

Interspecific Rice Hybrid of *Oryza sativa* × *Oryza nivara* Reveals a Significant Increase in Seed Protein Content

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Wild species offer a potential reservoir of genetic variation for crop improvement. Besides the valuable genes for disease resistance that the wild species have provided for rice improvement, recent studies have shown that these wild species could also provide favorable alleles for the improvement of yield and yield-related traits. The present study reports yet another potential of wild relatives of rice, which involves the improvement of seed protein content. A significant increase in seed protein content was observed in an interspecific hybrid between *Oryza sativa* ssp. *indica* and the wild species *Oryza nivara*. The hybrid showed a protein content of 12.4%, which was 28 and 18.2% higher than those of the parents *O. nivara* and IR 64, respectively. The increase in protein content was dependent on the genetic background of the rice variety used in the hybridization. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis of seed storage proteins demonstrated that a significant increase in prolamins and glutelins was mainly responsible for the elevated protein content of the hybrid. Amino acid analysis of seed proteins revealed that the hybrid had net gains of 19.5% in lysine and 19.4% in threonine over the *O. nivara* parent on a seed dry weight basis. Molecular analysis indicated that the increase in protein content of the hybrid was not a result of chromosomal rearrangements or transposable element activation, at least in the chromosomal regions containing seed storage protein genes. A preliminary genetic analysis of the F₂ segregating population showed that the inheritance of the increased protein content was polygenic in nature. The development of this interspecific hybrid offers a great potential for selecting new rice cultivars that combine the high yield and superior cooking quality of IR 64 with improved seed protein content.

KEYWORDS: Amino acid; interspecific hybrid; IR 64; *Oryza nivara*; prolamin; storage protein

INTRODUCTION

Strong efforts have been invested over the past five decades to improve the protein content of rice, mainly via conventional breeding techniques and induced mutations (1). Screening germplasm collections of cultivated rice for protein content has shown that protein content in cultivated rice ranges from about 5 to 18% with an average of 9.5%, indicating the presence of genetic variability for high protein content and suggesting the feasibility of breeding high-protein rice cultivars (2). However, these efforts have been largely unsuccessful, as indicated by the lack of modern high-protein rice cultivars (3). The lack of success may have been caused by the universal complexity of

the inheritance of endosperm traits. In addition, seed protein content tends to have low heritability due to the significant environmental effects and to show negative correlation with yield and some eating/cooking quality criteria (3). Nevertheless, the ability to combine high protein content and high yield has been reported in other cereals such as wheat and oats (3). In addition, with rice being a main source of protein for billions of people in developing countries, and with the increasing interest in high-protein food products of rice in developed countries, the protein content of rice seeds is still a very attractive target for genetic improvement.

Recent advances in biotechnology, particularly molecular marker technology and genetic transformation, hold great promise for crop improvement, including grain quality traits (4, 5). Crop improvement still, however, awaits the practical realization of the potentials of these new techniques. Another promising approach that has already proven to be effective for crop improvement is the exploitation of the tremendous reservoir of genetic variation present in the wild and cultivated relatives of

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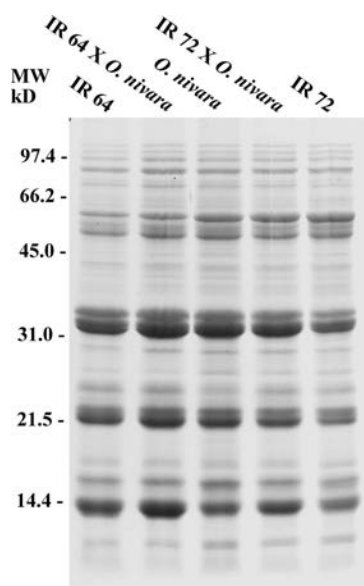


Figure 1. SDS-PAGE analysis of total seed proteins of parental lines and their hybrids. Total seed proteins extracted from equal amounts of seed powder from each genotype were separated by SDS-PAGE using a 13.5% acrylamide gel and stained with Coomassie Blue. MW, molecular mass marker in kilodaltons.

crop plants through interspecific hybridization (6, 7). Numerous traits that are potentially useful for rice improvement, particularly for disease resistance and tolerance to abiotic stresses, have been identified in many of the wild species of the genus *Oryza*. Efforts to incorporate some of these traits into rice cultivars have been already successful or are being actively pursued (8, 9). Although landraces and wild species are in general agronomically inferior to crop plants, the transfer of favorable alleles with positive effects on agronomic traits such as yield- and quality-related traits is still feasible and has already been documented in several crops, including rice (10–13).

The genus *Oryza* includes 20–21 wild species, depending on the classification, and two cultivated species, *O. sativa* L. of Asian origin and *O. glaberrima* Steud. of West African origin (14, 15). Of the 21 wild species, 7 species share the same genome (genome AA) with cultivated rice, and thus these species can be readily hybridized with cultivated rice and desirable traits can be transferred with relative ease. *Oryza nivara* Sharma et Shastry is a wild, annual diploid species of Asian origin that carries the AA genome. It has been suggested that *O. nivara* is the most closely related to *O. sativa* and probably its direct progenitor (14, 15). The first example of successful transfer of useful genes from the wild species into cultivated rice involved *O. nivara* and resulted in the introgression of a gene for grassy stunt virus resistance into cultivated rice varieties (16).

We have produced an interspecific hybrid between *O. sativa* and *O. nivara* with the initial objective of transferring the ability to tolerate drought from *O. nivara* to cultivated rice. Because we were also interested in rice grain quality, we examined seed protein content in these interspecific hybrids. Here, we report a significant increase in protein content in the interspecific hybrid compared to both parents.

MATERIALS AND METHODS

Plant Materials. Two cultivars of *O. sativa* spp. *indica*, IR 64 and IR 72, were crossed as the female parents to an accession of *O. nivara* (Acc. 104444) as the male parent at Tamil Nadu Agricultural University,

Table 1. Levels of Individual Amino Acids and Total Protein in Seeds of Parental Lines and Hybrid

amino acid ^a	IR 64	<i>O. nivara</i>	hybrid	LSD ^c
alanine	5.90	5.89	5.86	0.09
arginine	8.07	8.07	8.20	0.09
aspartic acid	9.07	9.54	9.41	0.12
cysteine	2.28	2.32	1.89	0.09
glutamic acid	18.16	16.62	18.01	0.21
glycine	4.55	4.86	4.56	0.13
histidine	2.58	2.76	2.71	0.23
isoleucine	4.51	4.37	4.64	0.06
leucine	9.09	8.62	9.35	0.16
lysine	3.82	4.50	3.85	0.12
methionine	2.80	3.09	2.30	0.43
phenylalanine	5.60	5.33	5.75	0.08
proline	4.70	4.72	4.66	0.36
serine	4.17	4.23	4.01	0.12
threonine	3.46	3.62	3.36	0.09
tyrosine	3.46	3.54	3.70	0.22
valine	6.43	6.39	6.57	0.16
total protein ^b	10.51	8.89	12.42	0.20

^a Expressed as a percentage of total amino acid content per 100 g of seeds.

^b Expressed as grams per 100 g of seeds. ^c Least significant difference at $P \leq 0.01$.

India. The F₁ and F₂ plants as well as the parental lines were grown side by side in the field to maturity, and the harvested seeds from the parents and the hybrid were used for this study.

Analysis of Total Seed Proteins. Total proteins were extracted from seeds of the parental lines and from a bulk of F₂ seeds or from individual F₂ seeds of the hybrid. Seeds were hulled manually and then ground to a fine powder using a mortar and pestle. Total proteins were extracted from 100 mg of seed powder from each genotype with 1 mL of sodium dodecyl sulfate (SDS)–sample buffer [60 mM Tris-HCl (pH 6.8), 2% SDS (w/v), 10% glycerol (v/v), 0.03% bromophenol blue (w/v), and 5% 2-mercaptoethanol (v/v)]. For extraction of total proteins from individual seeds, a total of 60 seeds from the hybrid were hulled and then ground individually to a fine powder between two sheets of weighing paper using a pestle. The powder of the individual seeds were transferred to microfuge tubes and the exact weight of each seed was recorded. Total proteins were extracted from individual seeds using SDS–sample buffer at a ratio of 60 μ L of extraction buffer/mg of seed powder. Samples were heated in boiling water for 5 min and then microcentrifuged at full speed for 5 min at room temperature. Ten microliters of the supernatant for each sample were subjected to SDS–polyacrylamide gel electrophoresis (SDS-PAGE) using a Hoefer SE-260 mini-gel apparatus (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's instructions. After the gels had been stained with Coomassie Blue and destained with a 50% methanol/10% glacial acetic acid mixture, gels were placed in 10% glacial acetic acid prior to visualization. Images of the gels were captured using Kodak Electrophoresis Documentation and Analysis System (EDAS) 290 (Eastman Kodak Co., Rochester, NY). Chemical quantification of total seed proteins was carried out by combustion analysis of nitrogen on three independent replicates of seed powder from each genotype.

Isolation of the Different Fractions of Seed Storage Proteins. The albumin, globulin, prolamin, and glutelin fractions of rice seed storage proteins were extracted from seed powder of the parents and the hybrid using the acetone precipitation procedure (17). Precipitated proteins were centrifuged at 12000g for 15 min, and protein pellets were dried briefly in a speed vacuum and resuspended in SDS–sample buffer. For each protein fraction, 10 μ L from each of the parents and the hybrid was subjected to SDS-PAGE, and the gels were processed as described above.

Densitometry Analysis of SDS-PAGE Gels. Images of the Coomassie-stained protein gels were captured with the EDAS 290 system using the least exposure time, to minimize the effect of signal saturation. Signal intensity measurements were carried out using the Kodak ID

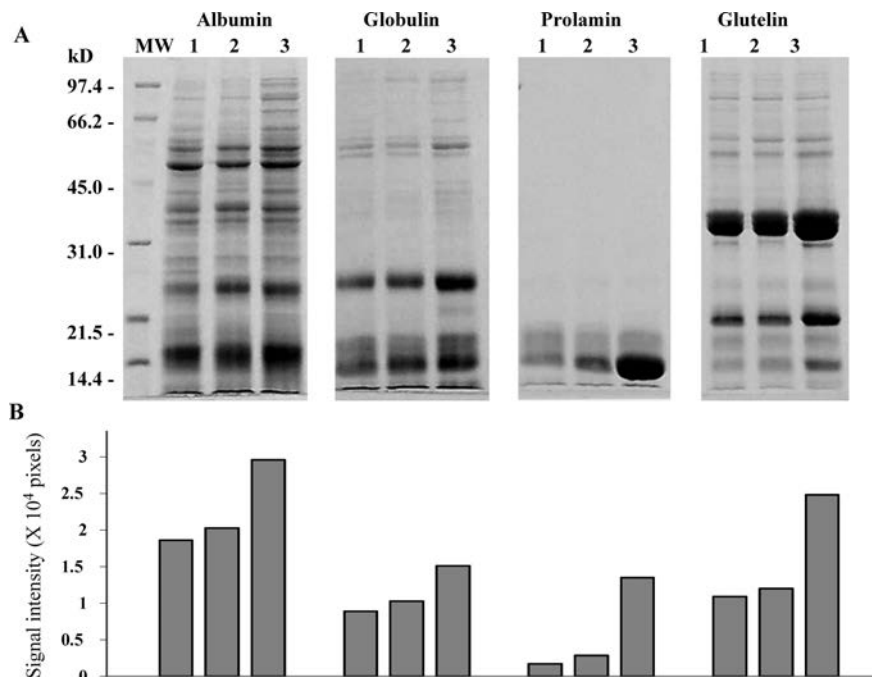


Figure 2. Analysis of different seed protein fractions from parental lines, *O. nivara* (lane 1) and IR 64 (lane 2), and their hybrid (lane 3): (A) purified seed protein fractions based on their solubility from equal amounts of seed powder fractionated by SDS-PAGE on a 13.5% acrylamide gel and stained with Coomassie Blue; (B) signal intensity measurements over the entire lane of each genotype quantified from the SDS-PAGE gels in (A). MW, molecular weight (mass) marker in kilodaltons.

Image analysis software, version 3.6.1 (Eastman Kodak Co.). Net signal intensity (the sum of background-subtracted pixel values) was measured for the two parental lines and the hybrid either over the entire lane for each genotype or of the bands corresponding to the individual major polypeptides corresponding to the different fractions of seed storage proteins.

Amino Acid Analysis. Contents of individual amino acids in seed powder of the parents and the hybrid were quantified in three independent replicates. Sulfur-containing amino acids, methionine and cysteine, were quantified by oxidation with performic acid. For quantification of other amino acids, samples were hydrolyzed in 6 N HCl at 155 °C for 16 h under a nitrogen atmosphere (18). Samples were then separated using a Beckman 6300 Amino Acid Analyzer with a postcolumn ninhydrin reaction detection system (Beckman Instruments, Fullerton, CA).

Tissue Preparation for Electron Microscopy (EM). Seeds from the parents and the hybrid were cut into 2–3 mm segments with a razor blade. The sliced segments were immediately fixed for 4 h at room temperature in 2.5% glutaraldehyde (v/v) buffered at pH 7.2 with 50 mM sodium phosphate. Samples were then thoroughly washed several times with excess of phosphate buffer. Postfixation with osmium tetroxide, embedding in Spurr's resin, and section preparation for EM examination were performed as described earlier (19–21). Sections were examined with a JEOL 1200 EX (Tokyo, Japan) transmission electron microscope at 80 kV.

Southern Hybridization. Total genomic DNA was isolated from leaf tissue using the cetyltrimethylammonium bromide (CTAB) method (22). Ten micrograms of total genomic DNA from the parents and the hybrid was digested with different restriction enzymes and separated by electrophoresis on a 0.8% agarose gel. After electrophoresis, DNA was transferred to nylon membranes by capillary transfer using 0.4 M NaOH. The DNA fragments used as probes for Southern hybridization included the genomic clone of the prolamin gene and a cDNA clone of rice α -globulin (23). For the glutelin probe, a DNA fragment corresponding to the coding region of the glutelin gene was amplified by reverse transcriptase PCR (RT-PCR) from total RNA that was extracted from developing seeds of cv. IR 64 using the Trizol reagent (Invitrogen, Carlsbad, CA). The RT-PCR was carried out using the OneStep RT-PCR kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. The primers used for the amplification were

as follows: forward, 5'- ATGGCAACCATCAAATTCCTATAG- 3'; reverse, 5'- TGCCTCATATCTATACAATTGTTGAC- 3'. The glutelin PCR fragment was cloned and sequenced to verify the authenticity of the PCR product. The prehybridization, hybridization, and washing of Southern blots were performed as described by Mahmoud et al. (24).

Statistical Analysis. Data were statistically analyzed using the SAS program, version 8.2 (SAS Institute, Inc., Cary, NC). Replicated measurements of total seed protein and individual amino acids were subjected to analysis of variance and comparison of means with the least significant difference test (LSD). For the densitometry measurements, parental means of three independent measurements from three different lanes of each parental line were compared using the *t* test.

RESULTS

Analysis of Total Seed Proteins of the Hybrid and the Parents. The SDS-PAGE analysis of total seed proteins from the two hybrids involving *O. nivara* and the two rice cultivars IR 64 and IR 72 revealed a genotype-dependent increase in total seed proteins in these hybrids (Figure 1). In the hybrid involving IR 64, the total protein content of the hybrid seeds appeared to clearly exceed those of both parents, with the most notable increase being in the accumulation of the 13–14 kDa polypeptides followed by the 34–39 and 21–23 kDa polypeptides. In the hybrid involving IR 72, the protein content of the hybrid seeds was more or less intermediate between those of two parents with the exception of the 13–14 kDa polypeptides, which appeared to accumulate to a level that exceeded those of both parents. It is worth noting that the cultivar IR 72 was clearly lower in seed protein content than IR 64 as well as the *O. nivara* parent (Figure 1). The pronounced increase in protein content in the IR 64 \times *O. nivara* hybrid compared to the hybrid involving IR 72 prompted its selection for further characterization to gain some understanding of the biochemical, genetic, and molecular basis of the increased protein content. The chemical quantification of seed protein content in IR 64, *O. nivara*, and their hybrid confirmed the observations from the

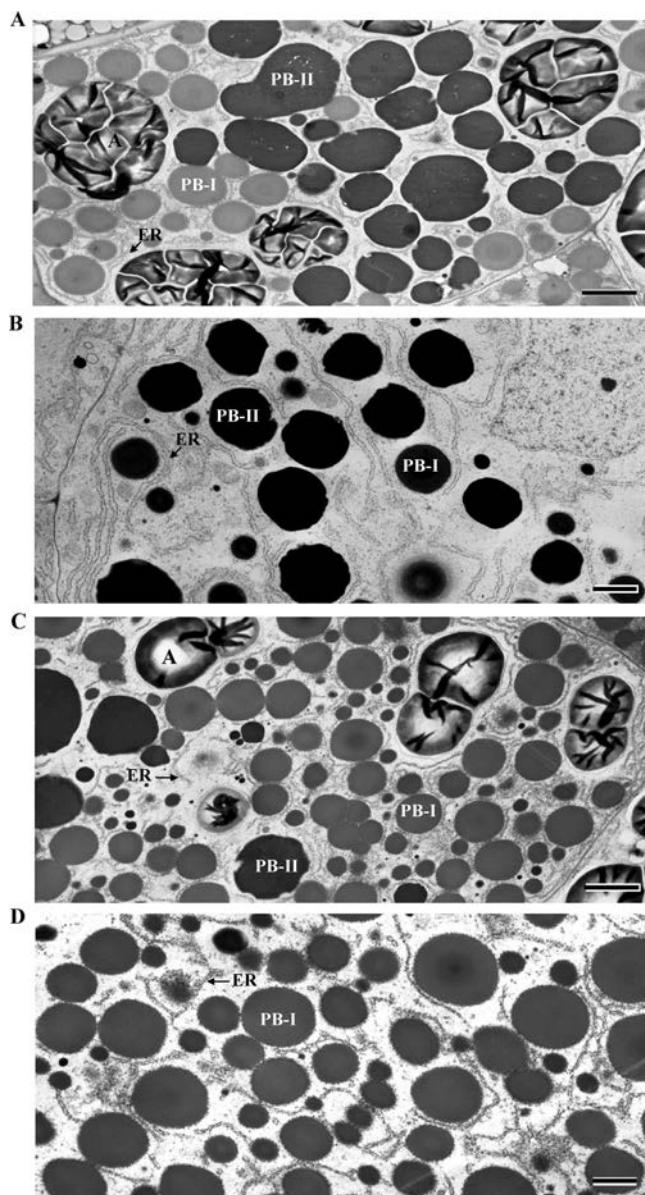


Figure 3. Ultrastructure of seed endosperm of parental lines and their hybrid. Electron micrographs of the parental lines, IR 64 (A) and *O. nivara* (B), and their hybrid (C, D) revealed the presence of two distinct populations of protein bodies, the spherical protein bodies type I (PB-I) and the irregularly shaped protein bodies type II (PB-II). Note the higher preponderance of the prolamin-containing PB-I in the hybrid compared to the parental lines. A, amyloplast; ER, endoplasmic reticulum. Bar = 2 μm in (A) and (C) and 1 μm in (B) and (D).

SDS-PAGE analysis (Table 1). The hybrid showed a protein content of 12.4%, which was 28% higher than the midparent value (9.7%) and 18.2% higher than that of the higher protein parent IR 64 (10.5%).

Characterization of the Different Fractions of Seed Storage Proteins. The four different fractions of rice seed storage proteins, the albumins, globulins, prolamins, and glutelins (17, 25), were purified and subjected to SDS-PAGE analysis to determine the relative contribution of each protein fraction to the elevated total protein content observed in the hybrid (Figure 2). The SDS-PAGE profiles clearly showed an increase in each of the four protein fractions in the hybrid compared to both parents. The extent of the increase, however, differed among the four protein components. The measurements of signal intensity per lane in the Coomassie-stained SDS-PAGE gels showed that the

IR 64 parent had slightly higher contents than *O. nivara* of the different protein fractions with the exception of the prolamins, in which IR 64 was up to 70% higher than *O. nivara* (Figure 2B). The densitometry measurements also showed that the prolamins had the highest increase among the four protein fractions in the hybrid (Figure 2B). The glutelin fraction showed the second highest increase, followed by the globulin and albumin fractions.

Comparison of Ultrastructure of Seed Endosperm. An electron microscopy analysis of rice endosperm was carried out to evaluate the impact of the increased protein content in the hybrid on the protein body distribution in the endosperm cells. The overall ultrastructural features of endosperm cells in the three genotypes examined were similar and corresponded well with previous reports on the structure of rice seed endosperm (17, 19–21) (Figure 3). Two distinctive populations of protein bodies were visible. Protein bodies type I (PB-I), in which prolamins are deposited, are spherical and surrounded by a membrane of endoplasmic reticulum (ER) origin. Protein bodies type II (PB-II), in which glutelins and globulins are deposited (21), are irregularly shaped. Microscopic examination revealed a distinctive difference in the distribution of PB-I and PB-II in the endosperm cells of the parents and the hybrid. The endosperm cells of the hybrid showed higher numbers of the prolamin-containing PB-I compared to the parents (Figure 3C,D).

Differences in Amino Acid Content between Parents and Hybrid. A comparison of the seed amino acid profiles of the parents and the hybrid was carried out to evaluate the quality of the increased seed proteins. The two parents differed statistically in 8 of the 17 amino acids examined (Table 1). The most pronounced differences between the parents were in lysine, where *O. nivara* was 17.8% higher than IR 64, and glutamic acid, where IR 64 was 9.3% higher. The glutamic acid content in the hybrid was similar to that of IR 64, whereas the threonine content was lower than those of the parents. Similarly, the sulfur-containing amino acids, cysteine and methionine, were lower in the hybrid (Table 1). The hybrid showed an increase in arginine over the parents, which had similar contents of arginine. The hybrid was also higher in leucine, isoleucine, and phenylalanine when compared to IR 64.

Molecular Variation in the Genes Encoding Seed Storage Proteins. Southern hybridization was carried out for the genes encoding the major storage proteins, the glutelins, prolamins and globulin, to examine the level of divergence at the DNA level for these genes among the parents and the hybrid. The probes for the glutelin and prolamin genes showed hybridization to numerous restriction fragments in each genotype, whereas the probe for the globulin showed hybridization to a single fragment (Figure 4). Such hybridization patterns are expected as the glutelin and prolamin proteins are known to be encoded by multigene families, whereas the globulin is encoded by single gene (25). Although the majority of the restriction fragments that hybridized to each of the glutelin and prolamin probes were common to both parents, several restriction fragments were polymorphic between the two parents and clearly distinguished them from one another (Figure 4). The single restriction fragment that hybridized to the globulin probe was also polymorphic between the two parents. The hybridization profiles of the hybrid for each of the three examined genes showed the combination of all the hybridized fragments, both the shared and the polymorphic, in the two parents, which confirmed its hybridity. No additional or novel restriction fragments were detected in the hybridization profiles of the hybrid compared to those of the parents for any of the three genes.

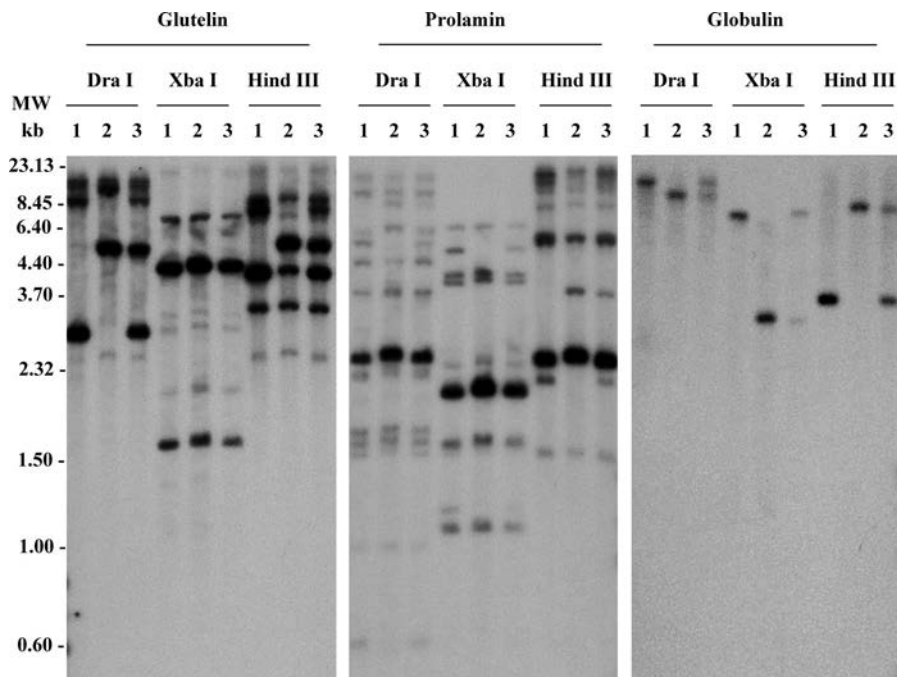


Figure 4. Southern blot analysis of seed storage protein genes of parental lines IR 64 (lane 1) and *O. nivara* (lane 2) and their hybrid (lane 3). Total genomic DNA from leaf tissue was digested with the restriction enzymes *Dra* I, *Xba* I, and *Hind* III, separated by electrophoresis on a 0.8% agarose gels, and then transferred to nylon membranes. Blots were hybridized with ^{32}P -labeled DNA probes corresponding to the glutelin, prolamin, and globulin genes and then exposed to X-ray films. MW, molecular weight (mass) marker in kilobase pairs.

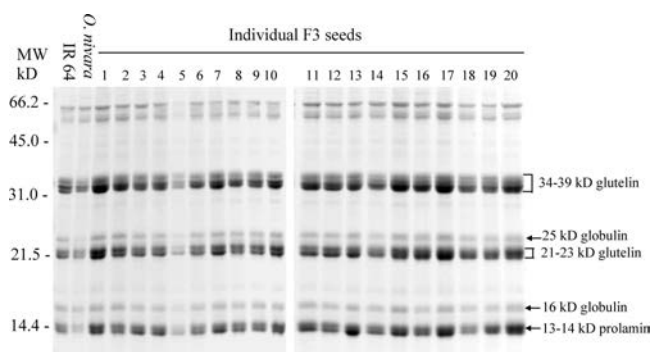


Figure 5. SDS-PAGE analysis of total seed proteins from parental lines and individual seeds of F_2 plants. Total seed proteins were extracted on an equal seed weight basis, fractionated by SDS-PAGE, and stained with Coomassie Blue. Major seed storage protein polypeptides that were subjected to densitometry analysis (see text) are indicated. MW, molecular weight (mass) marker in kilodaltons.

Inheritance of Increased Seed Protein Content. Segregation analysis of seed proteins in a total of 58 individual F_2 seeds was carried out to determine the nature of the genetic control of the increased protein content in the hybrid. SDS-PAGE analysis of seed proteins of individual F_2 seeds clearly showed segregation in their protein content, with a high proportion of seeds showing higher protein content than both parents (**Figure 5**). The SDS-PAGE protein profiles were transformed into quantitative measurements through quantification of signal intensities over the entire lane (total protein) and of four of the five major seed storage protein polypeptides. The signal intensity data are summarized in **Table 2**. As expected, the parental line IR 64 was higher than *O. nivara* in total protein content as well as individual major storage protein polypeptides except for the 16 kDa globulin polypeptide, which had similar levels of accumulation in both parents. The F_2 mean was higher than both parents in total protein content and the contents of the individual storage protein polypeptides (**Table 2**). The high F_2

means relative to those of the parental line IR 64 supported the earlier conclusion that IR 64 may carry dominant favorable alleles in the majority of the genetic loci involved in controlling seed proteins. The mean of the F_2 also reflected the presence of heterosis, which might be the result of additional favorable alleles for high seed proteins contributed by the *O. nivara* parent and/or the epistatic interaction of the different alleles from both parents.

DISCUSSION

Rice is a staple food and a main source of calories as well as protein intake for nearly half of the world's population; hence, it has become the world's single most important food crop. The improvement of seed protein content of rice will have a significant positive impact on millions of poor and malnourished people in developing countries.

In the present study, we report an interspecific hybrid of rice that has the potential to produce new rice cultivars with a significant increase in their protein content. The interspecific hybrid was generated by crossing the *indica* group cultivar IR 64 and the wild species *O. nivara*. *O. nivara* carries the same basic genome as cultivated rice (AA genome) and shows enough genetic similarity to *O. sativa* to be considered as its potential immediate progenitor (14, 15). Therefore, the possibility of transferring the favorable alleles for high seed protein from *O. nivara* into rice cultivars with little or no linkage drag through selection in segregating generations should be attainable.

The increase in seed protein content in the hybrid was dependent on the rice genotype used to produce the hybrid. The relatively higher seed proteins in IR 64 compared to IR 72 and the significant increase in seed proteins in the hybrid involving IR 64 compared to that involving IR 72 indicated that, unlike IR 72, IR 64 carried favorable alleles for high seed proteins, which were also able to interact with the alleles from the *O. nivara* parent in a synergetic manner leading to higher seed proteins in their hybrid. Similar cases of other traits being

Table 2. Mean Densitometry Measurements ($\times 10^4$ Pixels) on SDS-PAGE Gels of Total and Major Seed Proteins of Parents and F₂ Individual Seeds

variable	parental means		F ₂ , mean \pm SD	F ₂ confidence intervals ^a	
	<i>O. nivara</i>	IR 64 ^b		LL	UL
total protein	35.39	46.49**	64.46 \pm 22.85	58.45	70.47
34–39 kDa glutelin	8.88	13.80*	17.82 \pm 5.94	16.26	19.38
21–23 kDa glutelin	3.79	6.21**	5.87 \pm 2.68	5.17	6.58
25 kDa globulin	0.60	1.17*	1.47 \pm 0.71	1.28	1.66
16 kDa globulin	1.23	1.41ns	1.27 \pm 0.44	1.15	1.39
13–14 kDa prolamin	1.93	5.48**	6.62 \pm 2.20	6.05	7.20

^a LL, lower limit; UL, upper limit. ^b *, ** = significant at $P = 0.05$ and 0.01 , respectively; ns = not significant at 0.05 probability level.

dependent on the genetic background of the rice parent used to produce the interspecific hybrid have been reported (26). The rice cultivar IR 64 is an internationally recognized, disease-resistant, and high-yielding cultivar with superior cooking qualities and relatively high protein content ($\sim 10\%$). It was released by IRRI in 1985, and it is still widely grown in the tropics. Therefore, the hybrid involving IR 64 offers a great potential for selecting new rice genotypes that combine high yield and high cooking quality with improved seed protein content.

Glutelins and prolamins are the two most abundant rice seed storage proteins (20, 25). Analysis of the storage protein fractions in the hybrid showed that the prolamins had the highest increase among the four different fractions (Figure 2). In previous studies comparing low- and high-protein rice cultivars, it was found that the major increase in protein in the high-protein cultivars was in the endosperm, where prolamins and glutelins accumulate in the highest amount, whereas only modest increases were found in the embryo and the aleurone layer, where globulins and albumins are concentrated. Similar trends in the accumulation of the different protein fractions as a result of higher seed protein have been also reported in other cereals (27). Bradbury et al. (28) also found that the high-protein cultivars showed the presence of a greater number of the spherical, prolamin-containing PB-I in their endosperm. In the present study, the hybrid also showed a significant increase in the population of PB-I compared to the parents. Therefore, it appears that the increase in rice seed proteins generally involves the preferential increase in the accumulation of the prolamins. The molecular mechanism behind such a preferential increase in the prolamins in high-protein rice genotypes is yet to be determined.

The results of the Southern hybridization showed a high level of conservation between the two parents in the DNA sequence of the genes encoding seed storage proteins, as indicated by the majority of the hybridized DNA fragments being present in both parents. Our observation supports the previously reported close genomic similarity between cultivated rice and *O. nivara* (14, 15). Previous studies have demonstrated that some mammalian and plant interspecific hybrids, including interspecific hybrids of rice, undergo genomic changes after their production as a result of the activation of dormant transposable elements (TE) and their insertion in new locations in the genome, leading to alteration of gene expression (29, 30). In the interspecific hybrid reported here, the Southern hybridization pattern of the hybrid did not show loss of any parental DNA fragment or gain of novel fragments. Therefore, the increased seed protein in the hybrid was not the result of chromosomal rearrangements or TE activation and insertion, at least in the chromosomal regions containing the storage protein genes.

The inheritance of the increased seed protein appeared to be quantitative in nature, indicating the involvement of more than two genes in its genetic control. The quantitative or polygenic inheritance of seed protein content has been well documented

in both varietal and interspecific hybrids of rice (12, 13, 31). The quantitative inheritance of seed protein content is a manifestation of the numerous factors affecting the expression and accumulation of seed proteins. As an endosperm trait, seed protein content is determined by the triploid endosperm nuclear genes, the cytoplasmic and maternal genes, and the genotype \times environment interaction (31). In addition, the spatial- and temporal-specific expression of seed storage protein genes is exerted through a complex interaction among a number of cis- and trans-acting regulatory elements (32). Analysis of the F₂ population showed a wide range of phenotypic variation for protein content, higher means than those of the parents, and a high proportion of transgressive segregation, indicating the contribution of favorable alleles for high protein content by both parents and/or epistatic interaction between the different alleles from both parents. Recent studies have shown that the agronomically inferior wild relatives of rice such as the wild species *O. rufipogon* Griff., which is very closely related to *O. nivara*, could actually contribute favorable alleles for the improvement of yield and yield-related traits in rice (26, 33, 34). In the present study, the *O. nivara* parent, which was significantly lower than IR 64 in protein content, has also shown the potential of contributing favorable alleles for the improvement of protein content in rice. Therefore, the interspecific hybrid reported here demonstrates the potential of wild relatives of rice in general, and *O. nivara* in particular, for the improvement of seed protein content in rice. This interspecific hybrid could serve as the initial breeding material for the selection of new rice genotypes that combine the high yield potential and superior cooking quality of IR 64 with those of high seed protein or higher lysine content.

ACKNOWLEDGMENT

We thank Dr. R. Chandrababu for his critical reading of the manuscript and valuable suggestions.

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Received for review June 15, 2007. Revised manuscript received November 10, 2007. Accepted November 20, 2007. Names are necessary to report factually on available data; however, neither the University of Missouri nor USDA guarantees or warrants the standard of product, and the use of the name by the University of Missouri or USDA implies no approval of the product to the exclusion of others that may be suitable.

JF071776N