A RAV-like transcription factor controls photosynthesis and senescence in soybean

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Abstract

A cDNA library enriched for mRNAs encoding ESTs that increased in abundance during short days was constructed by SSH from leaf tissues of a photoperiod sensitive soybean. The proteins predicted to be encoded by the mRNAs were inferred to be involved in diverse functions. A full-length mRNA that encoded a soybean ortholog of the transcription factor RAV was isolated by RACE, containing an open reading frame of 1,056 bp. The GmRAV protein included an AP2/ERF domain and a B3 domain. GmRAV mRNA abundance was increased in SDs following leaf treatments with ABA and decreased following BR treatment. Transgenic tobacco overexpressing GmRAV showed morphological and physiological alterations such as slower plant growth rate (dwarfing), reduced root elongation, delayed fowering time and reduced photosynthetic rate, reduced chlorophyll contents in leaves. Therefore GmRAV may be a negative regulator acting on both photosynthesis and growth. Transgenic tobacco also showed accelerated senescence with both dark and ABA treatments versus the longer longevity compared to the wild type in LDs. The analyses of soybean leaf, root and stem organs showed that GmRAV mRNA abundances were higher in SDs than in LDs. Therefore, the enhanced expression of GmRAV in SDs compared to LDs may have caused the inhibited growth of soybean leaf, root and stem.

Media summary

GmRAV may be a negative regulator acting on both photosynthesis and growth.

Key words

Dark-induced, Photoperiod, GmRAV，Senescence， Soybean，Suppression substractive hybridization (SSH)

Introduction

Soybean (*Glycine max* (L.) Merrill.) is typically a short-day (SD) sensitive plant (Fehr and Caviness 1977; Fehr 1987). The time of flowering and date of maturity remain important reproductive characters of agronomic interest that both underlie seed yield (Yuan et al. 2002) and partial disease resistance (Njiti and Lightfoot 2006). Among many plant species (including *Arabidopsis thaliana* and *Nicotiana tabacum*) the exposure of plants to both short days and darkness will accelerate leaf senescence (Smart et al. 1995; Gan and Amasino 1997; Buchanan-Wollaston et al. 2003; Gepstein et al. 2003; Han et al. 2006). The metabolic

changes associated with leaf senescence included the attenuation of anabolic activities, such as photosynthesis and protein synthesis, and the acceleration of catabolic activities, such as nucleic acid breakdown and proteolysis by the induction of hydrolytic enzymes occurring during senescence (Smart 1994). In this study hundreds of ESTs that increased in abundance during SD treatments were isolated. Among these was an mRNA encoding a RAV ortholog (GmRAV). GmRAV encompassed an AP2/ERF domain and a B3 domain. Transcript abundances were measured during vegetative development, after treatment with plant growth hormones and during dark stress in soybean.

Methods

A cDNA library enriched for mRNAs encoding ESTs that increased in abundance during short days was constructed by SSH from leaf tissues of a photoperiod sensitive soybean. A full-length mRNA that encoded a soybean ortholog of the transcription factor RAV was isolated by RACE. The CaMV 35S promoter-GmRAV cDNA construct was used to transform tobacco, N. tabacum 'Petite Havana SR1', by Agrobacterium tumefaciens (LBA4404)-mediated transformation according to the procedures described by Horsch et al. (1985). Five transgenic lines (T2) and wild-type plants were grown in pots for further analysis. The mRNA abundance of the GmRAV in soybean different organs during the shift from SD to LD conditions was investigated by quantitative real-time RT-PCR analysis.

Results

Identification of differentially expressed ESTs subtracting long-day from short-day treated mRNA populations (collected from leaves at night's end) provided 76 unique cDNAs. Among these were 43 cDNAs with putative functional orthologs and 33 cDNAs that could not be assigned a function or ortholog (Genbank EH183310 to EH183340, EH183416 and DR007285). The 43 annotated ESTs were grouped into 9 categories according to the functions of their putative orthologs. Orthologs predicted to be involved in regulation encoded transcription, signal transduction and programmed cell death related proteins. Orthologs predicted to be involved in catabolism, particularly macromolecule degradation, encoded enzymes that could degrade proteins, nucleic acids and carbohydrates. Some of the orthologs encoded enzymes that were involved in anabolisms like cell wall modification, primary metabolism (such as carbohydrate and lipid synthesis), secondary metabolisms and stress response (such as detoxification and defense).

A full-length GmRAV cDNA (Soybean RAV-like DNA-binding protein gene, GenBank DQ147914) contained 1,380 bp with an open reading frame of 1,056 bp that was predicted to encode 351 amino acids. The GmRAV protein contained an AP2/ERF domain from amino acid 53 to 108 and a B3 domain from amino acid 172 to 286. Transgenic tobacco overexpressing GmRAV showed morphological and physiological alterations such as slower plant growth rate (dwarfing), reduced root elongation (Figure 1), delayed fowering time and reduced photosynthetic rate, reduced chlorophyll contents in leaves (Table 1). Therefore GmRAV may be a negative regulator acting on both photosynthesis and growth. Transgenic tobacco also showed accelerated senescence with both dark and ABA treatments versus the longer longevity compared to the wild type in LDs. The analyses of soybean leaf, root and stem organs showed that GmRAV mRNA abundances were higher in SDs than in LDs. Therefore, the enhanced expression of GmRAV in SDs compared to LDs may have caused the inhibited growth of soybean leaf, root and stem.

Table 1. Comparison of growth and photosynthesis parameters of transformed GmRAV tobacco plants and wild-type (WT) grown in the growth chamber at 25°C under 16-h light/8-h dark condition with 250 µmol $m^{-2}s^{-1}$ white light

The growth data were obtained from plants grown for five-month-old plants (ten plants). Photosynthesis was measured in middle leaves from node 4 counted upward from five-month-old plants. Values were presented as measurement means ± S.E.

Type of plant	Plant height (cm)	Internode length (mm)	Total leaves number	Root number	Chlorophyll content (µ g g^{-1} FW)	Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	Flowering time (day)
Wild type	95.2±5.3	28±2.4	28±0.7	76±5.8	2757±64	8.34±1.3	176±3.2
GmRAV4	47.5±2.3	11±2.0	27±0.8	52±5.4	1840±55	3.35±0.9	190±3.5
GmRAV15	51.8±1.8	12±1.8	27±1.0	49±3.9	1750±57	2.94±0.7	187±2.7

Figure 1. Phenotypes of transgenic tobaccos expressing GmRAV transcripts

Morphology of transgenic GmRAV-4, GmRAV-15 and wildtype plants in LDs. a Four-week-old transgenic plants grown on MS plates showed shorter internode and root length, as well as fewer numbers of roots compared to the wild-type plants. b Five-month-old transgenic plants grown on soil that were severely dwarfed, had smaller leaves and reduced internode distances compared to the wild-type plants

Conclusion

GmRAV may be a negative regulator acting on both photosynthesis and growth. Therefore, the enhanced expression of GmRAV in SDs compared to LDs may have caused the inhibited growth of soybean leaf, root and stem.

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