A more severe mutant allele at the *ls* locus

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During our ethylmethane sulphonate (1%) mutagenesis program (7), a severely dwarfed (nana) mutant, AF51, was isolated. The parental cultivar was Torsdag, a tall, quantitative long day pea. The AF51 mutant responded well to applied gibberellin A_3 (GA_3), and indeed treatment of young seedlings with GA_3 was required in order to obtain a reasonable seed yield (5 - 10 seeds per plant). This suggested the mutant might be deficient in GA_1, the biologically active gibberellin in peas (5). Currently, there are mutations at four described loci that block various steps in GA_1 biosynthesis (Fig. 1) and lead to a dwarf GA-responsive phenotype in peas; *le* which blocks 3P-hydroxylation of GA_{20} to GA_1 (3), *na* which appears to block the conversion of 7α-hydroxy-kaurenoic acid to GA_{12} - aldehyde (2), *lh* which blocks the oxidation steps from *ent*-kaurene to *ent*-karenoic acid (6) and *ls* which blocks the conversion of geranyl-geranyl pyrophosphate (GGPP) to copalyl pyrophosphate (CPP) by reducing kaurene synthetase A activity (1).

The cross Torsdag x AF51 produced a wild type tall F_1. The F_2 segregated to give 27 tall : 8 nana progeny, in good accordance with a 3 : 1 segregation ($\chi^2_1 = 0.09$) (Fig. 2). This indicates that a single gene recessive mutation caused the mutant AF51 phenotype.

Allelism tests were conducted between the new mutant, AF51, and standard lines possessing four known GA-synthesis mutations (NGB5839, allele *le-3;* NGB1766, allele *na-1;* K511, allele *lh-1,* HL181, allele *ls-1).* All mutations were on a Torsdag genetic background except *na.* The F_1 plants of all crosses were wild type (tall) in phenotype except for the F_1 plants of cross AF51 (nana) x HL181 (dwarf), which were dwarf in stature. The F_2 of cross AF51 x HL181 segregated to give 41 dwarf: 19 nana progeny, again in agreement with a 3 : 1 segregation ($\chi^2_1 = 1.4$) (Fig. 3).

The results indicate that the AF51 mutant possesses a mutation at the *ls* locus. Because two mutations at this locus have previously been described, *ls-1* (formerly known as ls^{K202}) from cv. Torsdag by Dr K. Sidorova (4), and *ls-2* (formerly known as ls^{M26}) from cv. Dippes Gelbe Viktoria by Professor W. Gottschalk (4), the mutant *ls* allele in AF51 has been designated *ls-3*. The *ls-1* and *ls-2* mutations are not markedly different in severity because no clear segregation in the F_2 was observable when the two mutants were crossed. In the present cross, *ls-1* x *ls-3* (HL181 x AF51; Fig. 3), the clear segregation in the F_2 indicates that *ls-3* is a more severe allele than *ls-1*. Previous genetic analyses had suggested that the *ls-1* allele was leaky (i.e. that it did not completely block GA biosynthesis) because the double mutant, *ls-1 lh-1,* was shorter than either single mutant (4). However, existance of an alternative synthesis pathway or another gene coding for kaurene synthetase A activity could not be ruled out. The discovery of a more severe mutation at the *ls* locus confirms the suggestion that *ls-1* is a leaky mutation. The *ls-3* allele is also recessive to both the wild type *Ls* allele and the less severe *ls-1* allele. The new *ls-3* allele should prove useful in analysing the molecular action of this gene, a question already under active examination (1).

1. Ait-Ali, T., Swain, S.M., Reid, J.B, Sun, T. and Kamiya, Y. 1997. Plant J. (in press).
2. Ingram, TJ. and Reid, J.B. 1987. Plant Physiol. 83:1048-1053.

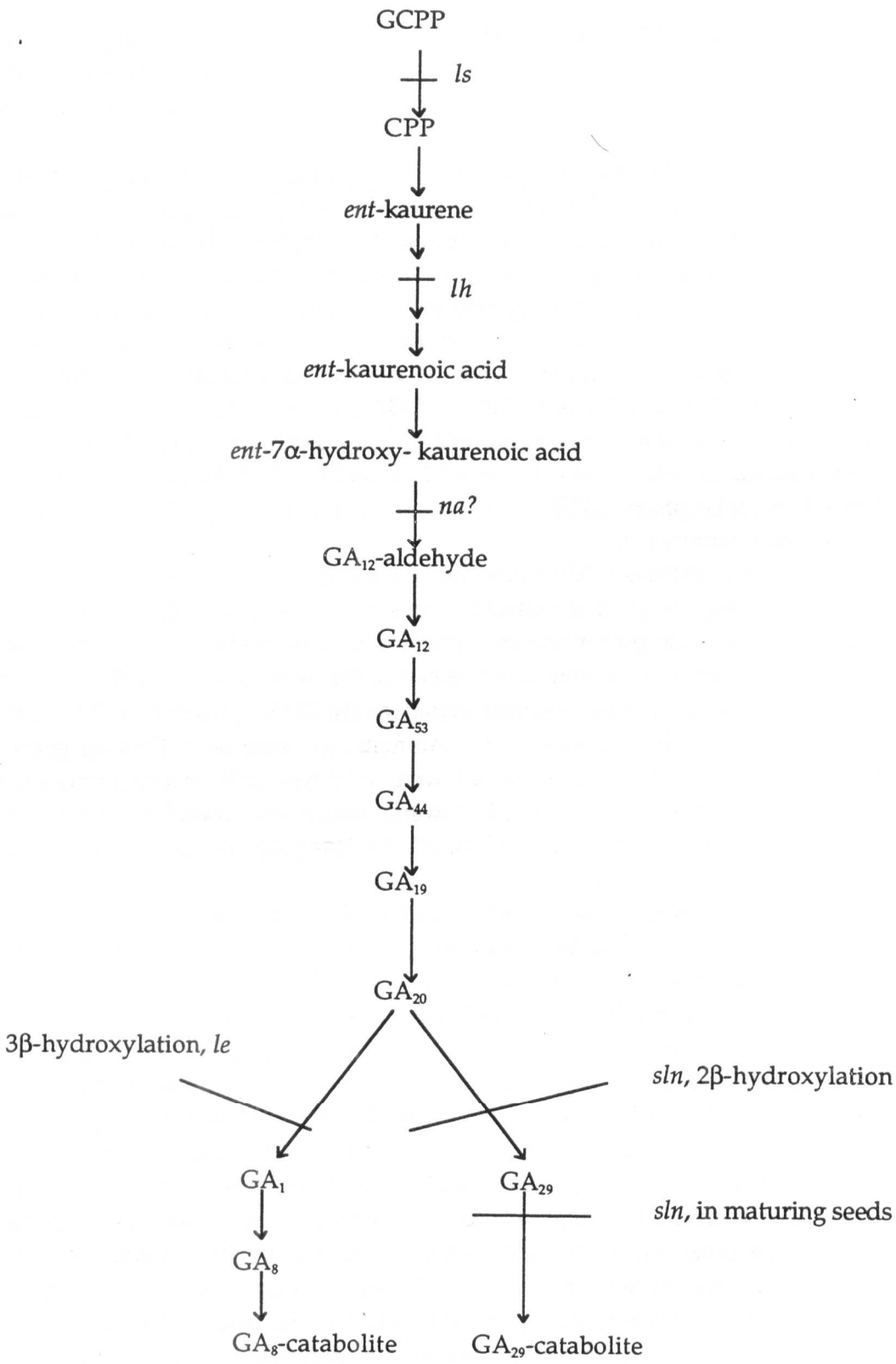

Fig. 1. The dominant GA biosynthetic pathway in the shoots of peas and the sites of action of the GA-biosynthesis mutations (1, 2, 3, 4, 6).

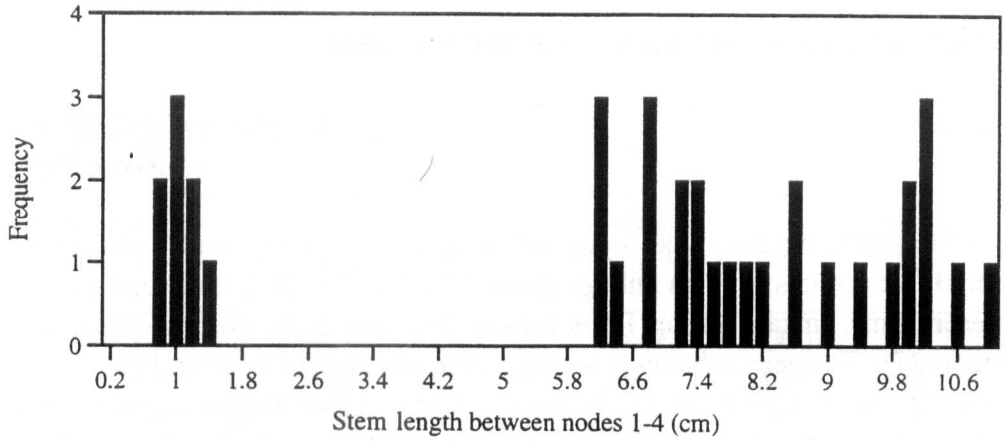

Fig. 2. Distribution of stem lengths between nodes 1 and 4 (to the nearest 2 mm) for the F_2 of the cross Torsdag x AF51. All plants were grown in a glasshouse under an 18 h photoperiod.

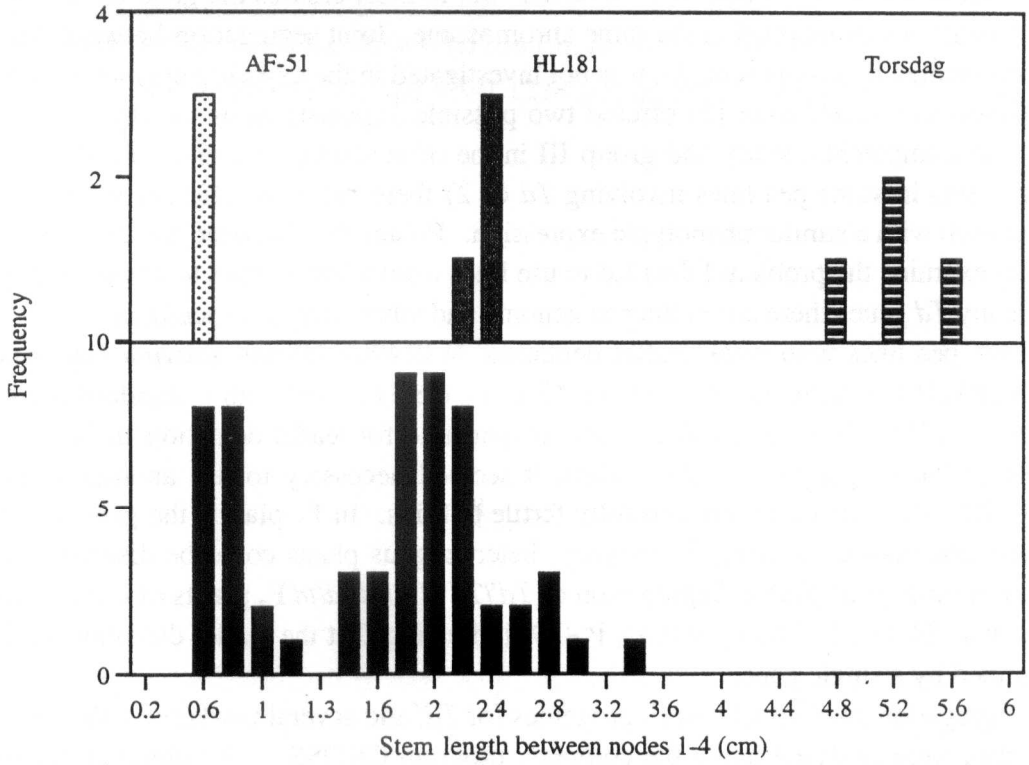

Fig. 3. Distribution of stem lengths between nodes 1 and 4 (to the nearest 2 mm) for parental lines HL181 (dwarf) and AF51 (nana) and the F_2 of the cross HL181 x AF51. As a control, Torsdag (tall) stem lengths are also shown. All plants were grown in a glasshouse under an 18 h photoperiod.

3. Ingram, T.J., Reid, J.B., Murfet, I.C., Gaskin, P., Willis, C.L. and MacMillan, J. 1984. Planta 160:455-463.
4. Reid, J.B. 1986. Ann. Bot. 57:577-592.
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6. Swain, S.M., Ross, J.J., Reid, J.B. and Kamiya, Y. 1995. 15th Int. Conf. Plant Growth Substances, Minneapolis, USA. Abstract 115.
7. Weller, J.L., Terry, M.J., Reid, J.B. and Kendrick, R.E. 1997. The phytochrome-deficient *pcd2* mutant of pea is unable to convert biliverdin IXα to 3Z-phytochromobilin. Plant J. (in press).