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PPF-1, a post-floral-specific gene expressed in short-day-grown G2 pea, may be important for its never-senescing phenotype

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Abstract

We cloned a developmentally regulated gene from a cDNA library constructed from short-day (SD) grown G2 pea tissue using cDNA representational difference analysis (cDNA RDA) and named it PPF-1 for the first *Pisum sativum* post-floral-specific gene. Sequence comparisons with various databases revealed that PPF-1 shares a substantial homology only at the deduced amino-acid level with the *Bacillus subtilis* gene SP3J, which is required for maintaining vegetative growth, and with other genes coding for bacterial inner membrane proteins. All five potential hydrophobic regions from the bacterial proteins were maintained in the PPF-1 sequence. A series of Northern blots showed that this gene was only expressed after floral initiation and was limited to the apical buds, with non-detectable levels in roots, stems and mature leaves. Under SD conditions, when G2 pea displays an unlimited growth habit, PPF-1 expression was sustained at a relatively high level long after floral initiation. Under long-day (LD) conditions, when G2 pea undergoes an apical senescence similar to wild-type plants with genotype *sn hr*, PPF-1 was only expressed very briefly after flower initiation. Interestingly, in day-neutral, wild-type Alaska pea, the PPF-1 level was hardly detectable under any growth conditions. Treatment of LD-grown G2 pea with gibberellin A3 (GA_3) was able to stimulate PPF-1 expression unless it was applied at a very late growth stage, at which time the process of apical senescence cannot be reversed. © 1998 Elsevier Science B.V.

Keywords: Cloning; G2 pea; Gibberellins; Senescence; PPF-1 gene

1. Introduction

Despite the genetic propensity for longevity within the plant kingdom, senescence usually occurs at a precise developmental stage after reproduction in many monocarpic species such as wheat, barley, rice, corn and garden pea. Relatively little is known about the cause or internal control mechanisms of senescence in whole plants, although many different theories have been proposed to account for this dramatic and striking phenomenon (Nooden and Leopold, 1978; Kelly and Davies, 1988a; Engvild, 1989). Murneek (1926), followed by Sinclair and DeWitt (1975), suggested that the diversion of nutrients from growing apex to the developing fruits was a main factor for senescence induction. This was challenged by McCollum (1934) and Leopold et al. (1959). They concluded that neither seed nor fruit development is a primary factor in maintaining vegetative growth.

Previous work reported the role of cytokinins (CTKs) and GAs in plant leaf senescence (Fletcher and Osborne, 1965; Carrasco and Carbonell, 1990; Gan and Amasino, 1995). However, among applied plant hormones, including N^6-benzyladenine (BA), α-naphthaleneacetic acid (NAA), GA_{20}, GA_3 and GA_1, only the bioactive GA_3 and GA_1 delayed senescence of LD-grown G2 pea (genotype *Sn Hr*) indefinitely, whereas GA_{20} had a moderate effect (Davies et al., 1977; Proebsting et al., 1978; Zhu and Davies, 1997). Moreover, the level of active GA in SD-grown, non-senescing G2 apical buds increased soon after the initiation of reproductive growth, whereas that of LD-grown apical buds was maintained at a very low level (Zhu and Davies, 1997). The amount of CTK and auxin (indoleacetic acid) in G2 vegetative tissue did not change very much during the same period (Zhu and Davies, 1997; Davies et al., 1986). It was suggested that the change of hormonal level may regulate, or at least contribute to, the continued growth or senescence of G2 pea apical buds.

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2. Experimental and discussion

2.1. The growth of various parts of LD- and SD-grown G2 pea

Comparisons of the growth habit of G2 peas have revealed a striking difference between SD and LD growth conditions (Fig. 1A; Marx, 1968; Proebsting et al., 1976; Kelly and Davies, 1986). Under SD conditions (less than 12 h of light per 24-h cycle), the apical bud of this plant will grow continuously for a very long period of time (longer than 13 months, Zhu and Davies, unpublished results) with a large number of flowers and fruits being produced three or four nodes down from the apex (Fig. 1A, left). Under LD conditions (18 h of light per 24 h cycle), G2 ceases to grow soon after the initiation of reproductive growth, and its apical bud undergoes full senescence 3–5 weeks later (Fig. 1A, right). At this time, the vegetative tissues of the apical buds of SD-grown G2 plants were still robust, whereas the LD-grown apical bud had rapidly turned yellow (Fig. 1B). This phenomenon was limited to the G2 pea shoots at the root systems of LD-grown plants exhibited vigorous growth long after growth cessation of their shoot apices (Fig. 1C). This observation is in full agreement with a previous report that there were no differences in the CTK levels of SD- and LD-grown G2 peas, since cytokinins are produced mainly by the roots (Davies et al., 1986).

2.2. Cloning, sequencing and hydrophobicity study of PPF-1 gene

To elucidate fully the molecular mechanisms of G2 pea senescence, we set out to clone genes that are developmentally regulated, and expressed specifically after GA treatment, with a method termed cDNA representational difference analysis (Hubank and Schatz, 1994). After three rounds of subtractive hybridization, we found both GA-suppressed and GA-up-regulated cDNAs from the same set of pea tissues (Zhu et al., 1997, and data not shown). One of the GA up- regulated fragments was used for probing a cDNA library that was constructed with SD-grown G2 pea apical tissue harvested 2 weeks after flower initiation. We obtained a full-length cDNA that possesses an open reading frame of 1326 bp with 48 bp as the 5′ untranslated region and 150 bp as a 3′ downstream sequence (EMBL Bank Accession Number Y12618). The deduced peptide contains 442 amino acids. An extensive database search showed that the PPF-1 gene

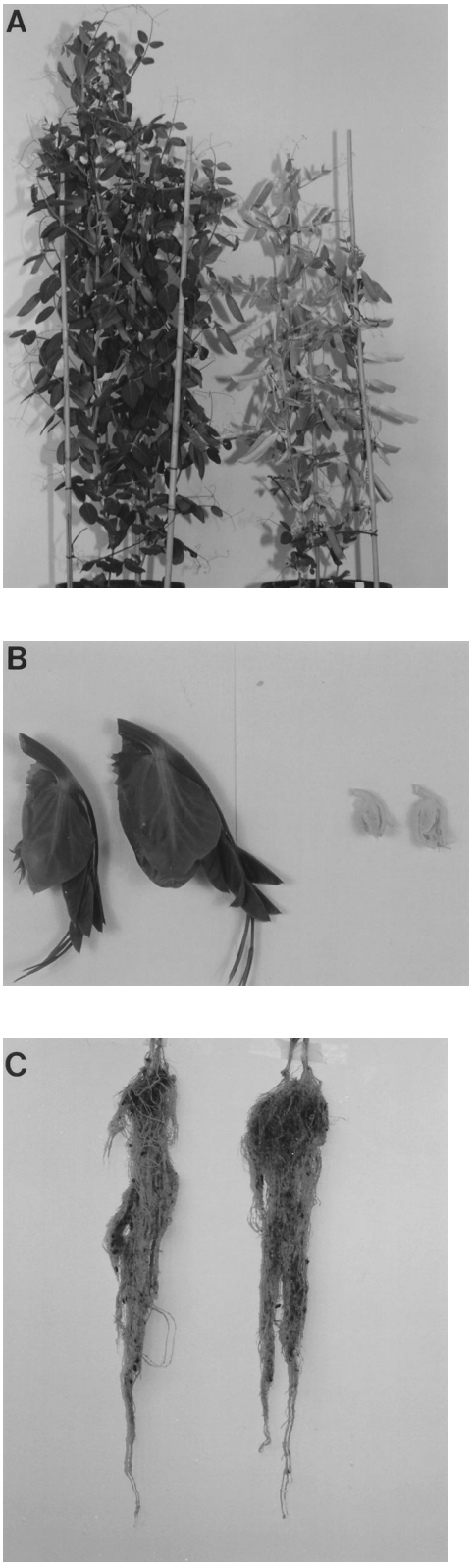

Fig. 1. Morphological comparisons of different parts of G2 pea plants grown under different growth conditions. (**A**) Photographs of whole plants grown in 20-cm plastic pots 8 weeks after germination [see Zhu and Davies (1997) for growth conditions]. Right, 9 h of light per 24 h cycle; Left, 18 h of light per 24-h cycle. (**B**) Close-up photo of the same plants showing the apical buds. (**C**) The root systems of the same plants. Note the differences in the size and the numbers of fruits born on SD and LD plants, the cessation of apical bud growth of LD grown plants, as well as the vigor of its root systems at the same time.

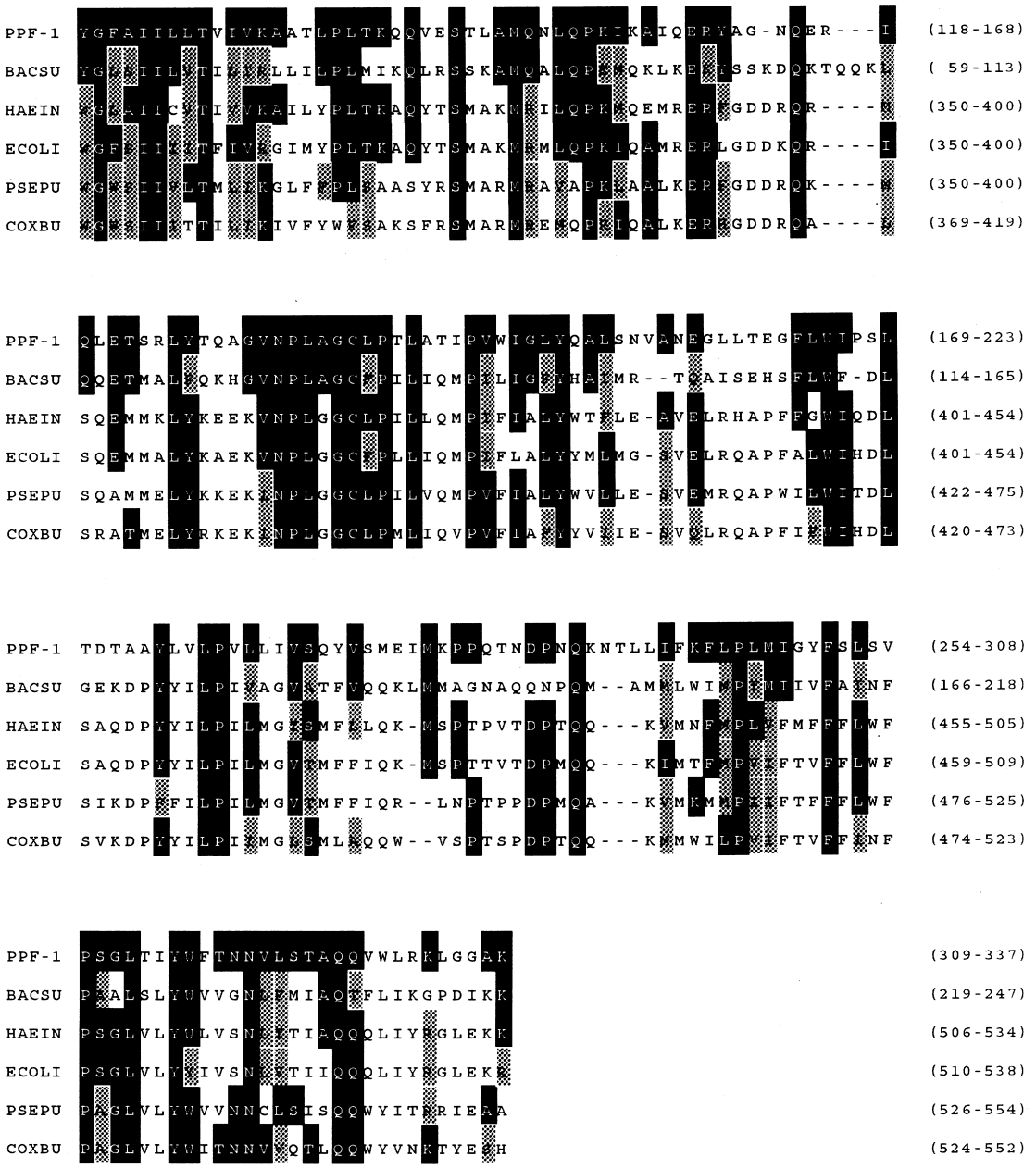

Fig. 2. Sequence comparison of the deduced PPF-1 peptide with five prokaryoticproteins: the sporulation protein J precursor from Bacillus subtilis (SWISS_PROT code SP3J_BACSU) and four inner-membrane proteins from *Haemophilus influenzae* (60IM_HAEIN), *Escherichia coli* (60IM_ECOLI), *Pseudomonas putida* (60IM_PSEPU) and *Coxiella burnetii* (60IM_COXBU). The comparison is based on the FASTA search, which yields the five highest optimized alignment scores (Pearson and Lipman, 1988). Residues 224–253 in PPF-1 are not shown. Complete identical amino-acid residues among PPF-1 and the other five sequences are shown in black regions. Gray background denotes that PPF-1 has at least one identical residue and one or more conserved substitutions within the other four sequences. The highest amino-acid sequence homology among these fragments is 54%, yet the similarity at nucleotide level is only 25–30%.

shares a significant homology with a *Bacillus subtilis* vegetative-growth-specific gene SP3J and also with several other genes that encode bacterial inner membrane proteins (Fig. 2). Hydropathy analysis showed that, with the exception of only a few minor regions, the N-terminal portion (from amino acid 1–313) of our deduced polypeptide is almost entirely hydrophobic, whereas the C-terminal portion (from amino acids 346–440) is highly hydrophilic. There were four or five putative transmembrane regions that showed a high degree of conservation among all five deduced proteins (Fig. 3). These results are taken as evidence for a possible membrane localization of the PPF-1 protein.

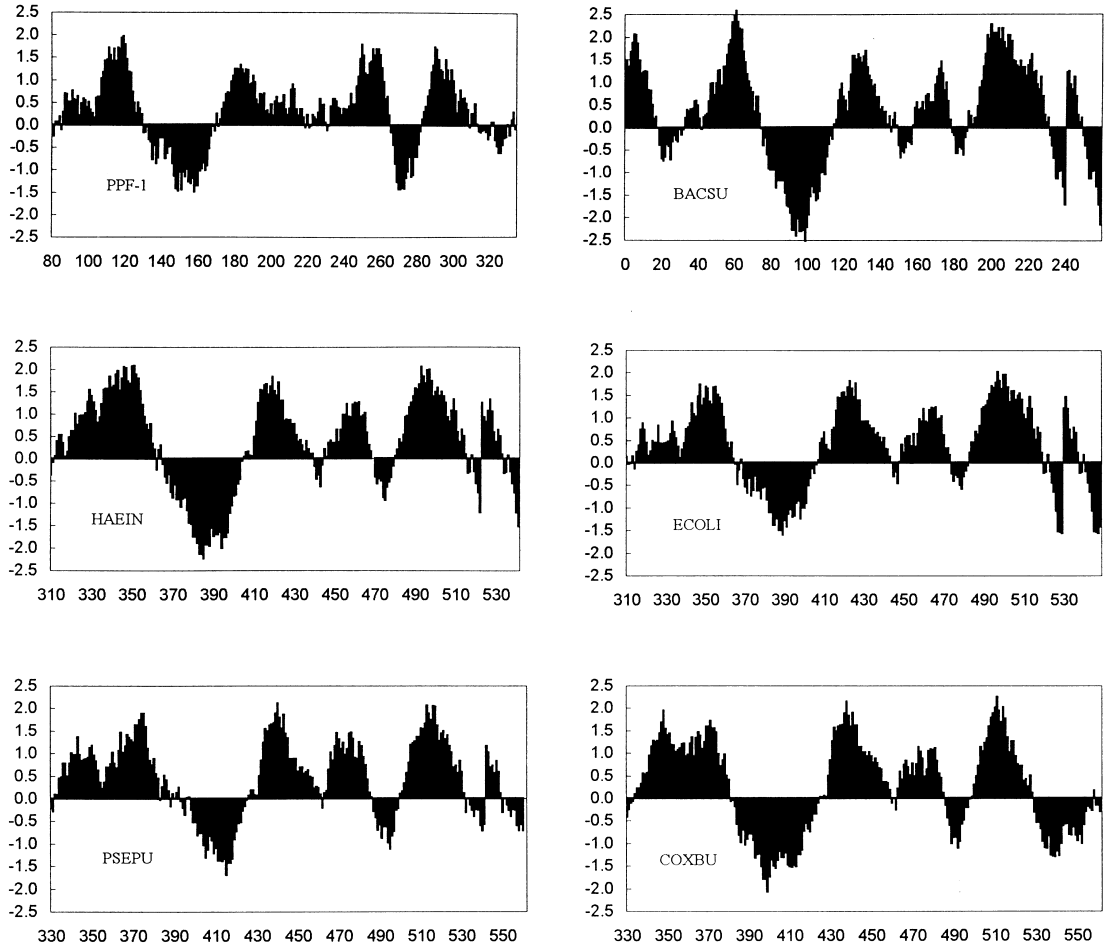

Fig. 3. Partial hydrophobicity analysis of PPF-1 based on the Kyte and Doolittle hydrophobicity index table. A window size of 19 residues was taken in the plot. The N-terminal part is composed mainly of five hydrophobic regions and is found in SP3J (BASCU) and in all four prokaryotic inner membrane proteins (HAEIN, ECOLI, PSEPU, COXBU). The x-axis designates amino-acid residue numbers and the y-axis designates hydrophobic scales.

2.3. The expressional patterns of PPF-1

Since the patterns of PPF-1 expression may have a key importance in the understanding of its function, we performed a series of Northern blots using RNAs prepared from different G2 pea tissues grown under different conditions. As shown in Fig. 4, we found that PPF-1 expression was limited mainly to the apical bud portion of G2 pea, with almost non-detectable levels in mature leaves, stems and roots. Further analysis demonstrated that PPF-1 was only expressed in substantial amounts in SD-grown G2 pea tissue after flower initiation (Fig. 5, lanes marked SD). It was increased only very weakly in LD-grown G2 pea tissue after flower initiation (Fig. 5, lanes marked LD). In the wild-type, day-neutral Alaska pea, senescence occurs under any growth conditions, and PPF-1 was not activated at any growth stage (Fig. 5B). Obviously, a spatially localized and developmentally regulated membrane protein could control, or at least contribute to, the vigour as well as the life spans of its target cells. Both CEN and TFL1 genes from *Antirrhinum* and *Arabidopsis* determinate inflorescence mutants were similar to animal phosphatidylethanolamine-binding proteins that are part of a membrane-bound complex (Bradley et al., 1997). In animal systems, Bcl-2-related proteins, which are known to be critical regulators of programmed cell death, were localized to the outer mitochondrial, outer nuclear and endoplasmic reticulum membranes (Minn et al., 1997; Yang et al., 1997).

2.4. The effects of GA_3 on PPF-1 expression

Previous work reported that among the externally applied plant hormones, including auxins and cytokinins, only GA_3 or GA_1 treatment was able to prevent apical senescence of LD-grown G2 pea to a large extent (Davies et al., 1977; Proebsting et al., 1978; Zhu and Davies, 1997). We therefore wanted to know whether GA_3 treatment would also cause an increase in PPF-1

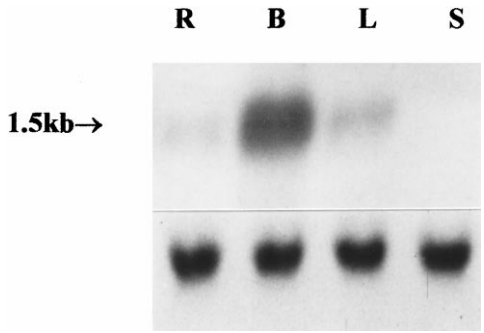

Fig. 4. Northern blot analysis of PPF-1 expression in different G2 pea tissues. R, roots; B, apical buds as shown in Fig. 1B; L, mature leaves; S, stems. Samples were harvested at the floral initiation stage. Forty nanograms of the whole length PPF-1 cDNA were labeled with α-^{32}P dCTP using a Stratagene Prime-it II kit to obtain 1×10^8 cpm of radiolabelled probe. Total RNA from different tissues was extracted using a Qiagen plant RNA kit. Twenty micrograms of RNA were loaded on to each lane of an electrophoretic gel. The transfer membranes were hybridized to the above probe for 20 h at 68°C before being washed several times and exposed to Kodak X-ray film for 36 h [see Hong et al. (1992) for detailed procedures]. A 5S rRNA cDNA (EMBL bank X95566) from pea was used to verify the loading (the bottom row).

expression in LD-grown G2 pea. Indeed, the PPF-1 level increased sharply only 3 h after GA_3 application and reached a maximum level within 6–12 h of GA treatment as determined by Northern blot analysis. In longer-term GA treatments, PPF-1 level dropped slightly but remained significantly higher than that of controls (Fig. 6). This GA_3 effect was not observed when GA_3 was applied at a very late growth stage, at which time the process of senescence cannot be reversed (data not shown).

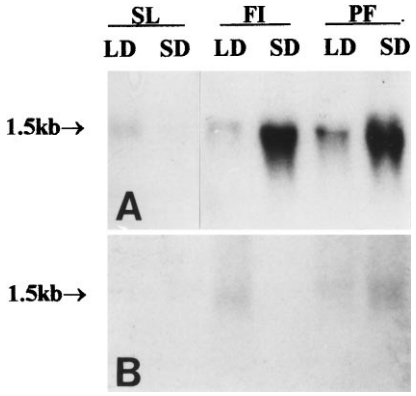

Fig. 5. Developmental regulation of PPF-1 expression under different growth conditions and in different genotypes of peas. PPF-1 expression patterns in G2 (A) and Alaska (B) pea apical buds were determined by Northern blots (as in Fig. 4). Apical buds included all vegetative tissues inside the enfolding stipules, with floral buds removed. LD, LD-grown plant materials; SD, SD-grown plant materials. SL, 2-week-old seedlings; FI, plant materials harvested at floral initiation stage; PF, plant materials harvested 2 weeks after the appearance of the first flower (post-floral stage).

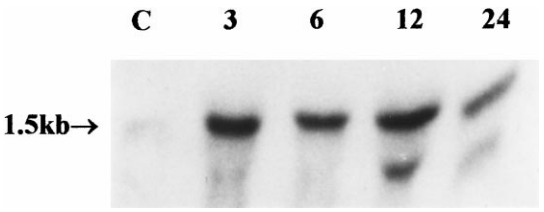

Fig. 6. Northern blots showing the accumulation of PPF-1 mRNA after GA_3 treatment. Different total RNA samples were extracted from prefloral LD-grown G2 pea apical buds 3, 6, 12 or 24 h after external applications of 30 μM GA_3 with 0.1% Tween 20 and probed as in Fig. 4. In lane 1, plants treated with dH_2O containing 0.1% Tween 20 for 12 h were used for RNA extraction. Fifty microliters of either GA_3 or H_2O were spread on to the apical tissue of the growing plant. The treatments were reinforced every 3 h with the same solution where applicable.

2.5. Conclusions

The delay of senescence in G2 peas was associated with a slower reproductive development under SD conditions, and the demise of LD-grown apical bud was found to be preceded by a decrease of GA_1 content in the shoot and an increase in auxin levels in the young flower buds (Proebsting et al., 1978; Kelly and Davies, 1986; Zhu and Davies, 1997). Our early work also argues that the transition to reproductive phase requires a redirection of photosynthate and other resources of the plant to the reproductive sinks and that such a strong commitment confers monocarpism (Kelly and Davies, 1988a,b). Taken together with the finding here that the non-senescing SD-grown G2 pea produces a vegetative-growth-specific polypeptide that may be associated with cell membranes, and which is also up-regulated by GA_3 treatment, we think that it is quite possible for this PPF-1 gene to play some regulatory role in maintaining the prolonged vegetative growth of the SD-grown G2 pea shoot apices. As senescence is likely a consequence of nutrient diversion to the young, developing fruits (Zhu and Davies, 1997; Kelly and Davies, 1988b), this hydrophobic and possibly membrane-associated peptide might be involved in the partitioning of photosynthate between the vegetative and reproductive structures within the apical bud. Alternatively, the maintenance of cell vigour via such a gene could result indirectly in an increased sink capacity leading to the maintenance of apical growth. Actual localization and functional elucidation of the PPF-1 protein may contribute significantly to the understanding of apical growth and senescence of G2 pea in particular and of monocarpic plants in general.

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