

DOSAGE EFFECTS AT THE R LOCUS ON THE PROPERTIES OF THE STARCH FRACTIONS<sup>1/</sup>

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Previous work in maize has shown that starch from endosperms homozygous for amylose-extender (ae) have an altered amylopectin in addition to increased amylose (Boyer and Preiss, Biochem. & Biophys. Res. Communications 80:169. 1978, and references therein). The starch from cotyledons of peas homozygous for rugosus (r) also contain increased amylose (Hilbert and McMasters, J. Biol. Chem. 162:229, 1946). In view of the similar effects of ae and r on the amylose contents of the starch, similar effects might also be expected in the amylopectin fractions of the starch. Therefore, the properties of the starch fractions of mature cotyledons with 0, 1, and 2 doses of the r allele have been reinvestigated. Starch granules were isolated as previously described for maize starch granules (Boyer et al., Cereal Chem. 83:327, 1976). Fractionation of dispersed starch was accomplished by gel filtration and butanol complexing followed by gel filtration. Two dosage series were investigated: commercial varieties 'Alaska' and 'Progress #9' (and crosses) and experimental lines provided by G. A. Marx. No reciprocal differences were observed so only the properties of a single heterozygote sample will be reported. In addition, no significant differences in the two dosage series were observed so only one will be reported at this time.

Properties of the starches and starch fractions from an r dosage series are shown in Table 1. Although increasing dosage of the r allele increased amylose content of the starch when measured by two methods, Blue Value (iodine absorbance at 615nm) or fraction into size classes, the properties of the amylose fraction appeared unaffected by genotype. Equally important, however, is the decrease in the amylopectin content with increased r dose. In addition, the increasing absorbance maxima and  $A_{660}/A_{540}$  ratio of the iodine spectra of these fractions with r dosage suggest an increasing linearity of the molecules. It is important to note that these methods fully separate the amylopectin from the low molecular weight amylose. Therefore, the amylopectin fractions of the starches examined are qualitatively different.

Table 1. Characterization of starches and starch fractions isolated from mature pea cotyledons of differing gene dosage at the R locus.<sup>a</sup>

Genotype	% Amylose		Iodine spectra					
	Blue Value	Column <sup>b</sup>	Unfractionated starch		Amylopectin		Amylose	
			Amax	A <sub>660</sub> /A <sub>540</sub>	Amax	A <sub>660</sub> /A <sub>540</sub>	Amax	A <sub>660</sub> /A <sub>540</sub>
R/R	32.6	29.8	620	1.30	560	0.74	640	1.74
R/r	36.0	32.5	615	1.25	570	0.78	630	1.72
r/r	66.2	60.5	620	1.53	580	0.81	635	1.74

<sup>a</sup>All data is the average of two or more replications

<sup>b</sup>Bio-Gel A<sub>50,m</sub> (1.5 x 30 cm)

Any model of molecular action of the gene product of the r locus must also account for the above observed qualitative changes in the amylopectin fraction of the starch. Although it is clear that soluble phosphorylase activities are different in R/R and r/r seeds (Williams, PNL 11:36), it is less clear how this difference can account for the observed differences in the amylopectins. We are presently examining the effects of the R locus on other starch metabolizing enzymes, namely starch synthase and branching enzyme.

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