Early Generation Selection for Amylose Content in Rice Grain: Heritability and Response to Selection

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ABSTRACT

Landraces of glutinous rice contain many desirable traits, but many consumers do not prefer its glutinous grain characteristic. Genetic variability in the glutinous grain type exists, suggesting that it should be feasible to select for non-glutinous grain type. The aim of the present study was to accelerate the selection of non-glutinous rice grain type by examining the amylose content of F_3 and F_4 lines.

The grain amylose content of two hybrids of rice combinations was investigated: Kao Dok Mali 105 (non-glutinous) x Kum Doi Saket (glutinous) and RD15 (non-glutinous) x Kum Doi Saket. The amount of amylose was measured as an extraction from the unmilled ground grains, using 2N NaOH in 95% ethyl alcohol. Fifty F_2 plants were selected randomly to build up the F_3 families and, after harvesting, seeds of the 50 F3-derived lines were divided into two halves. One half was used for analyzing amylose while the remainder was used to produce 50 F_4 families. In the F_4 generation, 20 plants were sown from each family and the amylose means and variance values of the 50 F4 families were calculated as a within family variance. Heritability was calculated based on the linear regression coefficient value (b), which was obtained from the regression of the within family mean of F_4 families on its relative derived F3 mean ($h_{ns}^2 = b \times 4/7$). The response to selection (R) for amylose content was then estimated by using a mathematical model, $R = i\sigma h^2$, with 5% of selected proportion (v =5%).

Variation in rice grain amylose content was detected in F3 and F4-derived lines in both combinations of parents, with a population mean intermediate between the two parents' values but closest to the value of the higher parent. This indicated the occurrence of the partial dominant action of gene segregation. Therefore, a selection for the character could be achieved in the F3 generation. In the F4 generation, family variance of the derived lines varied little with standard deviation (σ) = 2.24 and 2.35 in the two combinations. Regression of these variance values on its family mean showed a considerable number of families that regressed closely to the parents, thereby, indicating the homozygosity of genotypes in some F4-derived lines. There were 14 lines (28%) in Kao Dok Mali 105 x Kum Doi Saket combination and 2 lines (4%) in RD15 x Kum Doi Saket combination that showed homozygosity. A significant linear regression

of F_4 on F_3 means was detected only in the derived lines of RD 15 x Kum Doi Saket combination with b = 0.3135. Thus, the low narrow sense heritability for grain amylose content in this combination (h_{ns}^2 = 0.179) indicated a large environmental influence was involved. Furthermore, if high performing lines were selected in F_4, with v = 5% (i = 2.065), the achieved rate of response to selection for percent grain amylose content was +0.869 per generation, which is too low to change the population mean in advanced generations. Therefore, selection for high grain amylose content in rice should be performed at an early generation.

Keywords: Amylose content, Narrow-sense heritability, Response to selection, Rice grain

INTRODUCTION

In rice grain, a pair of homozygous recessive genes (wxwx) controls synthesis of amylopectin. In contrast, a dominant gene (Wx-) is responsible for synthesizing amylose. The action of this dominant gene when heterozygous (Wxwx) is additive with partial dominance (IRRI, 1975), which is segregated into intermediate type in the subsequent generations. Two groups of intermediate type are classified according to their level of amylose. Rice with a high grain amylose content belongs to the "indica" group and rice with a low amylose content to "japonica" rice (Matsuo et al., 1997). The endosperm is 3n with high or low amylose content dependant on a number of dominant genes accumulated in the genotype in each generation (IRRI, 1986). Therefore, the effectiveness of selection for amylose content by the pedigree method is difficult to predict unless the heritability has been evaluated. Furthermore, an estimated rate of response to selection could identify the selected generations with high response. In this report, the genetic variance versus the environmental variance of F_3 derived lines and F4 derived lines of two rice combinations (*Oryza sativa* L., indica type) of high and low amylose content parents was investigated. A narrow-sense heritabilty (h_{ns}^2) was then calculated. Moreover, 20 percent of F_3 derived lines with high amylose content was then selected and the difference between the original F_3 and its derived F_4 lines were used in calculating the response to selection.

MATERIALS AND METHODS

An individual plant of each of 250 F_3 hybrids of crosses between two high amylose white non-glutinous rice (Kao Dok Mali 105 (KDML 105) and RD 15) and a low amylose purple glutinous rice (Kum Doi Saket (KDK)) were planted in the 2008 growing season at the experimental field of the Agronomy Division, Department of Plant Science and Natural Resources, Faculty of Agriculture, Chiang Mai University, Thailand. After harvest, 50 families of the F_3 derived lines were chosen randomly, and the seeds were divided into two groups. One of the groups was analyzed for amylose content, while the other group was sown as the 50 families of F_4 generation in which 20 plants were sown individually from each family. Grain amylose content in F_4 was analyzed using the derived F_4 seeds of each plant separately. Amylose content was extracted from 0.1g of ground unthreshed grain in 8 ml of NaOH (2N) with 1 ml of 95% ethyl alcohol, and shaken for 10 minutes. Then, 5 ml of the extracted solution was transferred into the volumetric flask mixed with 2 ml iodine and 2 ml acetic acid (1N). This solution was diluted by 100 ml of distilled water before reading under spectrophotometer at wavelength 620 nm. Family variance was estimated in F_4 and the regression coefficient (b) was calculated by regressing the F_4 families' mean on its derived F_3 families' mean. Narrow-heritability (h_{ns}^2) was estimated by using a mathematical equation ($h_{ns}^2 = b \times 4/7$) proposed by Smith and Kinman (1965) and the rate of response to selection (R) was calculated as $R = i\sigma\ h_{ns}^2$ (Knight. 1979 , Roff. 1997), where i = intensity of selection, σ = standard deviation, and h_{ns}^2 = narrow-sense heritability

Seeds on individual F_3 plants developed after the third self-fertilization are referred to as "F_3-derived lines" while seeds on individual F_4 plants are referred to as "F_4-derived lines."

RESULTS

In both combinations of parents, the amylose content of F_3 and F_4-derived lines varied widely, covering the range of amylose contents found in the parents (Figure 1A and B, Figure 2A and B). The hybrid population mean moved toward the value of the higher parent in the F_3-derived lines. In the F_4-derived lines, the within family variance was 2.24 and 2.35 in KDML 105 x Kum Doi Saket and RD15 x Kum Doi Saket, respectively (Table 1). Distribution of amylose contents in F_3 was normal distributed and moved toward the higher parent (KDML 105), but in F_4 distribution showed a positive skew. However, transgress segregates were high in F_3, but in F_4, particularly in the cross KDML 105 x Kum Doi Sket, the effect diminished and the mean moved toward a lower parent (KDK), signifying a dominant effect manifested in this combination (Figure 1A and B, Figure 2A and B). The regression between the F_4 within family variance on the family mean was significant. The results indicated 14 F_4 derived lines in KDML 105 x Kum Doi Saket and 12 F_4 derived lines in RD 15 x Kum Doi Saket regressed close to a lower parent (KDK), and a few close to the higher parent (KDML 105 (Figure 3A and B).

The regression of amylose mean values of F_4 on derived F_3 lines' means gave a value for the coefficient regression (b) of 0.1654 in KDML 105 x Kum Doi Saket combination, which was not significant, but in RD 15 x Kum Doi Saket, the value (b) was 0.3135 and the regression was significant (Figure 4A and B). This was because RD 15, although derived as a mutant line of KDML 105, was shorter in height and maturity. Narrow-sense heritability was thus estimated only for this combination, with a result of $h_{ns}^2 = 0.179$. If selection of the top 5% of F_4 is made, the intensity (i) is 2.065 (v = 5%) and standard deviation (σ), thus the selection response rate ($R = i\sigma\ h_{ns}^2$) is 0.869 (Figure 4).

Table 1. Comparison of the hybrid population means to those of the mid-parent value for amylose content in grain.

Combination	Amylose content (%)	
	F3	F4
KDML 105 x Kum Doi Saket		
Mid-Parent	11.47	12.21
Mean	12.92	9.49
Standard deviation (σ)	-	2.24
RD 15 x Kum Doi Saket		
Mid-Parent	10.64	11.19
Mean	11.43	9.97
Standard deviation (σ)	-	2.35

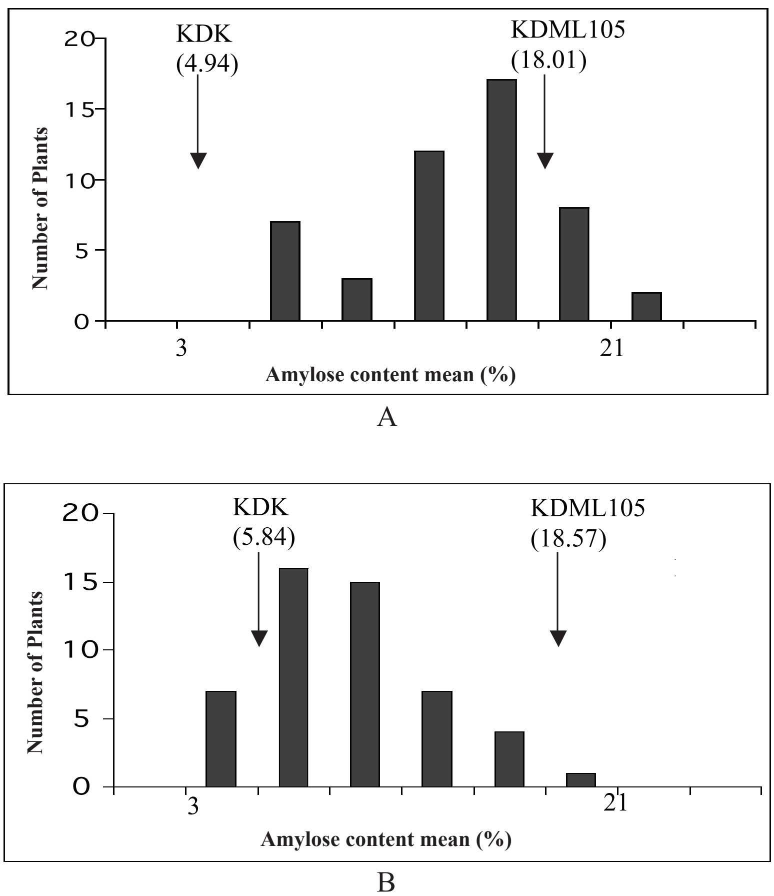
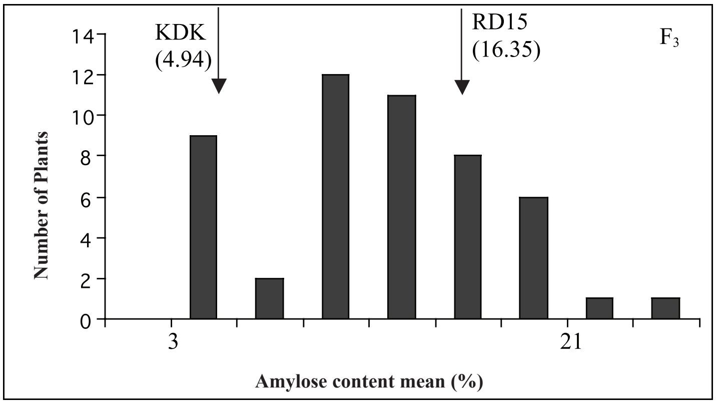


Figure 1. Amylose (%) distribution of rice grain within F_3 (A) and F_4 (B) progenies of a cross between KDML 105 and Kum Doi Saket. Values in parentheses represent amylose content of parents.



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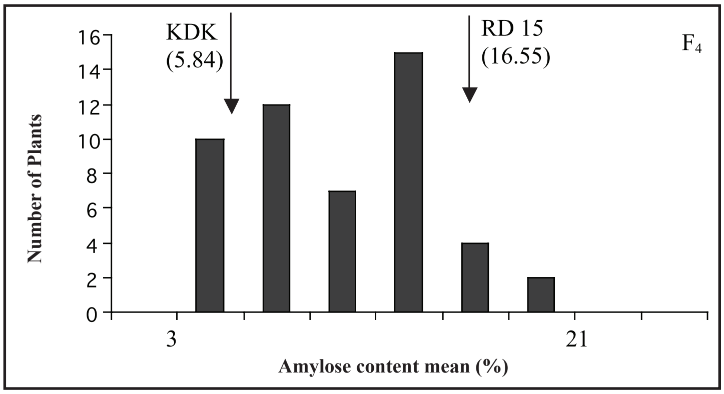

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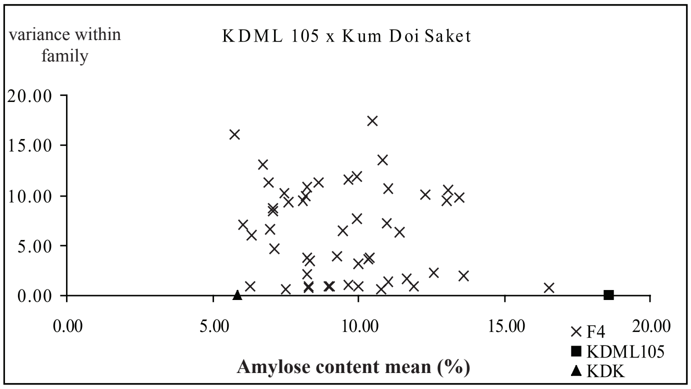
Figure 2. Amylose (%) distribution of rice grain within F_3 (A) and F_4 (B) progenies of a cross RD 15 and Kum Doi Saket. Values in parentheses represent amylose content of parents.



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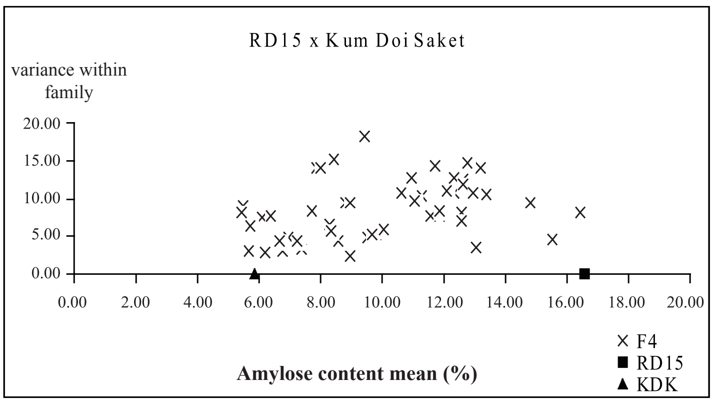

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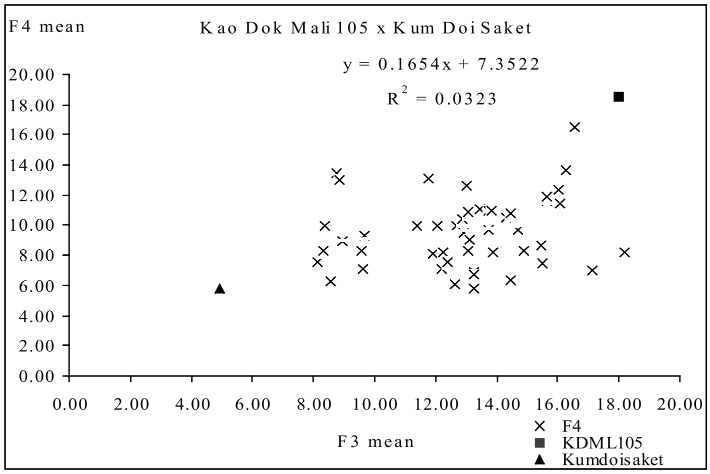
Figure 3. Regression of amylose of within family variance on the family mean of F_4 and parents in two combinations (A: KDML 105 x Kum Doi Saket, B: RD15 x Kum Doi Saket).



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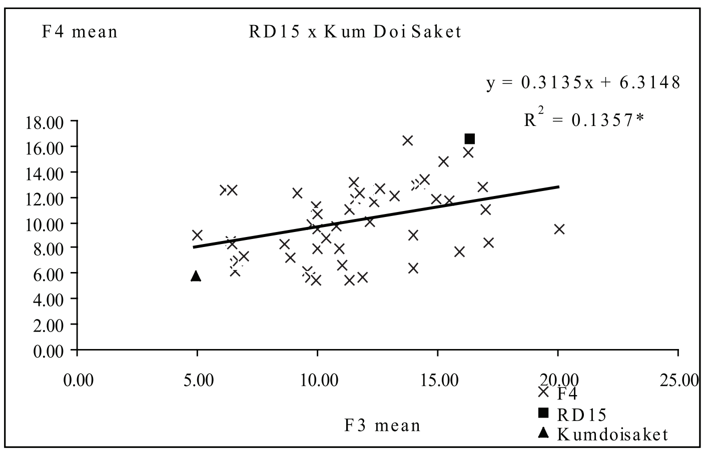

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Figure 4. Regression of F_4 means on F_3 means of amylose content in the two combinations

(A: KDML 105 x Kum Doi Saket, B: RD15 x Kum Doi Saket)

h^2 (RD 15 x Kum Doi Saket) = 4/7 × 0.3135 = 0.179

R (RD 15 x Kum Doi Saket) = $i\sigma\ h_{ns}^2 = 2.065 \times 0.368 \times 0.179 = 0.8690$

DISCUSSION

The small variation obtained in the parental populations when planted with F_3 and F_4 indicated that homogeneity in starch types existed in the endosperm of both glutinous and non-glutinous rice cultivars. This, therefore, brought high segregation of starch types in the F_3 and F_4-derived line populations of the two combinations. In F_3 populations, transgressive segregants were detected, but in F_4 the means were between the two parents' means but approaching the mean value of the higher parent when comparing to the combination's mid-parent value, indicating the additive gene effect with partial dominant action involved in the behavior of genes controlling amylose content. The result confirmed previous research reported by IRRI (1975). High segregation detected in F_3-derived lines in both combinations implied that selection for amylose content in rice can be manipulated efficiently in the early segregating generations. Kumar and Khush (1986) also suggested that when the segregants were attributed to gene dosage effects in the endosperm, selection for amylose level was effectively being done in an early generation. The low value of narrow-sense heritability obtained suggested the influence of environmental factors was high for expressing on amylose content in grain. These results support the work of Juliano and Villareal (1993) who reported that rice grain amylose content was positively related to temperature during seed formation. Thus, in a selection procedure, the methodology to reduce environmental variance must be taken into account. The low value of narrow-sense heritability and a rather low response rate of selection achieved when selection was made in F_4 also suggested that the F_5 population's mean would only narrowly differ from the mean of the F_4 population. This indicated that from an advanced population of F_4, genotypes become homogeneous, and a homogeneity population is thus obtained. Thus, selection for high grain amylose content in late generations would be less efficient.

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