The Pea DELLA Proteins LA and CRY Are Important Regulators of Gibberellin Synthesis and Root Growth[W][OA]

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The theory that bioactive gibberellins (GAs) act as inhibitors of inhibitors of plant growth was based originally on the slender pea (*Pisum sativum*) mutant (genotype *la cry-s*), but the molecular nature of this mutant has remained obscure. Here we show that the genes *LA* and *CRY* encode DELLA proteins, previously characterized in other species (Arabidopsis [*Arabidopsis thaliana*] and several grasses) as repressors of growth, which are destabilized by GAs. Mutations *la* and *cry-s* encode nonfunctional proteins, accounting for the fact that *la cry-s* plants are extremely elongated, or slender. We use the *la* and *cry-s* mutations to show that in roots, DELLA proteins effectively promote the expression of GA synthesis genes, as well as inhibit elongation. We show also that one of the DELLA-regulated genes is a second member of the pea GA 3-oxidase family, and that this gene appears to play a major role in pea roots.

It is well known that primary root growth is strongly influenced by the plant hormone GA (Davies, 2004). For example, the application of bioactive GA to roots treated with the growth inhibitor ancymidol completely restored growth to that of the untreated plants (Tanimoto, 1991). Yaxley et al. (2001) established the importance of GAs for root growth in peas (*Pisum sativum*) by using a variety of GA-deficient mutant plants. In the *na-1* mutant, for example, root GA_1 levels, and root elongation, were significantly reduced compared with wild-type plants, and when the GA_1 content was restored to wild-type levels, so too was root elongation.

The GAs act by destabilizing the growth inhibitory DELLA proteins (Peng et al., 1997; Harberd et al., 1998; Silverstone et al., 2001; Alvey and Harberd, 2005). In other words, GA acts as an "inhibitor of an inhibitor" (Harberd et al., 1998). Interestingly, there is also evidence that DELLA proteins promote the biosynthesis of active GAs. For example, in the Arabidopsis (*Arabidopsis thaliana*) DELLA mutant *rga*, the expression of the biosynthesis gene *GA4* is reduced, indicating that high DELLA protein levels are associated with an up-regulation of GA synthesis genes (Silverstone et al., 2001). More recently Zentella et al. (2007) provided evidence that the Arabidopsis GA synthesis genes *GA3ox1* (*GA4*) and *GA20ox2* are direct DELLA targets.

DELLA proteins display conserved amino acid sequences among both dicot (Arabidopsis) and monocot (rice [*Oryza sativa*], wheat [*Triticum aestivum*], and barley [*Hordeum vulgare*]) species (Silverstone et al., 1998; Gubler et al., 2002). However, the available evidence indicates greater redundancy in dicots compared with monocots (Ikeda et al., 2001; Thomas and Hedden, 2006). There have been five DELLA genes isolated from Arabidopsis (*GAI*, *RGA*, *RGL1*, *RGL2*, and *RGL3*), yet only one in rice (*SLR1*), barley (*SLN1*), and maize (*Zea mays*; *D8*; Peng et al., 1997, 1999; Silverstone et al., 1998; Ikeda et al., 2001; Chandler et al., 2002; Gubler et al., 2002), with the possibility of another DELLA gene in maize (*D9*; accession no. ABI84225). It should be noted, however, that DELLAs have been studied in fewer dicot model species than in monocot species. To date, DELLA-encoding genes from pea have not been reported, even though observations on the slender phenotype of pea triggered the early suggestion that GA acts an inhibitor of an inhibitor (Brian, 1957). The elongated slender phenotype, conferred by the gene combination *la cry-s*, has long been considered to show constitutive GA signaling (Potts et al., 1985), but the exact nature and function of the *LA* and *CRY* genes have not been reported before now.

In this investigation we identify DELLA-encoding genes and their associated mutants from pea. We then use those mutants to show that in roots DELLA proteins promote the expression of GA synthesis genes and inhibit the expression of GA deactivation genes. We also report on the discovery of a previously unidentified GA 3-oxidase gene (Fig. 1) from pea.

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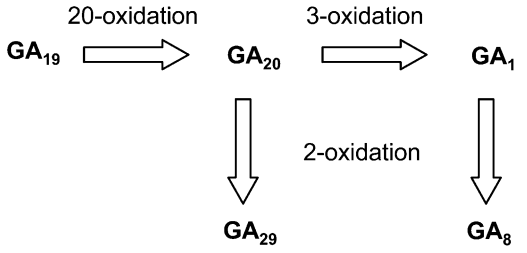

Figure 1. Late stages of GA biosynthesis in vegetative pea plants.

RESULTS

LA and *CRY* Encode DELLA Proteins

Although there has been much work on the involvement of DELLA proteins in shoots, there has been less emphasis on roots. We sought to clone pea DELLA genes to study their involvement with GAs in the regulation of root growth. Two partial sequences from PCR (see "Materials and Methods") were used to probe a pea-shoot complementary DNA (cDNA) library. Full-length clones, selected on the basis of a conserved amino-terminus, were obtained in both cases, and sequence analysis showed that they both encode DELLA-like proteins. Sequencing of PCR products from genomic DNA from mutant genotypes showed that the base sequence of one of the two clones was altered in *cry-s* and *cry-c* plants, whereas the other was altered in *la* plants (Fig. 2). The mutant sequence altered in *la* plants cosegregated with the slender phenotype in a progeny in which *LA* and *la* segregated on a *cry-s* background (Supplemental Fig. S1). On this background, *la* segregates were immediately recognizable as slender plants whereas plants carrying at least one *LA* allele were wild type in appearance. These data suggest that the first of the two cloned DELLA genes is *LA*.

In a separate cross segregating for both *la* and *cry-s*, each of the seven slender (*la cry-s*) F_2 plants carried the mutant form of the second DELLA gene, as well as the mutant form of the first DELLA gene (*LA*). The remaining 52 F_2 plants were tall (wild type) and thus carried *LA* and/or *CRY*. Because of the duplicate nature of *LA* and *CRY*, segregation for *cry-s* cannot be followed phenotypically in plants carrying *LA*. However, in this cross, *cry-s* was segregating in coupling phase with a tightly linked and easily seen leaf-wax marker (*wa*). Within the tall F_2 plants, an RFLP in the second sequence cosegregated with *wa* in 50 of the 52 plants. These data, taken together, strongly indicate that the second DELLA gene corresponds to *CRY*. Of 59 plants genotyped in this F_2, clearly two plants were recombinant between *CRY* and *WA*. This corresponds to a recombination frequency of approximately 3% (Stevens, 1939). In turn, this recombination frequency agrees well with the previously reported value for linkage between *WA* and *CRY* of 2.0 ± 1.1 (maximum likelihood method; Lamm, 1947). In a third cross, there was 100% cosegregation of *wa* and the second sequence (33 plants genotyped).

The predicted LA protein (Supplemental Figs. S1 and S2) is 56% identical (69% similar) to AtRGA1, 55% identical (69% similar) to AtGAI, and 54% identical (67% similar) to CRY. The predicted CRY protein (Supplemental Figs. S1 and S2) is 60% identical (71% similar) to AtRGA1 and 64% identical (77% similar) to AtGAI. Furthermore, in accordance with other known DELLA proteins, LA and CRY both contain the DELLA motif, the TVHYNP motif, the Leu zipper, the VHIID domain, and the LXXLL motif (Fig. 2; Ikeda et al., 2001; Itoh et al., 2002).

The nature of the *la* and *cry-s* mutations was established by comparing the *LA* and *CRY* DNA sequences with those of the *la* and *cry-s* mutants. The *la* mutant was found to result from a 190-bp insertion at position Gln-85 (Fig. 2), and the *cry-s* mutation involves a frame-shift deletion at position 152 (Fig. 2). Both of these mutations are, therefore, predicted to encode nonfunctional proteins as a result of the out-of-frame stop codons. Another mutation in *CRY*, *cry-c*, involves a G to A substitution at base 583, which results in a Gly to Gln substitution in the predicted protein. This, in turn, results in a reduced (but not abolished) capacity to inhibit growth; *la cry-c* plants are shorter than *la cry-s* plants (Reid et al., 1983).

As in the case of other multigene families, it would be expected that the gene pair, *LA* and *CRY*, arose from gene duplication. In fact, this gene pair is one of the first described examples of duplicate genes (Rasmusson, 1927). Interestingly, however, the duplication event

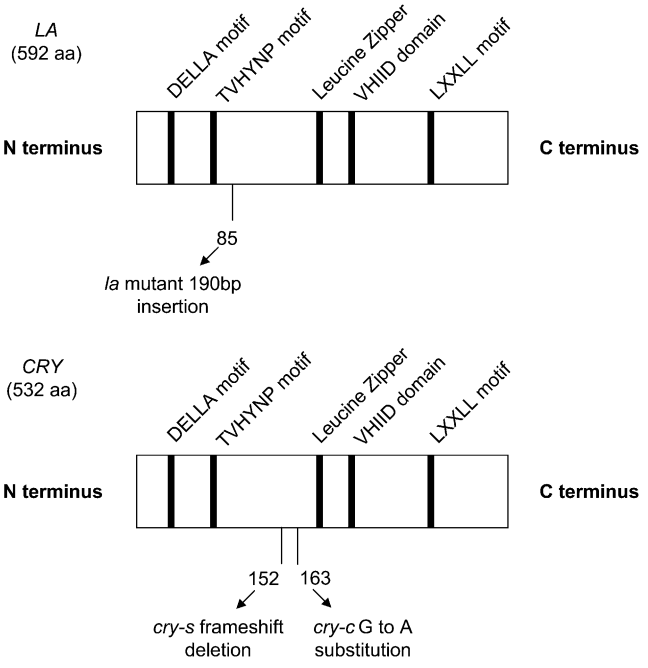

Figure 2. Structure of the genes *LA* and *CRY*.

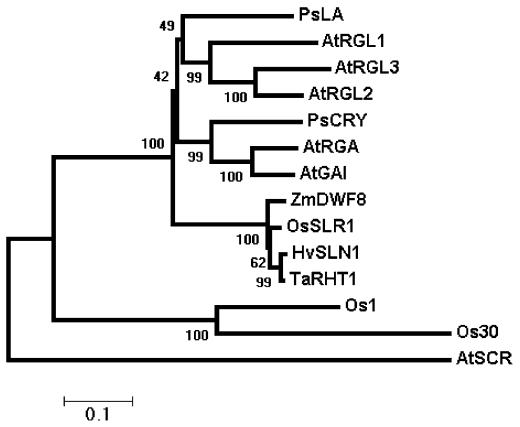

Figure 3. Relationship of PsLA and PsCRY to other DELLA proteins. Eleven DELLA proteins (HvSLN1, TaRHT1, AtRGL2, AtRGL3, AtRGL1, PsLA, ZmDWF8, OsSLR1, AtRGA, AtGAI, and PsCRY) and three related GRAS proteins (AtSCR, Os30, and Os1) were used to construct a consensus phylogram, showing the lack of a one-to-one relationship between the pea DELLA proteins, PsLA and PsCRY, and the key Arabidopsis DELLA proteins, AtGAI and AtRGA. Bootstrap percentages are shown at each branch.

appears to be quite ancient, having occurred prior to the divergence of Arabidopsis and pea (Fig. 3).

Our molecular studies were performed using mutant alleles derived from lines that date from the foundation reports on cryptodwarf (genotype *la cry-c le-1*; Rasmusson, 1927) and slender (de Haan, 1927) peas, and on their relationship (Lamm, 1937). Full sequences were obtained for the *la* allele from Rasmusson's 'Gray Dwarf' line (HL2, Lamm line 2), *cry-c* from a Rasmusson cryptodwarf (HL8, Lamm line 8a), and *cry-s* from Lamm line 7 (HL7, Vilmorin's acacia line). In addition, the *cry-s* allele from de Haan line 204.1 (HL6, Lamm line 6) was shown to have the frame-shift deletion at position 152.

To investigate the roles of *LA* and *CRY* on root development, we recombined the severely GA-deficient mutant *na-1* with *la* and/or *cry-s.* The *na-1* mutant has been crucial for establishing a role for GAs in root development (Yaxley et al., 2001). This mutant has an extremely short shoot phenotype, termed nana, and roots that are shorter, thicker, and less ramified than those of wild-type plants (Yaxley et al., 2001). We found (Fig. 4) that recombining *na-1* with *la* and *cry-s* essentially rescues the root phenotype of *na-1*. Interestingly, the *la* mutation on its own is able to largely rescue the *na-1* root phenotype (Fig. 4; Supplemental Table S1), whereas *cry-s* on its own does not. This suggests that LA is the main functioning DELLA protein in the roots of pea. The *la* mutation is also more effective at rescuing the shoot phenotype of *na-1* plants, compared with *cry-s* (Fig. 4). It was previously noted that gene *LA* is a more effective inhibitor of shoot elongation than is *CRY* (de Hann, 1927; Reid et al., 1983). This difference is pronounced in the shoots of *na-1* plants, and is especially clear in *na-1* roots (Fig. 4).

In contrast to the roots, both of the null mutations *la* and *cry-s* are required to fully rescue the shoot phenotype of *na-1* plants, and the shoot phenotype of *na-1 la cry-s* is slender (Fig. 4; Potts et al., 1985). The GA-saturated shoot phenotype of *la cry-s* plants indicates that *LA* and *CRY* are the only DELLA genes operative in the shoot. Results from genomic Southern blots probed with *PsLA* and *PsCRY* also did not provide evidence for more than two DELLA genes in pea. Here we found that *na-1 la* plants heterozygous for *CRY/cry-s* had internodes much longer than nana plants, and fitting within the range of Mendel's *le-1* dwarf. The dwarf phenotype of *na la CRY/cry-s* plants was not apparent in the previous investigation (Potts et al., 1985) because *le-1* was also segregating. In contrast, we found homozygous *na-1 la CRY* plants had internodes only around half as long as the *CRY/cry-s* heterozygotes and a phenotype consistent with the upper end of the nana phenotypic range.

A Second GA 3-Oxidase Gene, *PsGA3ox2*, Is Expressed Primarily in the Roots and Is Responsible for the Conversion of GA_{20} to GA_1

Before investigating the effects of DELLA proteins on GA synthesis gene expression, we sought to clone additional 3-oxidase genes from pea. The reasoning for this was that the roots of the GA biosynthesis mutants *le-1* and *le-2* (null) are phenotypically similar to wild type and contain similar levels of endogenous GA_{20} and GA_1 to wild type, in contrast to their dwarf shoot phenotype (Yaxley et al., 2001). It was therefore expected that another GA 3-oxidase must carry out substantial 3-oxidation in the roots (Yaxley et al., 2001). Indeed, another pea GA 3-oxidase gene, *PsGA3ox2* (Supplemental Fig. S2), was isolated using PCR primers based on *Medicago* sequence, 3′ RACE, and cDNA

Figure 4. Shoot and root phenotypes of pea genotypes. Left to right, *NA LA* and/or *CRY*; *na-1 LA cry-s*; *na-1 la CRY/cry-s*; *na-1 la cry-s*; *NA la cry-s*. The *na-1* mutation on a *LA* background gives rise to the characteristic nana phenotype (second from left), with a very short shoot and shortened roots. The homozygous presence of *cry-s* does not rescue the root phenotype, whereas *la* largely does.

clones. The expression product of *PsGA3ox2* converted [^{14}C]GA_{20} to [^{14}C]GA_1, as shown by HPLC and gas chromatography-mass spectrometry-selected ion monitoring, demonstrating its 3-oxidase activity (Supplemental Fig. S3).

The expression levels of the 3-oxidase genes *PsGA3ox1* (also known as Mendel's *LE*; Lester et al., 1997; Martin et al., 1997) and *PsGA3ox2* were measured in the shoot and root tissue of 6-d-old pea seedlings using real-time PCR. *PsGA3ox1* was more highly expressed in the shoot compared with root tissue, with an approximate 2-fold difference ($P < 0.001$; Fig. 5). Conversely, *PsGA3ox2* showed approximately 2-fold higher expression in the root tissue than the shoot tissue ($P < 0.02$; Fig. 5).

Pea DELLA Proteins Promote the Expression of GA Synthesis Genes and Inhibit That of GA Deactivation Genes

In Arabidopsis shoots, DELLA proteins feed-back regulate the GA biosynthesis genes *AtGA3ox1* and *AtGA20ox1* (Dill and Sun, 2001; King et al., 2001; Silverstone et al., 2001). To investigate whether the pea DELLA proteins are involved in the feed-back regulation of key GA biosynthesis genes in pea roots, we undertook real-time PCR on *LA* and the mutant *la* on a *cry-s* background. In the *la* mutant, there was a 4-fold and 6-fold down-regulation of *PsGA3ox1* ($P < 0.001$; Fig. 6A) and *PsGA3ox2* ($P < 0.05$; Fig. 6A), respectively. The greatest effect on gene expression was seen for *PsGA20ox1*, which was down-regulated 14-fold in the mutant ($P < 0.01$; Fig. 6A). In contrast, a more than 2-fold up-regulation of the 2-oxidase genes *PsGA2ox1* ($P < 0.02$; Fig. 6B) and *PsGA2ox2* ($P < 0.01$; Fig. 6B) was seen in the mutant roots. Similar results were obtained for *CRY* and the mutant *cry-s* on a *la* background (Supplemental Fig. S4).

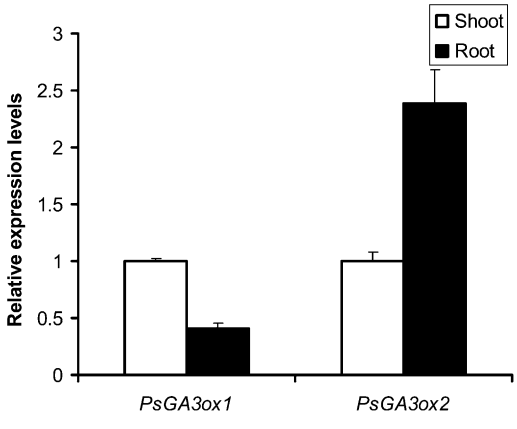

Figure 5. Expression of GA 3-oxidase genes in pea seedlings. Transcript levels of *PsGA3ox1* (*LE*) and *PsGA3ox2* in root and shoot tissue of 5-d-old pea seedlings (line 205+), measured by quantitative real-time PCR. For each gene, the shoot value was set to 1, and the level in the root sample was calculated relative to the corresponding shoot value. Shown are means with SE ($n = 4$). It should be noted that direct comparison between the expression levels of the two genes is not valid. Plants were grown in pasteurized potting mix.

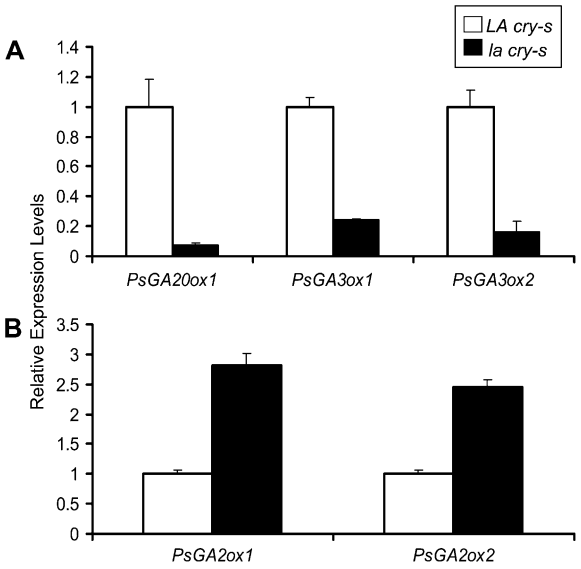

Figure 6. Effects of *la* on expression of GA genes. A and B, Transcript levels of GA biosynthesis (A) and deactivation (B) genes in *LA cry-s* and *la cry-s* pea roots, as measured by quantitative real-time PCR. Plants were grown in pasteurized potting mix for 6 d. Shown are means with SE ($n = 3$). For each gene, the transcript level in *LA* plants was set to 1, and the level in the *la* mutant was calculated relative to the *LA* value.

DISCUSSION

DELLA proteins play a pivotal role in GA signal transduction (Fu and Harberd, 2003). However, despite the historical importance of pea for physiological and genetic studies, DELLA genes have not previously been isolated from this species. In pea, the mutations *la* and *cry-s* together produce the slender phenotype, which played a pivotal role in the development of early theories on GA action. In 1957, Brian published the prescient theory that GAs, rather than positively promoting stem elongation, actually inhibit an inhibitor of that process. This was supported by genetic studies using GA-deficient mutants (Potts et al., 1985). Next came the discovery of growth-repressing DELLA proteins, and the finding that DELLAs are destabilized by GA (Harberd et al., 1998; Silverstone et al., 2001). Here we complete the picture by showing that *LA* and *CRY* encode DELLA proteins in pea. The mutant alleles *la* and *cry-s* both appear to encode nonfunctional proteins, and the stem elongation of *la cry-s* plants is similar to that of wild-type plants given a saturating dose of bioactive GA (Brian, 1957; Potts et al., 1985). It appears, therefore, that at least with respect to shoot elongation, *LA* and *CRY* are the only DELLA-encoding genes in pea.

It was shown previously that although the *na-1* mutation dramatically reduces GA levels and leads to the very short nana phenotype, the *la cry-s* gene combina-

tion is completely epistatic to *na-1* in shoots (Potts et al., 1985). Here we show that *la cry-s* also rescues (Fig. 4) the distinctive *na-1* root phenotype (Yaxley et al., 2001). Interestingly, *la* on its own largely rescues the *na-1* root phenotype, at least in terms of elongation of lateral roots (Fig. 4). This indicates that LA may be the main functioning DELLA protein in roots.

We then used the pea DELLA mutations to examine the effects of these proteins on the expression of GA synthesis and deactivation genes in roots. In the DELLA slender mutants *sln* in barley (Chandler et al., 2002), *slr1* in rice (Itoh et al., 2002), and *la cry-s* in pea (Potts et al., 1985; Martin et al., 1996), the synthesis of bioactive GAs is reduced, but this has only been shown for shoots. We therefore monitored the expression of GA biosynthesis and deactivation genes in the roots of wild-type and slender pea plants. When both DELLA genes were null (*la cry-s*), there was a strong reduction in expression of the biosynthesis genes *PsGA20ox1*, *PsGA3ox1*, and *PsGA3ox2*, and a strong promotion of the deactivation genes *PsGA2ox1* and *PsGA2ox2*, compared with *LA cry-s* or *la CRY* plants. Therefore, DELLA proteins promote the expression of GA synthesis genes and inhibit that of GA deactivation genes, indicating that in roots, DELLAs are an integral part of the feedback and feed-forward phenomena, whereby bioactive GA reduces GA synthesis and speeds up GA deactivation (Dill and Sun, 2001; Zentella et al., 2007).

Another key GA gene from pea is Mendel's *LE*, also known as the 3-oxidase gene *PsGA3ox1* (Lester et al., 1997; Martin et al., 1997). Mendel exploited the dwarf stature of mutant *le-1* shoots in his original genetics experiments, but it is interesting to note that the roots of *le-1* plants (and of other *le* mutants) are indistinguishable from the wild type, and contain normal GA levels (Yaxley et al., 2001). The identification of a second GA 3-oxidase gene (*PsGA3ox2*) from pea provides an explanation for these observations. It appears that *PsGA3ox2*, which is relatively strongly expressed in roots, can compensate for the reduction in PsGA3ox1 activity in *le-1* roots, and even for the complete loss of that activity in roots of the null mutant *le-2* (Martin et al., 1997; Lester et al., 1999). The capacity for compensation by *PsGA3ox2* (and possibly by other, as yet unknown, 3-oxidase genes) is clearly reduced in the shoots and consequently *le-1* and *le-2* shoots are dwarfed. However, it appears that there is some compensation even in the very short *le-2* shoots because they do produce a trace amount of GA_1 (Ross et al., 1989).

In conclusion, we have isolated the *LA* and *CRY* genes of pea and have shown that they encode DELLA proteins. This provides valuable support for the inhibitor-of-an-inhibitor model of GA action, which was based originally on the slender *la cry-s* mutant (Brian, 1957). Of the two DELLA genes, *LA* appears to be the major one operating in roots, as indicated by the capacity of *la* on its own to largely rescue the root phenotype of the GA-deficient *na-1* mutant. We have used the *la* and *cry-s* mutations to show that in pea roots, DELLA proteins can be viewed as positive regulators of the expression of GA biosynthesis genes, including the second pea 3-oxidase gene, *PsGA3ox2*. Our studies on the pea DELLA proteins LA and CRY further demonstrate the importance of GA signaling in the regulation of root growth.

MATERIALS AND METHODS

Plant Material

Experiments involving gene expression studies and quantification of endogenous GAs were conducted with the tall (wild type) Hobart line HL205+ (*LA CRY*; Ross and Reid, 1989). Progenies segregating for *LA*/*la* and/or *CRY*/*cry-s* were derived from the following crosses: HL133 (*la cry-s NA*) × NGB1766 (*LA CRY na-1*; Potts et al., 1985); HL6 (*LA cry-s wa*) × HL73 (*la CRY WA*); and HL6 (*LA cry-s wa*) × line K524 (*LA CRY WA*). Genotypes *LA cry-s* and *la cry-s* (Supplemental Fig. S2) were derived from the same F_4 plant from cross HL133 × NGB1766. Other genotypes were selected from a cross between HL107 (*LA CRY NA*) and HL188 (*la cry-s na-1*; HL188 was selected from cross HL133 × NGB1766). The foundation lines HL2 (Lamm line 2), HL6 (Lamm line 6), HL7 (Lamm line 7), and HL8 (Lamm line 8a) were kindly provided in 1957 by Dr. Robert Lamm.

Plant Growth and Chemical Treatments

Plants to be raised to maturity for genetic studies were grown in a 1:1 mixture of dolerite chips and vermiculite, topped with pasteurized peat/sand potting mix. Plants for gene expression experiments were grown in 100% potting mix for 4 to 5 d. Gene expression material was immediately immersed in liquid nitrogen and stored in a −70°C freezer.

Cloning of *LA*, *CRY*, and *PsGA3ox2*

A partial *PsLA* sequence was obtained by amplification of genomic DNA using two independent degenerate PCR primer pairs: 5′-GCTAATCAAGCGATHYTGGARGC-3′ and 5′-CCAACCAAGCATAARRCANCCRT-3′; and 5′-TTAGCTGTAKTWGGTTAYAARGT-3′ and 5′-ACATACTCGCYTGYTTRAANGC-3′, based on the *Medicago* contigs TC43628 and TC51390 and the Arabidopsis *RGA* gene.

A partial *PsCRY* sequence was obtained using nested PCR on cDNA, using primers based on the gene *LS*, a GRAS gene from tomato (*Solanum lycopersicum*; DELLAs belong to the GRAS protein family). The primary PCR was conducted with primers 5′-ATTCAACTGAACGGTTAGTCCA-3′ and 5′-GCAATGTAGCTTCCAGTGAATC-3′, followed by a secondary PCR with primers 5′-GTTTACTCAATTAACCGCTAATCA-3′ and 5′-AATGTAGCTTCCAGTGAATCAAA-3′. Using a CAPS marker between the lines Terese and Torsdag (Laucou et al., 1998), *PsCRY* was mapped between the two RAPD markers Q4_450 (at 2 cM) and X17_500 (10 cM) on LGVII, not far from *RMS4* (Rameau et al., 1998). The remainder of the sequences of *PsLA* and *PsCRY* was obtained by screening a cDNA library made from the shoots of 7-d-old deetiolated pea (*Pisum sativum*) shoots (Clontech).

The initial portion of *PsGA3ox2* was isolated using primers based on a partial *Medicago* bacterial artificial chromosome sequence (gi89514974). The 3′ end of the *PsGA3ox2* sequence was isolated by 3′ RACE (Frohman et al., 1988). The partial sequence thereby obtained was used as a probe to screen approximately 350,000 clones of a pea seedling shoot library. A single clone containing the 5′ end of the gene was isolated, sequenced, and then ligated into pGem-T Easy (Clontech) and expressed in *Escherichia coli*. The functional activity of the expression product was tested as before (Lester et al., 1997), using $[^{14}C]GA_{20}$ as a substrate.

Segregation Studies

The segregation of *PsLA* was followed using two PCR primers that flanked the deletion in the *la* mutant, 5′-CTTAGCTGTATTAGGTTATAAGGTTCGTT-3′ and 5′-TCTTCACGAGTCTATCAGCAATCTT-3′, giving a 542-bp band in the wild type, and a 727-bp band in the *la* mutant. The segregation of *PsCRY* was followed using two PCR primers, 5′-CTTGAACAAGCTATGGGTAATTTTCA-3′ and 5′-ATCCCTTTCTCCTGCGTT-3′, which amplified a PCR product

containing several *Bcc*I sites near the *cry-s* mutation, one of which was polymorphic between *cry-s* and the *CRY* gene in line 107 (Torsdag).

Phylogenetic Analysis

ClustalX within MEGA2 was used to align 14 GRAS proteins (HvSLN1, 75161835; TaRHT1, 75207630; AtSCR, 15232451; Os1, 115438851; Os30, 115465589; AtRGL2, 15228553; AtRGL3, 15237971; AtRGL1, 15777857; PsLA, DQ848351; ZmDWF8, 75207626; OsSLR1, 109287736; AtRGA, 2569940; AtGAI, 2569938; and PsCRY, DQ845340) using the minimal evolution method.

RNA Extraction, cDNA Synthesis, and Quantitative PCR

Plant material was ground to a fine powder with a mortar and pestle in liquid N_2. Approximately 100 mg of ground tissue was used for RNA extraction, as carried out by Wolbang et al. (2004). One to 2 μg of total RNA was used to synthesize single-strand cDNA using the QuantiTect reverse transcription kit (QIAGEN). cDNA samples were diluted to a total volume of 100 μL.

For gene expression quantification, the following primer pairs were used: *PsGA20ox1*, 5′-CATTCCATTAGGCCAAATTTCAAT-3′ and 5′-CTGCCCTATGTAAACAACTCTTGTATCT-3′; *PsGA3ox1*, 5′-TTCGAGAACTCTGGCCTCAAG-3′ and 5′-ATGTTCCTGCTAACTTTTTCATGGTT-3′; *PsGA3ox2*, 5′-ATCATGGGGTCACCGTCTAA-3′ and 5′-GCTAGTGTCTTCATTTGCTTTTGA-3′; *PsGA2ox1*, 5′-CACAACCAATCAAGAACACAATTTC-3′ and 5′-CCCTTCTGCATCAAATCAAG-3′; *PsGA2ox2*, 5′-CCCTCCTGACCCCAGTGAAT-3′ and 5′-CTCACACTCACAAATCTTCCATTTG-3′; and *actin*, 5′-GTGTCTGGATTGGAGGATCAATC-3′ and 5′-GGCCACGCTCATCATATTCA-3′. All primers were acquired from Geneworks.

Two microliters of cDNA was used for quantitative real-time PCR using Bio-Rad iQ Sybr master mix (Bio-Rad) following the manufacturer's recommendations and run on a Rotorgene 2000 dual-channel machine (Corbett Research). Mean expression levels of the gene of interest were calculated relative to the expression of actin.

Sequence data from this article can be found in the GenBank data libraries under accession number(s) DQ845340 (*PsCRY*), DQ848351 (*PsLA*), and DQ864759 (PsGA3ox2).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Cosegregation of the slender mutant and the *LA/la* gene pair.

Supplemental Figure S2. Amino acid sequences of PsLA, PsCRY, and PsGA3ox2.

Supplemental Figure S3. GA 3-oxidase activity of *PsGA3ox2*.

Supplemental Figure S4. Effects of *cry* on expression of GA genes.

Supplemental Table S1. Length of lateral roots of pea genotypes.

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