correlation coefficients between Protein Content (PC) and Amylose Content (AC), Husking Yield (HY), Milling Yield (MY), Head Rice Ratio (HRR), Thousand Grain Weight (TGW), Grain Length (L), Grain Width (W), Grain Thickness (T), Length/Width ratio (L/W), Thickness/Width ratio (T/W), Chalkiness (Chlk), Translucency (Trans), Whiteness (White), Peak Viscosity (PV), Minimum Viscosity (MIN), Breakdown viscosity (BD), Final Viscosity (FV) and Setback viscosity (SB)

	1997	1998	1999
PC	1	1	1
AC	-0.293*	0.075	-0.018
HY	-0.166	-0.097	-0.451**
MY	0.245	0.325*	0.077
HRR	0.215	0.162	0.042
1000GW	-0.083	-0.253*	-0.360**
L	-0.127	0.087	-0.099
W	0.185	0.201	0.020
T	-0.014	0.029	-0.115
L/W	-0.219	-0.143	-0.084
T/W	-0.204	-0.275*	-0.100
Chlk	0.086	0.113	0.023
Trans	-0.103	-0.038	-0.101
White	-0.001	0.001	0.087
PV	0.061	-0.103	-0.093
MIN	0.102	0.142	0.373*
BD	0.031	-0.159	-0.285*
FV	-0.034	0.240	0.263*
SB	-0.051	0.205	0.230

\* Indicates significance at  $p < 0.05$ . \*\* Indicates significance at  $p < 0.01$

Table 3: Comparison of Amylose Content (AC), Husking Yield (HY), Milling Yield (MY), Head Rice Ratio (HRR), Thousand Grain Weight (TGW), Grain Length (L), Grain Width (W), Grain Thickness (T), Length/Width ratio (L/W), Thickness/Width ratio (T/W), Chalkiness (Chlk), Translucency (Trans), Whiteness (White), Peak Viscosity (PV), Minimum viscosity (MIN), Breakdown viscosity (BD), Final Viscosity (FV) and Setback viscosity (SB) between 10 high and 10 low Protein Content (PC) interspecific progenies

	PC (%)	AC (%)	HY (%)	MY (%)	HRR (%)	1000GW (g)	L (mm)	W (mm)	T (mm)	L/W	T/W	Chlk	Trans	White	PV (BU)	MIN (BU)	BD (BU)	FV (BU)	SB (BU)
1997																			
High PC	9.03	22.8	80.4	87.4	53.3	28.5	6.7	2.6	1.9	2.65	0.73	3.8	2.27	46.0	644	421	224	939	295
Low PC	7.76	25.2	80.6	86.4	46.9	29.1	6.8	2.5	1.9	2.73	0.75	2.8	2.35	46.0	653	424	230	966	313
	***																		
1998																			
High PC	9.76	25.4	80.7	88.7	68.2	26.4	7.2	2.7	1.9	2.69	0.71	3.2	2.65	42.6	620	394	227	942	322
Low PC	8.29	23.9	80.7	87.1	62.1	29.7	7.2	2.6	1.9	2.78	0.74	2.9	2.66	43.5	642	382	260	851	209
	***			*		*													
1999																			
High PC	11.18	24.6	76.8	85.3	51.0	28.2	7.2	2.7	1.9	2.73	0.71	2.5	2.98	45.2	610	398	212	969	358
Low PC	8.90	23.7	78.1	84.7	48.7	31.9	7.4	2.7	1.9	2.80	0.72	2.4	3.01	45.6	631	364	267	854	223
	***		*			*													**

\* Indicates significant difference at  $p < 0.05$ . \*\* Indicates significant difference at  $p < 0.01$ . \*\*\* Indicates significant difference at  $p < 0.001$ . † BU-Brabender unit

content varied in the ranges from 7.4 to 9.4%, from 7.8 to 10.2% and from 8.5-11.8% in 1997, 1998 and 1999, respectively (Table 1). Those variations always exceeded the range between the parents in the three seasons (Table 1).

A simple correlation with protein content was performed for all other grain quality traits (Table 2). In each year, the top 10 and bottom 10 interspecific lines were selected in relation to protein content and the other grain quality traits were compared between those high and low protein interspecific progenies by T-test (Table 3).

For the milling characteristics, protein content had a negative correlation with HY in general though the correlation was significant only in 1999 (Table 2). On the other hand, a significant positive correlation was observed between protein content and MY in 1998 (Table 2). There was no significant correlation between protein content and HRR in any of the three years (Table 2). The difference of HY in 1999, when a significant negative correlation with protein content was observed, is as little as 1.3% between the high and low protein interspecifics (Table 3). In 1997 and 1998, no difference was observed in HY between the high and low protein interspecifics (Table 3). High protein content will not be associated with the considerable reduction of HY. Generally, protein content was remotely related to the milling characteristics in the varietal variation of the interspecific progenies.

Thousand Grain Weight (TGW) of paddy showed a significant negative correlation with protein content in 1998 and 1999 (Table 2) and also its values were significantly different between the high and low protein interspecifics in those years (Table 3). Interspecifics with high protein content had a tendency to have smaller grains. Regarding the appearance of milled rice, i.e., grain dimensions, chalkiness, translucency and whiteness, no

Table 4: Correlation coefficients between Amylose Content (AC) and Husking Yield (HY), Milling Yield (MY), Head Rice Ratio (HRR), Thousand Grain Weight (TGW), Grain Length (L), grain Width (W), grain Thickness (T), Length/Width ratio (L/W), Thickness/Width ratio (T/W), Chalkiness (Chlk), Translucency (Trans), Whiteness (White), Peak Viscosity (PV), Minimum viscosity (MIN), Breakdown viscosity (BD), Final Viscosity (FV) and Setback viscosity (SB)

	1997	1998	1999
PC	-0.293*	0.075	-0.018
AC	1	1	1
HY	0.118	0.026	-0.328*
MY	0.320*	0.048	-0.309*
HHR	-0.203	-0.229	-0.049
1000GW	-0.444**	-0.501***	-0.297*
L	0.294*	0.319*	0.347**
W	-0.256*	-0.289*	-0.237
T	-0.238	-0.175	-0.101
L/W	0.362**	0.409**	0.359**
T/W	0.085	0.260*	0.235
Chlk	-0.080	-0.047	-0.050
Trans	-0.207	0.031	-0.081
White	0.412**	0.195	0.182
PV	-0.782***	-0.807***	-0.860***
MIN	-0.319*	-0.072	-0.147
BD	-0.824***	-0.861***	-0.822***
FV	0.801***	0.863***	0.700***
SB	0.888***	0.927***	0.839***

\* Indicates significance at  $p < 0.05$ . \*\* Indicates significance at  $p < 0.01$ . \*\*\* Indicates significance at  $p < 0.001$

Table 5: Effects of protein and amylose contents on viscosity parameters: peak viscosity (PV), minimum viscosity (MIN), breakdown viscosity (BD), final viscosity (FV) and setback viscosity (SB)

	PV	MIN	BD	FV	SB
1997					
r	0.802***	0.319*	0.853***	0.828***	
$r^2$	0.915***				
SPRC for PC	-0.185	0.102	0.728	0.686	0.837
SPRC for AC	-0.837	-0.317	-0.892	0.866	0.955
1998					
r	0.808***	0.164	0.866***	0.881***	
$r^2$	0.936***				
SPRC for PC	-0.042	0.148	-0.095	0.177	0.136
SPRC for AC	-0.804	-0.083	-0.853	0.850	0.916
1999					
r	0.867***	0.399**	0.875***	0.753***	
$r^2$	0.874***				
SPRC for PC	-0.109	0.371	-0.300	0.276	0.245
SPRC for AC	-0.862	-0.140	-0.827	0.705	0.843

\* Indicates significance at  $p < 0.05$ . \*\* Indicates significance at  $p < 0.01$ ; \*\*\* Indicates significance at  $p < 0.001$ ; † SPRC-Standardized partial regression coefficient

significant correlation was observed except W/T in 1998 (Table 2). However, there was no significant difference in W/T between the high and low protein lines in that year (Table 3) and protein content was not associated with the milled grain appearance.

A significant negative correlation between protein and amylose contents was obtained only in 1997 (Table 2), though the difference in amylose content

between high and low protein content lines was not significant. Instead, in 1998 and 1999, there was an adverse relationship between them although that was not significant; high protein interspecifics depicted high amylose content (Table 3). In 1999, protein content showed a significant correlation to three viscosity parameters, MIN, BD and FV (Table 2). However, the significant difference between the high and low protein interspecific lines was observed only in MIN (Table 3). Since protein content was known to affect texture of rice, stronger correlations with the viscosity parameters had been anticipated. However, the influence of protein content on viscosity and eventually on rice texture was small.

#### Comparison of protein content and amylose content in terms of influence on other grain quality traits:

Since amylose content, like protein content, is known to be a dominant factor affecting rice texture, simple correlation coefficients of amylose content with the viscosity parameters and also the other quality traits are shown in (Table 4). For the most part a consistent relationship of amylose content with viscosity, i.e., highly significant negative correlations with PV and BD and highly significant positive ones with FV and SB, was obtained. The clear effects of amylose content on the viscosity parameters were indicated by the correlation coefficients. Significant correlations were also observed with some traits in grain dimension and size; high amylose content interspecific lines showed a tendency to have longer (high L values), more slender (high L/W values) and lighter (low TGW values) grains (Table 4), whereas protein content depicted no influence on grain dimension (Table 2 and 3).

Effects on the viscosity parameters were compared between protein and amylose contents using multiple regression analysis (Table 5). Varietal variations of all parameters except NIM were sufficiently explained by the variations of protein and amylose contents, coefficients of determination of which were from 0.567-0.877. Protein and amylose contents showed the same positive or negative code with regression coefficients for the respective viscosity parameters excluding MIN, suggesting that they had the same type of influence on viscosity. In general, their low concentration corresponded to stickier rice texture. However, the absolute values of the standardized partial regression coefficient were much larger in amylose content than in protein content. The effect of amylose content on the viscosity was much more dominant than that of protein content.

## DISCUSSION

In *O. sativa*, a negative correlation between protein content and Thousand Grain Weight (TGW) has been often reported (HilleRisLambers *et al.*, 1973; Higashi *et al.*, 1974; Kataoka, 1978; Tsuzuki and Furusho, 1986). However, higher protein content in smaller grain rice could occur through non-genetic regulation since a given weight of small rice grains has more surface area than the same weight in larger grains and because protein is concentrated more in the surface layer of the endosperm than in the core. Kambayashi *et al.* (1984) suggested they are genetically independent and selection for protein content and grain size could be made separately. The same relationship was obtained between protein content and TGW in the interspecific progenies although we cannot from the current data comment specifically on the genetic linkage between them. In West African countries, slender grain rice is highly preferred by consumers in general; for instance, IDSA 85, which has an L/W value of around 4.0 (MINAGRA, 1998), is favored in Côte d'Ivoire for this aspect. Protein content was not influential on grain dimensions in the interspecific progenies (Table 2 and 3). Nor was there a close relationship between protein content and grain appearance, i.e., chalkiness, translucency and whiteness, in the interspecific progenies (Table 2 and 3). Similar observations have also been reported in *O. sativa* (Tsuzuki and Furusho, 1986). The selection for high protein content may be made independently on grain dimensions and appearance in interspecific breeding. By contrast, amylose content influenced grain dimensions and high content seemed to be associated with high L and L/W values (Table 4). This may cause some difficulty when breeders attempt to develop a variety with slender grains, which is a highly desirable trait, in a country where soft texture is preferred.

Regarding milling characteristics, protein content constantly showed negative, positive and positive correlations, respectively, to HY, MY and HRR during the three years (Table 2). However, correlation coefficients only with HY in 1999 and MY in 1998 depicted significance and their differences between the high and low protein interspecific progenies were small: 1.3% in 1999 HY and 1.6% in 1998 MY (Table 2 and 3). Protein is more concentrated in the surface layer of endosperm than the core. Therefore, protein content of milled rice decreases with the increase in thickness of the kernel removed by milling when milling degree is varied (Chen *et al.*, 1998). However, when protein content and milling yield are contrasted with various genotypes at a fixed milling machine setting, correlation is lacking

(Delwiche *et al.*, 1996). In trials where the amount of nitrogen application was changed, positive correlation was observed between protein content and head rice ratio (Perez *et al.*, 1996). However, nitrogen application can increase grain protein content and also reduce grain breakage (Wopereis-Pura *et al.*, 2002) and the correlation between protein content and head rice ratio could result from the separate effects of nitrogen application on those 2 traits. As protein content was remotely related to milling characteristics in the interspecific progenies, the development of high protein rice through interspecific breeding could also produce good milling characteristics.

It is noted that protein in milled rice disturbs the swelling and gelatinization of milled grain powder (Taira, 1998) and has an influence on rice texture and consequently on taste (Ishima *et al.*, 1974; Matsue *et al.*, 1994; Wada *et al.*, 2006). Regarding the effects of protein content on the viscosity parameters, however, significant correlations were obtained only in the 1999 trials with some parameters (Table 2). Protein content depicted much less effect on the viscosity parameters than did amylose content (Table 5). Amylose content of officially-released varieties in West African countries showed a clear tendency from one country to another; for example, the content of most varieties in Nigeria was above 25%, while many varieties in Côte d'Ivoire had their content between 20 and 25% and some showed content of less than 20 (unpublished data). This could partly suggest that amylose content is important in the determination of rice taste and the released varieties adequately correspond to people's preferences. If amylose content maintains an appropriate value, high protein content interspecific progenies with acceptable texture to the intended consumers could be developed.

Juliano and Villareal (1993) have reported a significant positive correlation at the 0.05 probability level between protein and amylose contents in *O. glaberrima* although the number of lines tested was 15. On the other hand, no correlation between them was found in the trial by Koutroubas *et al.* (2004) with 318 European rice varieties of *O. sativa*. In case of the interspecific progenies, a negative correlation was obtained in 1997, although there was no correlation in the other years (Table 2). Apart from overall trends in the relationship between the 2 traits, high protein and high amylose interspecific progenies were identified as well as high protein and low amylose interspecifics. WAB450-11-1-2-P41-HB was an example of the former. Its protein content was 8.9, 9.8 and 11.2% in 1997, 1998 and 1999, respectively and the amylose content was 26.1, 27.0 and 26.5% in 1997, 1998 and 1999, respectively. The averages of all interspecifics for protein content in 1997, 1998 and

1999 were 8.3, 9.0 and 9.9, respectively and those for amylose content in 1997, 1998 and 1999 were 24.1, 24.3 and 23.7. Another example was WAB450-I-B-P-131-HB; its protein content was 9.4, 9.6 and 11.1% in 1997, 1998 and 1999, respectively; and the amylose content was 26.3, 26.7 and 26.0% in 1997, 1998 and 1999, respectively. The example of the latter was WAB450-B-1A1.1, protein and amylose contents of which were 9.2 and 15.5%, respectively in 1997; 9.6 and 15.9%, respectively in 1998 and 11.8 and 14.1%, respectively in 1999. It will be possible to develop high protein content interspecific rice possessing a range of amylose content responding to various eating preferences in West Africa.

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