Internode length in *Pisum: le5839* is a less severe allele than Mendel's *le*

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Mutant alleles at the *le* locus, particularly Mendel's *le* (5, 16), have proved valuable in unravelling the role of gibberellins (GAs) in the control of internode length in *Pisum sativum* (1, 13). The allele *le* partially blocks the conversion of GA_{20} to GA_1 the bioactive gibberellin in peas (1, 2), and consequently confers the dwarf phenotype. Quantification of GA_1 levels in the apical portions of isogenic *LeLe* and *lele* lines (using an internal standard and gas chromatography-mass spectrometry) has shown that tall (*Le Le*) plants typically contain 10-18 times more GA_1 than comparable dwarf plants (14). However, similar determinations on another isogenic pair of lines, Torsdag (tall, *LeLe*) and NGB5839 (dwarf, $le^{5839}le^{5839}$) yielded a somewhat smaller difference in GA_1 level (5-6 fold; 11). This suggests that the mutant allele in NGB5839, le^{5839} (an induced mutation, 3) may be "leakier" than Mendel's *le*. However, this is not supported by the very short internode length of NGB5839 (3). Here we examine this question further at the phenotypic level. The evidence comes from a linkage study in which a gene pair linked to the *le* locus (*V*, normal pods / *v*, sugar pods) was used to monitor the inheritance of the mutant allele present in NGB5839. The *v* and *le* loci are linked (10) with an overall RCV of 12.6 ± 0.47% (4). The effect of a photoperiod extension with incandescent light on internode length in *lele* and $le^{5839}le^{5839}$ plants is also examined.

Materials and Methods

The pure lines used were Nordic Gene Bank line 5839 (NGB5839) (*VV* $le^{5839}le^{5839}$), NGB463 (*vv lele*) and cv. Dippes Gelbe Viktoria (*VV lele*). NGB5839 and Dippes Gelbe Viktoria carry allele *Lf* (minimum flowering node 11) while NGB463 carries lf^a (minimum flowering node 5; 6). NGB5839 was produced by mutagenesis from cv. Torsdag, by Dr K.K. Sidorova (Novosibirsk, U.S.S.R.).

The plants were grown in a heated glasshouse. The day temperature was usually 20-25°C and the night temperature was 15-18°C. The growing medium was a 1:1 mixture of dolerite chips and vermiculite, topped with 3-4 cm of potting mix. For generations F_1 to F_6 the light regime consisted of natural daylight extended with mixed fluorescent (Thorn 40 W cool white tubes) and incandescent (Mazda 100 W pearl globes) light (intensity ca. 30 µmol m^{-2} s^{-1} at pot top) to give a photoperiod of 18 h. Certain F_6 plants were grown in either an 8 h photoperiod (8 h natural light) or a 24 h photoperiod (8 h natural light extended to 24 h with weak incandescent light at an intensity of ca. 3 µmol m^{-2} s^{-1} at pot top). The flowering node is defined as the number of the node bearing the first initiated flower, counting from the cotyledons as zero.

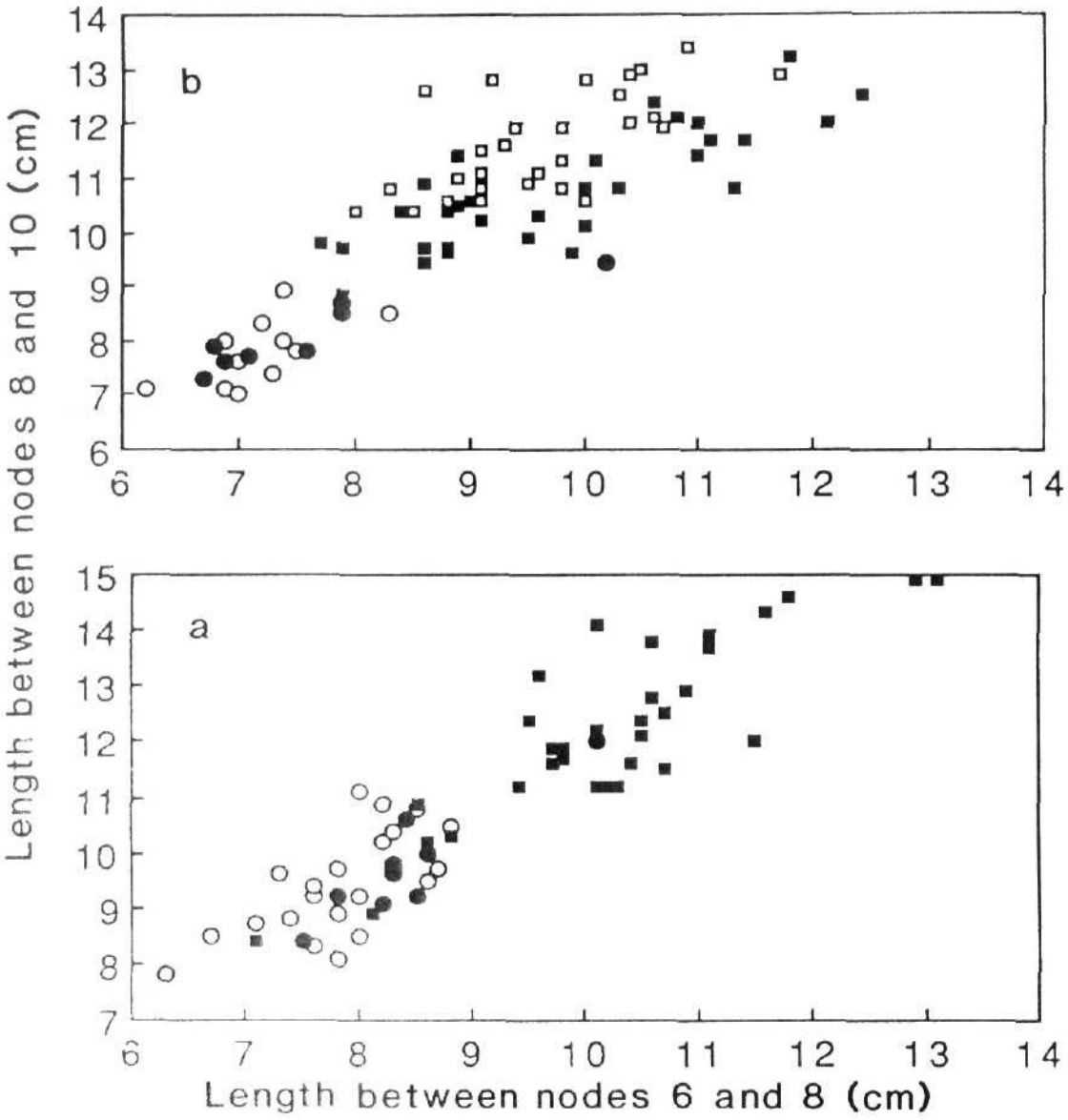

Fig. 1. Stem length between nodes 8 and 10 plotted against stem length between nodes 6 and 8 for F_5 (a) and F_6 (b) plants from cross NGB463 x NGB5839. Key: ○, *vv* plants from *vv* parents; ●, *vv* plants from *Vv* parents; ■, *V* - plants from *Vv* parents; □, *VV* plants from *VV* parents. The F_5 was generated from 4 *vv* and 3 *Vv* F_4 plants, and the F_6 from 2 *vv*, 3 *Vv* and 2 *VV* F_5 plants.

Results

The F_1 of cross NGB463 x NGB5839 was dwarf, as were all plants in subsequent generations. Generations F_3 to F_6 were produced by single plant selection beginning with an F_2 segregate of genotype $lf^a lf^a$ *Vv*. Plants of genotype *vv* (sugar pod) possessed, on average, significantly ($P < 0.001$) shorter internodes than *V*- (normal pod) plants in all generations from F_2 to F_6 (e.g. Table 1, Fig. l, a and b). This suggests that the mutant allele in NGB5839, le^{5839} (which entered the cross linked to *V*), results in longer internodes and is, therefore, a leakier allele than *le*.

Table 1. Stem length (cm) between nodes 6 and 9 for lines NGB463 and NGB5839, and for *Lf- V-* and *Lf- vv* segregates in the F_2 generation of crosses NGB463 x NGB5839 and NGB463 x Dippes Gelbe Viktoria. The data are shown as mean ± SE with n in parentheses. Photoperiod 18 h.

Line or Cross	Genotype	Stem length
NGB463	*lf*^{a} *lf*^{a} *vv*	16.10 ± 0.50 (6)
NGB5839	*LfLf VV*	6.32 ± 0.29 (6)
NGB463 x NGB5839 F_2	*Lf- V-*	10.41 ± 0.35 (50)
	Lf- vv	7.50 ± 0.35 (11)
NGB463 x Dippes Gelbe Viktoria F_2	*Lf-V-*	16.33 ± 0.46 (43)
	Lf- vv	16.18 ± 1.28 (10)

Table 2. Stem length (cm) between nodes 6 and 9 for *le*^{5839}*le*^{5839} and *lele* segregates from cross NGB463 x NGB5839 grown in either an 8 h or a 24 h photoperiod. Data are shown as mean ± SE of 10 replicates.

Genotype	Stem length	
	8 h photoperiod	24 h photoperiod
le^{5839}*le*^{5839}	11.91 ± 0.30	18.01 ± 0.56
lele	8.14 ± 0.17	10.97 ± 0.24

While there was some overlap of internode length values between the presumed *lele* and *le*^{5839}- plants in earlier generations, this was minimal by F_6 (Fig. 1, b). From F_2 to F_6, short plants of genotype *vv* produced only short offspring (e.g. Fig. 1, a and b). Plants of genotype *V*- were usually taller, while the vast majority (e.g. 89% in F_5 and 88% in F_6) of *vv* segregates from *Vv* parents were short. However, several taller *vv* segregates from *Vv* parents were observed; in one such case from the F_4, the progeny was grown and comprised 5 short and 12 taller types (data not shown). This suggests that this F_4 plant was of genotype *vv le*^{5839}*le* (a recombinant). The F_5 generation included 5 short *V*- segregates (Fig. 1, a). The F_6 generation from one of these plants was grown and consisted of 1 short *vv* plant and 13 taller *V*- plants. Thus this F_5 plant was clearly not a recombinant; the reason for its short stature is not clear. However, it is noteworthy that all *VV* F_6 plants possessed considerably longer internodes than all *vv* F_6 plants from *vv* parents (Fig. 1, b).

In contrast to the results from cross NGB463 (*vv lele*) x NGB5839 (*VV le*^{5839}*le*^{5839}) segregation of the gene pair *V/v* was not associated with differences in internode length in cross NGB463 (*vv lele*) x Dippes Gelbe Viktoria (*VV lele*) (Table 1).

Although the number of individuals available for comparison was small, the internode length of heterozygous *le*^{5839}*le* plants was intermediate between that of homozygous *le*^{5839}*le*^{5839} and *lele* plants. For example, in F_3 the mean values for the stem length between nodes 6 and 9 for *le*^{5839}*le*^{5839}, *le*^{5839}*le* and *lele* plants were (in cm) 17.4 ± 1.40 (n=3), 14.43 ± 0.92 (n=4) and 11.67 ± 0.03 (n=3), respectively. It therefore appears there is very little dominance of either allele over the other.

When *le*^{5839}*le*^{5839} and *lele* plants with a similar genetic background (F_6 segregates descended from a single F_4 plant from cross NGB463 x NGB5839) were grown in 8 h and 24 h photoperiods, the internodes of both genotypes were longer in 24 h (8 h natural light plus 16 h incandescent light) than in 8 h (Table 2), in accordance with previous results (e.g. 8, 11). However, the response shown by *le*^{5839}*le*^{5839} plants (a 51 % increase) was greater than that of *lele* plants (a 35% increase, Table 2). There was no evidence that this difference was due to factors other than the genotype at the *le* locus. For example, the flowering behaviour of both *le*^{5839}*le*^{5839} and *lele* plants was similar. Both groups initiated flowers at nodes 6-8 in both photoperiods and in the 24 h photoperiod flower development ensued either at the node of initiation or at one node higher. In the 8 h photoperiod substantial flower abortion occurred, but to a similar extent in both genotypes. (The flowering genotype of these plants therefore appears to be *lf*^{a} *lf*^{a} *EE SnSn DneDne,* see 7).

Discussion

In cross NGB463 (*vv lele*) x NGB5839 (*VV le*^{5839}*le*^{5839}), *V le*^{58399}- segregates, on average, possessed longer internodes than did *vv lele* segregates. In NGB5839, GA_1 levels were not reduced to the same extent as in *lele* lines (compared with isogenic *LeLe* lines; 11,14).

Table 3. Effect of segregation for the *le*^{5839}/*le* pair of alleles on the number of seeds per plant in the F_5 and F_6 generations of cross NGB463 x NGB5839. The data are shown as mean ± SE with n in parentheses.

Generation	Genotype	
	le^{5839}-	*lele*
F_5	28.47 ± 0.78 (30)	23.55 ± 0.60 (33)
F_6	18.53 ± 0.36 (59)	15.63 ± 0.41 (19)

Considered together, these results strongly suggest that allele *le*^{5839} imposes a less severe block on GA_1 biosynthesis than does *le*. Clearly *le*^{5839} is a different allele from *le* and the designation *le*^{5839} should remain to indicate this. On the basis of measurements of true-breeding *le*^{5839}*le*^{5839} and *lele* F_6 families (descended from a single F_4 plant) allele *le*^{5839} increases stem length between nodes 6 and 9 by ca. 40% in an 18 h photoperiod, compared with *lele* plants. The paradoxial aspect of the present and previous work is that NGB463 (*lele*) possesses much longer internodes than NGB5839 (*le*^{5839}*le*^{5839}) (Table 1). Clearly the two pure lines differ with respect to other loci which affect internode length (e.g. possibly at the *Cry* locus). The presence of *lf*^{a} in NGB463 would most likely also result in longer internodes. However, this cannot alone explain the stature of NGB463, since this line was considerably taller than *lf*^{a} *lf*^{a} *lele* (and *lf*^{a} *lf*^{a} *le*^{5839}-) segregates in F_2-F_6 (data not shown).

The existence of at least one proven recombinant (genotype *le*^{5839} *le vv;* phenotype long internodes, sugar pods) in cross NGB463 x NGB5839 strongly indicates that the short stature of *vv* plants in F_2 to F_6 of this cross is not due to a pleiotropic effect of *v*. This is confirmed by the lack of effect on internode length of segregation for *V/v* in cross NGB463 x Dippes Gelbe Viktoria, which, furthermore, is consistent with the presumption that both NGB463 and Dippes Gelbe Viktoria possess the "normal" *le* allele.

The identification of allele *le*^{5839} increases to four the number of alleles at the *le* locus (in order of increasing length, *le*^{d}, *le, le*^{5839} and *Le,* see 12). It seems possible that allele *le*^{5839} may be of some agronomic value since it has the effect of increasing internode length compared with *le.* In this context it is of interest that in the F_5 and F_6 generations of cross NGB463 x NGB5839 (on a *lf*^{a} *lf*^{a} *EE SnSn DneDne* genetic background in an 18 h photoperiod, see 9), *le*^{5839}- plants produced ca. 20% more seeds than did *lele* plants ($P < 0.001$; Table 3). However, this effect cannot for certain be attributed to the difference at the *le* locus since the plants also differed at the *v* locus.

It is well known that in pea internode elongation is enhanced by photoperiod extensions with incandescent or far-red-rich light (3, 8, 11, 15). It has also been shown (3, 11) that line NGB5839 (*le*^{5839}*le*^{5839}) responds to such extensions to a lesser extent than does its tall (*LeLe*) progenitor, Torsdag. In contrast, some *le* dwarf lines or selections are at least as responsive as Torsdag (Table 1 from 8; Torsdag = L107). However, in the present study *le*^{5839}*le*^{5839} plants responded to an incandescent photoperiod extension to a greater extent than did *lele* plants (Table 2). Therefore the difference in responsiveness between NGB5839 and *le* lines referred to above is probably attributable to differences in genetic

background. The results shown in Table 2 support the suggestion (3) that on a constant genetic background the response to an incandescent (or far-red-rich) photoperiod extension decreases as the severity of the genetic block in GA_1 biosynthesis increases (see also 11).

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