Photoresponses of transgenic tobacco plants expressing an oat phytochrome gene

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Abstract. The physiological responses of transgenic tobacco (Nicotiana tabacum L.) plants that express high levels of an introduced oat (Avena sativa L.) phytochrome (phyA) gene to various light treatments are compared with those of wild-type (WT) plants. Seeds, etiolated seedlings, and light-grown plants from a homozygous transgenic tobacco line (9A4) constructed by Keller et al. (EMBO J, 8, 1005--1012, 1989) were treated with red (R), far-red (FR), or white light (WL) with or without supplemental FR light, revealing major perturbations of the normal photobiological responses. White light stimulated germination of both WT and transgenic seed, but addition of FR to the WL treatment suppressed germination. In the WT, all fluence rates tested inhibited germination, but in the transgenics, reduction of fluence rate partially relieved germination from the FR-mediated inhibition. It is suggested that the higher absolute levels of the FR-absorbing form of phytochrome (Pfr) in the irradiated transgenics, compared to the WT, may be responsible for the reduced FR-mediated inhibition of germination in the former. Hypocotyl extension of dark-grown seedlings of both WT and transgenic lines was inhibited by continuous R or FR irradiation, typical of the high-irradiance response (HIR). After 2 d of de-etiolation in WL, the WT seedlings had lost the FR-mediated inhibition of hypocotyl extension, whereas it was retained in the transgenics. The FR-mediated inhibition of hypocotyl extension in the transgenic seedlings after de-etiolation may reflect the persistence of an, FR--HIR response mediated by the overexpressed oat phyA phytochrome. Light-grown WT seedlings exhibited typical shade-avoidance responses when treated with WL supplemented with high levels of FR radiation.

Internode and petiole extension rates were markedly increased, and the chlorophyll a:b ratio decreased, in the low-R:FR treatment. The transgenics, however, showed no increases in extension growth under low-R:FR treatments, and at low fluence rates both internode and petiole extension rates were significantly decreased by low R:FR. Interpretation of these data is difficult. The depression of the chlorophyll a:b ratio by low R:FR was identical in WT and transgenic plants, indicating that not all shade-avoidance responses of light-grown plants were disrupted by the over-expression of the introduced oat phyA gene. The results are discussed in relation to the proposal that different members of the phytochrome family may have different physiological roles.

Key words: Gene (oat phyA) – Light (red:far red ratio) – Nicotiana (transgenic, photoresponses) – Phytochrome – gene (in transgenic plant)

Introduction

Phytochrome is a family of plant photoreceptors, encoded by at least three types of nuclear genes (Sharrock and Quail 1989). The members of the phytochrome family are responsible for acquiring from the natural light environment information that is crucially important for the normal development of the plant at all stages of its life-cycle (for a review, see Smith 1982). The realisation that phytochrome is encoded by a family of genes has led to the proposal that the members of the family may have differential roles in the regulation of development and metabolism (Sharrock and Quail 1989; Smith and Whitelam 1990; Smith et al. 1991). This concept offers the prospect of resolving some of the conflicts created by physiological investigations which have identified several different modes of phytochrome regulation of development, a multiplicity of control irreconcilable on the basis of a single photoreceptor. Direct evidence on whether or not different phytochromes have different roles is, how-
However, as yet unavailable. In this article, we address this question by the analysis of the physiological responses of transgenic tobacco plants that constitutively express an introduced cereal phytochrome gene.

We use here the terminology recently adopted regarding the naming of the different phytochrome genes and the gene products (see Thomas and Johnson 1991). Thus, phyA refers to the gene, and PhyA to the functional protein encoded by that gene. Of the three phy genes characterised by Sharrock and Quail (1989) in Arabidopsis, phyA was shown to be homologous with the genes previously characterised in oats (Hershey et al. 1985), rice (Kay et al. 1989a) and peas (Sato 1988) and which are reasonably assumed to code for the species of phytochrome that accumulates in etiolated tissues, is down-regulated by exposure to light, and is unstable in the far-red absorbing (Pfr) form. This form of phytochrome is variously known as Type I, or etiolated-tissue phytochrome, and can be distinguished from other forms of phytochrome on immunochemical grounds (Abe et al. 1978; Shimazaki and Pratt 1985; Tokuhisa and Quail 1985). It is not justified, however, to use the terms PhyA and Type I interchangeably, except where the identity of the gene product has been demonstrated; this restriction is adhered to here.

Three reports have appeared of transgenic plants that express introduced cereal phyA genes. Keller et al. (1989) introduced an oat phyA gene into transgenic tobacco, Boylan and Quail (1989) introduced an oat phyA gene into transgenic tomato, and Kay et al. (1989b) introduced a rice phyA gene into transgenic tobacco. In all three cases, constitutive promoters were used, and high levels of expression of the introduced phyA genes were observed. In the oat gene transformations, major morphological phenotypic changes were observed, with transgenic plants exhibiting marked dwarfism and increased leaf area, for tomato, fruit pigmentation (Keller et al. 1989; Boylan and Quail 1989). Transgenic tobacco expressing the rice phyA gene did not exhibit a similar morphological phenotype, although alterations in photoregulated gene expression were observed (Kay et al. 1989b).

The dwarfism caused by expression of an introduced phyA gene was interpreted by Keller et al. (1989) and Boylan and Quail (1989) as probably being a consequence of the normally high levels of functional Pfr established in the light-grown transgenic plants. This view is consistent with recent speculation (see, for example, Smith and Whitelam 1990; Smith et al. 1991) that Type I phytochrome, which accumulates in etiolated tissues to high levels, operates as an antenna, allowing rapid and sensitive detection of the approach of a seedling tip to the soil surface and the initiation of the developmental change towards the photoautotrophic growth habit. If this is so, then forms of phytochrome other than Type I may be responsible for the perception of the ratio of red to far-red light (R:FR) in light-grown plants, and for the phenomena of neighbour detection, proximity perception and shade avoidance that are of major importance in adaptation to the natural light environment (Holmes and Smith 1975; Morgan and Smith 1978; Smith 1982; Ballaré et al. 1987, 1990; Casal and Smith 1989; Smith et al. 1990). On this basis, the light-lability of Type I Pfr, and the down-regulation of Type I synthesis in the light, result in the rapid loss of Type I on de-etiolation to levels at which it presumably cannot interfere with R:FR perception by other phytochromes normally present at very low concentrations. The heterologous PhyA in transgenic plants appears not to be subject to down-regulation of synthesis (Keller et al. 1989; Boylan and Quail 1989), presumably because of the constitutive promoter used, and therefore its level remains high even after de-etiolation.

The fact that introduced PhyA is functional in an heterologous transgenic situation provides an opportunity to probe the physiological role of this form of phytochrome, and to begin to address the question of where all phytochromes have interchangeable, or distinct, roles. In the experiments reported here, seeds, etiolated seedlings, and light-grown plants from a transgenic tobacco line expressing high levels of oat phytochrome (Keller et al. 1989) have been subjected to physiological tests which reveal major perturbations of the normal photobiological responses, some of which are difficult to interpret on the basis of current models.

Materials and methods

Plant material. Seed used were of the wild-type (WT) Nicotiana tabacum L., cv. xanthi, and an homozygous isogenic line which had been transformed with the oat phyA gene fused to the constitutive cauliflower mosaic virus (CaMV-35S) promoter using standard Agrobacterium procedures (Keller et al. 1989), and referred to in the following text as the 9A4 line. Seed used in these experiments had been stored at room temperature for at least three months prior to sowing and were allowed to imbibe at 25°C in white light (WL) on water-saturated filter paper. For use in experiments with light-grown plants, germinated seed were transferred to potting compost and grown under continuous WL from fluorescent tubes (photosynthetically active radiation (PAR) = 130 μmol photons m⁻² s⁻¹) at 25°C until the third true leaf pair had expanded (approx. five weeks after sowing). At this stage plants were potted into 300-cm³ pots and transferred to the light treatments indicated.

Light sources. Red light (R) (total fluence rate 600--700 nm = 4.8 μmol m⁻² s⁻¹) for inhibition of seedling hypocotyl growth was provided by filtering the radiation from Thorn EMI (Birmingham, UK) Deluxe Natural 40-W fluorescent tubes through 1-cm-deep copper sulphate solution (1.5% w/v) and one red (No. 14) Cinemoid sheet (Rank Strand, Isleworth, Middlesex, UK). Far-red light (FR) (total fluence rate 700--800 nm = 11 μmol m⁻² s⁻¹) was provided by water-cooled 100-W incandescent bulbs with a black acrylic filter (Plexiglas Type FFR 700; West Lake Plastics, Lem, Penn., USA).

Two pairs of R:FR-treatment cabinets of similar design were used in this work, one used at low and one at high irradiances. In both pairs of cabinets, background WL was provided by banks of fluorescent tubes, and additional FR by filtering the radiation from interspersed banks of incandescent bulbs through combinations of red and green plastic filters, the radiant infra-red being removed by flowing "water windows". The design allows for uniform levels of PAR (400--700 nm) and wide ranges of R:FR.

In the high-PAR cabinets, background WL was provided by 24 80-W Cool White fluorescent tubes, and FR was provided by filtering the radiation from 24 500-W Philips (Turnhout, Belgium) 7785R quartz-halogen lamps through 4 cm of cooled flowing water, one layer (3 mm) of red (Number 4400), and one layer of green (Number 6600) Perspex (SIBA, Leicester, UK). In the low-PAR cabinets, WL was from eight 40-W Cool White fluorescent tubes,