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# Genetics of amylose content in rice (*Oryza sativa* L)

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**Abstract.** Inheritance of amylose content was studied in crosses involving very low-, intermediate-, and high-amylose parents. The single-grain analysis of parents, $F_1$, $F_2$, $B_1F_1$, and $B_2F_1$ seed from a single-season harvest, showed that the parental mean difference of 14–17% in IR37307-8/BPI 121–407 or IR37307-8/IR24632–34 and about 20% in the cross IR37307–8/IR8 were controlled by a single gene with major effect, along with some minor genes and/or modifiers. The appearance of segregants inbetween the two parents was attributed to gene dosage effects in the endosperm. The results indicate that selection for amylose level can effectively be done in early segregating generations. Selection for intermediary segregants would be ineffective because the dosage effects would dissipate in further generations.

**Keywords.** *Oryza sativa*; amylose content; inheritance; dosage effect; endosperm; chemical composition; cooking quality.

## 1. Introduction

After yield, grain quality is one of the most important objectives of most rice breeding programs. Consumer acceptance of a variety depends primarily on its cooking quality. Varieties with desirable cooking qualities are at a premium in the market. Of the cooking quality components, amylose content is the most important because it determines cooked rice texture. Rice varieties with very low amylose content become very sticky, moist and tender on cooking, whereas varieties with intermediate amylose content become fluffy, soft, moist and tender, and those with high amylose content become fluffy and dry and harden on cooling. Different regions of the world prefer rice varieties with different cooking characteristics. Breeders often have to cross varieties of different amylose content to transfer some other desirable characters. Such crosses may yield segregants with poor grain quality. The inheritance of very low amylose content has not been investigated. The present work was undertaken to study the mode of inheritance of very low amylose content in crosses with intermediate- and high-amylose parents.

## 2. Materials and methods

One very low-amylose line, IR37307–8, was crossed with an intermediate-amylose variety BPI 121–407, an intermediate-amylose line IR24632–34 and one high-amylose variety IR8 (including reciprocal crosses) in the 1984 wet season (WS) (September–October). The parents and $F_1$ were grown in the 1985 dry season (DS) at the

International Rice Research Institute (IRRI), Los Baños, Philippines, to obtain $F_2$ seeds (seeds borne on $F_1$ plants). These $F_1$'s were crossed with both their parents to produce $B_1F_1$ and $B_2F_1$ seeds for all the crosses. A fresh set of $F_1$ seeds to these crosses was produced, also during DS '85. Thus, seeds of 3 parents, their 6 crosses ($F_1$), 6 $F_2$'s, and 12 backcrosses (BC) were produced in the same season to minimize seasonal effects. The dehulled grains of all the above parents, $F_1$'s, $F_2$'s, and BC's were milled in a test tube mill. The remnants of embryo from each grain were removed with a blade and the single-grains were ground in a wig-L-bug. Single-grain analysis for amylose content was done by taking 20 seeds from each parent and each $F_1$, about 400 seeds from each $F_2$ and 50–100 seeds from each backcross. The analysis was done on a Technicon autoanalyser (Juliano 1971).

## 3. Results

### 3.1 *Cross* 1 (*IR*37307–8/*BPI* 121–407)

The average amylose content in $F_1$ seeds (crossed seed) in cross IR37307–8/BPI 121–407 was 13·84% compared to 18·65% in the reciprocal cross. Taking amylose content of the intermediate-amylose parent as 100, its first dose has increased amylose in $F_1$ endosperm by about 26%. Two of its doses increased the amylose further by about 19% over the first dose.

On the basis of single-grain analysis, the amylose content of the $F_2$ seeds of cross IR37307–8/BPI 121–407 varied from 3 to 25%. On the basis of dosage effect, the segregants were classified into four categories, viz, $\leqslant 9\%$, 10–14%, 15–20%, and $> 20\%$ (table 1). There were 103 seeds in the $\leqslant 9\%$ category, 99 in the 10–14%, 107 in the 15–20%, and 91 in the $> 20\%$, with 0, 1, 2, and 3 doses of intermediate-amylose gene, respectively (table 1, figure 1a). This fits the expected 1:1:1:1 ratio ($X^2 = 1{\cdot}40$). If we broadly consider two categories, viz, very low and higher amylose containing seeds, the segregation pattern was 103 seeds having 3–9% amylose and 297 having 10–25% amylose in a 1:3 ratio ($X^2 = 0{\cdot}12$). The $B_1F_1$ seeds clearly segregated into two classes (table 1, figure 1b) with 22 seeds in category 1 (1–7%) and 21 seeds in category 2 (12–18%) in 1:1 segregation ($X^2 = 0{\cdot}02$). In $B_2F_1$ seeds, segregation into the two categories has also been observed because of dosage effects (table 1, figure 1c). Twenty-five seeds were observed in category 1 (9–17%) having 1 dose of intermediate-amylose gene and 24 seeds in category 2 (18–24%) having 3 doses of intermediate-amylose gene in 1:1 ratio ($X^2 = 0{\cdot}02$).

In the reciprocal cross, in the $F_2$ seed analyses also, 97 seeds with no dose of intermediate-amylose gene were observed in the category of 1–7% amylose. On the other hand, without considering dosage effects, 292 seeds with 1, 2, or 3 doses of intermediate-amylose gene were found to occur in the 8–25% amylose content category. On the basis of dosage effect, this class could be further divided into 3 sub-classes: those with 8–12%, 13–18%, and 19–25% amylose content, with 100, 100, and 92 seeds, respectively. This suggests a satisfactory fit to the 1:1:1:1 ($X^2 = 0{\cdot}44$) ratio. The $B_1F_1$ seeds and $B_2F_1$ seeds also showed a satisfactory fit to the 1:1 ratio confirming the above results (see table 1, figures 2a–c).

**Table 1.** Frequency distribution of the amylose content in cross IR37307-8/BPI 121–407 and its reciprocal.

| Population | | Amylose (%) Mean | Range |
|---|---|---|---|
| IR37307-8 | $(P_1)$ | 7·41 | 5–9 |
| BPI 121-407 | $(P_2)$ | 24·89 | 24–25 |
| $F_1$ | $(P_1/P_2)$ | 13·84 | 10–16 |
| $F_1$ | $(P_2/P_1)$ | 18·65 | 16–20 |

| Population | Amylose class (%) | Observed frequency | $X^2$ 1:3 | 1:1:1:1 | 1:1 |
|---|---|---|---|---|---|
| $F_2$ $(P_1/P_2)$ | 3–9 | 103 | 0·12 | — | — |
| | 10–14 | 99 ⎤ | | | |
| | 15–20 | 107 ⎥ 297 | — | 1·40 | — |
| | 21–25 | 91 ⎦ | | | |
| $B_1F_1$ $(P_1/P_2//P_1)$ | 1–7 | 22 | — | — | 0·02 |
| | 12–18 | 21 | | | |
| $B_2F_1$ $(P_1/P_2//P_2)$ | 9–17 | 25 | — | — | 0·02 |
| | 18–24 | 24 | | | |
| $F_2$ $(P_2/P_1)$ | 1–7 | 97 | 0·00 | — | — |
| | 8–12 | 100 ⎤ | | | |
| | 13–18 | 100 ⎥ 292 | — | 0·44 | — |
| | 19–25 | 92 ⎦ | | | |
| $B_1F_1(P_2/P_1//P_1)$ | 5–12 | 49 | — | — | 0·35 |
| | 13–24 | 35 | | | |
| $B_2F_1$ $(P_2/P_1//P_2)$ | 12–19 | 51 | — | — | 0·01 |
| | 20–28 | 50 | | | |

### 3.2 *Cross* 2 (*IR*37307–8/*IR*24632–34)

In the cross between a very low-amylose line IR37307–8 (7·41 %) and an intermediate-amylose line IR24632–34 (21·75 %), the amylose content in the $F_1$ endosperm was 17·45 %. The reciprocal $F_1$ seeds had, on the average, 15·10 % amylose. Although with the second dose of the intermediate amylose gene, the amylose content in the $F_1$ endosperm was lower than that with one dose, the difference was not significant, indicating only slight dosage effect (table 2).

In the analyses of $F_2$ single grains of cross IR37307–8/IR24632–34, amylose values ranged from 1 to 25 %. The seeds could be classified into 3 categories (table 2, figure 3a). Category 1 with 1–7 % amylose had 105 seeds while category 2 (8–19 %) (which encompasses seeds having 1 or 2 doses of gene for intermediate amylose content) had 197 seeds. Category 3 (20–25 %) having three doses of intermediate-amylose gene had 94

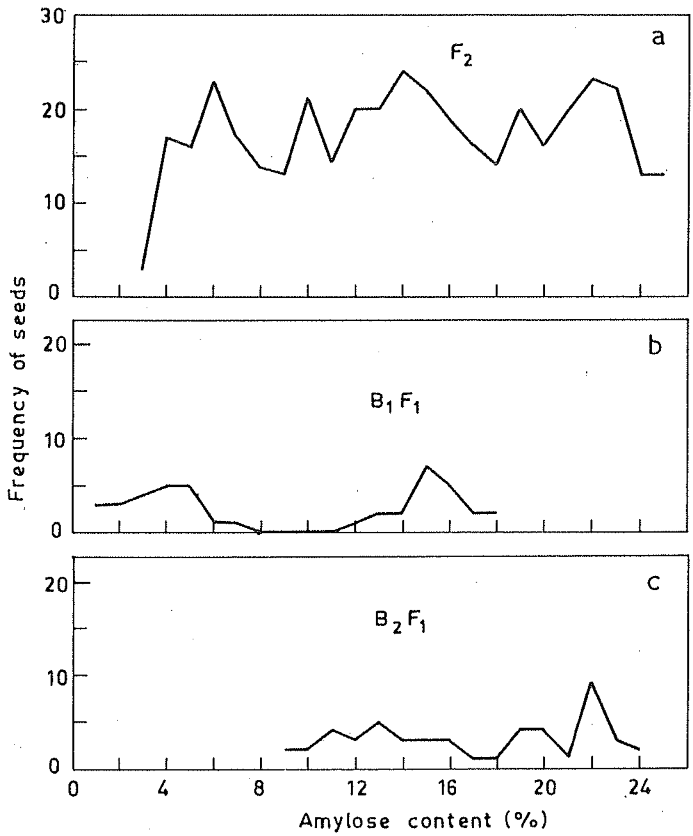


**Figure 1.** Frequency distribution of amylose content in cross IR37307–8/BPI 121–407.

seeds. This suggests a segregation of 1:2:1 ($X^2 = 0{\cdot}62$). In $B_1F_1$ seed analyses, 37 seeds with very low amylose (1–8 %) and 41 seeds with 13–22 % amylose were observed (table 2, figure 3b) in 1:1 ratio ($X^2 = 0{\cdot}21$). The $B_2F_1$ seeds also segregated into two categories (table 2, figure 3c) with 37 seeds in category 1 (8–18 %) and 38 in category 2 (19–26 %) in a ratio of 1:1.

In the $F_2$ of reciprocal cross analyses (table 2, figure 4a), three categories with 1–9 %, 10–19 %, and 20–27 % were observed to have 108, 207, and 105 seeds, respectively, in a ratio of 1:2:1 ($X^2 = 0{\cdot}13$). The $B_1F_1$ seeds segregated in a 1:1 ratio with 57 seeds in the 1–8 % amylose category and 56 seeds in 12–21 % category (table 2, figure 4b). The analysis of $B_2F_1$ seeds also confirmed the above results as they segregated into two classes (figure 4c), 6–15 % (39 seeds) and 18–27 % (35 seeds) in a ratio of 1:1 ($X^2 = 0{\cdot}22$).

### 3.3 *Cross* 3 (*IR*37307–8/*IR*8)

The very low-amylose parent IR37307–8 (7·41 %) and the high-amylose parent IR8 (27·26 %) indicated a difference of about 20 % in the amylose content of the parents. $F_1$ seeds of cross IR37307–8/IR8 which have only one dose of high-amylose gene in the endosperm have 23·47 % amylose. The reciprocal cross seeds have 26·88 % amylose

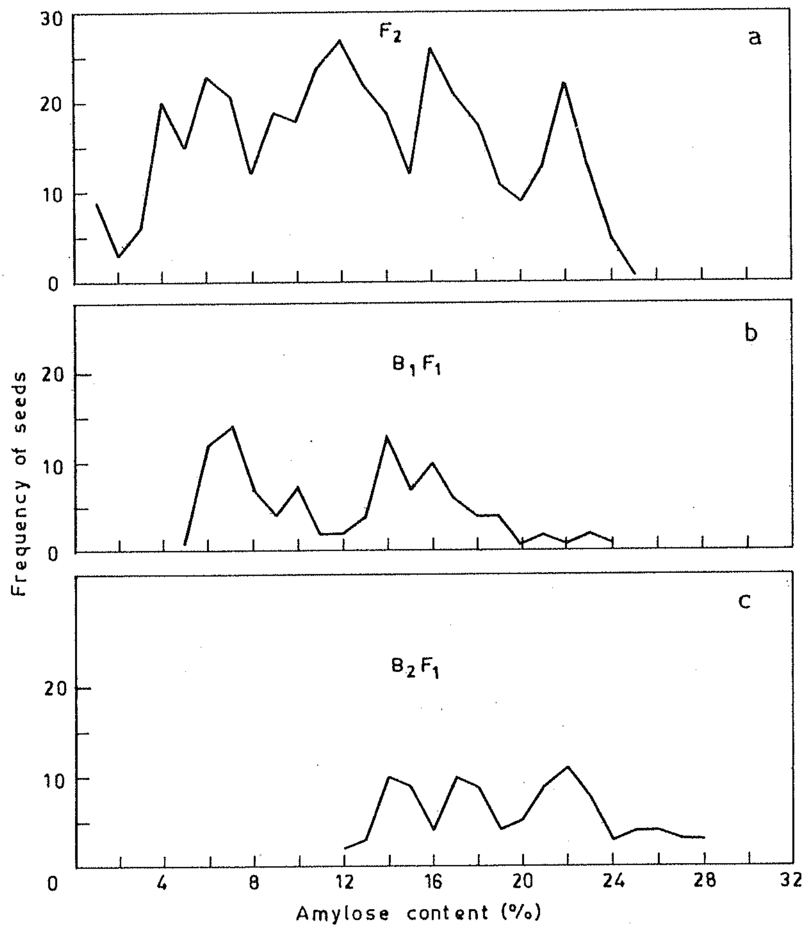


**Figure 2.** Frequency distribution of amylose content in cross BPI 121–407/IR37307–8.

which is almost equal to that of the high-amylose parent. The significant difference in the amylose level of both IR37307–8/IR8 and its reciprocal $F_1$ seeds indicated dosage effects.

The amylose content of $F_2$ seeds varied from 3 to 34 %. The $F_2$ seeds could essentially be classified into two distinct categories, i.e. 3–14 % and 18–34 %. The distribution of the grains into these classes showed a bimodal pattern (table 3, figure 5a) with 93 seeds in the first category and 311 in the second category. All the grains in the first category had a dull appearance like the very low-amylose parent. This indicated a satisfactory fit to a ratio of 1:3 ($X^2 = 0{\cdot}84$). Among the second category (from 18–34 %), seeds with one dose of gene for high-amylose could be grouped into a separate class rather than seeds containing 2 or 3 doses of high-amylose gene in the endosperm. The overall ratio thus fits well a value 1:1:2 in $F_2$ ($X^2 = 1{\cdot}44$). The $B_1F_1$ seeds also clearly segregated into 2 distinct classes. One class with very low-amylose gene, i.e., having no dose of gene for high amylose, had 55 seeds and the other having two doses of gene for high amylose had 59 showing a segregation of 1:1 ($X^2 = 0{\cdot}14$) (see also table 3, figure 5b). In $B_2F_1$ seed

**Table 2.** Frequency distribution of the amylose content in cross IR37307–8/IR24632–34 and its reciprocal

| Population | | Amylose (%) Mean | Amylose (%) Range |
|---|---|---|---|
| IR37307–8 | $(P_1)$ | 7·41 | 5–9 |
| IR24632–34 | $(P_2)$ | 21·75 | 19–22 |
| $F_1$ | $(P_1/P_2)$ | 17·45 | 14–19 |
| $F_1$ | $(P_2/P_1)$ | 15·10 | 13–18 |

| Population | Amylose class (%) | Observed frequency | $X^2$ 1:3 | $X^2$ 1:2:1 | $X^2$ 1:1 |
|---|---|---|---|---|---|
| $F_2(P_1/P_2)$ | 1–7 | 105 | 0·48 | — | — |
| | 8–19 | 197 | — | 0·62 | — |
| | 20–25 | 94 | | | |
| $B_1F_1$ $(P_1/P_2//P_1)$ | 1–8 | 37 | — | — | 0·21 |
| | 13–22 | 41 | | | |
| $B_2F_1$ $(P_1/P_2//P_2)$ | 8–18 | 37 | — | — | 0·01 |
| | 19–26 | 38 | | | |
| $F_2$ $(P_2/P_1)$ | 1–9 | 108 | 0·11 | — | — |
| | 10–19 | 207 | — | 0·13 | — |
| | 20–27 | 105 | | | |
| $B_1F_1$ $(P_2/P_1//P_1)$ | 1–8 | 57 | — | — | 0·01 |
| | 12–21 | 56 | | | |
| $B_2F_1$ $(P_2/P_1//P_2)$ | 6–15 | 39 | — | — | 0·22 |
| | 18–27 | 35 | | | |

analyses, the frequency of seeds in the 13–21 % and 22–30 % amylose categories showed a bimodal pattern (table 3, figure 5c) with 50 seeds in the 13–21 % category and 46 in the 22–30 % category in a 1:1 ratio ($X^2$ = 0·17).

In the analysis of $F_2$ seeds of the reciprocal cross IR8/IR37307–8, the seeds distinctly segregated into 2 broad categories, 2–12 % and 16–31 % amylose content. There were 93 seeds in category 1 and 272 in category 2. This distribution shows a bimodal pattern (table 3, figure 6a) in a 1:3 ratio ($X^2$ = 0·04). Further classification in category 2 shows overall segregation in a 1:1:2 ratio ($X^2$ = 0·51). The $B_1F_1$ seeds segregated into two categories with 45 and 52 seeds (table 3, figure 6b). $B_2F_1$ seeds could also be categorized into two classes on the basis of dosage in a 1:1 ratio confirming the above results (table 3, figure 6c).

## 4. Discussion

In the present study, $F_1$ seeds of crosses IR37307–8/BPI 121–407, IR37307–8/IR8, and their reciprocals showed significant differences in amylose content. The endosperm in

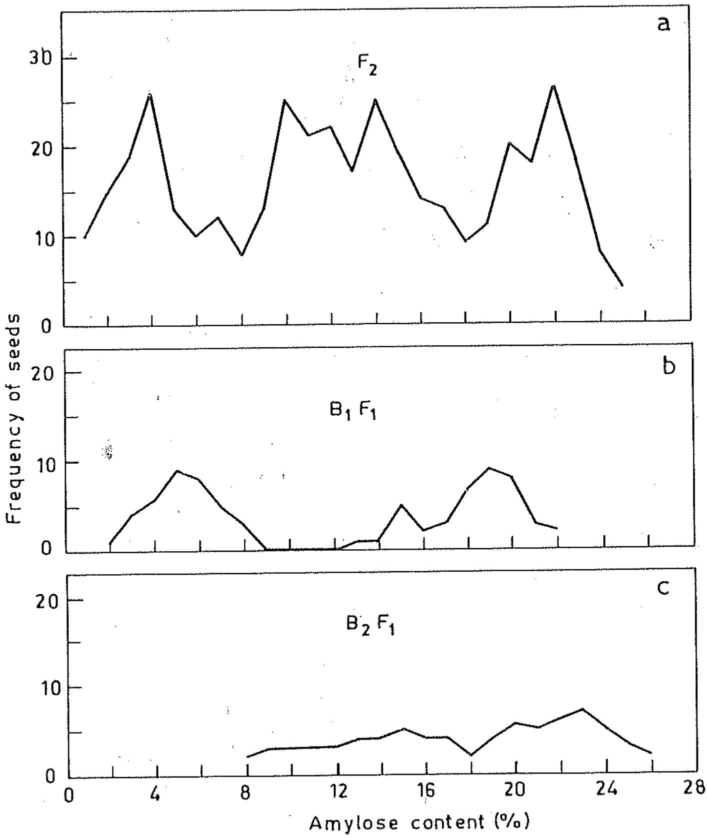


**Figure 3.** Frequency distribution of amylose content in cross IR37307–8/IR24632–34.

rice being triploid tissue has two doses of maternal and one dose of paternal allele. Because of the differences in doses of amylose genes in the endosperm, a dosage effect for amylose content is expected. The amylose content in the $F_1$ seeds of the crosses studied increased with an increase in gene dosage of the higher-amylose parent, although not always in linear order. No report is available in the literature on dosage effects in the crosses among varieties having very low and intermediate or high-amylose content. In the cross IR37307–8/IR24632–34, although the amylose content increased significantly with addition of one dose, the second dose did not increase it further by a significant margin. The dosage effects for amylose content in rice in waxy/nonwaxy and among nonwaxy crosses have been observed in some earlier studies (IRRI 1976; Heu and Park 1976a, b; Okuno 1978; Chang and Li 1981; Okuno *et al* 1983; Okuno and Yano 1984) but such information has not been utilized in analyzing the inheritance pattern of amylose content. In earlier studies on the inheritance of amylose content, the analyses for amylose content were done on bulk $F_2$ seeds, i.e., seeds borne on $F_1$ plants (Seetharaman 1959; Stansel 1966; Ghosh and Govindaswamy 1972; Bollich and Webb 1973; Chauhan and Nanda 1983; McKenzie and Rutger 1983), and dosage effects were not considered (Puri *et al* 1980). In fact, every seed of $F_2$ is genetically different from the other and bulk seed analysis would yield biased information.

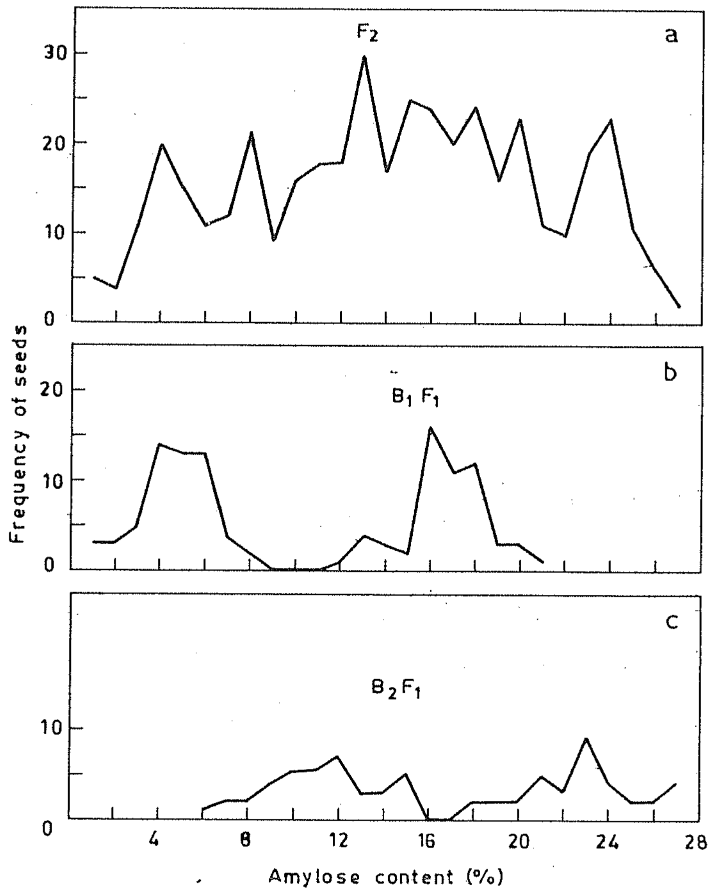


**Figure 4.** Frequency distribution of amylose content in cross IR24632–34/IR37307–8.

In the present studies with single-grain analyses, the segregation for amylose content has been found to be in accordance with gene dosage of the intermediate- or high-amylose gene. In crosses between very low- and intermediate-amylose parents, four types of endosperm constitutions with 0, 1, 2, and 3 doses of intermediate amylose are expected in equal proportion. In the cross IR37307–8/BPI 121–407 and its reciprocal, these four types of segregants with different amylose contents occurred in a ratio of 1:1:1:1. The ratio between the very low amylose category and the other three categories was 1:3. This suggests that a major gene controls very low amylose content. In the cross IR37307–8/IR24632–34 and its reciprocal, lack of significant dosage effect by the intermediate-amylose gene and almost intermediary amylose level of $F_1$ seeds between the two parents resulted in a 1:2:1 ratio. This also suggests that the difference between very low and intermediate amylose contents is under single gene control.

In the cross IR37307–8/IR8 and its reciprocal, segregants which did not inherit any allele for high amylose content had very low amylose content. Segregants with one dose of high amylose allele had amylose content almost equal to $F_1$ seeds of cross IR37307–8/IR8. The other two types of segregants, e.g., having either 2 or 3 doses of

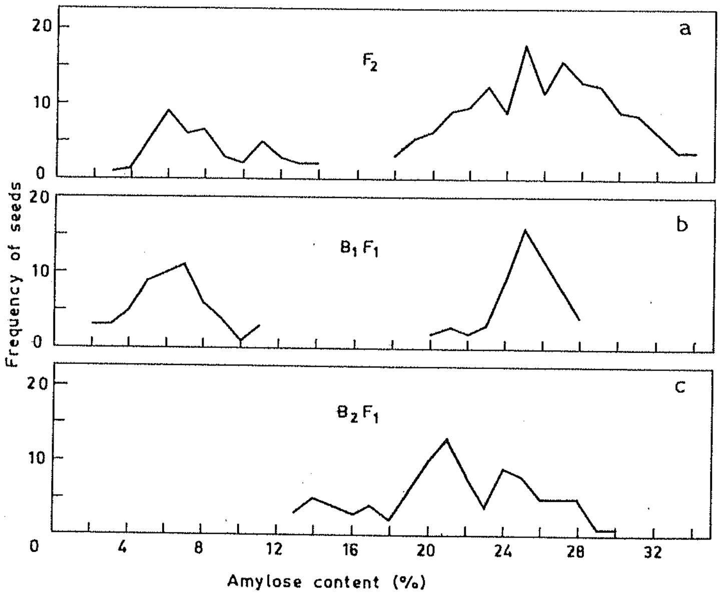


**Figure 5.** Frequency distribution of amylose in cross IR37307–8/IR8.

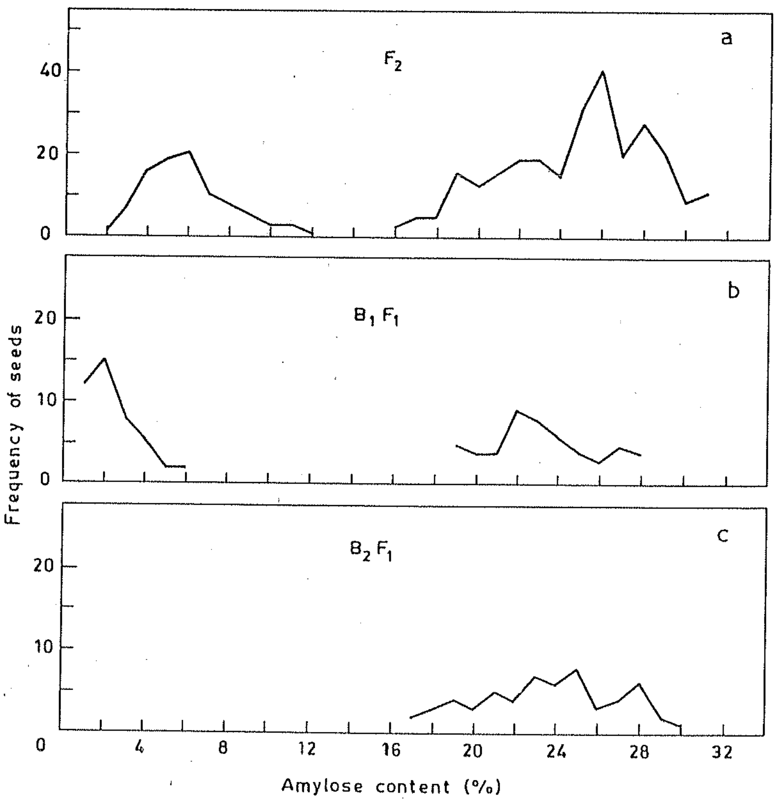


**Figure 6.** Frequency distribution of amylose in cross IR8/IR37307–8.

**Table 3.** Frequency distribution of the amylose content in cross IR37307–8/IR8 and its reciprocal

| Population | | Amylose (%) Mean | | Range | |
|---|---|---|---|---|---|
| IR37307–8 | ($P_1$) | 7·41 | | 5–9 | |
| IR8 | ($P_2$) | 27·26 | | 26–29 | |
| $F_1$ | ($P_1/P_2$) | 23·47 | | 21–25 | |
| $F_1$ | ($P_2/P_1$) | 28·88 | | 24–28 | |

| Population | Amylose class (%) | Observed frequency | $X^2$ 1:3 | $X^2$ 1:1:2 | $X^2$ 1:1 |
|---|---|---|---|---|---|
| $F_2$ ($P_1/P_2$) | 3–14 | 93 | 0·84 | — | — |
| | 18–24 | 110 } 311 | — | 1·44 | — |
| | 25–34 | 201 } | — | — | — |
| $B_1F_1$ ($P_1/P_2//P_1$) | 2–11 | 55 | — | — | 0·14 |
| | 20–28 | 59 | | | |
| $B_2F_1$ ($P_1/P_2//P_2$) | 13–21 | 50 | — | — | 0·17 |
| | 22–30 | 46 | | | |
| $F_2$ ($P_2/P_1$) | 2–12 | 93 | 0·04 | — | — |
| | 16–23 | 96 } 272 | — | 0·51 | — |
| | 24–31 | 176 } | | | |
| $B_1F_1$ ($P_2/P_1//P_1$) | 1–6 | 45 | — | — | 0·50 |
| | 19–28 | 52 | | | |
| $B_2F_1$ ($P_2/P_1//P_2$) | 17–23 | 28 | — | — | 0·07 |
| | 24–30 | 30 | | | |

high amylose allele, had almost similar amylose content (table 3), thus resulting in a 1:1:2 segregation ratio. This confirms the role of a single major gene in imparting very low amylose content. Results in the backcross progenies confirm these conclusions. The presence of a few individuals having higher or lower levels of amylose content as compared to high- and very low-amylose containing parents suggests the role of polygenes or modifiers.

This study emphasizes the need for single grain analysis and study of dosage effects for a precise understanding of the inheritance pattern of amylose content in each cross. In the absence of such information, the apparent continuous variation may lead to erroneous conclusions such as polygenic inheritance. The results also emphasize the importance of screening for amylose content during several segregating generations. Lines tending to show amylose content different from both the parents may not breed true because of the dissipation of dosage effect.

## References

Bollich C N and Webb B D 1973 Inheritance of amylose content in two hybrid populations of rice. *Cereal Chem.* 50: 631–636

Chang W L and Li W Y 1981 Inheritance of amylose content and gel consistency in rice. *Bot. Bull. Acad. Sinica* 22: 35–47

Chauhan J S and Nanda J S 1983 Inheritance of amylose content and its association with grain yield and yield contributing characters in rice. *Oryza* 20: 81–85

Ghosh A K and Govindaswamy S 1972 Inheritance of starch iodine blue value and alkali digestion value in rice and their genetic association. *Il Riso* 21: 123–132

Heu M H and Park S Z 1976a Dosage effect of waxy allele on the amylose content of rice grain. I. Amylose content of hybrid seeds obtained from isogenic lines for glutinuous and base colour. *Korean J. Breed.* 8: 48–54

Heu M H and Park S Z 1976b Dosage effect of waxy gene on the amylose content of rice grain. II. Amylose content of hybrid seeds obtained from male sterile stocks. *Seoul Natl. Univ. Coll. Agric. Bull.* 1: 39–46

IRRI 1976 Annual report for 1975, International Rice Research Institute, Los Baños, Philippines, pp. 479

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

McKenzie K S and Rutger J N 1983 Genetic analysis of amylose content, alkali spreading and grain dimension in rice. *Crop Sci.* 23: 306–313

Okuno K 1978 Gene dosage effect of waxy alleles on amylose content in endosperm starch of rice. *Jpn. J. Genet.* 53: 219–222

Okuno K, Fuwa H and Yano M 1983 A new mutant gene lowering amylose content in endosperm of rice, *Oryza Sativa* L. *Jpn. J. Breed.* 33: 387–394

Okuno K and Yano M 1984 New endosperm mutants modifying starch characteristics of rice, *Oryza sativa* L. *Jpn. Agric. Res. Q.* 18: 73–84

Puri R P, Siddiq E A and Mehra R B 1980 Studies on cooking and nutritive qualities of cultivated rice *Oryza Sativa* L. II. Quantitative genetic characterisation of amylose content. *Genet. Agrar.* 34: 15–34

Seetharaman R 1959 The inheritance of iodine value in rice and its association with other characters. *Diss. Abstr. Int.* 20: 856

Stansel J W 1966 The influence of heredity and environment on endosperm characteristics of rice (*Oryza sativa*). *Diss. Abstr. Int.* 27: 48B