Internode length in *Pisum*

A new, slender mutant with elevated levels of C\textsubscript{19} gibberellins

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Received 20 April; accepted 18 June 1992

Abstract. A new, elongated mutant of garden pea (*Pisum sativum* L.) is described, and shown to be conferred by a recessive allele of a new gene, *sln*. At the seedling stage, the mutant resembles the previously described slender type (*genotype* *la cry*\textsuperscript{a}), possessing markedly longer basal internodes than the wild-type. Furthermore, as for *la cry*\textsuperscript{a} plants, application of gibberellin (GA)-biosynthesis inhibitors to the dry seeds (before sowing) did not markedly affect internode length in the mutant. However, the inheritance of the new slender phenotype is unusual, since in crosses between *sln* and *Sln* plants the mutant phenotype is absent in the F\textsubscript{2} generation, reappearing in the F\textsubscript{3}. Young shoots possessing the new slender phenotype (*sln*) contained much higher levels of GA\textsubscript{1}, GA\textsubscript{8}, GA\textsubscript{20} and GA\textsubscript{29} than did wild-type shoots. Mature, near-dry seeds from slender plants contained very high levels of GA\textsubscript{20}, marginally more GA\textsubscript{29}, and very little (if any) GA\textsubscript{29}-catabolite, compared with seeds harvested from wild-type (*Sln*) plants. It is suggested that *sln* may impair the catabolism of GA\textsubscript{20} in maturing seeds. As a result, GA\textsubscript{20} accumulates and on germination may move into the seedling where it is converted to GA\textsubscript{1}, promoting elongation growth. A model is proposed to explain the inheritance of the *sln* phenotype and its physiological implications are discussed. The new *sln* slender mutation has a different mode of action from the established *la cry*\textsuperscript{a} slender gene combination.

Key words: Gibberellin levels - Internode length - Material effect (genotype) - Mutant (stem elongation) - *Pisum* (mutant)

Introduction

Elongated mutants are rare by comparison with dwarf mutants, but have been isolated in a number of species, including tomatoes (Koornneef et al. 1985; López-Juez et al. 1990), *Arabidopsis* (Koornneef et al. 1980; Chory et al. 1989), peas (Potts et al. 1985; Reid and Ross 1988), cucumber (Adamse et al. 1987), *Brassica rapa* (Rood et al. 1990a, b) and *Sorghum bicolor* (Beall et al. 1991). The enhanced elongation has been attributed to several causes, including gibberellin (GA) over-production (Rood et al. 1990a; Beall et al. 1991), gibberellin hypersensitivity (Reid and Ross 1988) and deficiency of one form of phytochrome (Koornneef et al. 1985; Parks et al. 1987; Peters et al. 1991; López et al. 1992). Such mutations have been of considerable use in exploring the control of plant development and in particular for defining a regulatory role for the gibberellins and phytochrome (see Reid 1990).

In the garden pea (*Pisum sativum* L.) there are two well-defined mutant gene systems which confer an elongated phenotype. Firstly, the *lv* mutant (Reid and Ross 1988) shows an enhanced response to GA\textsubscript{1}, the major native GA with biological activity in this species (Ingram et al. 1984, 1986). In *lv* plants photomorphogenetic responses are impaired, possibly due to a lesion in the transduction chain for light-stable phytochrome (Nagatani et al. 1990). Secondly, the duplicate gene combinations *la cry*\textsuperscript{a} and *la cry*\textsuperscript{c} result in the elongated slender and *crypto* phenotypes, respectively (de Haan 1930; Rasmussen 1927; Reid et al. 1983). Slender (*la cry*\textsuperscript{a}) plants behave phenotypically as if saturated with biologically active GAs. However, Potts et al. (1985) and Ingram and Reid (1987) obtained evidence that *la cry*\textsuperscript{a} plants do not contain elevated levels of endogenous GAs. Instead it has been suggested that the *la cry*\textsuperscript{a} gene combination may act by modifying the GA-receptor system, although action at some step beyond this point cannot be precluded (Potts et al. 1985).

In the present paper we show that a new, elongated slender mutant in peas possesses elevated levels of several
GAs, thus differentiating this type from the la cry*, la cry*, and lb mutants. The inheritance of the mutation is examined and a model is developed to explain its physiology.

Materials and methods

*Plant material.* The pure lines of peas used during this work are held in the collection at Hobart. The new, slender mutant, NGB6074, was derived by J. Jarawanski, Poyon, Poland. Other internode-length lines used during this work were cv. Torsdag (wild-type, tall internode-length phenotype), line 177* (cryptotal, la cry*), line 197 (slender, la cry*) and NEU3 (tall, le cry* lb). These lines are homozygous recessive for the genes indicated and homozygous for the dominant wild-type genes at the other internode-length loci. Further details about the genotypes and phenotypes of these lines may be found in Reid and Ross (1988, 1989) and Murfet (1988). For comparisons of endogenous GA levels, wild-type (tall) and slender (NGB6074-type) plants produced from advanced (B2, B3) generations from the backcross (NGB6074 × Torsdag F1) × NGB6074 (female) were harvested.

Growing conditions. All plants were grown in a heated glasshouse as previously described (Reid and Potts 1986). Nodes were counted starting from the cotyledons as zero. The natural photoperiod was extended to 18 h with light from mixed fluorescent (Thorn, 40-W cool-white tubes) and incandescent (Mazda 100–W pearl globes) lamps (~30 μmol m–2 s–1 at pot top).

The GA-synthesis inhibitors, AMO1618 (2-isopropyl-4-dimethoxy-amino-5-methylphenyl-1-piperidine-carboxylate methyl chloride; 100 μg) and paclobutrazol (1-[4-chlorophenyl]-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-penta-3-ol; 20 μg) were applied to the dry nicked seeds in 5 μl of ethanol before planting.

Extraction and quantification of GAs. Harvest details are shown in Tables 1, 2 and 3. Harvested material was immersed in cold (~–20 °C) methanol and placed in a freezer. After homogenisation, GAs were extracted for 24 h at 4 °C. The extracts were filtered and the residue washed with 80% methanol. Internal standards were then added to the extracts. The internal standards used were [17,17-2H2]GA1, [17,17-2H2]GA2o, [17,17-2H2]GA19, [17,17-2H2]GA8, (provided by Professor L. Mander, Australian National University, Canberra) and [17,17-13C,2H2]GA2o (provided by Professor B.O. Phinney, University of California, Los Angeles, USA). For certain GAs (e.g. GA2o) the use of Sep-Pak C18 cartridges provided sufficient purification prior to analysis by gas chromatography-selected ion monitoring (GC–SIM). In other cases, purification was by solvent partitioning, Sep-Pak C18 cartridges or high-performance liquid chromatography (HPLC), or a combination of these techniques, which have been described previously (Reid et al. 1990). The GC–SIM procedure followed Reid et al. (1991). Samples containing GAs were analysed as the methyl ester trimethylsilyl ethers. Extracts were methylated in a 4:1 mixture of etheral diazomethane and methanol, dried, and then trimethylated at 60 °C for 10 min in 10 μl of bis(trimethylsilyl)trifluoroacetamide, with 3 μl of dry pyridine to aid dissolution. The ion pairs monitored for quantification of the respective GAs were: 506/508 (GA1), 594/596 (GA2o), 434/436 (GA19), 418/420 (GA2o), 506/507 (GA19) and GA20 and GA44 (GA20). Additional ions were monitored to confirm identification. Endogenous GA levels were calculated as described by Lawrence et al. (1992). For comparison of GA2o-catabolite levels in seeds of wild-type and slender plants, the intensities of the molecular ions of two forms of derivatised GA2o-catabolite were compared with the intensity of the molecular ion (508) corresponding to a known amount of [2H2]GA1 (Table 3).

Results

Phenotype of the mutant line NGB6074. Line NGB6074 has long, thin basal internodes (Fig. 1) and at the young seedling stage is similar in overall appearance to the slender types described by de Haan (1927, 1930). These internodes are up to 3 times as long as internodes from comparable wild-type tall plants. Slender plants of genotype la cry* have been shown to be insensitive to the application of GA-synthesis inhibitors and possess long internodes regardless of endogenous levels of the active gibberellins, GA1 (Potts et al. 1985; Ingram and Reid 1987). The internode length of line NGB6074 was likewise not markedly altered by the application of the GA-synthesis inhibitors, AMO1618 or paclobutrazol to the dry seed before planting (Fig. 2). However such treatment caused a dramatic reduction in comparable wild-type plants of cv. Torsdag (Fig. 2). This indicates a distinct similarity at the phenotypic level between the mutant line NGB6074 and the standard slender types (Potts et al. 1985; Ingram and Reid 1987).

Fig. 1. Eight-day-old seedlings of the new slender mutant (left) shown with wild-type pea seedlings (right). The seedlings are segregates from an advanced generation from the backcross (NGB6074 × Torsdag F1) × NGB6074 (female).

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Table 1. Harvest details of 10-d-old pea seedlings grown for quantification of GA levels. The apical portion consisted of all tissue above node 3. Photoperiod 18 h.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Height from soil to tip of plant (cm)</th>
<th>Number of expanded leaves</th>
<th>Number of plants</th>
<th>Plant portion</th>
<th>FW of harvested tissue (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>6.0 ± 0.3</td>
<td>4.0 ± 0.1</td>
<td>33</td>
<td>Apical</td>
<td>9.14</td>
</tr>
<tr>
<td>Slender</td>
<td>17.5 ± 0.5</td>
<td>3.9 ± 0.1</td>
<td>55</td>
<td>Internode 2–3</td>
<td>2.27</td>
</tr>
</tbody>
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