Indoleacetic Acid and Abscisic Acid Antagonism

I. ON THE PHYTOCHROME-MEDIATED ATTACHMENT OF MUNG BEAN ROOT TIPS ON GLASS

T. Tanada
United States Soils Laboratory, Soil and Water Conservation Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705

ABSTRACT

The phytochrome-mediated attachment of mung bean (Phaseolus vulgaris L., var. Oklahoma 612) root tips on glass is quickly affected by indoleacetic acid and abscisic acid at concentrations of 10 nM or less. Indoleacetic acid induces detachment, whereas abscisic acid induces attachment. Both plant regulators rapidly antagonize the action of the other. None of several cytokinins, gibberellins, and ethylene tested over a wide range in concentration had any effect on either attachment or detachment of root tips. It is postulated that phytochrome could control the endogenous levels of indoleacetic acid and abscisic acid and perhaps other hormones under certain circumstances, that this action is the first process initiated by phytochrome, and that indoleacetic acid and abscisic acid act on the plasmalemma to bring about opposing changes in the surface electric charges of plant cells.

A recent study on the phytochrome-mediated attachment of mung bean and barley root tips on a wet glass surface has indicated that this rapid photo-reversible process could be modified by prior exposure of imbibed seeds or seedlings to red or far red light (20). Since the effects of the pretreatment become manifest only after several hours following light exposure, this modification could be due to a buildup of one or more substances of a hormonal nature through phytochrome action. Since low concentrations of IAA influence the photo-reversible attachment of both mung bean and barley root tips (18–20), it is possible that IAA could be one of the hormones whose level is controlled by phytochrome. This investigation was undertaken to see whether other growth substances could also affect the photo-reversible attachment of mung bean root tips at low concentrations. A preliminary report of this study has been published recently (21).

MATERIALS AND METHODS

The methods and materials used were essentially the same as reported earlier (20). In brief, mung bean seeds (Phaseolus aureus L. var. Oklahoma 612), steeped in aerated water for 8 hr were exposed to 5 min of red light (65 μW cm⁻² at 600–700 nm) or 10 min of far red light (190 μW cm⁻² at 700–750 nm). They were then germinated in darkness at 24 C over aerated deionized water. Three days later, 10 root tips 2 mm long were excised from seedlings and placed in a glass beaker containing 10 ml of solution of the following composition: KCl, CaCl₂, MgCl₂ of 0.1 mM; MnCl₂, 2 μM; L-ascorbic acid, 1 μM; and 3-IAA, 0.2 nM. To this solution were added various amounts of ABA, IAA, GA₅, mixture of GA₅ (54%), and GA₇ (46%), kinetin, zeatin, benzyladenine, or ethylene. (The ABA was supplied by Dr. J. C. Bauerfeind, Hoffman-La Roche, Inc.) The beaker was placed immediately on a phonograph turntable in a beam of red light (4.0 μW cm⁻², 650–680 nm), and the root tips were arranged about 2.5 cm from the center of the turntable with a stainless steel wire. After 1 min of red irradiation, the turntable was gradually accelerated to a speed of 33 rpm for a few revolutions. The number of root tips remaining attached was counted while the turntable was gradually slowed down. Tips were scored as detached if they were displaced more than 2 mm. This procedure of scoring once every minute was carried out for 5 min in red light and for 7 min in far red light (8.9 μW cm⁻², 710–750 nm). Except for arranging them at the beginning of each new light exposure, the root tips were subjected to a minimum of handling. After each run, the beaker was washed with a detergent containing phosphates. All operations were done under very dim light and at 24 C.

RESULTS

Some typical results are presented in Figures 1 to 6. Throughout this investigation the variations among replicates were usually less than three root tips. Experiments dealing with IAA and ABA were repeated 10 times or more with different batches of seedlings. Those involving other hormones were repeated at least three times.

Figure 1a illustrates the behavior of root tips excised off seedlings from imbibed seeds exposed to red light. These root tips attach to the glass surface in red light and detach in far red light. Photoreversibility of attachment is maintained for at least three cycles. As reported earlier, increasing the IAA concentration to 10 nM quickly results in poor tip attachment in red light and complete detachment in far red light (Fig. 1b). Few tips show photoreversibility of attachment in this concentration of IAA. The data in Figure 2a show that the depressing effect of IAA on attachment is effectively reversed by ABA. In the presence of 10 nM IAA, the addition of ABA to a concentration of 1 nM at the beginning of the second cycle restores attachment in red light. At this ABA concentration, the root tips show detachment in far red light, indicating restoration of photoreversibility. At the higher concentration of 4 nM ABA, however, attachment becomes accentuated, and the root tips remain attached even in far red light (Fig. 2b).
and larger amounts preventing attachment in both red and far red light.

Some representative findings of similar studies concerning the effects of zeatin, kinetin, benzyladenine, \( GA_3 \), \( GA_4 \), \( GA_5 \) and ethylene on the photoreversible attachment of mung bean root tips are given in Figures 5 and 6. These growth substances were added to the assay solution over the concentration range from 0.3 nM to 0.3 \( \mu M \). At all concentrations employed and as depicted for 10 nM concentration, none of these substances had any effect on either attachment or detachment of mung bean root tips regardless of the light treatment given to the imbibed seeds.

![Graph 1: MUNG BEAN (5' R to seeds)](image1)

**Fig. 1.** Effect of IAA on the photoreversible attachment on glass of mung bean root tips excised off seedlings grown from seeds irradiated with red light. R: red light; FR: far red light.

![Graph 2: MUNG BEAN (5' R to seeds)](image2)

**Fig. 2.** Reversal of IAA-induced detachment of mung bean root tips by ABA. The ABA was added at the times indicated. R: red light; FR: far red light.

In contrast to root tips from seedlings irradiated in the seed stage with red light, the root tips of seedlings from seeds irradiated with far red light manifest poor attachment in red light (Fig. 3a). Attachment in red light is increased when ABA is added to the assay medium to a concentration of 0.2 nM (Fig. 3b). At this concentration of ABA, the root tips attach in red and detach in far red light. At the higher concentration of 0.5 nM ABA, however, the root tips tend to remain attached in both red and far red light (Fig. 4a). The results presented in Figure 4b show that the ABA-promoted attachment is counteracted by IAA. The degree of reversal of the ABA-promoted attachment by IAA depends upon the concentration of added IAA—smaller amounts allowing attachment in red light only.

![Graph 3: MUNG BEAN (10' FR to seeds)](image3)

**Fig. 3.** Effect of ABA on the photoreversible attachment on glass of mung bean roots tips excised off seedlings grown from seeds irradiated with far red light. R: red light, FR: far red light.

![Graph 4: MUNG BEAN (10' FR to seeds)](image4)

**Fig. 4.** Reversal of ABA-induced attachment of mung bean root tips by IAA. IAA was added at the time indicated. R: red light; FR: far red light.
The data presented in this communication show that IAA and ABA have specific and opposing influences on the phytochrome-mediated attachment of mung bean root tips on glass: IAA inducing detachment and ABA inducing attachment. Since the effects of these hormones are observed within a short time after application and at very low concentrations, they raise the interesting possibilities that these hormones are responsible for the generation of the photoreversible electric charges on the cell surfaces, that their endogenous level in some tissues could be under phytochrome control, and that the latter action could be the initial process mediated by phytochrome. An early effect of IAA on the plasma membrane during growth stimulation has been suggested by Rayle et al. (15), possibly through a membrane-bound ATPase or proton pump (8). Changes in the concentrations of IAA or auxin-like substances have been observed in plants following red or far red exposure (3, 4, 6). Barnes and Light (2) have found more ABA in pea plants grown in red light than those grown in darkness.

From the preceding surmises, the mechanism of the phytochrome-mediated attachment is postulated to proceed in the following manner. On the one hand, after red irradiation, phytochrome could increase the endogenous concentration of ABA relative to that of IAA. The excess ABA would then somehow produce a more positive charge on the cell membrane, causing attachment of root tips on the negatively charged glass surface. On the other hand, after far red irradiation, phytochrome could increase the endogenous concentration of IAA relative to that of ABA. The excess IAA would then produce a more negative charge on the cell membrane, causing detachment of root tips from the negatively charged glass surface. Stolarek (17) and Etherton (5) have shown that exogenous IAA induces a more negative bioelectric potential in plant cells. If a long dark period follows a light exposure, the concentration of one of the hormones could rise to such a high level that the rate of photoreversible attachment of root tips could be substantially affected (Fig. 3a).

Beside controlling the endogenous levels of IAA and ABA, it is possible that phytochrome could control levels of other hormones under certain circumstances. The mimicking of phytochrome action by some growth hormones, especially GA, is indicative of the existence of such a control in some plant tissues. Several investigators (13, 16) have found that the GA concentration increases shortly after red light exposure of barley and wheat leaves. Endogenous levels of cytokinins have been noted to rise after red irradiation of light-sensitive Rumex seeds (23). Recently, Jaffe (10) reported that the amounts of acetylcholine in mung bean root tips were higher immediately following red irradiation. However, his claim that acetylcholine promotes attachment of mung bean root tips on glass through phytochrome action is probably not valid since it is effective only at high concentrations, and its effect is reversed by K+ (22). In addition, acetylcholine appears to take no part in several phytochrome-mediated processes in mustard seedlings (11).

If phytochrome regulates the endogenous levels of plant hormones, it could do so through several means. In one form it could release them from vesicles or bound forms, activate or promote synthesis of hormone-destroying enzymes, or it could induce formation of substances that inhibit these enzymes (9, 14). Needless to say, further studies are necessary to identify the correct control mechanism.

Antagonism of ABA to the actions of other growth-promoting hormones appears to be fairly common (1, 7, 12). However, since in all of these investigations the time lag between
application of ABA and the observed effect was usually long, the process or reaction under ABA influence could be different from that promoted by the growth promoters. In this study it has been shown that IAA counteracted the effect of ABA and ABA counteracted the effect of IAA within a few minutes after application of either substance. Consequently, in this particular case, these two growth regulators could have been acting, if not on the same site, on the same process or system that controls the surface electric potentials of plant cell membranes.

LITERATURE CITED