Growth Regulators Have Rapid Effects on Photosynthetic Unloading from Seed Coats of Phaseolus vulgaris L.¹

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ABSTRACT
Of nine plant growth regulators (indoleacetic acid, 1-naphthaleneacetic acid, gibberellic acid, gibberelin 4/7, 6-benzylaminopurine, 6-furfurylaminopurine, abscisic acid, and 1-aminocyclopropane carboxylic acid) tested, only 6-benzylaminopurine and abscisic acid affected ¹⁴C-photosynthetic unloading from excised seed coats of Phaseolus vulgaris L. Unloading, in the presence of KCl, was stimulated by 25 to 40%. Stimulation occurred immediately for 6-benzylaminopurine and for abscisic acid within 10 to 12 minutes of application.

MATERIALS AND METHODS
Plant Material and Selection of Seeds. Dwarf bean plants (Phaseolus vulgaris L. cv Redland Pioneer) raised under greenhouse conditions (14) in the summer months in Australia (September–March) were pruned to a single pod at the third trifoliate node supported by the second trifoliate and primary leaves. Plants were selected for experiments when their pods contained seeds at one of the two stages of development defined as: stage 1—pale-green seeds, fresh weight embryo = 0.43 ± 0.03 g; fresh weight seed coat = 0.18 ± 0.006 g; and stage 2—light-gray to brown seeds, fresh weight embryo = 0.66 ± 0.03 g; fresh weight seed coat = 0.22 ± 0.008 g. These stages correspond closely to stages 4 and 6, respectively, of the seed developmental stages described for Phaseolus (24). The fresh weight values reported may be related to other seed growth characteristics (10). Before use in experiments, plants were transferred to a growth room (14-h photoperiod, constant temperature 24°C, quantum flux density at plant level 200 μmol m⁻² s⁻¹ (400–700 μm)) for a 16-h equilibration period.

Preparation of Seed-Coat Halves and Measurement of ¹⁴C-Photosynthetic Unloading. The terminal leaflet of trifoliate leaf 2 was supplied with 0.375 MBq ¹⁴CO₂ generated from Na₂¹⁴CO₃ (37 MBq/mmol from Radiochemical Centre Amersham) and pods harvested 2 h later. Seeds were removed from pods and the seed coat was cut laterally and gently pulled from the embryo. Matched seed-coat halves from each seed were used as a control and treatment. Aerated bathing solutions with or without growth regulator and containing 150 mM sorbitol, 20 mM KCl, 10 mM sucrose, and 0.5 mM CaCl₂ in 5 mM Mes-Tris buffer at pH 6.0 were dispensed into the cavities of seed-coat halves. In some experiments, KCl concentration was varied and the osmolality maintained at a constant value by altering the concentration of sorbitol in the bathing medium. Bathing solutions were discarded/replaced 3 times over a 0 to 10 min period to remove free space label and thereafter collected/replaced every 2 or 5 min depending on the experiment. Collected solutions were bulked when required, reduced to dryness at 80°C, and ¹⁴C activity assessed using liquid scintillation counting. Full details of methods used to prepare seed-coat halves and measure ¹⁴C-photosynthetic unloading have been described previously (12).

Plant Growth Regulators. These were obtained from the following sources: ACC³, BAP, IAA, and NAA from Sigma Chemical Co.; ABA (racemate), GA₃, and KIN from Calbiochem-Behring Corp.; GA₄/7, a gift from Schering Aust. Pty. Ltd. All growth regulators (except GA₄/7 supplied as a liquid concentrate containing 20 g active ingredient/L) were dissolved in a minimum volume of 0.5 M KOH before being prepared as appropriate stock solutions.

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³ Abbreviations: ACC, 1-aminocyclopropane carboxylic acid; BAP, 6-benzylaminopurine; GA₄/7, gibberellin 4/7; NAA, 1-naphthalene acetic acid; KIN, 6-furfurylaminopurine.
RESULTS AND DISCUSSION

Any data showing that growth regulators alter photosynthetic unloading in the legume seed-coat experimental system arguably provide an unequivocal demonstration that they influence unloading in sink regions in a direct manner. Such a demonstration was shown by the results for BAP and ABA, two of the growth regulators used in this study (Table I). For these growth regulators, we did not observe promotory effects of more than 40% above the control. At stage 2 of seed development, BAP effects on unloading were readily reproducible with best promotion observed at 10⁻⁵ M BAP without effect or slight promotion at 10⁻⁶ M, and no effect or inhibition at 10⁻⁴ M (cf. Table I). In contrast, ABA effects on unloading varied from experiment to experiment. In many experiments best promotion occurred at 10⁻⁵ M, but in some experiments promotions were highest at 10⁻⁶ M and, in a single experiment, at 10⁻⁴ M. For 35% of experiments, an ABA effect was completely absent. Such variability in response to ABA is possibly due to variations in its endogenous levels in the seed coats both between plants and between batches of plants (cf. 15). For stage 1 seeds, 10⁻³ M BAP gave a significant (P = 0.05) 10 to 15% promotion of unloading, but ABA was nonaffective at this stage of development (data not shown). Photosynthetic unloading was not significantly affected by IAA, NAA, GA₃, GA₄/7, KIN, and ACC applied at concentrations of 10⁻⁴, 10⁻³, and 10⁻² M to seed coats excised from seeds at the two stages of development (data not shown). We recognize that the latter results do not preclude the possibility that these chemicals could influence unloading. For instance, extension of the treatment times could elicit responses (e.g. 8). Alternatively, endogenous levels could have been optimal as indicated by the finding that 2-chlorophenoxyisobutyric acid, 4-chlorophenoxyisobutyric acid, and 2,4,6-trichlorophenoxyacetic acid (compounds related to 2,4-dichlorophenoxyacetic acid and thought to be auxin agonists [GF Katekar, personal communication]) were all capable of promoting unloading in the order of 10 to 15% above the control (data not shown). We tentatively suggest that the reason for no significant response to KIN while the synthetic cytokinin BAP produced both inhibition and promotion is due to the higher physiological activity of BAP compared with KIN (18).

Further investigation was confined to examination of the effects of BAP and ABA on photosynthetic unloading from seed coats of stage 2 seeds. Clearly, promotory effects of both BAP and ABA on photosynthetic unloading are rapid as shown by time-course data for these growth regulators (Fig. 1). For BAP (10⁻⁵ M), promotion of unloading was immediate and reached

Table I. Effect of BAP and ABA on ¹⁴C-Photosynthetic Unloading from Excised Seed Coats of Developmental Stage 2 Seeds of Phaseolus vulgaris L.

<table>
<thead>
<tr>
<th>Concentration of Growth Regulator</th>
<th>BAP % Unloading (no</th>
<th>ABA % Unloading (no</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BAP = 100% at</td>
<td>BAP = 100% at</td>
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<tr>
<td></td>
<td>10-30 min 30-50 min</td>
<td>10-30 min 30-50 min</td>
</tr>
<tr>
<td>10⁻⁴ M</td>
<td>72* 75*</td>
<td>107* 99*</td>
</tr>
<tr>
<td>10⁻³ M</td>
<td>122b 126b</td>
<td>131b 125*</td>
</tr>
<tr>
<td>10⁻² M</td>
<td>108c 106a</td>
<td>131b 137*</td>
</tr>
</tbody>
</table>

Fig. 1. Time course of BAP- or ABA-stimulated ¹⁴C-photosynthetic unloading from excised seed coats of developmental stage 2 seeds of Phaseolus vulgaris L. BAP or ABA was added at 10 min, after removal of free space label. Bathing solutions were collected/replaced every 2 min with ¹⁴C activity assessed for each collection. Unloading is expressed as a percentage of the control value. Values are the mean of 10 replicates. BAP and ABA were supplied at 10⁻⁵ M and 10⁻⁶ M concentrations, respectively. For each experiment the LSD P = 0.05 is indicated.

Fig. 2. Effect of KCl concentration on BAP- or ABA-stimulated ¹⁴C-photosynthetic unloading from excised seed-coats of developmental stage 2 seeds of Phaseolus vulgaris L. Osmolality was maintained at a constant value (200 mOsm/kg) with changing KCl concentration by altering the concentration of sorbitol. BAP or ABA was added at the start of the experiment and KCI at 10 min, after removal of free space label. Bathing solutions were collected/replaced every 5 min and ¹⁴C activity assessed for 10 to 30 (○) and 30 to 50 (□) min collecting periods. Unloading is expressed as a percentage of the control value. Values are the mean of 8 replicates. Both BAP and ABA were supplied at a 10⁻⁵ M concentration. For each experiment the LSD P = 0.05 is indicated.
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maximal levels within 4 to 6 min. Thereafter, unloading declined to control levels before rising rapidly again by 18 to 20 min. For ABA (10^{-4} M) treatment, however, unloading was apparently inhibited between 0 to 6 min and increased only with further time so that promotion neared maximum by 10 to 12 min. Interpretation of these contrasting time-course patterns for BAP and ABA need further study. However, the effects of both growth regulators are suggestive of direct action on membrane permeability. Certainly, for ABA it is well documented that it closes stomata within minutes (27) and it is now generally accepted that many growth regulators including cytokinins and ABA can change the properties of cell membranes without the need for changes in enzyme synthesis (28).

We also examined the possible interaction of BAP and ABA with external KCl concentration since, in Phaseolus seed coats, K^+ is a major osmotic component of the apoplast (13) and K^+ stimulates proton extrusion (21) and photosynthetic unloading (20). For BAP and ABA (both at 10^{-5} M), promotion of ^14C-photosynthetic unloading was maximal between 20 to 50 mM KCl with unloading in the case of ABA falling to control levels when 100 mM KCl was present in the bathing solution (Fig. 2). The KCl-dependence of the growth regulator effects on photosynthetic unloading is suggestive of their interaction with a membrane protein (27) which appears to be directly involved in the transfer (20). Thus, BAP could stimulate K^+ influx across the plasmalemma in the same way that cytokinins stimulate K^+ accumulation by guard cells (18) which, in turn, would increase photosynthetic unloading (20). Dependence of ABA promotion of unloading upon K^+ is more difficult to interpret, especially in view of reports that ABA causes K^+ efflux from guard cells (7) and K^+ release into the xylem (4). A number of possible modes of ABA action on photosynthetic unloading are currently being examined. These include, an indirect effect of ABA mediated by its inhibition of proton extrusion through a plasmalemma-ATPase (21) and possible interaction with Cl^- rather than K^+ ions (23).

It is especially interesting that testing a range of growth regulators for their short-term effects on photosynthetic unloading revealed promotions with a cytokinin and ABA. Current evidence suggests that a large proportion of both these growth regulators are not synthesized in the seeds themselves but are transported to seeds after synthesis elsewhere in the plant. For example, cytokinins synthesized in roots are transported to developing seeds via the xylem (22), while ABA synthesized in photosynthesizing leaves is transported to seeds via the phloem (2). It is possible, therefore, that assimilate supply from source leaves could influence indirectly the amounts of cytokinins, and influence directly the amounts of ABA, reaching the tissues of the seed coat. Some control of sink unloading residing in the source itself would provide the means for source leaves to influence sink unloading according to total photosynthetic supply. Circumstantial evidence that such a mechanism operates in the whole plant for ABA is provided by a report that soybean varieties with larger seed size contain higher ABA levels in seed coats (17). Interestingly, preliminary results of our own examining the effects of BAP and ABA injection into developing Phaseolus seeds in situ indicate that higher seed size is associated with both growth regulators only when plants are severely defoliated in order to create a limited photosynthetic supply for seed growth.

In conclusion, it has been demonstrated that the synthetic cytokinin BAP and ABA are capable of rapid and significant promotions of ^14C-photosynthetic unloading from Phaseolus seed coats provided that 20 to 50 mM KCl is included in the bathing medium. Our study gives direct evidence that BAP and ABA influence unloading of phloem-derived photosynthetic into the apoplasm of the Phaseolus seed coat. The possibility that cytokinins and ABA can regulate the energized component of plasmalemma transfer to the apoplast in the legume seed coat and the significance of such regulation for seed growth are worthy of further investigation.

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