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*Rapid Communication*

# Phytochrome B Affects the Levels of a Graft-Transmissible Signal Involved in Tuberization[1]

**Stephen D. Jackson*, Pat James, Salomé Prat, and Brian Thomas**

Horticulture Research International, Wellesbourne, Warwick CV35 9EF, United Kingdom (S.D.J., P.J., B.T.); and Departamento de Genética Molecular, Centro de Investigación y Desarrollo, Consejo Superior de Investigaciones Científicas, Jordi Girona 18–26, 08034 Barcelona, Spain (S.P.)

**Grafting experiments between phytochrome B antisense and wild-type potato (*Solanum tuberosum* L. subsp. *andigena* [line 7540]) plants provide evidence that phytochrome B is involved in the production of a graft-transmissible inhibitor of tuberization, the level of which is reduced in the antisense plants, allowing them to tuberize in noninducing photoperiods.**

In the 1950s grafting experiments suggested that a tuber-inducing stimulus is produced in the leaves of potato (*Solanum tuberosum* L.) plants that were grown in inductive short photoperiods (Gregory, 1956). In these experiments a leaf from an induced plant grown under SD conditions grafted onto a plant grown in noninducing, LD conditions was capable of promoting tuberization in the noninduced plant. These results were repeated with different varieties and species of potato (Chapman, 1958). In *S. tuberosum* L. subsp. *andigena*, it has been shown that the graft-transmissible signal can move acropetally as well as basipetally (Kumar and Wareing, 1973).

There are many similarities between the tuber-inducing signal and the flower-inducing florigen, and in fact there is evidence that common signaling factors are involved in daylength-induced flowering and tuberization. This evidence also comes from grafting experiments. A leaf from a tobacco (*Nicotiana tabacum* L.) plant induced to flower grafted onto a potato stock induced the potato stock to tuberize, whereas a leaf from a noninduced tobacco plant did not induce tuberization. This was true even if a leaf from LD-requiring tobacco (*Nicotiana sylvestris* L.) was used, in which case tuberization of the potato stock was induced under LD conditions and did not occur under SD conditions as it normally would. Similar results have also been obtained in other interspecific grafting experiments between sunflower and Jerusalem artichoke and are thus not unique to tobacco and potato (Nitsch, 1965; Chailakhyan et al., 1981; Martin et al., 1982; for review, see Ewing, 1995).

Grafting experiments between LD and day-neutral tobacco species (Lang et al., 1977) and between different flowering time mutants of pea (Taylor and Murfet, 1996) have also indicated the existence of a floral inhibitor or "antiflorigen." There appears to be a direct relationship between the inducing signal and an inhibitor in tobacco. When shoots with differing numbers of leaves from the SD plant *N. tabacum* L. cv Maryland Mammoth and the LD plant *N. sylvestris* were grafted together onto a receptor cv Maryland Mammoth plant and kept in SD conditions, flowering of the receptor was advanced with increasing numbers of leaves from the SD plant and delayed with increasing numbers of leaves from the LD plant (Lang, 1980). The response to daylength thus appears to be determined by the relative levels of the inducing and inhibitory substances, the levels of at least one of which is affected by photoperiod. The involvement of inducing and inhibitory substances in a multifactorial control is one reason that the isolation and identification of these factors still has not been achieved.

Phytochrome B has been shown to be involved in the photoperiodic control of tuberization in *S. tuberosum* subsp. *andigena* (Jackson et al., 1996). Plants with reduced levels of phytochrome B as a result of antisense inhibition have lost the control of tuberization by photoperiod. These phytochrome B antisense plants tuberize in LD as well as SD conditions, whereas wild-type plants will only tuberize in SD conditions. Phytochrome B is therefore involved in the inhibition of tuberization caused by LD conditions. The tuberization of the antisense phytochrome B plants in LD conditions could be explained either by the production of an inducer in both LD and SD conditions or by the absence of an inhibitor normally present in LD conditions. In this paper we describe grafting experiments designed to determine whether phytochrome B is involved in the production of a graft-transmissible signal and whether this signal is an inducer or an inhibitor.

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* Corresponding author; e-mail stephen.jackson@hri.ac.uk; fax 44–1789–470552.

Abbreviations: LD, long-day; SD, short-day.

## MATERIALS AND METHODS

The photoperiodic potato (*Solanum tuberosum* L. subsp. *andigena*) wild-type line 7540 was obtained from the Institute für Pflanzenbau und Pflanzenzüchtung Bundesforschungsanstalt für Landwirtschaft Braunschweig-Volkenrode (Braunschweig, Germany). The antisense phytochrome B transgenic lines were produced as described by Jackson et al. (1996). Plants were derived from in vitro grown plantlets that had been planted in soil and subsequently propagated through stem cuttings. The plants were grown in soil in growth cabinets (Sanyo, Itasca, IL) under cool-white fluorescent tubes (Pluslux 3500, Thorn, UK) in 16-h light (100 $\mu$mol$^{-2}$ s$^{-1}$), 8-h dark photoperiods at a constant 70% humidity and 22°C.

### Grafting

The scions were prepared by cutting the stem with a new razor blade below the second or third leaf from the apex. They were immediately put in water to prevent air bubbles from forming in the xylem. The stocks were prepared by removing all of the lower leaves if necessary, tying the stem firmly to a supporting stick, and then cutting the stem transversely approximately 2 cm above soil level with a clean razor blade. A drop of water was applied to the cut stem to prevent its drying out. The scion to be grafted was then placed on top of the stock and the two sections were held in place by binding them together with tape (Micropore, 3M) to the supporting stick. The grafted plants were then placed in a tray with a transparent cover and returned to 16-h photoperiods in the growth cabinets.

## RESULTS

In a typical experiment, five or six grafted plants were prepared for each combination. A high proportion of the grafts did not take and died within 1 to 2 d. However, one or two grafts for each combination did take successfully, and it is only the results of the successful grafts that have been taken into consideration. Because of the low success

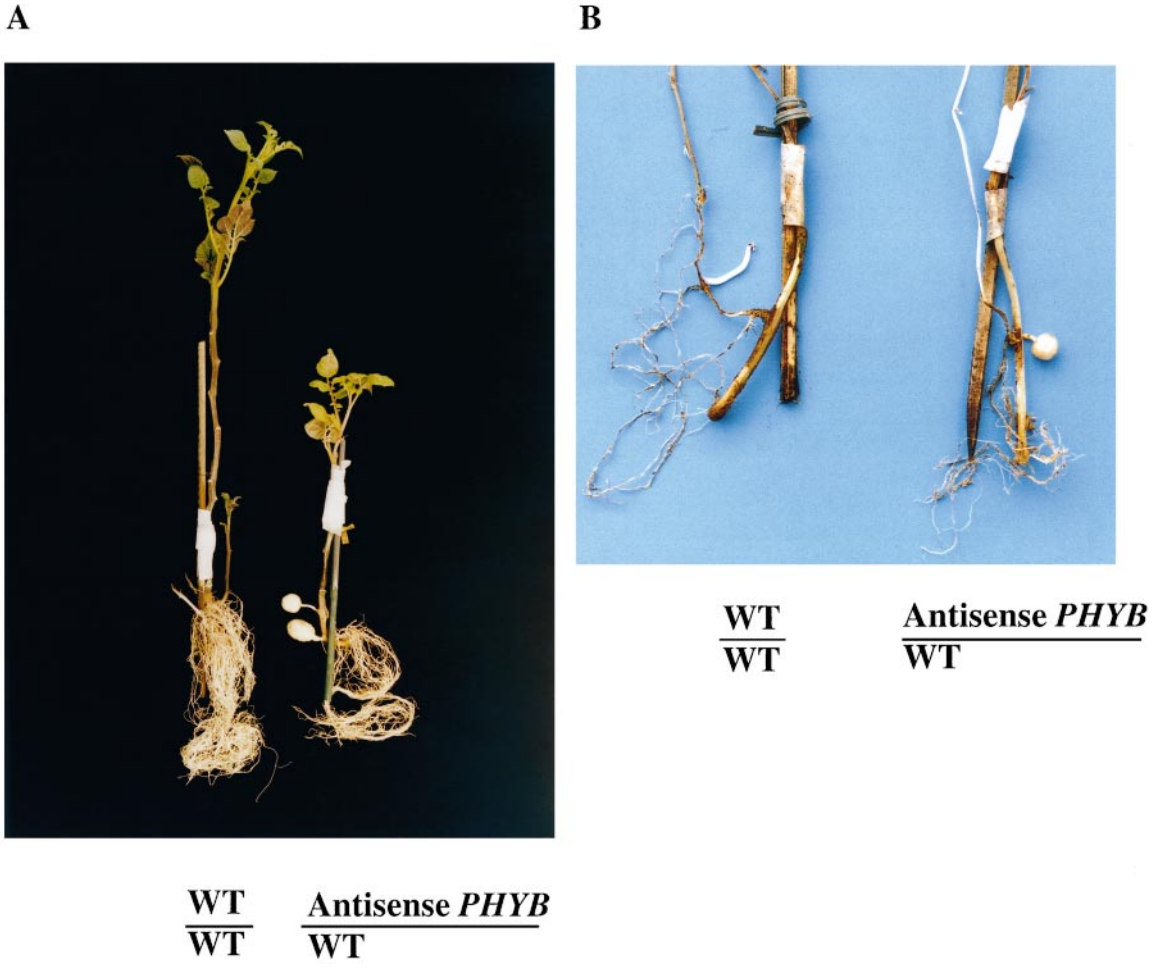


**Figure 1.** A, Antisense phytochrome B (*PHYB*) scion grafted onto a wild-type (WT) stock (right) 1 month after grafting, and a control wild-type scion on a wild-type stock (left) 2 months after grafting. B, Antisense phytochrome B plant scion on a wild-type stock (right) and a control wild-type scion on a wild-type stock (left). At the time of the photograph, 3 weeks after grafting, there were approximately five or six leaves on the antisense phytochrome B and wild-type scions. In both cases the grafted plants were maintained in a 16-h photoperiod.

rate the experiment was repeated three times. The total number of successful grafts for each combination were five antisense phytochrome B/wild-type, five wild-type/wild-type, and four wild-type/antisense phytochrome B, with consistent results being obtained from all graftings. In the graftings in which tuberization occurred, tubers normally formed after 2 to 3 weeks.

### Antisense Phytochrome B Graft on a Wild-Type Plant Stock

When apex scions from antisense phytochrome B plants were grafted onto wild-type stocks from which all of the leaves had been removed, the scion from the antisense plant could induce tuberization of the wild-type plant under LD conditions (Fig. 1). The stolon from the wild-type stock that can be seen to be developing into a shoot in the antisense plant/wild-type plant grafting shown in Figure 1B had just emerged above the soil surface and developed a few very small leaves when the photograph was taken.

If the apex scions from antisense plants were grafted onto wild-type plants from which all leaves and side shoots had not been removed, tuberization did not occur under LD conditions even several weeks after nongrafted control antisense phytochrome B plants kept in the same conditions had tuberized (data not shown).

The effect of the reduced levels of phytochrome B in the antisense plants can thus be transmitted across a graft union from a transformed plant to a leafless, nontransformed plant, indicating that phytochrome B must be involved in the production of a graft-transmissible signal. The presence of leaves on the wild-type stock annuls the effect of the antisense graft and tuberization was not induced under LD conditions.

### Wild-Type Graft on a Wild-Type Plant Stock

Wild-type scions were grafted onto wild-type stocks to serve as controls to prove that the stress suffered by the plants during the grafting procedure was not the cause of the tuberization observed in the antisense phytochrome B/wild-type graft unions. Wild-type scions did not cause tuberization on wild-type stocks under LD conditions (Fig. 1). The wild-type/wild-type grafting shown in Figure 1A is larger than the antisense phytochrome B/wild-type grafting because it had been grown for a longer period of time; despite being older, it still had not tuberized by the time the antisense phytochrome B/wild-type grafting had tuberized.

Since all of the leaves had been removed from the wild-type stock plants, the inhibitory effect of LD conditions on tuberization must have been transmitted across the graft union, from the grafted section possessing the leaves to the stock section where tuberization was inhibited. Whereas tuberization was inhibited, stolon growth was not and occurred as in normal wild-type plants grown under LD conditions.

### Wild-Type Graft on an Antisense Phytochrome B Plant Stock

Because the influence of an antisense phytochrome B graft on a wild-type stock plant could be negated if leaves were left on the wild-type section, the reciprocal grafting was performed to address the question of whether the tuberization response was a proximity effect, i.e. whether the response (tuberization or not) simply depended on the type of leaves (antisense phytochrome B or wild type) closest to the prospective site of tuberization. Figure 2 shows a wild-type apical section grafted onto one of two shoots of an antisense phytochrome B plant. In this particular case we can see that the antisense phytochrome B plant has initiated tuber formation under LD conditions, as indicated by the small, pinkish swelling of the stolon not far from the base of the buried stem. As the wild-type graft grows, however, its influence becomes stronger and tuber

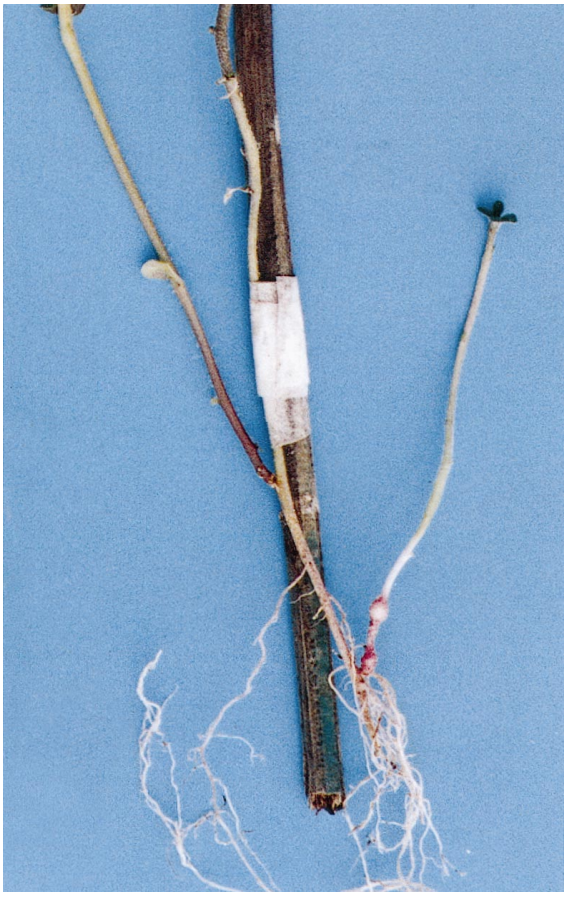

**Figure 2.** A scion from a wild-type plant grafted onto the right of two shoots of an antisense phytochrome B plant. The grafted plant was maintained in a 16-h photoperiod and tuberization of the antisense phytochrome B plant had started (visible as a small, pink swelling of the stolon) but ceased after the wild-type graft took and its influence became dominant. Stolon growth resumed and this eventually became a new shoot—a typical wild-type response to LD conditions. At the time of the photograph, 3 weeks after grafting, there were 10 leaves on the shoot from the antisense phytochrome B plant and 8 on the wild-type shoot.

formation ceases and stolon growth resumes. The stolon eventually forms a new shoot after it emerges above the soil surface. Other graftings of this type did not form tubers, although stolon formation did occur.

These grafting experiments demonstrate that an inhibitor of tuberization is present in the leaves of wild-type plants under LD conditions and that this inhibitor is dominant over the influence of leaves from an antisense phytochrome B plant. This also explains the observation that an antisense phytochrome B graft will induce tuberization in a wild-type stock plant in LD conditions only if all of the leaves and side shoots of the stock plant have been removed.

## DISCUSSION

In this paper we show that phytochrome B affects the levels of a graft-transmissible factor involved in the control of tuberization. Our observations support the conclusion that this factor is an inhibitor of tuberization that is produced in the leaves of wild-type plants in noninducing, LD conditions and that this inhibitor is absent or ineffective at preventing tuberization in the phytochrome B antisense plants.

There is an accumulating amount of evidence that this tuberization inhibitor may be a GA. GAs are known to inhibit tuberization, and treating wild-type *S. tuberosum* subsp. *andigena* potato plants with an inhibitor of GA biosynthesis enables them to tuberize in LD conditions (Jackson and Prat, 1996). Photoperiod is known to affect certain steps in the GA biosynthetic pathway in various species, e.g. spinach, pea, and willow (Davies et al., 1986; Gilmour et al., 1986; Olsen et al., 1995), and phytochrome B has been shown to affect GA levels or sensitivity (Devlin et al., 1992; Foster et al., 1994; Weller et al., 1994; Lopez-Juez et al., 1995).

Although the tuberization of the antisense phytochrome B plants in LD conditions can be explained solely on the basis of a reduction in the level of an inhibitor, we cannot say anything about the role of an inducer of tuberization in these plants, the level of which may be constant or vary depending on the photoperiod. It is possible that the absence of or reduced levels of inhibitor caused by reduced levels of phytochrome B is an unnatural situation that would not normally occur and that the photoperiodic control of tuberization in wild-type plants is normally mediated solely by changes in the level of the inducer. Most current theories, however, propose that the levels of both the inducer and the inhibitor can vary and that the tuberization/flowering response is determined by the relative levels of the two. Our data argue strongly for the existence of an inhibitor in wild-type plants in LD conditions, the levels of which are controlled by phytochrome B, and thus are consistent with the inducer/inhibitor hypothesis.

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