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Genetic analysis of waxy locus in rice (*Oryza sativa* L.)

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Summary. Inheritance of waxy locus was studied in crosses of a waxy variety with four non-waxy parents having high-, intermediate-, low- or very low-amylose content. The analysis for amylose content was done on a single grain basis in parents, F_1, F_2, B_1F_1, and B_2F_1 seeds. The waxy parent lacking synthesis of amylose content was found to differ from the ones having high-, intermediate-, low- or very low-amylose content by one gene with major effect. Dosage effects for amylose content were observed to have great influence on segregation pattern and efficiency of selection. Selection efficiency for amylose content can be enhanced by selecting for endosperm appearance in early segregating generations.

Key words: Inheritance – Amylose content – Dosage effects – Endosperm-cooking quality

Introduction

The waxy locus in rice is known to have an important influence on the properties of starch produced in the endosperm. Rice starch, in general, is composed of two types of polysaccharides, amylose and amylopectin.

Waxy allele was identified by Ikeno (1914) and located on linkage group I by endosperm appearance or pollen grain analysis (Nagao and Takahashi 1963; Iwata and Omura 1971; IRRI 1976). The waxy allele produces primarily amylopectin while the non-waxy alleles produce both amylose and amylopectin. Waxy rices are the staple food of people in northern and northeastern Thailand and Laos. These rices are also used for special preparations like sweets, pudding desserts, and others. Non-waxy rices, on the other hand, may have a very low (3–9%), low (10–19%), intermediate (20–25%) or high (> 25%) amylose content. Varieties with different amylose content are preferred in different countries. Sano (1984), on the basis of an analysis of a *Wx* gene product, (major protein bound to starch granule), reported a monogenic difference in two crosses between waxy and low amylose content parents.

No information is available on the genetics of amylose content of waxy endosperm in crosses with all other amylose content classes. The purpose of this paper is to present the results of crosses among such parents.

Materials and methods

One waxy indica rice variety, IR29, which lacks the synthesis of amylose content, was crossed with four non-waxy varieties (IR8, BPI 121-407, IR24, and IR37307-8) having high-, intermediate-, low- and very low-amylose content respectively. To produce F_2 seeds, these crosses, along with their parents, were grown during the 1985 dry season (DS) at the International Rice Research Institute (IRRI), Los Baños, Philippines. All the F_1's were crossed to both of their parents in the same season to produce backcross seeds. A fresh set of F_1 seeds of all crosses (including reciprocals) was also produced during DS 85. The seeds of 5 parents, 8 F_1's, 8 F_2's, and 16 backcrosses were produced in the same season to minimize environmental effects. The seeds of the F_1 and backcrosses were dehulled by hand and those of the parents and F_2, by a Satake rice dehulling machine. Dehulled seeds were milled in a Wig-L-Bug (if numbers of seeds were few) or a test tube mill. The embryos were carefully removed from all the seeds. Twenty single seeds from each parent and F_1, about 400 single seeds from each F_2, and about 60–100 single seeds from each backcross were analyzed for amylose content following Juliano's procedure (1971) with the help of a Technicon Autoanalyser. An X^2-test was applied to test the segregation pattern.

Results

Cross 1: IR29/IR8

The difference in amylose content of F_1 seeds of the cross IR29/IR8 and its reciprocal was significant, sug-

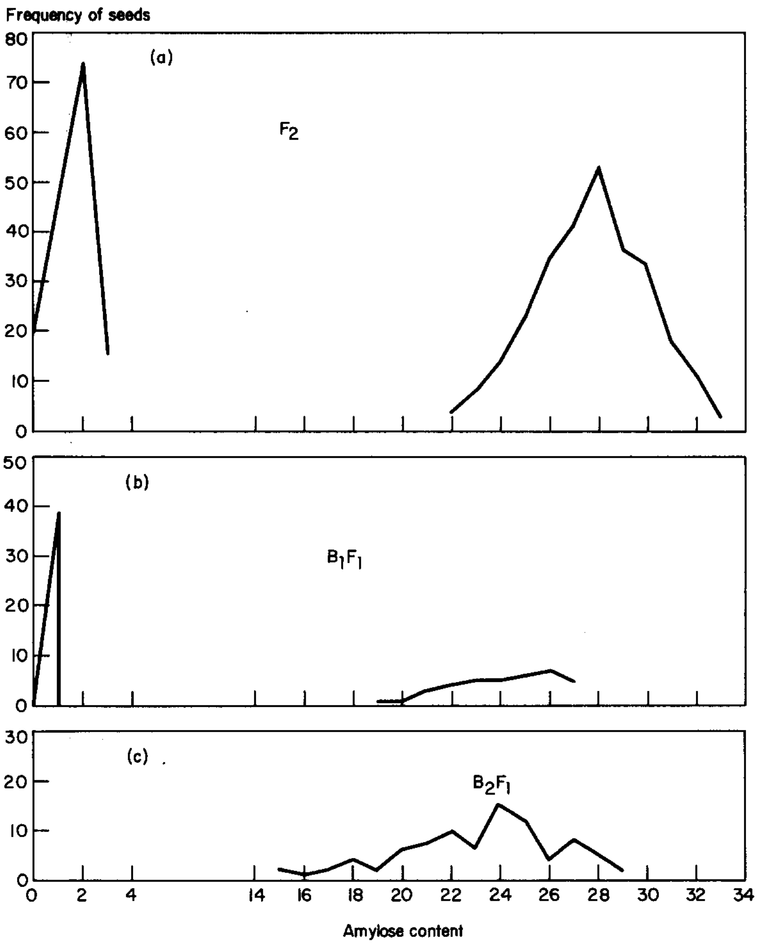

Fig. 1. Frequency curve showing distribution of amylose content in cross IR29/IR8

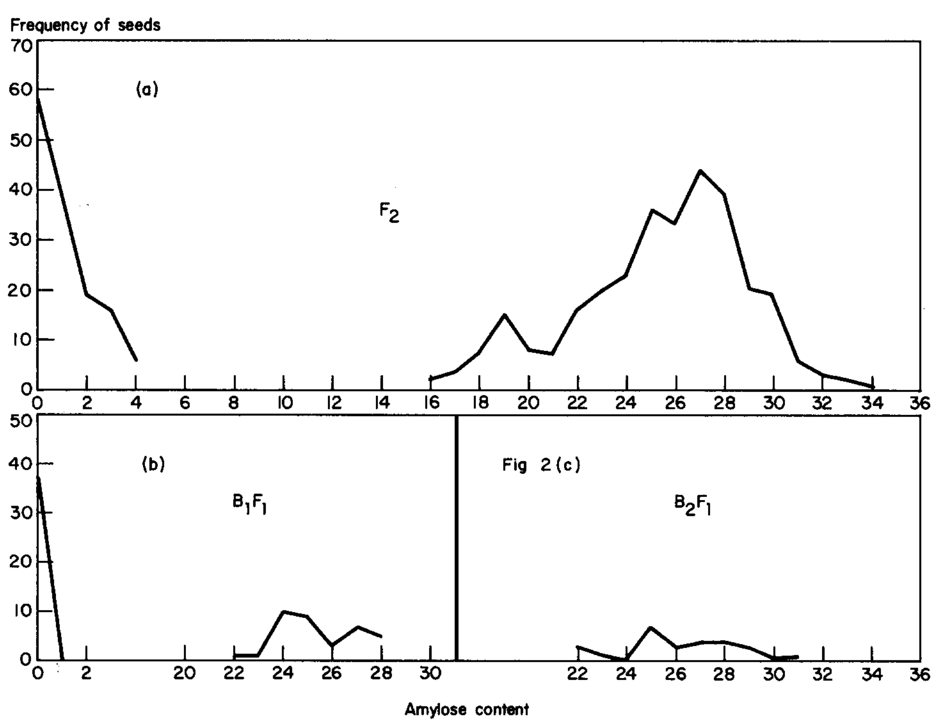

Fig. 2. Frequency curve showing distribution of amylose content in cross IR8/IR29

Table 1. Frequency distribution of amylose content in cross IR29/IR8 and its reciprocal

Population		Amylose (%) Mean	Range
IR29	(P_1)	0.01	0.0– 0.2
IR8	(P_2)	27.26	26 –29
F_1	(P_1/P_2)	23.88	20 –27
F_1	(P_2/P_1)	27.54	24 –29

Population		Amylose class (%)	Observed frequency	X^2 1:3	1:1
F_2	(P_1/P_2)	0– 2	110	2.13	–
		22–33	280		
B_1F_1	($P_1/P_2//P_1$)	0– 2	42	–	0.32
		19–27	37		
B_2F_1	($P_1/P_2//P_2$)	15–23	40	–	0.29
		24–29	45		
F_2	(P_2/P_1)	0– 2	99	0.05	–
		16–33	305		
B_1F_1	($P_2/P_1//P_1$)	0– 2	39	–	0.22
		22–28	35		
B_2F_1	($P_2/P_1//P_2$)	22–26	14	–	0.04
		27–31	13		

Table 2. Frequency distribution of amylose content in cross IR29/BPI 121-407 and its reciprocal

Population		Amylose (%) Mean	Range
IR29	(P_1)	0.01	0.0– 0.2
BPI 121-407	(P_2)	24.89	24 –25
F_1	(P_1/P_2)	22.92	20 –24
F_1	(P_2/P_1)	22.40	22 –25

Population		Amylose class (%)	Observed frequency	X^2 1:3	1:1
F_2	(P_1/P_2)	0– 2	103	0.34	–
		19–27	289		
B_1F_1	($P_1/P_2//P_1$)	0– 1	58	–	0.59
		20–27	50		
B_2F_1	($P_1/P_2//P_2$)	17–27	121	–	–
F_2	(P_2/P_1)	0– 1	102	0.03	–
		20–28	300		
B_1F_1	($P_2/P_1//P_1$)	0– 1	42	–	0.00
		17–26	42		
B_2F_1	($P_2/P_1//P_2$)	17–25	69	–	–

gesting dosage effects. All seeds in F_1 were translucent in appearance. Variation in amylose content in F_2 seeds (0–33%) was observed. The F_2 segregation pattern was essentially bimodal with 2 distinct categories. There were 110 seeds in category 1 with 0–2% amylose content and 280 seeds in category 2 with 22–33% amylose content (Table 1, Fig. 1a). These data showed a satisfactory fit to the ratio 1 waxy:3 high amylose content segregants. All the seeds with 0–2% amylose content had an opaque endosperm appearance similar to that of the waxy parent. The remaining seeds with high amylose content were translucent. In B_1F_1, a segregation ratio of 1:1 was observed with 42 waxy and 37 high amylose seeds with translucent endosperm (Fig. 1b). In B_2F_1, all the seeds had translucent endosperm but two categories for amylose content were observed in a ratio of 1:1 (Table 1, Fig. 1c). In the analysis of reciprocal cross seeds, a ratio of 1 waxy:3 high amylose in F_2, and 1 waxy:1 high amylose in B_1F_1 and B_2F_1 were observed (Table 1, Figs. 2a, b, c).

Cross 2: IR29/BPI 121-407

The difference in amylose content of varieties IR29 and BPI 121-407 was 24.89%. One dose of gene for intermediate amylose content was as efficient in producing the same amylose content as 2 or 3 doses of this allele. The F_1 seeds from the direct and reciprocal crosses were translucent in appearance.

In the analysis of the F_2 seeds of cross IR29/BPI 121-407, two categories were observed. There were 103 seeds in category 1 with 0–2% amylose content, and 289 seeds in category 2 with 19–27% amylose content (Table 2). This suggested a bimodal pattern of distribution (Fig. 3a) in the ratio of 1:3 ($X^2=0.34$). In the analysis of B_1F_1 seeds, there were 58 seeds in category 1 (0–1%) and 50 seeds in category 2 (20–27%) (Fig. 3b). This gave a good fit to a 1:1 ratio ($X^2=0.59$). All seeds with 0–1% amylose were opaque in appearance and the ones with 20–29% amylose were translucent. In B_2F_1, because of a lack of dosage effect, no clear cut classes were observed (Fig. 3c). All the B_2F_1 seeds were translucent.

In the analysis of the reciprocal cross, two categories in the ratio of 1 waxy:3 non-waxy were observed ($X^2=0.03$) (Table 2, Fig. 4a). In B_1F_1, a 1:1 ratio ($X^2=0.00$) was also noted (Fig. 4b). No clear cut classes were observed in B_2F_1 (Fig. 4c).

Cross 3: IR29/IR24

The parents in this cross differed in amylose content by a margin of about 15%. The dosage effect for amylose content was conspicious from the F_1 seeds of the direct

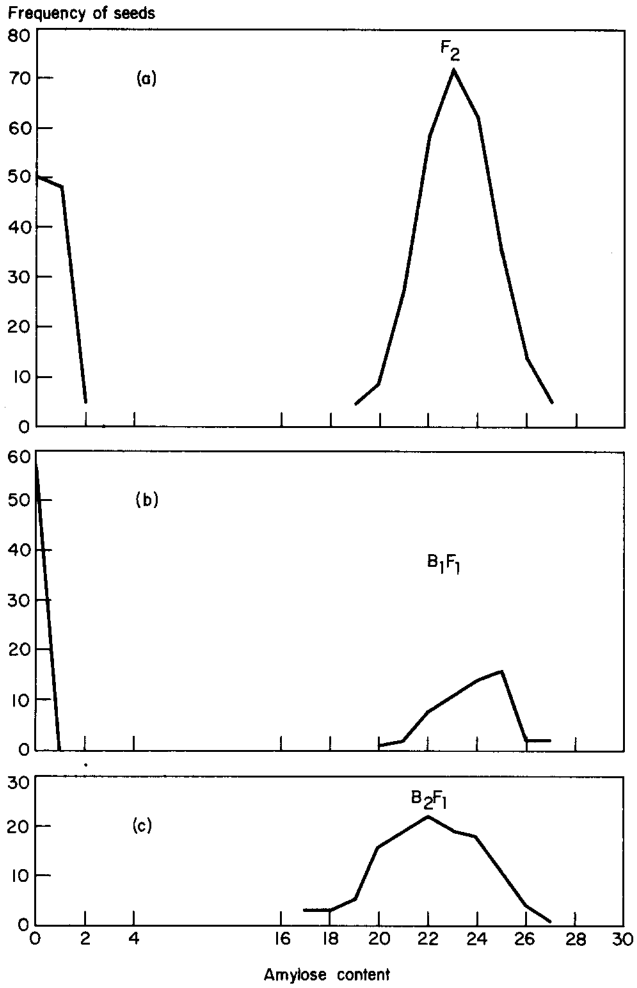

Fig. 3. Frequency curve showing distribution of amylose content in cross IR29/BPI 121-407

and reciprocal crosses. The F_1 seeds of cross IR29/IR24 were hazy, whereas those in the IR24/IR29 cross were translucent. In F_2 seeds of the cross IR29/IR24, three categories could be distinguished on the phenotypic basis. The class having 3 doses of *wx* gene had opaque endosperm, while the seeds having two doses of *wx* allele had hazy endosperm like the F_1 seds of the cross IR29/IR24. These seeds could be easily distinguished from the seeds of waxy and translucent parents. The third category of seeds having one or zero dose of the *wx* allele was translucent in appearance but could not be distinguished from each other phenotypically. On the basis of amylose content, however, four categories could be distinguished. These were 0–2% amylose content (107 seeds), 5–9% (112 seeds), 10–14% (115 seeds), and 15–20% (97 seeds) (Table 3, Fig. 5 a). These data agree with the 1 : 1 : 1 : 1 ratio (X^2 = 0.73). In the B_1F_1 analysis, there were 48 seeds with opaque endosperm and 0–1% amylose content; 49 seeds had translucent endosperm and 4–14% amylose content. These data fit the 1 : 1 segregation ratio (Table 3, Fig. 5 b). On the basis of endosperm appearance, two classes (one with hazy, the other with translucent endosperm) were observed in B_2F_1, and on the basis of amylose content, two categories were obtained. Thirty seeds have 0–6% and 33 seeds have 7–16% amylose content (Table 3, Fig. 5 c).

The F_2 seeds of the reciprocal cross could also be classified into three phenotypic classes with opaque, hazy, and translucent endosperm, in a ratio of 1 : 1 : 2. On the basis of amylose content, four classes were observed with 105 seeds having 0–1% amylose content, 101 seeds with 5–9%, 119 seeds with 10–14%, and 101 seeds with 15–21% (Table 3, Fig. 6 a). These data agree with a ratio of 1 : 1 : 1 : 1. The B_1F_1 seeds had two types of endosperm, opaque, and translucent, in a 1 : 1 ratio. Forty-two seeds with 0–2% amylose content and 38 seeds with 4–10% amylose content were observed in a ratio of 1 : 1 (Table 3, Fig. 6 b). Similarly, B_2F_1 seeds were observed to be hazy and translucent 1 : 1 ratio (Table 3). On an amylose content basis, these seeds were classified into two classes with 42 seeds in the 0–5% category and 35 seeds in the 6–13% category (Fig. 6 c).

Cross 4: IR29/IR37307-8

There was a 7% difference in amylose content of the two parents. IR37307-8 had a dull endosperm which is relatively less white or opaque than that of the waxy parent IR29. The F_1 seeds in the cross IR29/IR37307-8 had 1.51% amylose content and the reciprocal cross seeds had 3.09%, compared with 0.01% and 7.41% of the parents (Table 4). F_1 seeds of both crosses had an appearance almost similar to the waxy parent.

In the analysis of F_2 seeds of cross IR29/IR37307-8 and its reciprocal, a variation of 0–11% in amylose content was observed. Because of very small differences in the endosperm appearance of segregants, it was difficult to differentiate between waxy and non-waxy categories. In the cross IR29/IR37307-8, the F_2 seeds were classified into 2 classes: 195 seeds with 0–3% and 191 seeds with 4–11% amylose content (Table 4, Fig. 7 a). The amylose content in B_1F_1 seeds varied from 0–6%. There were 48 seeds in the category with 0–1% amylose content and 44 seeds in the 2–6% category (Fig. 7 b). In B_2F_1 also, as expected, segregation into 0–4% and 5–11% amylose content categories was observed with 45 and 39 seeds, respectively in each class (Table 4, Fig. 7 c).

Similarly in the reciprocal cross, two classes were observed in the F_2, B_1F_1, and B_2F_1 populations, all in the 1 : 1 ratios, thereby confirming the above results (Table 4, Fig. 8 a–c).

Table 3. Frequency distribution of amylose content in cross IR29/IR24 and its reciprocal

Population		Amylose (%) Mean	Range
IR29	(P_1)	0.01	0.0– 0.2
IR24	(P_2)	15.11	13 –16
F_1	(P_1/P_2)	5.59	3 – 7
F_1	(P_2/P_1)	12.22	10 –14

Population		Amylose class (%)	Observed frequency		X^2 1:3	1:1:1:1	1:1
F_2	(P_1/P_2)	0– 2	107		0.01	–	–
		5– 9	112	324			
		10–14	115		–	0.73	–
		15–20	97				
B_1F_1	($P_1/P_2//P_1$)	0– 1	48		–	–	0.01
		4–14	49				
B_2F_1	($P_1/P_2//P_2$)	0– 6	30		–	–	0.14
		7–16	33				
F_2	(P_2/P_1)	0– 1	105		0.02	–	–
		5– 9	101	321	–	2.05	–
		10–14	119				
		15–21	101				
B_1F_1	($P_2/P_1//P_1$)	0– 2	42		–	–	0.20
		4–10	38				
B_2F_1	($P_2/P_1//P_2$)	0– 5	42		–	–	0.64
		6–13	35				

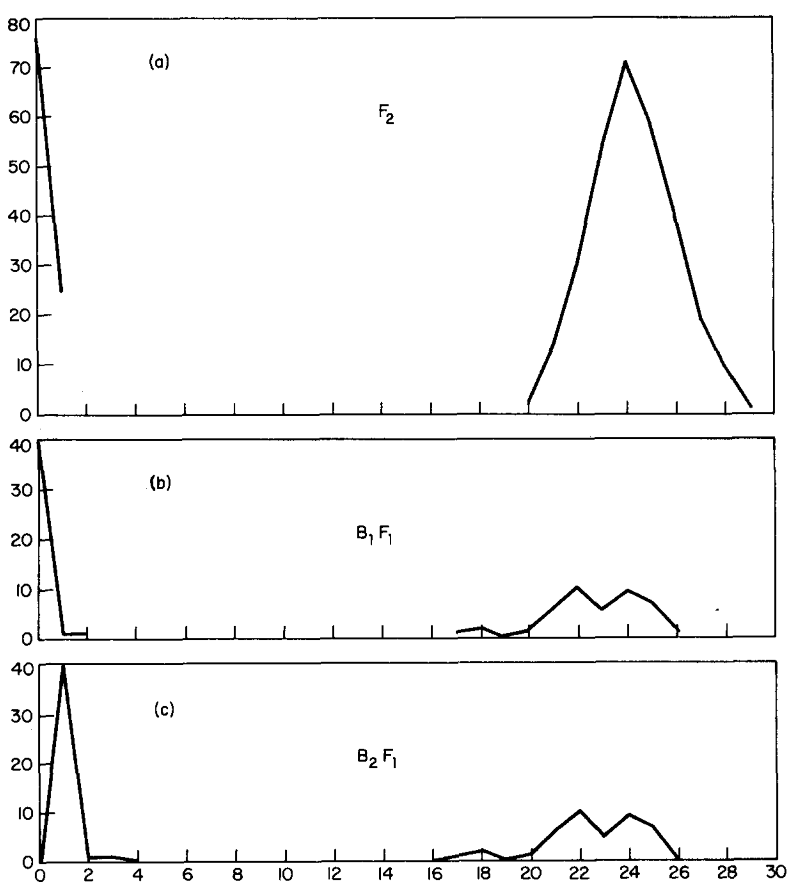

Fig. 4. Frequency curve showing distribution of amylose content in cross BPI 121-407/IR29

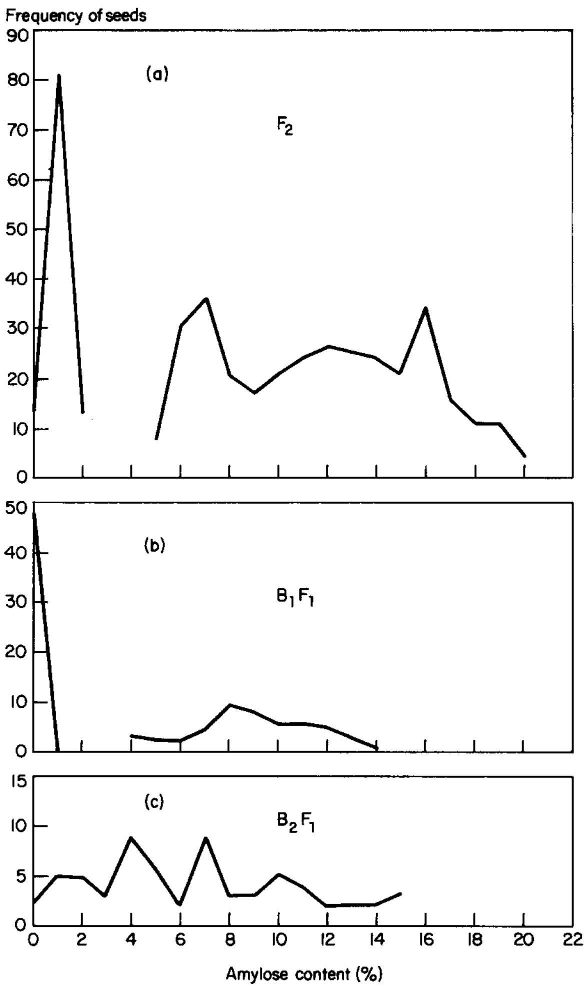

Fig. 5. Frequency curve showing distribution of amylose content in cross IR29/IR24

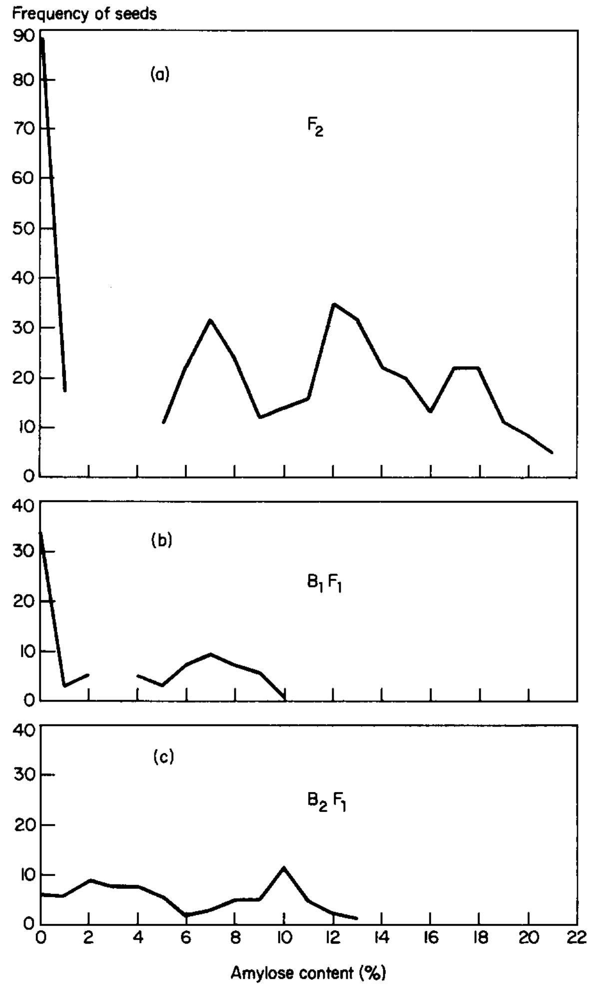

Fig. 6. Frequency curve showing distribution of amylose content in cross IR24/IR29

Discussion

Endosperm in cereals is a triploid tissue having two doses of a gene from the female parent and one dose from the male parent. Because of unequal dosage from the male and female gametes in the endosperm, the synthesis of amylose content and the appearance of endosperm have been found to be affected. In the present study, one gene dose for high- or intermediate-amylose content was capable of producing translucent endosperm and an amylose content almost equal to the level of the higher amylose parent. The segregation (in the ratio of 1 zero amylose content seeds with opaque endosperm to 3 higher amylose content seeds with translucent endosperm) suggests the role of a major gene in controlling the opaque versus translucent endosperm and in differentiating the seeds having no amylose content from intermediate or high amylose content seeds.

The effect of varying the genetic constitution on endosperm appearance and synthesis of amylose content in the endosperm has been examined in crosses having female or pollen parents with different amylose contents. As the amylose content in the pollen parent decreased, a dosage effect rather conspicuously appeared. The F_2 distribution in such cases showed segregation for amylose content according to 0, 1, 2, and 3 doses of gene for waxy endosperm in a 1 : 1 : 1 : 1 ratio. This suggests that a major gene controls waxy endosperm appearance with no synthesis of amylose. Because of dosage effects of gene on low amylose content, variation from 0 to 20% has been observed with all intermediary levels in crosses of waxy and low amylose content parents. In such cases, occurrence of seeds having very low amylose content is due to the dosage effect and the effect of some modifiers, Such seeds would not breed true in the next segregating generations as these dosage effects would disappear. Selection for amylose content in such crosses can only be done within the limits of the modifiers. On the other hand, in crosses between waxy and high or intermediate amylose content parents, dosage effects were very small or almost absent. The segregation for waxy and translucent grains in the ratio of 1 : 3 indicates the role of

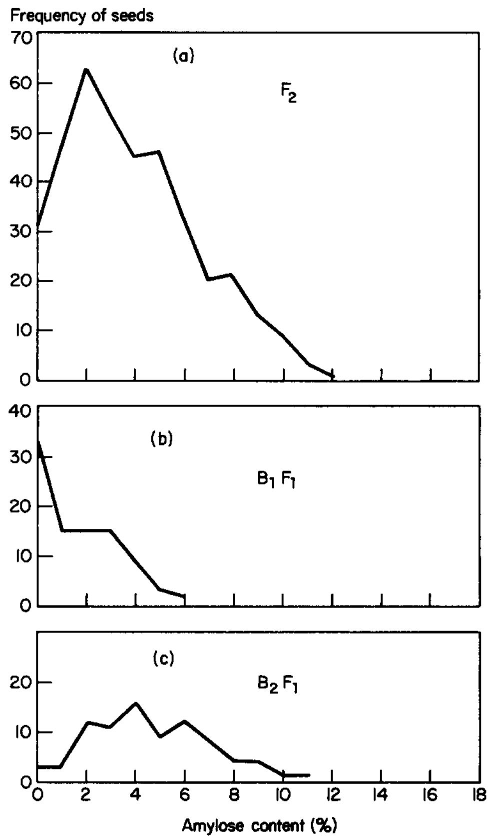

Fig. 7. Frequency curve showing distribution of amylose content in cross IR29/IR37307-8

Table 4. Frequency distribution of amylose content in cross IR29/IR37307-8 and its reciprocal

Population		Amylose (%) Mean	Range
IR29	(P_1)	0.01	0.0–0.2
IR37307-8	(P_2)	7.41	5 –9
F_1	(P_1/P_2)	1.51	1–3
F_1	(P_2/P_1)	3.09	2–4

Population		Amylose class (%)	Observed frequency	X^2 1:1
F_2	(P_1/P_2)	0– 3	195	0.04
		4–11	191	
B_1F_1	($P_1/P_2//P_1$)	0– 1	48	0.17
		2– 6	44	
B_2F_1	($P_1/P_2//P_2$)	0– 4	45	0.43
		5–11	39	
F_2	(P_2/P_1)	0– 2	210	1.22
		3–10	188	
B_1F_1	($P_2/P_1//P_1$)	0– 1	42	0.18
		2– 6	46	
B_2F_1	($P_2/P_1//P_2$)	0– 3	34	0.14
		5– 8	31	

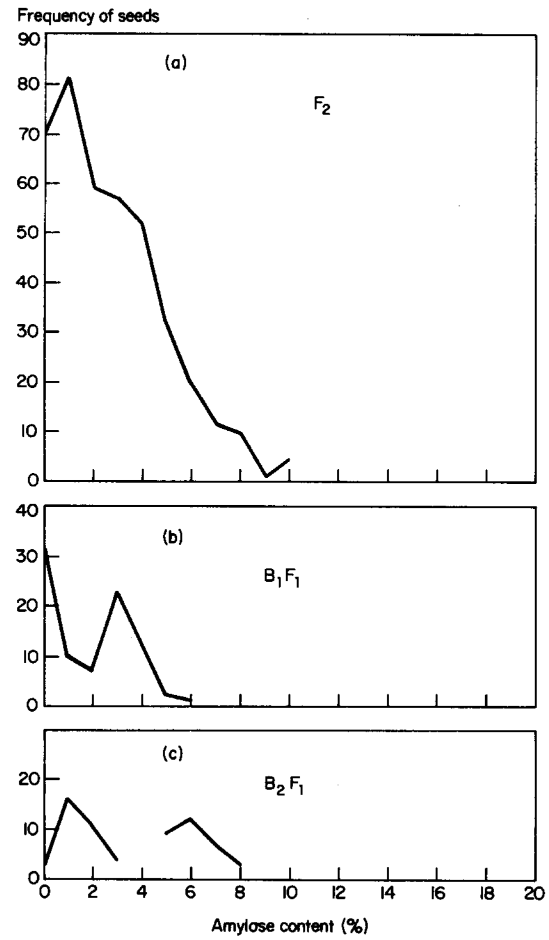

Fig. 8. Frequency curve showing distribution of amylose content in cross IR37307-8/IR29

a major gene. The transgressive segregants obtained suggest the role of modifiers as well.

In the cross IR29/IR37307-8, the effect of one dose of gene for very low amylose content is very small. Therefore, in segregating generations, such seeds cannot be differentiated from the ones having 0 dose of gene for very low amylose content. Similarly, because of the slight difference in amylose content and the effect of modifiers, it was difficult to differentiate the seeds containing 2 or 3 doses of gene for low amylose content from each other. Because of small differences in the amylose content of parents, and also among F_1's and parents and the effect of modifiers, the degree of overlap for amylose content in the segregating generations increased progressively. In such situations, it became difficult to distinguish among genotypes on the basis of amylose content, and the data showed a pseudo-normal distribution. Because of relatively greater differences among backcrossed seeds, the 1 : 1 segregation pattern suggests that a major gene governs the inheritance of waxy locus which does not produce amylose.

The present study suggests that in breeding waxy varieties, selection of F_2 seeds having opaque endosperms can be done in crosses involving waxy and translucent endosperm parents. All the seeds having opaque or waxy endosperm would be devoid of amylose. Opaque endosperm, being recessive, would breed true. The translucent seeds in crosses between waxy and translucent but low amylose content parents can be separated based on the degree of translucence. Seeds with hazy appearance in such crosses are in fact heterozygous for one or two alleles for amylose content and can be discarded in early segregating generations. Though affected by modifiers, completely translucent seeds would have an amylose content almost equal to the higher amylose content parent. In crosses between waxy and intermediate- or high-amylose content parents, two categories, opaque and translucent, can be sorted out in early segregating generations. All the opaque seeds would be devoid of amylose but the translucent seeds having 1, 2, or 3 doses of alleles for intermediate- or high amylose content are not phenotypically separable because they have the same degree of translucence. Plants from such seeds when continuously selfed would result in segregants having an amylose content almost equal to that of the non-waxy parent. Thus, the segregants with slightly higher or lower level of amylose content can be selected because of the effect of modifiers. Selection on a phenotypic basis in waxy/dull parents having a very low amylose content does not seem possible because of the almost similar endosperm appearance in segregating generations.

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