Endosperm Mutants in Rice: Gene Expression in Japonica and Indica Backgrounds

R. P. KAUSHIK and G. S. KHUSH[1]

Cereal Chem. 68(5):487–491

ABSTRACT

Dull, sugary, shrunken, floury, white-core, and amylose extender endosperm mutants induced in japonica rice varieties were transferred to IR36, an indica variety, after two backcrosses. Differential pollen staining was used to select heterozygotes among low-amylose (dull) mutants and the high-amylose recurrent parent (IR36). Segregation distortion was observed in some mutants during gene transfer from japonica to IR36. Phenotypic expressions of mutant genes in IR36 and japonica background were almost similar. However, shrunken-1, which showed partly chalky and partly translucent endosperm in japonica, was completely chalky in indica background. Floury-2 showed variation in phenotype, depending on the degree of distribution of the translucent fraction. Scanning electron photographs showed no starch granules in the endosperm cells of sugary mutants. All the floury-1 and white-core mutants had intermediate amylose (20–25%) in the IR36 background. Floury-2, on the other hand, produced low amylose in both backgrounds. In general, the amylose content of dull mutants was slightly higher in IR36 background. The amylose extender gene increased the amylose content of IR36 from 25% to as high as 40%, an increase of about 60%.

A number of loci at which mutations affect the quality and quantity of starch synthesized are known in maize. These loci are amylose extender (*ae*), brittle-1 (*bt*-1) and brittle-2 (*bt*-2); shrunken-1 (*sh*-1) and shrunken-2 (*sh*-2); sugary-1 (*su*-1) and sugary-2 (*su*-2); and dull (*du*), and waxy (*wx*) (Creech 1965, Shannon and Creech 1973, Boyer and Shannon 1983). Recently, similar endosperm mutations (except brittle mutants) were induced in japonica rice varieties with the use of chemical mutagens and radiations (Satoh and Omura 1981, Okuno et al 1983, Yano et al 1985, Rutger et al 1986). Such mutations must occur in nature but at very low frequency. Because of selective disadvantage, such mutants are lost in nature. These mutations, which directly influence the starch biosynthesis and alter the relative proportions of amylose to amylopectin, have a direct bearing on the cooking, eating, and processing quality of a variety. The mutants can serve as useful genetic markers and may be used in developing high-yielding, semidwarf indica varieties with great diversity in amylose content. Rice varieties are generally divided into indica and japonica groups. Indicas are most widely grown in tropics and subtropics and are planted in approximately 90% of the world's rice-growing area. Japonica varieties tolerate cold and are planted in temperate regions. This study was undertaken to transfer these endosperm mutant genes from japonica to an indica background and to compare their expression in the two backgrounds.

MATERIALS AND METHODS

The endosperm mutants used in the study are listed in Table I. The seeds of mutants were obtained from K. Okuno and H. Satoh, Kyushu University, Fukuoka, Japan; seeds of the opaque mutant were from J. Neil Rutger, University of California, Davis. These mutants were induced in Kinmaze by chemical mutagen *N*-methyl *N*-nitrosoinrea (MNU), in Sasanishiki by ethyl methane sulfonate (EMS), and in Norin 8 by ^{32p} β rays (Table I). These three varieties are japonicas, as is ESD7-3, from which the opaque mutant was selected.

The sugary mutants are characterized by wrinkled and translucent endosperm; shrunken mutants have wrinkled and chalky white endosperm. The floury mutants and the opaque mutant have soft white endosperm that breaks easily into a fine powder. In fact, the recessive gene of opaque mutant ESD7-3(0) is allelic to floury gene *fl*-1 (Kaushik and Khush 1987). The endosperm appearance of the amylose extender mutants ranges from completely vitreous grains to floury white grains. The white-core mutants have a central white portion consisting mainly of loosely packed starch. The endosperm of dull mutants is between waxy and translucent and is compactly packed and hard.

To transfer these mutants to an indica background, we crossed them as females to improved indica variety IR36. Two backcrosses were made with IR36 as the recurrent parent. Backcross 2 F_1 plants were dehulled by hand to avoid damaging the embryo. BC_2F_2 seeds with mutant phenotype were grown to recover the mutants in homozygous state.

The dull mutants have low amylose content, and the pollen of low-amylose mutants stains reddish brown with diluted iodine solution (0.05% I_2 in 2% KI), compared with the blue-black staining of pollen of high-amylose varieties. Thus, the heterozygous plants could be identified by the reddish brown and blue-black staining pollen in about equal proportions. The heterozygous plants identified in this way were used to make the second backcross. Heterozygous BC_2F_1 plants and plants homozygous for dull mutation in BC_2F_2 were identified using this differential

TABLE I
Rice Endosperm Mutants Used in the Present Study

Mutant	Gene Symbol	Parent Variety	Mutagen Treatment
Sugary			
82 GF	*Sug*	Norin 8	32_P β-rays
EM5	*Sug*	Kinmaze	MNU[a]
Shrunken			
EM20	*Shr*-1	Kinmaze	MNU
Amylose extender			
EM16	*ae*	Kinmaze	MNU
2064	*ae*	Sasanishiki	EMS
Floury			
EM17	*fl*-1	Kinmaze	MNU
EM28	*fl*-1	Kinmaze	MNU
Em36	*fl*-2	Kinmaze	MNU
2047	*fl*-1	Sasanishiki	EMS
Opaque			
ESD7-3(o)	*o*	ESD7-3	...
White-core			
EM3	...	Kinmaze	MNU
EM24	...	Kinmaze	MNU
EM66	...	Kinmaze	MNU
Dull			
2035	...	Sasanishiki	EMS
2057	...	Sasanishiki	EMS
2077	...	Sasanishiki	EMS
2078	...	Sasanishiki	EMS
2091	...	Sasanishiki	EMS
2120	...	Sasanishiki	EMS
EM12	*du*-1	Kinmaze	MNU
EM15	*du*-2	Kinmaze	MNU
EM47	...	Kinmaze	MNU

[a] MNU = *N*-methyl *N*-nitrosourea, EMS = ethyl methane sulphonate.

[1]Postdoctoral scientist and principal scientist-head, Division of Plant Breeding, Genetics, and Biochemistry, International Rice Research Institute, Box 933, Manila, Philippines.

staining procedure. However, at all stages, the selfed seeds of each heheterozygous plant were also harvested separately and dehulled to confirm segregation for mutant phenotypes.

The mutants in indica background obtained this way were grown, along with the japonica mutants and the parents, during the 1987 dry and wet seasons at the International Rice Research Institute, Los Baños, Philippines. After threshing, the seeds were stored in an air-conditioned laboratory before analysis to stabilize the moisture content of the seeds. Ten to twenty single grains of each were analyzed for amylose content on an autoanalyzer (Technicon) according to the procedures of Juliano (1971). Transverse sections of the mutants in IR36 background were observed under a scanning electron microscope according to the procedure described by Postek et al (1980).

RESULTS AND DISCUSSION

Segregation Distortion During Gene Transfer

After two backcrosses to IR36, the BC_2F_1 plants were selfed to recover the mutants. The segregation data of BC_2F_2 seed for three white-core mutants and six dull mutants (Table II) revealed a significant shortfall in the mutant phenotype expected on monogenic recessive inheritance of the mutants. Recovery of the two white-core mutants (EM3 and EM24) and four dull mutants (2057, 2077, 2078, and 2091) was less than 15%; that of one white-core mutant (EM66) and two dull mutants (2035 and 2120) was 15–20% (expected frequency, 25%). However, dull mutants EM12 and EM47, shrunken mutant EM20, and floury mutants 2047, EM17, EM28, ESD7-3(o), and EM36 segregated as expected (Table III).

Gametophytic genes are reportedly responsible for differential pollen fertilization resulting in distorted segregation in F_2 of crosses between distantly related rice varieties. Nakagahra (1986) reported segregation distortion for the *wx* marker on chromosome 6 in indica-japonica crosses. The extent of skewness in segregation is expressed by the tightness of the linkage between the marker and the gametophytic gene. Ten gametophytic abortion genes (*ga*-1–10) have also been reported in indica-japonica crosses (Sato et al 1987).

TABLE II
Segregation Ratios and χ^2 Values for F_2 Seed Produced on BC_2F_1 Heterozygous Plants for Some Dull and White-Core Mutants

Cross	Plant	Segregation			χ^2	Mutant
		Normal	Mutant	Total	(3:1)	Frequency
Dull						
2035/IR36[3]	1	415	101	516	8.1[a]	19.6
	2	249	42	291	17.3	14.4
2057/IR36[3]	1	675	70	745	96.7	9.4
	2	161	25	186	13.2	13.4
2077/IR36[3]	1	308	33	341	42.6	9.7
	2	114	12	126	16.0	9.5
2078/IR36[3]	1	733	70	803	113.5	8.7
2091/IR36[3]	1	980	141	1,121	92.2	12.6
	2	651	82	733	74.5	11.2
2120/IR36[3]	1	275	57	332	10.8	17.2
	2	648	121	769	35.2	15.7
White-core						
EM 3/IR36[3]	1	548	65	613	67.7	10.6
	2	447	41	488	71.7	8.4
EM 24/IR36[3]	1	595	94	689	47.4	13.6
	2	558	61	619	75.7	9.9
EM 66/IR36[3]	1	523	124	647	11.8	19.2
	2	461	110	571	10.0	19.3

[a] All values in this column significant at 0.01 level.

TABLE III
Normal Segregation for Shrunken, Floury, and Dull Mutants on Heterozygous BC_2F_1 Plants

Cross	Segregation				χ^2
			Mutant		
	Total	Normal	No.	%	(3:1)
Shrunken					
EM20/IR36[3]	1,023	776	247	24.1	0.4
Floury					
2047/IR36[3]	440	347	93	21.1	3.5
EM17/IR36[3]	1,254	925	329	26.2	1.0
EM28/IR36[3]	897	660	237	26.4	1.0
ESD7-3(o)/IR 36[3]	426	316	110	25.8	0.2
EM36/IR36[3]	590	466	124	21.0	5.0[a]
Dull					
EM12/IR36[3]	404	314	90	22.2	1.6
EM47/IR36[3]	486	354	132	27.1	1.2

[a] Significant at 0.05 level.

Gene Expression in Japonica and Indica Background

IR36 and the japonica varieties in which mutants were induced have translucent endosperm. Phenotypes of mutants in IR36 background are compared with japonica counterparts in Figure 1. Shrunken mutant EM20, which showed partly chalky and partly translucent endosperm in japonica background, was completely chalky in indica background. Similarly, both sugary mutants were slightly more chalky in indica background (Fig. 1a).

The white-core mutants exhibited a chalky portion in the center of grains enclosed by a thick translucent region (Fig. 2b). Floury-1 mutants had a chalky appearance in both backgrounds (Fig. 2c). We observed a variation in *fl*-2 phenotype of EM36 mutant during transfer of the *fl*-2 gene to IR36 and also during seed multiplication of EM36, depending on the degree of distribution of the translucent fraction in the endosperm of floury-2 grains (Fig. 1d). The amount of translucency varied from a very small fraction on the top of the grain to almost normal with only a small floury portion. A similar variation in the phenotype of opaque-2 maize reported by Vasal (1972) and others has been attributed to modifier genes.

The phenotype of the amylose extender mutants varied from chalky to translucent in both backgrounds (Fig. 1e). The endo-

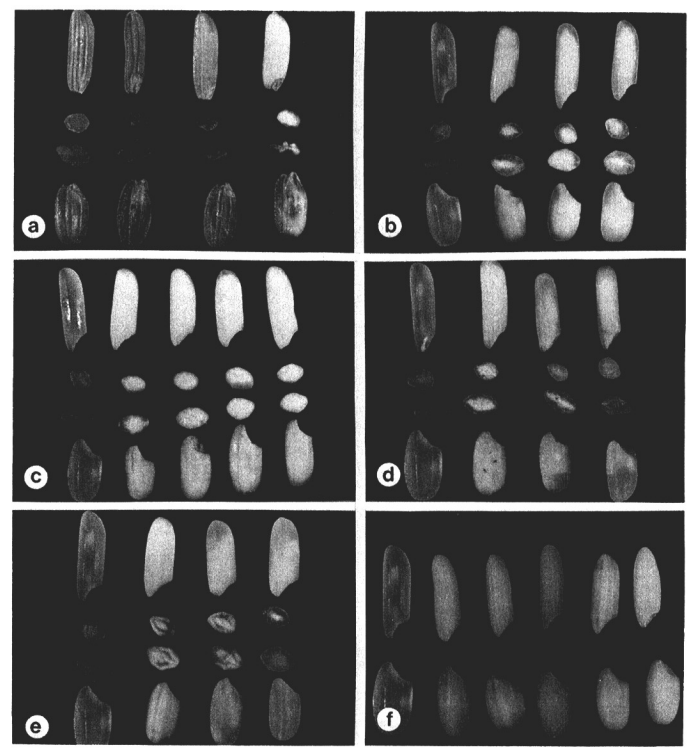

Fig. 1. Milled grains of indica (IR36) and japonica varieties and endosperm mutants. Upper row, indica background; middle, transverse sections; lower, japonica. The following descriptions are from left to right. **a,** normal, sugary 82GF, sugary EM5, shrunken EM20; **b,** normal, white-core mutant EM3, EM24, EM66; **c,** normal, floury mutants 2047, EM17, EM28, ESD7-3-(o); **d,** normal, different grains of floury mutant EM36; **e,** normal, different grains of amylose extender mutant 2064; **f,** normal, dull mutants 2035, 2057, 2077, EM12, EM47.

sperm of dull mutant 2077 was close to translucent in both backgrounds, whereas five other nonallelic dull mutants appeared close to waxy (Fig. 1f).

Scanning electron microscope photographs of the transverse sections of endosperm of the mutants in IR36 background showed distinct differences in the shape and packing of the starch granules and endosperm cells (Fig. 2). Starch in the endosperm cells of mature brown rice of IR36 consisted of compound polyhedral, tightly packed starch granules (Fig. 2a). Sugary mutant 82GF showed narrow rectangular endosperm cells in the central portion; the cells were squarish towards the periphery, and all the cells were devoid of starch granules (Fig. 2b). The other sugary mutant, EM5, showed loosely packed round starch granules (Fig. 2c). The endosperm cells of shrunken mutant (EM20) were filled with small, round, loosely packed starch granules (Fig. 2d).

In floury mutant ESD7-3(o), the compound starch granules were round and loosely packed (Fig. 2e), similar to the starch packing of crumbly rice reported by Evers and Juliano (1976). The central white portion of the white-core mutants consisted of loosely packed round starch granules (Fig. 2f), whereas the peripheral translucent portion showed tightly packed polyhedral starch granules (Fig. 2g). The starch granules in (EM36), a floury mutant, showed packing similar to that in white-core mutants.

Endosperm cells of the amylose extender mutant contained a large number of irregularly shaped starch granules (Fig. 2h) similar to those reported in the japonica amylose extender by Yano et al (1985) and in maize by Boyer et al (1976). However, the endosperm cells with loosely packed and comparatively tightly packed starch granules were interspersed in the chalky and translucent grains of the amylose extender mutant.

Starch granules of the dull mutants were polyhedral and tightly packed, like those of IR36, and had small cavities on the starch granules (Fig. 2i,j) similar to those reported in waxy rices by Utsunomiya et al (1975). However, the dull mutants had fewer such cavities than did the waxy mutants.

Amylose Content of Mutants

The amylose content of japonica and indica mutants during the 1987 dry and wet seasons are listed in Tables IV–VI. The endosperm starch of japonica cultivars Norin 8, Sasanishiki, and Kinmaze contains, on average, 13–15% amylose (range, 10–17%), whereas indica cultivar IR36 has an average amylose content of 24–25% (range, 22–27%).

The sugary mutant 82GF had no measurable amylose content in japonica background during either season (Table IV). In IR36 background, it had about 2.9% amylose during the dry season but none in the wet season. This corresponds with the scanning electron microscope results, which also showed no starch granules in this mutant. According to Zimmerman (1960), the main transport material in higher plants is sucrose, the first free sugar after photosynthesis. Bell et al (1983) reported that the reason for the high amount of sucrose in the *su*-2 endosperm in maize appears to be "a genetic block that occurs early in the synthesis of starch. Thus, very little of the sucrose translocated into the kernel after pollination is converted to starch or any of its precursors, giving rise to a kernel packed with sucrose and a very sweet taste." This seems to be the case with sugary mutant 82GF also.

Sugary mutant EM5 had 5–6% amylose in japonica background and 12.9% in indica in the wet 1987 season. Amylose content of shrunken mutant (EM20) was 8–9% in japonica and 18–19% in indica background.

The amylose content of three white-core mutants (Table V) was 10–15% in japonica but 23–24% in indica background. Similarly, the amylose content of floury mutants 2047, EM17, and EM28, all of which have the *fl*-1 gene (Kaushik and Khush 1987), was 10–13% in japonica and 19–23% in IR36 background (Table V). ESD7-3(o) had 6–9% amylose in parental background but increased to 22–24% when transferred to IR36. Like floury mutants 2047, EM17, and EM28, the floury appearance of ESD7-3(o) is conditioned by the *fl*-1 gene (Kaushik and Khush 1987). The amylose content of floury mutant EM36, which has the *fl*-2 gene, was 5–6% in japonica and 9–11% in IR36.

The amylose content of the amylose extender mutants 2064 and EM16 was 26% and 30–32%, respectively (Table IV), about twice as high as that of the nonmutant japonica counterparts. The amylose content of mutant 2064 in IR36 increased to 39–41% (range, 34–42%), or about 60%–the first report of a rice endosperm amylose level this high. Juliano (1979) observed varietal differences in the amylose content of rice endosperm starch. High amylose mutants have occurred in maize (Vineyard and Bear 1952), barley (Walker and Merritt 1969), and pea (Hilbert and MacMasters 1946). The amylose extender mutant of maize contains about 60% amylose content.

The amylose content of dull mutants varied among the mutant

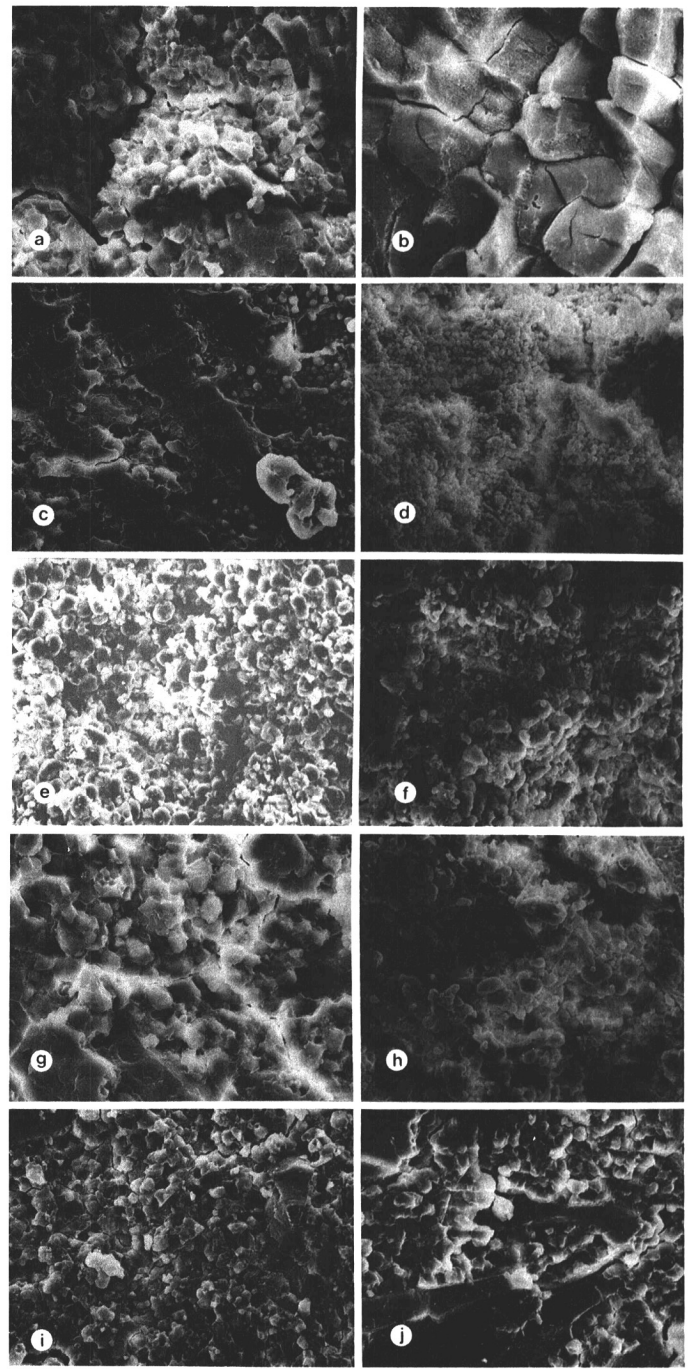

Fig. 2. Scanning electron microscopic photographs of transverse sections of IR36 and endosperm mutants in IR36 background (503X). **a,** IR36; **b,** sugary 82GF; **c,** sugary EM5; **d,** shrunken EM20; **e,** floury ESD7-3(o); **f,** white-core EM66 (central floury portion); **g,** white-core EM66 (peripheral translucent section); **h,** amylose extender 2064; **i,** dull 2035; **j,** dull EM47.

lines (Table VI). In Sasanishiki mutants it ranged from 1.9% in mutant 2091 to 6.2% in mutant 2078. In IR36 background, it ranged from about 2% in mutant 2057 to 11% in mutant 2078. Dull mutant 2035 of Norin 8 had 4–5% amylose in japonica background; its amylose content in IR36 was 3.0 and 1.9% in the dry and wet seasons, respectively. The amylose content of Kinmaze dull mutants varied from 0.7% in EM15 to 2.9% in EM12. In IR36 it ranged from 1.3% in EM47 to 8.5% in EM15. In general, the amylose content of dull mutants was higher in IR36 background.

The availability of various mutants with modified endosperm carbohydrates in japonica and indica backgrounds opens up possibilities for studying carbohydrate metabolism and starch biosynthesis. Considerable insight into the complex processes of starch biosynthesis can be gained through in vivo and in vitro studies of normal and mutant developing grains. These materials should prove useful to molecular geneticists for obtaining valuable information on gene action in biological processes. Moreover, these mutants would be useful markers for preparing the saturated linkage map of rice.

In maize, waxy and amylose extender mutants are commercially grown for use in industrial processes that require amylopectin and high-amylose starches, respectively. Possibilities of such industrial uses for rice starch should be explored.

Most of the improved indica varieties have intermediate or high amylose content. Varieties with very low amylose content or with waxy or dull endosperms are a staple food of people in northern and northeastern Thailand and Laos. They are also preferred in parts of Burma and are used for preparing specialty foods in other countries. Some of the mutants with very low amylose content may be useful in developing high-yielding semidwarf indica varieties.

TABLE IV
Amylose Content (%) of Sugary, Shrunken, and Amylose Extender Japonica Mutants and Their Parents and After Backcrossing to IR36

	Japonica Background, 1987 Seasons			IR36 (Two Backcrosses), 1987 Seasons		
Mutant	Dry (Mean ± SE)	Wet (Mean ± SE)	Mean	Dry (Mean ± SE)	Wet (Mean ± SE)	Mean
Sugary						
82GF	0.0 ± 0.0	0.0 ± 0.0	0.0	2.9 ± 0.3 (1.8 - 3.6)[a]	0.0 ± 0.0	1.4
EM5	5.9 ± 0.3 (4.0 - 8.0)	6.2 ± 0.5 (4.2 - 9.6)	6.0	...	12.9 ± 0.5 (10.8 - 15.8)	12.9
Shrunken						
EM20	8.7 ± 0.2 (8.2 - 9.8)	9.8 ± 0.4 (8.4 - 11.0)	9.3	18.9 ± 0.3 (17.4 - 21.8)	18.4 ± 0.2 (17.0 - 18.8)	18.7
Amylose extender						
2064	26.2 ± 0.2 (24.4 - 28.4)	26.3 ± 0.6 (24.4 - 28.8)	26.2	38.6 ± 0.4 (38.4 ± 39.8)	40.7 ± 0.4 (38.0 - 41.8)	39.4
EM16	30.9 ± 0.2 (29.2 - 33.0)	32.0 ± 0.6 (28.2 - 33.6)	31.3	... ...	41.1 ± 0.5 (38.0 - 43.8)	41.1
Parents						
Norin 8	14.0 ± 0.3 (11.6 - 16.4)	13.5 ± 0.3 (12.0 - 15.5)	13.8	...	...	...
Sasanishiki	15.1 ± 0.2 (12.6 - 16.6)	12.6 ± 0.5 (10.2 - 16.2)	14.2	...	...	...
Kinmaze	11.7 ± 0.3 (10.0 - 13.6)	14.9 ± 0.5 (12.2 - 16.2)	12.9	...	...	...
IR36	...	...	...	25.8 ± 0.3 (23.4 - 27.4)	24.2 ± 0.3 (22.6 - 25.6)	25.3 ...

[a] Numbers in parentheses are ranges.

TABLE V
Amylose Content (%) of Floury and White-Core Mutants in Japonica and IR36 Background

	Japonica Background, 1987 Seasons			IR36 (Two Backcrosses), 1987 Seasons		
Mutant	Dry (Mean ± SE)	Wet (Mean ± SE)	Mean	Dry (Mean ± SE)	Wet (Mean ± SE)	Mean
Floury						
2047	11.8 ± 0.3 (9.0 - 13.8)[a]	12.7 ± 0.5 (11.2 - 15.6)	12.1	21.3 ± 0.2 (19.0 - 22.6)	19.9 ± 0.5 (16.4 - 22.6)	20.8
EM17	11.3 ± 0.4 (8.2 - 14.0)	12.2 ± 0.3 (10.2 - 13.2)	11.6	22.1 ± 0.3 (19.6 - 23.8)	21.5 ± 0.4 (19.8 - 23.4)	21.9
EM28	9.8 ± 0.2 (8.2 - 11.2)	11.6 ± 0.5 (8.0 - 13.0)	10.4	22.8 ± 0.3 (19.6 - 24.4)	19.2 ± 0.6 (15.6 - 22.0)	21.6
ESD7-3(o)	6.3 ± 0.2 (4.2 - 8.6)	8.7 ± 0.3 (7.2 - 10.6)	7.1	22.5 ± 0.2 (19.8 - 24.0)	24.1 ± 0.3 (22.8 - 26.0)	23.1
EM36	5.1 ± 0.3 (3.4 - 9.0)	6.4 ± 0.7 (4.0 - 12.0)	5.6	10.9 ± 0.3 (8.4 - 13.0)	9.7 ± 0.6 (6.6 - 12.4)	10.5
White-core						
EM3	11.0 ± 0.4 (7.0 - 13.0)	14.5 ± 0.5 (10.4 - 16.2)	12.2	...	23.8 ± 0.3 (23.0 - 25.0)	23.8
EM24	10.4 ± 0.4 (6.4 - 12.0)	12.8 ± 0.5 (9.4 - 14.6)	11.3	...	22.8 ± 0.4 (21.2 - 24.6)	22.8
EM66	10.4 ± 0.4 (6.6 - 13.8)	11.0 ± 0.4 (9.6 - 12.8)	10.6	...	24.1 ± 0.5 (21.2 - 26.4)	24.1

[a] Numbers in parentheses are ranges.

TABLE VI
Amylose Content (%) of Very Low-Amylose (Dull) Mutants in Japonica and IR36 Background

Mutant	Japonica Background, 1987 Seasons			IR36 (Two Backcrosses), 1987 Seasons		
	Dry (Mean ± SE)	Wet (Mean ± SE)	Mean	Dry (Mean ± SE)	Wet (Mean ± SE)	Mean
Dull Mutants						
2035	4.5 ± 0.2 (3.2 - 6.0)[a]	4.7 ± 0.6 (2.8 - 8.0)	4.6	3.0 ± 0.2 (2.0 - 4.4)	1.9 ± 0.4 (1.0 - 4.6)	2.6
2057	2.4 ± 0.1 (1.4 - 3.4)	2.0 ± 0.2 (1.2 - 2.8)	2.3	2.6 ± 0.1 (1.8 - 3.4)	2.0 ± 0.4 (0.0 - 4.0)	2.4
2077	5.9 ± 0.2 (4.2 - 7.6)	5.8 ± 0.4 (3.2 - 7.4)	5.9	8.6 ± 0.3 (7.2 - 11.2)	7.3 ± 0.4 (4.8 - 9.0)	8.2
2078	6.2 ± 0.2 (5.0 - 8.0)	5.8 ± 0.4 (3.8 - 7.6)	6.1	11.0 ± 0.2 (9.2 - 12.8)	10.3 ± 0.5 (7.6 - 12.4)	10.8
2091	3.5 ± 0.1 (3.2 - 4.4)	1.9 ± 0.2 (0.6 - 2.4)	2.7	5.2 ± 0.2 (3.6 - 7.2)	7.1 ± 0.2 (6.4 - 8.2)	5.8
2120	5.1 ± 0.2 (3.6 - 6.4)	4.6 ± 0.3 (2.0 - 5.8)	4.9	5.3 ± 0.2 (3.8 ± 8.2)	7.7 ± 0.4 (6.2 - 10.2)	6.1
EM12	2.9 ± 0.1 (1.6 - 4.0)	2.1 ± 0.2 (0.8 - 3.0)	2.6	5.3 ± 0.2 (3.6 - 6.4)	3.5 ± 0.3 (1.8 - 5.4)	4.7
EM15	0.7 ± 0.1 (0.0 - 2.4)	1.9 ± 0.2 (1.0 - 2.6)	1.1	6.7 ± 0.4 (3.0 - 9.2)	8.5 ± 0.4 (5.8 - 10.0)	7.3
EM47	1.6 ± 0.2 (0.0 - 3.0)	2.4 ± 0.2 (1.0 - 3.4)	1.9	2.4 ± 0.2 (1.6 ± 3.8)	1.3 ± 0.2 (0.2 - 2.0)	2.0

[a] Numbers in parentheses are ranges.

ACKNOWLEDGMENTS

We thank H. Satoh and K. Okuno, Kyushu University, Fukuoka, Japan, and J. N. Rutger, University of California, Davis, for providing the seeds of the mutants used in the study; Normita dela Cruz, Rice Quality Laboratory, IRRI, for help in chemical analysis; H. Hibino, Plant Pathology, IRRI, for help in scanning electron microscope photography; and B. Juliano of Cereal Chemistry, IRRI, for valuable suggestions.

LITERATURE CITED

BELL, R. D., DARRAH, L. L., and ZUBER, M. S. 1983. Progress from mass selection for field emergence and seed weight in a *sh*-2 population of maize. Crop Sci. 23:461-464.

BOYER, C. D., and SHANNON, J. C. 1983. The use of endosperm genes for sweet corn improvement. Pages 139-161 in: Plant Breeding Reviews. Vol 1. J. Janick, ed. AVI Publishing Co., Inc.: Westport, CT.

BOYER, C. D., DANIELS, R. R., and SHANNON, J. C. 1976. Abnormal starch granules formation in *Zea mays* L. endosperms possessing the amylose-extender mutant. Crop Sci. 16:298-301.

CREECH, R. G. 1965. Genetic control of carbohydrate synthesis in maize endosperm. Genetics 52:1175-1186.

EVERS, A. D., and JULIANO, B. O. 1976. Varietal differences in surface ultrastructure of endosperm cells and starch granules of rice. Starch/Staerke 28:160-166.

HILBERT, G. E., and MacMASTERS, M. M. 1946. Pea starch, a starch of high amylose content. J. Biol. Chem. 162:229-238.

JULIANO, B. O. 1971. A simplified assay for milled rice amylose. Cereal Sci. Today 16:334-338,340,360.

JULIANO, B. O. 1979. The chemical basis of rice grain quality. Pages 69-90 in: Proc. of workshop on chemical aspects of rice grain quality. International Rice Research Institute, Los Banos, Philippines.

KAUSHIK, R. P., and KHUSH, G. S. 1987. Allelic relationships among floury and opaque endosperm mutants in rice. Rice Genet. Newsl. 4:81-82.

NAKAGAHRA, M. 1986. Geographic distribution of gametophyte genes in wide crosses of rice cultivars. Pages 73-82 in: Rice Genetics. International Rice Research Institute: Los Baños, Philippines.

OKUNO, K., FUWA, H., and YANO, M. 1983. A new mutant gene lowering amylose content in endosperm starch of rice. Jpn. J. Breed. 33:387-394.

POSTEK, M. T., HOWARD, K. S., JOHNSON, A. H., and McMICHAL, K. L. 1980. Scanning electron microscopy. A student's handbook. Ladd Research Laboratories, Inc.: Burlington VT.

RUTGER, J. N., AZZINI, L. E., and BROOKHOUZEN, P. J. 1986. Inheritance of semi-dwarf and other useful mutant genes in rice, *Oryza sativa* L. Pages 261-271 in: Rice Genetics. International Rice Research Institute: Los Baños, Philippines.

SATO, Y., SANO, Y., and NAKAGAHRA, M. 1987. Gene symbols for gametic effect, sterility and weakness. Rice Genet. Newsl. 4:46-51.

SATOH, H., and OMURA, T. 1981. New endosperm mutations induced by chemical mutagens in rice, *Oryza sativa* L. Jpn. J. Breed. 31:316-326.

SHANNON, J. C., and CREECH, R. G. 1973. Genetics of storage polysaccharides in *Zea mays* L. Ann. N.Y. Acad. Sci. 210:279-289.

UTSUNOMIYA, H., YAMAGATA, M., and DOI, Y. 1975. Scanning electron microscopy of the endosperm of cereal crops. IV. Starch cell layer of imperfect grain of rice (non-glutinous) and glutinous rice. Yamaguchi Daigaku Nogakubu Gakujutsu Hokoku 26:19-44.

VASAL, S. K. 1972. Use of genetic modifiers to obtain normal-type kernels with the *opaque*-2 gene. Pages 197-203 in: CIMMYT-Purdue Intl. Symp. on Protein Quality in Maize. CIMMYT: El Batan, Mexico.

VINEYARD, M. L., and BEAR, R. P. 1952. Amylose content. Maize Genet. Coop. Newsl. 26:5.

WALKER, J. T., and MERRITT, N. R. 1969. Genetic control of abnormal starch granules and high amylose content in a mutant of glacier barley. Nature 221:482.

YANO, M., OKUNO, K., KAWAKAMI, J., and OMURA, T. 1985. High amylose mutants of rice, *Oryza sativa* L. Theor. Appl. Genet. 69:253-257.

ZIMMERMAN, M. H. 1960. Transport in phloem. Annu. Rev. Plant Physiol. 11:167-190.

[Received August 30, 1990. Accepted March 1, 1991.]