Seed mutants in *Pisum: lam* (*l*ow *am*ylose) a new locus affecting starch composition

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The two *rugosus* loci, *r* and *rb,* are known to have many pleiotropic effects on seed development but their primary effect is on starch biosynthesis (5). Recently, three new *rugosus* loci (*rug-3, rug-4* and *rug-5*) were identified (6) following chemical mutagenesis. These genes all affect starch content and composition and, like *r* and *rb,* cause the dry seed to be wrinkled. A model has been proposed that links the physiological and biochemical changes brought about by a reduction in starch content with the wrinkling of the dry seed (5). This model predicts that an increase in embryo osmotic pressure is required for wrinkling and, in the case of the *rugosus* mutants, such an increase is brought about by a decrease in starch content. Thus mutants which have changes in starch composition or structure without a concomitant decrease in starch content should not possess wrinkled seeds. Hence, to isolate such mutants a screen is required that is based on a phenotype other than the shape of the seed and more directly on the composition of the starch. We chose, therefore, a method derived from the one used to isolate the *amf* (amylose-free) mutant of potato (2) that was based on the colour of the tuber following iodine staining and was related to the properties of the starch polymers.

Starch is composed of two polymers, amylose and amylopectin, which have different staining properties. Amylose, the un- or poorly-branched polymer, normally binds iodine strongly producing an indigo colour, whereas amylopectin, the highly-branched polymer, binds iodine more weakly with a lower absorption maximum for the complex. Consequently, granules from mutant lines with a very low amylose content were expected to stain poorly or with a red-brown coloration, whereas those from wild-type lines with normal amylose content would be expected to stain indigo.

Chemically-mutagenised M_2 and M_3 round-seeded populations of the BC1/RR line (4, 6) were screened for differences in starch composition by transferring granules from an abraded surface of the seed (cotyledons) onto moist glass fibre paper (1). Five lines were identified (SIM 501, 502, 503, 504, 512) which showed segregation for brown or lighter-coloured staining within the population of seed. These putative mutant seed were analysed for starch grain shape, starch amount and composition, and then sown and backcrossed to the parental BC1/RR line. The remaining indigo-staining seed from the population were also analysed and sown. The putative mutants all showed a very low amylose content, but starch amounts similar to indigo-staining seeds in the same population indicating that there was a compensatory increase in amylopectin content. Starch grains from the putative mutants were very characteristic, having large blue cores and a pale periphery when stained with iodine solution. In some granules, this periphery was a reddish-brown colour. The surrounding solution was similarly coloured, which accounts for the coloration when the grains were stained *en masse* on the filters. Presumably there is a difference in the solubilities of the two granule components when stained which accounts for this phenotype, the majority of the blue being retained in the granules. Four lines were recovered following backcrossing (SIMs 501, 502, 503, 512) and one was recovered (SIM 504) after the identification of heterozygous plants in selfed material from the original segregants, all of which were phenotypically wild type for starch grain staining.

Mutants segregating in the F_2 of the backcrosses, or the selfed heterozygote, were again identified by having abnormally staining grains. Furthermore, the putative mutant material which was selfed was found to have inherited the low amylose/normal starch amount and granule phenotype characteristic of the seed identified as mutant in the screen. The pooled values of mutant to WT segregants were 33 seed with abnormal staining out of 140 examined for the F_2 of the backcrosses (Chi-square = 0.15) and 10 out of 50 for the selfed heterozygotes (Chi-square as 0.67) which is consistent with them being monogenic recessive mutations. The lines were also test-crossed to each other and the F_1 seed found to possess starch grains that stained abnormally with iodine, giving the characteristic dark core and light periphery, indicating that the mutants were all at one locus. We have assigned the gene symbol *lam* (*l*ow *am*ylose) to this locus.

Preliminary evidence indicates the lack of the major granule-bound isoform of starch synthase in all but one of these mutants. This characteristic and the low-amylose phenotype indicate that the *lam* mutants are analogous to the waxy mutants of cereals and potato (3) which also have a near-normal amount of starch with a low amylose content in their storage organs. The isolation of the *lam* mutants increases the number of loci affecting starch composition in pea seeds to 6 - *r, rb, rug-3, rug-4, rug-5* and *lam.* The *lam* seed, however, are round unlike the other pea mutants affecting starch production which is in accordance with the original hypothesis relating the wrinkled nature of the seed to its starch content (5). Full details of the isolation and biochemical characteristics of these mutants will be reported elsewhere (1).

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