NAA Restores Apical Dominance in the axr3-1 Mutant of Arabidopsis thaliana

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Strong evidence for a role of auxin in apical dominance is provided by the classic Thimann-Skoog experiment (Proceedings of the National Academy of Sciences, USA 19: 714–716, 1933) wherein exogenous auxin applied to a decapitated shoot represses outgrowth of the next lower lateral bud. Although apical dominance in most herbaceous species can be restored by this auxin treatment, such is not the case with wild-type Arabidopsis thaliana. In the present study, it has been demonstrated that apical dominance can be partially or fully restored with exogenous auxin (1% naphthaleneacetic acid, NAA) applied to the decapitated shoot of the axr3-1 mutant which is thought to be hypersensitive to auxin. A similar repressive response to auxin (1 μM NAA) was also shown in an in vitro assay with detached nodes. The role of AXR3 as a gene mediating auxin response is thus supported.

Key words: Arabidopsis thaliana, axr3-1, auxin, naphthaleneacetic acid, apical dominance, lateral bud, decapitated shoot.

INTRODUCTION

Auxin plays an important role in a number of growth and developmental responses including cell elongation, tropistic responses, lateral root formation, vascular tissue development and apical dominance. Although it has been more than 70 years since the discovery of this plant hormone, a precise understanding of how the auxin signal is perceived and transduced to cellular response is lacking. A current hypothesis is that auxin acts by enhancing the turnover of regulatory proteins via ubiquitination (del Pozo and Estelle, 1999). This hypothesis is derived largely from the phenotypic and molecular characterization of Arabidopsis mutants with defective auxin responses. For example, mutants in the AXR1 gene have reduced auxin sensitivity in assays tested to date (Lincoln et al., 1990; Timpte et al., 1995). The AXR1 protein has been demonstrated to play an important role in a ubiquitination pathway (Leyser et al., 1993; del Pozo et al., 1998).

Although the targets for this ubiquitination pathway are unknown, it is tempting to speculate that one such target could be the AXR3 protein. Gain of function mutations in AXR3 result in increased amplitude in auxin responses (Leyser et al., 1996) and in increased stability of the AXR3 protein (Worley et al., 2000). AXR3 is a member of the Aux/IAA protein family (Rouse et al., 1998). The founding members of the Aux/IAA family are low abundance, highly labile nuclear proteins that act as transcriptional regulators (Abel and Theologis, 1996). The axr3-1 phenotype is highly pleiotropic with defects in many auxin-related processes. The mutants are small with increased apical dominance and anthocyanin content, curled leaves and no root gravitropism (Leyser et al., 1996).

The increased amplitude in auxin responses observed in the axr3 gain-of-function mutants has been of particular interest to us because of our previous studies on apical dominance. The restoration of apical dominance via auxin treatment has been demonstrated in a variety of herbaceous and woody species (Cline, 1996, 2000). An exception was Arabidopsis, which was found to be insensitive to auxin, i.e. exogenous auxin applied to the decapitated shoots did not repress lateral bud outgrowth. However, when auxin was applied to the end of isolated Arabidopsis nodes grown in vitro, it was able to repress bud outgrowth (Stirnberg et al., 1999). This suggests that some factor in the decapitated plant that is not present in isolated nodes can overcome the effects of apical auxin applications. One possibility is that root-derived compounds might have this effect and an obvious candidate is cytokinin. Cytokinin is known to promote bud outgrowth (Cline, 1991; Cline et al., 1997) and decapitation has been reported to increase cytokinin export from the roots (Bangerth, 1994). Furthermore, the addition of cytokinin to the basal end of excised nodes can overcome the effects of apical auxin (Chatfield et al., 2000).

Because of the interest in the mechanism of auxin action in Arabidopsis for apical dominance, it was considered necessary to determine whether the axr3 axr over-responsive mutant would indeed respond to exogenous auxin treatments with the subsequent inhibition of branching.

MATERIALS AND METHODS

Seeds of the axr3-1 mutant of Arabidopsis thaliana and the Columbia wild-type were germinated, propagated and grown in growth rooms (23–25°C) of the Ohio State University of York, Box 373, York YO10 5YW, UK

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University Arabidopsis Biological Resource Center under continuous light (130 μmol m⁻²) following 3 d of cold treatment (1–3°C). When they were 3 weeks old, after bolting had occurred, the main shoots were decapitated about 1 cm above the base of the shoot and immediately treated with either the natural auxin, indoleacetic acid (IAA), 1 % (60 mM) or the synthetic auxin, naphthaleneacetic acid (NAA), 1 % (45 mM) in lanolin. Release of apical dominance was determined by periodically measuring the number of basal branches (secondary inflorescences) growing out at the base of the main shoot. The IAA experiments were carried out three times with axr3-1 and once with wild-type plants. The NAA experiments were carried out five times with axr3-1 and three times with the wild-type.

Experiments with the in vitro assay system were carried out in growth rooms at the University of York (UK) under light and temperature conditions as previously described (Stirnberg et al., 1999). Excised cauline nodes (with laterals 0–1.5 mm long) were placed between two separate media blocks contained in upright Petri dishes. The medium at the apical end of the nodes was pre-treated with or without 1 mM NAA. The length of the buds was measured every 24 h thereafter.

RESULTS

The effect of apical auxin on decapitated plants

The Columbia wild-type is a large bushy plant with three or four basal branches (secondary inflorescences) growing out from the base of the main shoot by the age of 5–6 weeks (Fig. 1, Table 1). This number increased to four or five following decapitation of the main shoot (Fig. 2A, Table 1). Hence, apical dominance of the wild-type is weak. In contrast, there was little or no basal branching in the axr3-1 mutant and apical dominance was strong (Fig. 1, Table 1). However, decapitation of the mutant shoot released a mean of 1.14 basal branches (Table 1, Fig. 2B). Neither IAA nor NAA applied to decapitated shoots of the wild-type repressed basal branch outgrowth (Table 1, Fig. 2B). When IAA was applied to the decapitated shoots of the axr3-1 plants, there appeared to be some temporary repression before full growth of basal branches resumed (Table 1, Fig. 2B). However, the more persistent NAA treatment completely inhibited outgrowth during the first week (22–26 July) and then partially inhibited it during the second week (30 July–2 August, Table 1, Fig. 2B). The data for the NAA treatments of axr3-1 reported here are from one of five experiments. Results from other experiments were similar (date not shown). There was a problem with fungal growth on the soil surface of pots in this experiment, presumably due to overwatering. This may have contributed to the somewhat less vigorous appearance of these plants as compared with those of the earlier experiments. Although there were some minor variations in the procedures employed in the various experiments, the relative effects of IAA and NAA were the same.

An anomalous result occurred in one experiment (data not shown) when some auxin repression of basal branch outgrowth was observed in small-potted (5 × 5 cm) decapitated wild-type plants. It was noticed that when plants were grown in these small pots, there was a lack of basal branching even in the intact wild-type plants. When the plants were grown in larger (10 × 10 cm) pots in two successive experiments, basal branching was prolific and could not be repressed by auxin treatments to decapitated main shoots. Sufficient soil nutrients were presumably needed for basal branch outgrowth. Previous studies with other species have consistently demonstrated the need for adequate nutrition and light for apical dominance release (Cline, 1996).

The effect of apical auxin on isolated nodes

A similar increase in auxin response was observed in the detached nodes of axr3-1 and wild-type plants in the in vitro assay (Fig. 3). When 1 μM NAA was applied to the apical end of excised nodes, it almost completely repressed the outgrowth of the lateral buds over a 250 h period. In contrast, the buds of the wild-type, after some temporary inhibition, began to grow vigorously after 160 h.
Mutations in the \textit{AXR3} gene affect many responses to auxin, and its phenotype suggests a general increase in the amplitude of auxin responses (Leyser et al., 1996). One of the most widely known and best documented auxin responses is that of apical dominance. In the classical Thimann-Skoog experiment (Thimann and Skoog, 1933), exogenous auxin applied to the stump of a decapitated shoot represses lateral bud outgrowth in most plants (Cline, 1996). It is puzzling why this experiment does not result in bud repression in wild-type \textit{Arabidopsis}, particularly in light of Romano et al.'s (1993) report of increased apical dominance in transformed auxin overproducing \textit{Arabidopsis}. The availability of the \textit{axr3-1} mutant provides an opportunity, not only to test the increased auxin response hypothesis for the mutant, but also to analyse further the role of auxin in apical dominance, which has yet to be precisely elucidated (Cline, 1994).

The results of this study indicate that apical dominance in \textit{Arabidopsis} can be partially or completely restored in the \textit{axr3-1} mutant by treatment with the persistent synthetic auxin, NAA (45 mM). In previous studies with other species, we have generally found NAA to be a more potent inhibitor of lateral bud outgrowth than IAA (Cline, 1996). This may be due in part to the increased permeability of the tissue to NAA since it appears to be less dependent on auxin influx carriers for uptake into cells (Delbarre et al., 1994; Imhoff et al., 2000). A lower concentration (4.5 mM) of NAA had no effect on this response in the \textit{in vivo} experiments (data not shown).

The \textit{axr3-1} mutant was also more responsive than the wild-type to auxin in the detached node assay. This is consistent with the idea that both assays measure essentially the same response, but additional factors, not present in detached nodes, can overcome the inhibitory effect of apical auxin. The fact that 45 000-times more NAA is required to inhibit bud outgrowth in decapitated plants than in isolated nodes indicates that these factors must be extremely potent. One such factor could be root-derived cytokinin (Bangerth, 1994). In the detached node assay, basal cytokinin can overcome the inhibitory effects of apical auxin (Chatfield et al., 2000). However, attempts in the present study to rescue the branching phenotypes of \textit{axr3-1} mutants with exogenous cytokinin treatments were inconclusive (data not shown). This may reflect the method by which the treatment was administered. The orientation of cytokinin application has certainly been found to be critical in the detached node assay, where apical cytokinin actually increased the inhibitory effects of apical auxin. This result is similar to that obtained by other workers, e.g. Ali and Fletcher (1971) and Tamás et al. (1989).

Alternatively, Beveridge et al. (2000) have found evidence with \textit{rms} pea mutants for a graft-transmissible unidentified substance other than cytokinin that interacts with exogenous auxin in the control of axillary bud outgrowth.
following decapitation. Some of the complexities involved in interpreting the role of auxin and cytokinin in the regulation of bud growth and the need for a developmental perspective are thoughtfully articulated in a recent review by Napoli et al. (1999).

Another reason for the different response of buds in the two assays could be differences in auxin sensitivity. The detached node assay involves cauline nodes, whereas the decapitation assay involves rosette nodes, and these may show different auxin sensitivities. However, it is unlikely that this could account for the 45,000-fold difference in auxin response, especially since one might predict that cauline buds would be less sensitive to auxin, as reflected by their earlier release during Arabidopsis development.

CONCLUSIONS

The postulated increase in response to auxin in the axr3-1 mutant was confirmed here by exogenous auxin treatment to decapitated shoots, which resulted in the partial or complete restoration of apical dominance. The role of AXR3 as a gene mediating auxin response is thus supported.

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LITERATURE CITED