Internode length in *Pisum.* Interaction of genes *lh^i*, *la* and *cry^S*.

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In peas, a range of internode length mutations that affect either gibberellin (GA) synthesis or the response to GA have been identified. GA synthesis mutants have been identified at the *Le, Na, Ls* and *Lh* loci, and are characterised by a dwarf stature (reduced internode lengths) resulting from a reduction in the level of the biologically active GA_1 in the shoot compared with wild-type plants (5, 9, 10). At the *Lh* locus, two mutant alleles have now been identified: *lh* (7) and *lh^i* (11). The *lh^i* allele also reduces GA levels in the developing seeds, compared with its wild-type progenitor, Torsdag (*Lh*) (S.Swain, J.B. Reid and J.J. Ross, unpublished data). The reduced levels of GA_1 and GA_3 in young ovules is believed to account for the increased seed abortion and reduced seed yield of homozygous *lh^{i}lh^i* plants (11).

Pea genotypes with altered response to GA include the *la cry^S* gene combination. Plants homozygous for both the *la* and *cry^S* alleles exhibit the slender phenotype (long, thin internodes, 1, 2, 8) regardless of endogenous GA levels (3, 6). Hence, the *La* and *Cry* gene-products are thought to act at or after GA perception in the transduction chain linking GA_1 levels to changes in internode length (6).

A GA response mutant that is epistatic to the *lh^i* allele in developing seeds would lend support to the hypothesis that GA's play an important role in seed development Therefore, in an attempt to provide further evidence for a physiological role of the GAs in shoots and developing seeds, the interaction between the *la cry^S* gene combination and the *lh^i* allele was investigated.

Materials and Methods

The pure lines of *Pisum sativum* L. used during this work are held in the collection at Hobart, Australia. The dwarf line NGB5843 (*lh^i La Cry*) was derived from the wild-type tall cv. Torsdag (*Lh La Cry*) by Dr K. K. Sidorova (Novosibirsk, Russia). The slender line 197 (*Lh la cry^S*) was derived from a cross between Hobart line 133 and NGB1766. Lines NGB5843 and 197 are both homozygous dominant at the internode length loci *Le, Ls, Na, Lka, Lkb, Lkc, Lkd, Lv, Lk, Lm* and *Sln.* Further details about the phenotypes and genotypes of these lines can be found in papers 7 and 11.

Plants were grown in a 1:1 (v:v) mixture of vermiculite and 10 mm dolerite chips topped with 2-3 cm of potting mix in 14 cm slim-line pots (2 per pot) or plastic tote boxes (41 x 32 cm). All plants were grown in an 18 h photoperiod and provided with nutrient solution (Aquasol) once a week. Counting of nodes started from the first scale leaf as node 1.

All F_1, F_2, F_3, F_4 and F_5 plants resulting from the cross between lines 197 (*Lh la cry^S*) and NGB5843 (*lh^i La Cry*) were initially grown in a heated glasshouse (9). Some of the F_3 plants were transferred 3 weeks after planting to a controlled environment cabinet with a day/night temperature regime of 25/20°C where light was provided by a mixed fluorescent (Thorn 40W cool white tubes) and incandescent (Mazda 100W pearl globes) source (200 μmol m^{-2} s^{-1} at pot top).

Results and Discussion

The F_1 of the cross between lines NGB5843 (dwarf; *lh*^{i} *La Cry*) and 197 (slender; *Lh la cry*^{S}) had a tall phenotype and the F_2 (Fig. 1) segregated into three classes: tall, dwarf and slender. Seeds from some of the tall F_2 plants (solid circles, Fig. 1) and some of the slender F_2 plants (solid squares, Fig. 1) were grown-on to confirm their genotypes. One phenotypically slender F_2 plant was found to be genetically tall since its progeny segregated to give 5 tall and 1 slender F_3 plant (data not shown). Three phenotypically slender F_2 plants bred true in F_3. The remaining four slender F_2 plants could not be progeny tested because they produced only parthenocarpic pods and no seeds. This is a pleiotropic effect of the slender gene combination (6) and is consistent with these plants having genotype *la cry*^{S}. All seeds from the four dwarf F_2 plants were grown on in the F_3. One dwarf F_2 plant produced in F_3 13 dwarf and 1 slender offspring. Hence, the genotype of this slender F_3 plant was *lh*^{i} *la cry*^{S}, demonstrating that the slender phenotype is expressed on a *lh*^{i} background. This result is similar to that obtained for the *na la cry*^{S} and *le la cry*^{S} genotypes (3, 4, 6) and demonstrates that the *la cry*^{S} gene combination is epistatic to the *na, le* and *lh*^{i} genes in young shoots, and is expressed regardless of GA levels in this tissue. Plants of genotype *lh*^{i} *la cry*^{S} also developed parthenocarpic pods in subsequent generations.

Having established that plants homozygous for *lh*^{i}, *la* and *cry*^{S} have a slender phenotype, the expected F_2 ratio is 45 tall: 15 dwarf: 4 slender. The observed F_2 numbers of 74 tall, 4 dwarf and 7 slender plants (Fig. 1) are not in agreement with the expected ratio (χ^2 = 16.64, P<0.001). However, the number of slender plants agreed with expected results when compared with the total number of F_2 plants (χ^2 for 1:15 = 0.57, P>0.3). In contrast, there was a significant deficiency in the observed number of dwarf compared with tall F_2 plants (χ^2 for 1:3 = 16.42, P<0.001) as observed in other crosses segregating for the *Lh* and *lh*^{i} alleles (11).

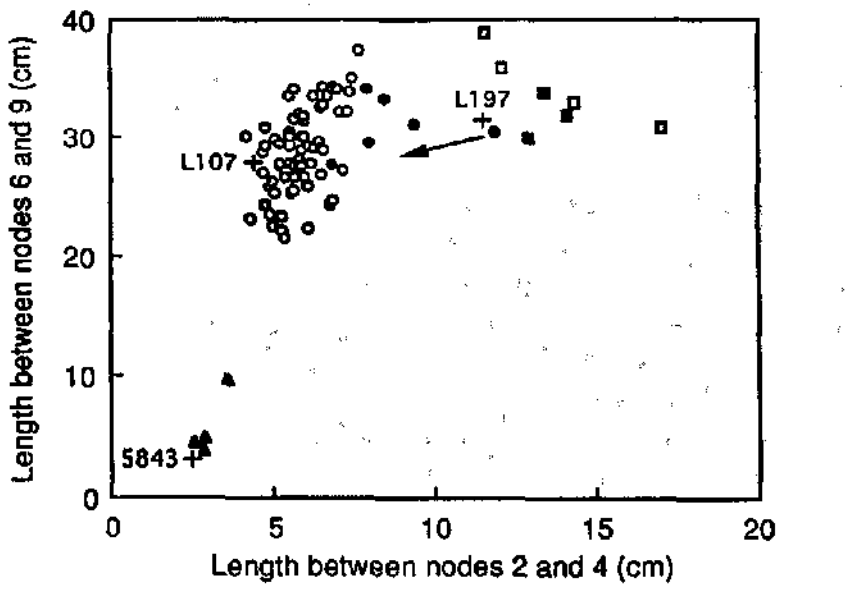

Fig. 1. Internode lengths for the F_2 resulting from a cross between line 197 (*Lh la cry*^{S}) and NGB5843 (*lh*^{i} *La Cry*), showing segregation into slender (□, ■), tall (○, ●) and dwarf (▲) phenotypic classes. Solid symbols represent plants bred-on to confirm their genotype. Means for parental lines 197 and NGB5843, and for the wild-type cv. Torsdag (*Lh La Cry*), are indicated by crosses (n≥5). Photoperiod 18 h.

Eleven of the dwarf F_3 plants, from the family containing 1 slender plant, were allowed to self-pollinate in an 18 h photoperiod with a day/night temperature regime of 25/20°C. No significant deviation from expected results was observed in any of the resulting progeny. Four F_4 families segregated in accordance with a ratio of 3 dwarf to 1 slender plant (totals: 83 dwarf, 26 slender, χ^2 for 3:1 = 0.08, P>0.7). Three F_4 families segregated in accordance with a ratio of 15 dwarf to 1 slender plant (totals: 73 dwarf, 4 slender, χ^2 for 15:1 = 0.15, P>0.7). One F_4 family segregated in accordance with both a 3:1 and a 15:1 ratio (16 dwarf, 2 slender). Three F_4 families bred true.

Seeds possessing genotype $lh^i lh^i$ have previously been shown to weigh less than *Lh*- seeds (11). Since the *la cry*^{s} gene combination is epistatic over the lh^i allele in shoots, the possible epistasis over final seed weight of lh^i seeds was examined. Six dwarf plants from F_4 families containing approximately 1/4 slender segregates were allowed to self-pollinate in a glasshouse during winter (day and night temperatures of between 20-25 and 10-15°C, respectively). The resulting seeds were weighed immediately prior to sowing to determine if the slender F_5 plants (segregating in four families) developed from heavier seeds than the dwarf F_5 plants. However, no significant difference (t=0.41, P>0.5) was found between the weight of seeds which gave rise to slender plants (0.298±0.010 g/seed, n=13) and those which gave rise to dwarf plants (0.293±0.007 g/seed, n=48). Hence, no evidence was found for epistasis of the *la cry*^{s} gene combination over the lh^i allele in developing seeds.

In conclusion, the *la cry*^{s} gene combination is epistatic over the lh^i allele in young shoots. The results support the hypothesis that the slender phenotype (long, thin basal internodes) is independent of endogenous GA levels since the lh^i allele is thought to block GA production before GA_{12}-aldehyde, the precursor to all GAs in peas (11).

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