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Dose-Dependent Effect of Salicylates in a Moss, *Funaria hygrometrica*

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ABSTRACT

Plant growth regulators now include more than the classic examples auxin, cytokinin, ethylene, and gibberellin, but little is known about the activity of these additional classes of molecules in nonvascular plants. The formation of buds by protonema of the moss *Funaria hygrometrica* is perhaps the best known and most fully characterized developmental system in the nonvascular plants. Examination of the effects of exogenously supplied salicylic acid and acetylsalicylate on this bioassay system shows that salicylates can regulate growth and development in mosses, producing a dose-dependent inhibition of bud formation. Other experiments show that this action is distinct from any direct effect on the well-known cytokinin stimulation of bud formation, occurs late in the process of bud formation, occurs prior to the stable commitment of nascent buds, and is not an effect on the outgrowth of young shoots. Because mosses are the sister clade to the vascular plants, these results suggest that the ability to perceive and transduce salicylate signals is an ancient feature of land plant physiology.

Key words: Development; Evolution; Hormones; Moss; Salicylate

INTRODUCTION

The ''classic'' plant hormones, auxin, cytokinin, gibberellic acid, abscisic acid, and ethylene were discovered and first characterized as regulators of plant growth in studies using angiosperms (Kende and Zeevaart 1997). Soon thereafter, however, studies using gymnosperms, ferns, liverworts, and mosses showed that all land plants used (or could use) these hormones to regulate growth and development (reviewed, Christianson 2000b). More recently, this knowledge of the presence of these hormones has been extended with phylogenetically comprehensive surveys of hormone metabolism (Auer 1997; Sztein and others 1995, 1999). These surveys demonstrate that hormone complexity, both the rates of metabolism and the diversity of metabolites, correlates with morphological complexity. A consideration of the amounts of free in-

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dole acetic acid (IAA), the percentages of the total IAA present as conjugates, and the chemical nature of the conjugates themselves, for example, finds that liverworts have relatively large amounts of free IAA with slow metabolism into amide and ester conjugates, whereas mosses have relatively low amounts of free IAA with metabolism to amide derivatives. Although vascular plants have relatively low amounts of free IAA, they also have rapid IAA metabolism and very large amounts of conjugated IAA (Stzein and others 1999). Similar overall conclusions have been drawn for cytokinin metabolism (Auer 1997). As one reviewer (Stzein and others 1999) says so succinctly, the complexity of hormone metabolism in vascular plants ''should, in theory, allow for more precise spatial and temporal regulation of free IAA levels with a reduced need for synthesizing new IAA moieties.''

Beyond this complexity in the metabolism of the ''classic'' plant hormones, the angiosperms are now known to use additional growth regulators, including salicylates, jasmonates, brassinosteroids, oligosaccharins, and peptide hormones. These are conveniently referred to as the ''neoclassic'' plant hormones, and descriptions of their roles in the growth and development of angiosperms now appear in major textbooks of plant physiology (for example, Salisbury and Ross 1992). However, it is not yet known if the ''neoclassic'' hormones can regulate growth and development in mosses or liverworts. Resolving this uncertainty would add to our knowledge of the basic hormone biology of the bryophytes, underscoring the utility of mosses as model systems for basic studies in plant biology (Cove and others 1997; Reski 1998; Schumaker and Dietrich 1997). Because the idea that increased complexity of hormone metabolism facilitates increased morphological complexity is so appealing, determining if neoclassic hormones can regulate development in moss will also help focus hypotheses about the underlying role of hormones and hormone diversity as causal forces in the evolution of plant morphology.

It is certain that increasingly complex plant morphology requires increasingly numerous ways to independently control the spatial and temporal action of hormones within and between plant organs and tissues (Sztein and others 1999). The addition of neoclassical hormones to the physiological repertoire of vascular plants would allow for many novel hormonal combinations. The neoclassic hormones could effect regulatory control directly, as has been shown for salicylate and thermogenesis (Raskin 1992), could exert effects through cross-talk between signal transduction pathways (Hooley 1998; Iten and others 1999) or could act more indirectly by affecting biosynthesis or metabolism of one of the classic plant hormones (for example, Cowan and others 1999). Experiments with vascular plants characterizing such mechanisms, however, do not and cannot provide evidence on whether the neoclassic hormones might represent physiological innovation that facilitated the reliable development of complex morphology.

Although certain kinds of chemical signals can be recovered directly from the fossil record (Niklas 1981), the prospect of unraveling the evolutionary history of plant hormones directly from the fossil record is a daunting one. Fortunately, the record of the successive addition of hormones in land plant phylogeny can be reconstructed by a cladistic or phylogenetic analysis of the physiology and biochemistry of the living land plants. For salicylate as a hormone, such an analysis would include documenting the ability to make salicylates, the origin of the receptor for salicylates, and the co-option or recruitment of components to form the salicylate signal transduction pathway now known from contemporary angiosperms. In its most simple form, the analysis begins with a survey of representatives of the major clades of land plants for their ability to respond to exogenous application of salicylate. If the neoclassic hormones such as salicylate are as ancient as the classic hormones, salicylate, like auxin and cytokinin, will be able to regulate growth and development in bryophytes, ferns, and gymnosperms.

Because DNA-based plant phylogeny (Mishler and others 1992) corroborates the suggestions drawn from analysis of morphological characters (Crandall-Stotler 1980; Mishler and Churchill 1985), the broad relationships of the land plants are now firmly established (Kendrick and Crane 1997). Because moss is the bryophytic sister clade to the vascular plants, information of the response of moss to salicylate and other neoclassic hormones is particularly instructive. Fortunately, the induction of shoot buds from protonema of the moss *Funaria hygrometrica* is a well-known and sensitive bioassay. Although originally developed as a quantitative bioassay for cytokinin (Hahn and Bopp 1968), this bioassay can also be used to measure the action of other plant growth regulators, as reductions in the numbers of buds formed by the protonema under standard conditions (Christianson 2000a; Valadon and Mummery 1971).

This paper examines one of the neoclassic plant hormones, salicylates, for effects on bud formation in the moss *Funaria* and reports experiments showing that exogenous application of salicylates does cause dose-dependent effects on the growth and development of the moss *Funaria hygrometrica.*

Characterization of the salicylate inhibition shows that the effect can be localized to a discrete period of time in the process of bud formation, after the initial triggering of development by cytokinin but before the subsequent cytokinin-mediated commitment of nascent buds. Direct examination of the formal kinetics of the interaction between salicylate and cytokinin reveals the mixed-competitive kinetics expected for cross-talk between components of separate signal perception-transduction pathways. The present study does not claim to show that mosses actually use salicylates as authentic plant growth regulators. It does show, however, that the ability to perceive and respond to salicylate is present in moss as well as in the vascular plants, suggesting that the ability to respond to salicylate was already present in the common ancestor of mosses and vascular plants during the Silurian. At least one of the ''neoclassic'' plant hormones is an ancient feature of land plants, originating in that burst of biochemical innovation as green plants made the transition to the land.

Materials and Methods

Plant Material

The isolates of *Funaria hygrometrica* used in these experiments were the ''Duke'' clone originally isolated and described by Shaw (1991) and the ''Stream'' clone, collected in northern Alberta by MLC. Aseptic cultures were established from spores (Saunders and Hepler 1983) and are maintained by serial subculture.

As described previously (Christianson 1998, 2000a; Christianson and Hornbuckle 1999), rather than select light-grown colonies of moss just as the first caulonemal (cytokinin-responsive) filaments appear (Brandes and Kende 1968), experiments in our laboratory use the cytokinin-responsive protonema generated as dark-grown caulonema (Doonan and others 1986). Briefly, three small pieces of a stock culture are inoculated onto sterile 7 cm Whatman number 1 filter papers placed on basal medium, and incubated in the dark for approximately 14 d. Culture plates are oriented edgewise, and gravitropism keeps protonemal filaments growing over the surface of the filter papers. The collection of filaments derived from each spot of inoculum is termed a ''colony,'' and bud formation after exposure to cytokinin and continuous light (Sylvania GroLux bulbs) is quantified by counting the number of buds formed by 7 d after the initial exposure to cytokinin. Because mean numbers of buds and the variances associated with those means are not independent, statistical comparisons between treatments use data transformed to achieve homogeneity of variance (details in Christianson and Warnick 1983); significance was judged at the 5% level.

The amount of inoculum and the duration of growth in the dark can be varied, resulting in larger colonies that form larger numbers of buds, or smaller colonies that form fewer numbers of buds. Because replicate experiments using colonies with differing amounts of bud-forming potential give identical results when converted to percentage of the mean for the benzyl adenine (BA) standard, we are confident that our results report a general property of moss biology and not some special condition such as mass or age of the protonema being assayed. Although all experiments reported were replicated, data presented in this paper are mean numbers of buds counted in one experiment, rather than the averages as % control, over all replications of the experiment. Accordingly, the error bars shown in the graphs, ± SEM, are easily understood as numbers of buds rather than a variation in a percentage.

Culture Media

Basal medium consisted of Brandes and Kende's (1968) formulation of Knop's major salts, including iron supplied as FeNaEDTA, and the microelements used by Fred Sack (Ohio State University, Columbus Ohio): 70 μM H_3BO_3, 14 μM $MnCl_2$, 10 μM NaCl, 1 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.2 μM $NaMoO_4$, and 10 μM $CoCl_2$. Medium was usually solidified with 8 g/L Difco BactoAgar; the increased gel strength of 18 g/L was needed for plates to be incubated edgewise in the dark. All media included sucrose at 10.2 g/L. Medium for maintenance of stock cultures included parachloroisobutyric acid at 20 μmol/L to retard the formation of caulonema (Sood and Hackenberg 1979). Medium for induction of buds included cytokinin, 1 μM benzyladenine, added before autoclaving. When used, the cytokinin, diphenylurea (DPU) was at 1 μM. Bud-inducing medium was supplemented with salicylic acid (SA) or acetyl-salicylic acid (ASA) (Sigma Co) immediately before pouring using stock solutions prepared in DMSO. Control experiments show no effect of these levels of DMSO (to 0.01%) on bud formation.

Results

Salicylic Acid and Acetylsalicylate are Dose-dependent Inhibitors of Bud Formation

The formation of buds along filaments of moss protonema can be inhibited when the standard

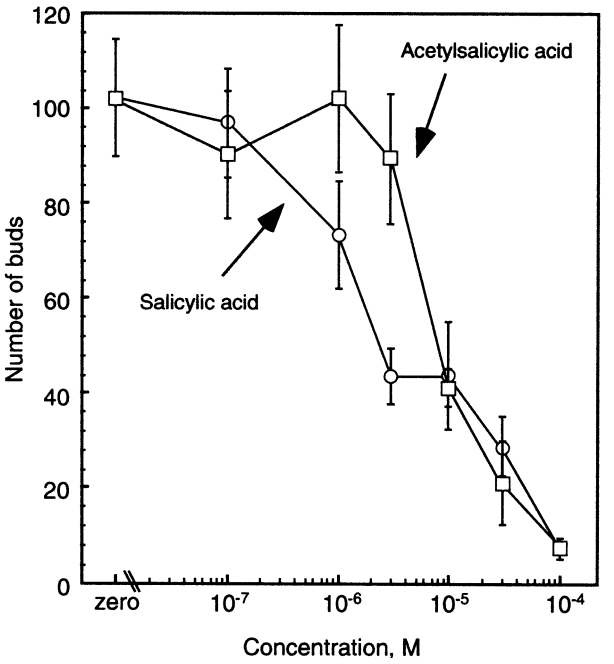

Figure 1. Numbers of buds of *Funaria hygrometrica* after exposure to salicylates. Dark-grown protonema were transferred to 1 μM BA, bud-inducing medium, supplemented with various concentrations of salicylic acid or acetylsalicylate. Buds were counted after 7 days. N = 9; error bars represent ± SEM.

bioassay medium is supplemented with sufficient concentrations of salicylates (Figure 1). As reported in studies with vascular plants (Du and Klessig 1997; Raskin 1992), both salicylic acid and acetylsalicylic acid are equally effective as inhibitors. The addition of salicylates to standard medium gives slight reductions in numbers of buds at concentrations as low as 1 μM, approximately 50% reduction at 10 μM and maximal amounts of inhibition (to 95%) at concentrations of 30–100 μM (Figure 1). Salicylates inhibit the formation of shoot buds from angiosperm tissues cultured *in vitro* at the same concentrations (1–10 μM) (Christianson and Warnick 1984). Unlike the complete suppression of bud formation observed in those experiments with angiosperm tissue cultures, we did not observe complete suppression of bud formation from *Funaria* protonemata even at the highest concentration of salicylate tested, 100 μM (Figure 1), or even when the effects of salicylates were assayed at suboptimal concentrations of cytokinin (0.2 vs 1.0 millimolar) (data not shown). It is possible to imagine surveys of bud formation in the presence of salicylate at millimolar or higher concentrations, but the physiological relevance of such experiments would be open to question. However, since the inhibition of bud formation produced by abscissic acid is also not complete even at very high levels of ABA (Valadon and Mummery 1971), the similar lack of complete inhibition by salicylates we report here may simply reflect the robust nature of bud formation in moss.

Salicylates Do Not Inhibit Early in the Process of Bud Formation

The process of bud formation by moss protonemata is known to involve sequential developmental events, from initial perception of the triggering hormone cytokinin to the final commitment of nascent buds; these events occur at discrete times over a period of several days (Brandes and Kende 1968; Christianson 2000; Saunders 1986; Saunders and Hepler 1982). Salicylates could disrupt bud formation at any point in this process either by blocking some event that should occur or by allowing some event that shunts development into an alternate pathway. Fortunately, experiments to locate the developmental time(s) at which salicylates act are possible. Just as the time of action for a gene product, the phenocritical period (Goldschmidt 1935), can be located using temperature-sensitive mutants and a series of shifts from permissive to nonpermissive conditions (Suzuki 1970), a series of timed transfers of tissue between media with and without an inhibitor can localize the inhibitory effects of an applied compound to a particular time period during a developmental process (Christianson 1998, 2000a). If, for example, initial exposure to both salicylate and cytokinin disrupted the quick influx of calcium known to initiate bud formation, protonema would be able to produce buds as long as salicylate was introduced a few hours after the cytokinin.

When such an experiment was performed, salicylates were not found to inhibit early in the process of bud formation, but late in the process (Figure 2, note log scale). Protonema placed on cytokinin plus salicylate medium for 1, 2, or even 3 days before washing and transfer to cytokinin-alone medium, produced as many buds as protonema exposed only to cytokinin (that is, transfer to cytokinin-alone at day 0). Conversely, protonema placed on cytokinin-alone medium for 1, 2, or 3 days before transfer to medium with salicylate made as few buds as protonema cultured in the presence of salicylate from day 0. Salicylate inhibits bud formation only if present during day 3–4 of bud formation.

Salicylates Inhibit Before, Not As Nascent Buds Commit

The phenocritical period for salicylate is very late in the process of bud formation. Indeed, by days 3–4

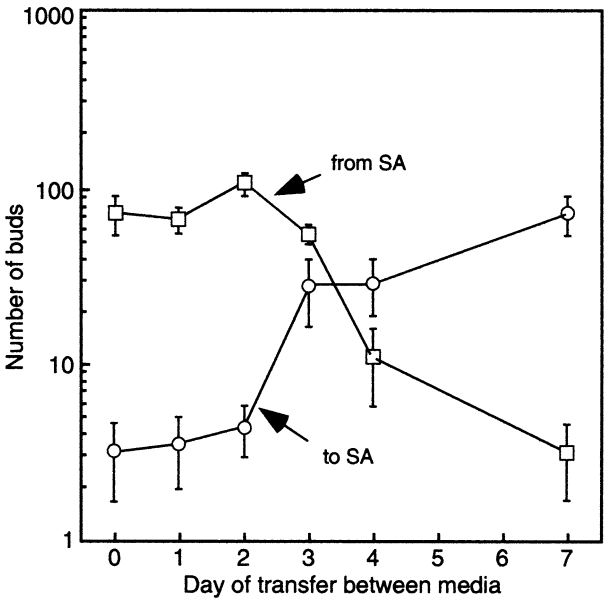

Figure 2. Numbers of buds from protonema transferred between media containing 1 μM BA and 1 μM BA supplemented with 10 μM SA. Dark-grown protonema on filter paper discs were placed on one of the two types of media; after various amounts of time, filters with the protonema were removed, washed with 3 approximately 25 ml aliquots of liquid basal medium to reduce hormone carryover, and placed on the other type of growth-regulator supplemented medium. Protonema ''transferred on day 0'' were placed directly on their final medium and cultured for 7 days, while protonema ''transferred at day 7'' remained on their first medium for 7 days; these four sets of protonema are internal controls and give replicate, independent estimates of the number of buds from continuous exposure to BA or BA + SA. Buds were counted 7 days after initial transfer to growth-regulator supplemented medium; note log scale of ordinate. N = 9; error bars represent ± SEM.

nascent buds are becoming stably committed as buds, exiting the process of bud formation and beginning the process of bud growth. Characterizing the role of salicylate as a growth regulator in moss must distinguish between an action during the process of shoot bud formation from some action on the growth of newly committed shoot buds. Because bud formation in a culture is asynchronous, newly committed shoot buds begin to grow out even as nascent buds continue to commit; the characterization of the action of salicylate requires particularly fine experimental precision.

Transfer of protonema to basal medium (BM) allows further development of only commited buds, therefore direct assay for the effect of salicylate on newly committed buds is possible. In simple experiments that compare the numbers of buds produced by cytokinin-stimulated protonema moved after various durations of culture to BM or to BM supplemented with salicylate, there are no differences in numbers of buds from the two treatment series: transfer to BM mimics transfer to BM + SA (data not shown). Salicylate does not act on the population of newly committed buds. The salicylate inhibition observed in asynchronous bud-forming cultures (Figure 1) then means that salicylate acts as an inhibitor during bud formation *per se.* These results extend the observation that leafy shoots grow with no obvious abnormality in the presence of salicylates (Christianson 2000b) to include the earliest stages of shoot growth, the first growth of a newly committed bud. These results also confirm the conclusion made in the previous section that salicylates inhibit late in the process of bud formation (experiments transferring protonema between cytokinin-containing media with or without salicylate supplements (Figure 2).

This scheme of transfers to BM with or without salicylate does not, however, distinguish between a salicylate inhibition of some penultimate event just prior to the commitment of nascent buds and a salicylate inhibition of the cytokinin-mediated commitment event itself. Fortunately, the recent description of the ability of the phenylurea cytokinins to stimulate bud formation in mosses (Christianson and Hornbuckle 1999) permits fine-scale experiments that can distinguish between these two options.

The cytokinin diphenylurea (DPU) is able to trigger the initial events of bud formation in protonema. Unlike substituted adenine cytokinins (BA in these experiments), however, DPU is unable to trigger the final cytokinin-requiring event in bud formation—the commitment of nascent buds (Christianson and Hornbuckle 1999). Protonema exposed to DPU can and do begin bud formation; with a subsequent transfer to BA, these protonema are able to complete the process, producing committed buds. Using this sequence of media separates the events prior to the final commitment of nascent buds from the cytokinin-mediated commitment process itself. If salicylates inhibit bud formation prior to the final commitment of nascent buds, protonema cultured on DPU plus salicylate for a sufficient amount of time will be inhibited by the salicylate and such protonema, although moved to BA to finish the process of bud formation, will only produce small numbers of buds. However, if salicylates inhibit by disrupting the adenine-cytokinin-mediated final commitment of nascent buds, salicylate supplied in the DPU medium will be unable to inhibit bud formation.

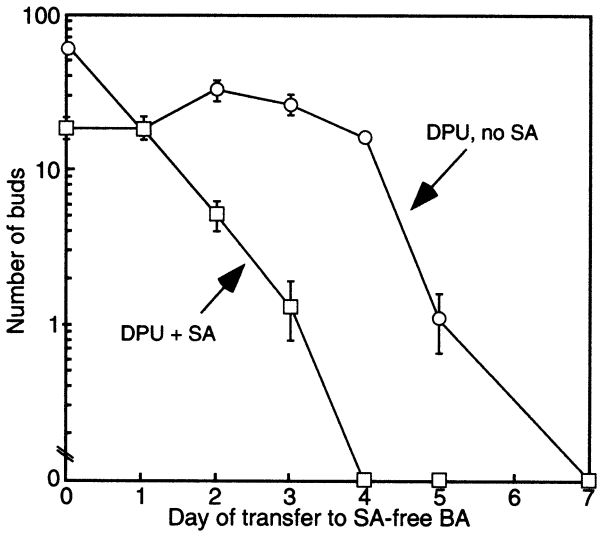

Figure 3. Numbers of buds from protonema using DPU to preclude any effects of SA on committed buds. Dark-grown protonema were placed on 1 μM DPU with or without 10 μM SA; after various amounts of time, filters with the protonema were removed, washed with 3 approximately 25 ml aliquots of liquid basal medium to reduce hormone carryover, and placed on 1 μM BA, a medium which allows nascent buds to commit and complete bud formation. Buds were counted 7 days after initial placement on growth-regulator supplemented medium; note log scale of ordinate. N = 9; error bars represent ± SEM.

When such an experiment was performed, salicylate was found to interfere with an event prior to the final commitment of nascent buds (Figure 3). The series of transfers of protonema from DPU to BA media measures the bud forming capacity of the protonema (Figure 3, open circles). Comparison of numbers of buds produced by protonema moved immediately to BA (day 0) with numbers of buds from protonema moved to BA after 1–4 days on DPU shows how effectively DPU functions as a cytokinin for the events of bud formation prior to final commitment (Figure 3). The series of transfers of protonema from DPU + SA to BA without salicylate is a specific assay for effects of salicylate on events prior to the actual commitment of nascent buds (Figure 3, open squares). Exposure to salicylate in the presence of DPU does result in inhibition of bud formation (Figure 3, open squares). As predicted by experiments indicating that salicylate acts late in the process of bud formation (Figure 2), protonema transferred from salicylate early during the process of bud formation produce numbers of buds similar to those from protonema without any exposure to salicylate. Protonema transferred from salicylate after 2 or more days, however, made distinctly fewer buds than protonema whose culture history did not include any salicylate (compare open square, open circle, days 2, 3, 4, Figure 3). These experiments provide evidence that salicylate exerts its growth regulatory effect late in the process of bud formation *per se*, but prior to the cytokinin-mediated commitment of nascent buds.

No Formal Kinetic Interaction Between SA and BA

The sets of experimental data presented above link the inhibitory effects of SA to neither the initial perception nor the second involvement of the hormonal signal for bud formation, cytokinin. Carefully considered, however, those experiments focus only on time of action and do not substitute for experiments that directly measure any formal kinetic interaction between salicylate and BA. Fortunately, such an assessment is straightforward: protonemata are exposed to a range of cytokinin concentrations at several levels of salicylate, and bud production is counted and analyzed for evidence of interaction by an adaptation of classic Lineweaver-Burk plots (Christianson 2000a; Dixon and Webb 1979).

Data from such an experiment illustrate the well-known dependence of number of buds on the concentration of cytokinin, as well as the increasing degree of inhibition with greater amounts of salicylate (note log scale of abscissa, Figure 4, upper panel). As might be expected given the disjunction between the temporal dependence for an external supply of BA and that for an inhibition by salicylate, double reciprocal plots of these data (Figure 4, lower panel) give lines that appear not to intersect along the *x-axis* (evidence of non-competitive interaction) nor at the *y-axis* (evidence of competitive interaction). This appearance is confirmed by statistical test for similarity: the two x-intercepts, -6.7×10^{6} and -15.8×10^{6}, and the two y-intercepts, 1.02×10^{-2} and 0.71×10^{-2}, are each statistically distinct, $p < 0.05$.

Discussion

SA and Direct Hormone Interactions

Salicylates have been suggested as hormonal regulators of three seemingly distinct processes in vascular plants: flowering, thermogenesis, and disease resistance (Raskin 1992). At appropriate concentrations, salicylates can also act as allosteric effectors of enzymes, for example, inhibit the conversion of ACC to ethylene (Romani and others 1989) and act as AMP analog (Marcus 1976). Although the evi-

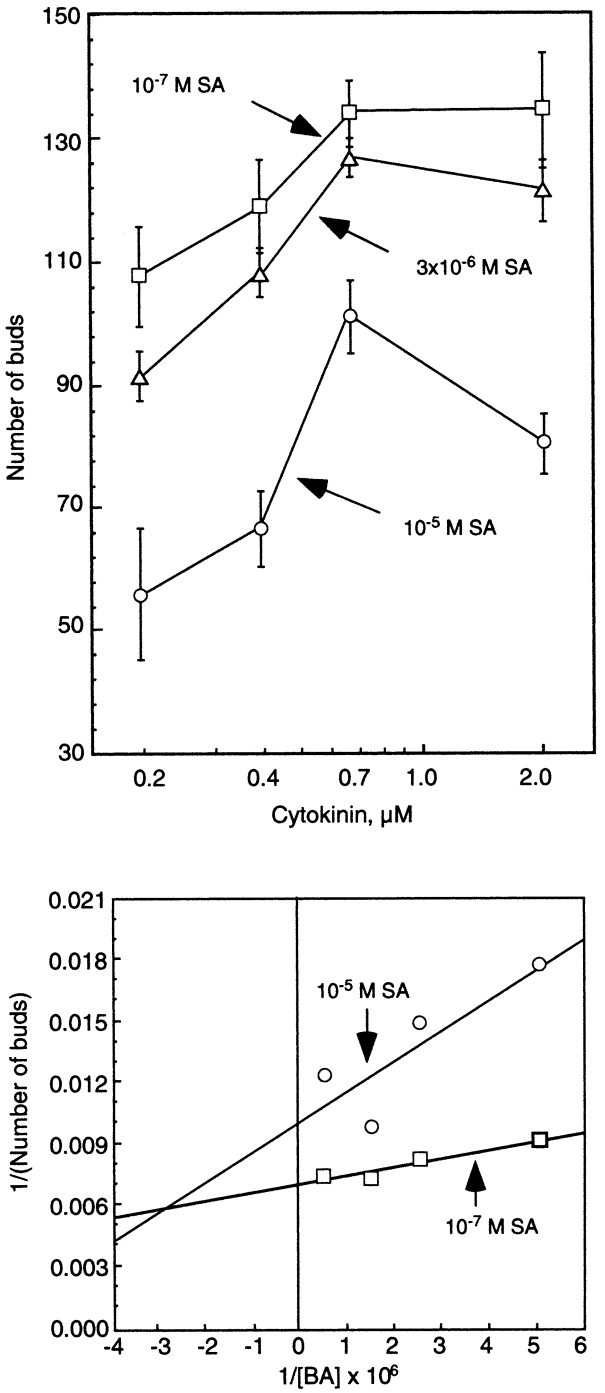

Figure 4. Assessing the interaction between SA and cytokinin. (Upper panel) Dark-grown protonema were placed onto medium with one of four concentrations of cytokinin (BA) and one of three concentrations of salicylic acid (SA). Buds were counted after 7 days; note log scale of ordinate. N = 9; error bars represent ± SEM. (Lower panel) A double reciprocal plot, 1/[cytokinin] vs. 1/mean number of buds, of the data shown in the upper panel, treatments with 10^{-5} and 10^{-6} M SA.

dence for the natural involvement of salicylate in the regulation of flowering is weak, there is no doubt that salicylate plays a key role in thermogenesis and the disease response in plants (Raskin 1992). In both these cases, exposure to salicylate, either endogenous or exogenous, results in the induction of specific proteins (alternative oxidase, pathogenesis-related proteins, respectively). A putative salicylate receptor has been isolated; it is a low abundance soluble protein that binds ligands in proportion to the ability of the ligands to induce PR proteins and with a K_d appropriate for the levels to which salicylate accumulates in responding vascular plants (Du and Klessig 1997). Other salicylate binding proteins have been isolated (for example, Chen and Klessig 1991), and salicylate interactions with these SA-effector proteins may play roles in the diverse responses of vascular plants to salicylate (Du and Klessig 1997).

The experiments reported in this paper show that salicylates can control a developmental process in a nonvascular plant: salicylates are a negative regulator of bud formation in the moss *Funaria hygrometrica*. Interestingly, using a moss, *Anoectangium thomsonii*, remarkable for its inability to make shoot buds under conditions where other mosses bud freely, a brief report by Saxena and Rashid (1980) demonstrated a stimulation of bud formation in the presence of lower concentrations of exogenous salicylate (10^{-8} to 10^{-6} M) and inhibition of this stimulation by concentrations of salicylate above 10^{-6} M. The inhibition of bud formation we report for *Funaria* also occurs at concentrations above 10^{-6} M (Figure 1). Such concentrations of salicylate are similar to the salicylate concentrations that accumulate at the sites of pathogen infection (0.5–9 µm) and which trigger defense mechanisms in vascular plants (Du and Klessig 1997).

Given the diversity of responses to salicylate in vascular plants, including the possibility that the higher levels of salicylate that accumulate during systemic acquired resistance have a physiological function as direct inhibitors of enzymes (for examples, catalase, shown to bind salicylate with appropriate affinity) (Du and Klessig 1997), extending the observations made in this paper will require much further work. Though demonstration that moss can and does make endogenous salicylate would certainly use traditional biochemical techniques, the description of the response pathway will likely use reporter gene constructs based on salicylate-responsive genes isolated from vascular plants. Such an approach has already shown the conservation of the ABA signaling pathway between moss and vascular plants (Knight and others 1995).

Parallel Effects of SA on Bud Formation in Angiosperm Tissue Cultures

Although it is not yet known if salicylates induce defense proteins or altered respiration in moss as they do in angiosperms, the inhibition of shoot bud formation in mosses by salicylate does have a parallel in the angiosperms: salicylates have been shown to inhibit *de novo* shoot bud formation in tissue cultures of both *Nicotiana* and *Convolvulus* (Christianson and Warnick 1984, 1988). Unlike the formation of somatic embryos from cultured plant tissue, where there is abundant evidence for a program of gene activity that mimics the formation of zygotic embryos (Bonner 1965; Zimmerman 1993), as well as evidence of a stimulatory effect for salicylate (Shillito and others 1989), it is not exactly clear what shoot bud formation in tissue cultures represents. Two things are certain: that plants have existed for hundreds of millions of years without any selection for the ability to regenerate shoots *in vitro*, and that this ability is yet another example of the characteristic flexibility and resilience of plants (Yusuvof 1982).

The idea that a developmental program for shoot bud formation in the gametophyte of a contemporary moss and a developmental program for shoots in contemporary vascular plants represent alternate evolutionary divergence of a single developmental pathway in the common ancestor of mosses and the vascular plants would explain a salicylate-sensitive step in both processes. Such an idea may be easier to accept than postulating a salicylate inhibition developed by convergence or by mere coincidence in both processes of shoot formation.

Given the multiple kinds of salicylate response pathways demonstrated in angiosperms, each with several distinct components for their signal transduction pathways encoded in the genome, it will be possible to couple genomics approaches with the common sensitivity to salicylate to test the hypothesis that shoot buds formed in moss gametophytes and sporophytic tissues of angiosperms both result from the ordered action of gene products derived from a single ancestral developmental pathway. Finding congruence in the salicylate transduction component of moss and angiosperm shoot formation will not reveal the morphology of the common ancestor, either as gametophyte or sporophyte, nor reveal whether the common ancestor had shoots or merely some regulatory interactions that could be recruited into a pathway that elicits shoots; finding discordance, however, would weaken support for any ''deep homology'' between shoots in these two clades.

Salicylate as a Signaling Molecule in Land Plants

Salicylate is known to affect growth and development in vascular plants (Raskin 1992), so the discovery that it can also affect development in mosses enlarges the phylogenetic compass of salicylate. The data presented in this paper do not show that mosses can and do synthesize salicylate nor that ordinary development routinely uses endogenous salicylate as a growth regulator. This paper does present clear evidence that exogenous salicylate can affect at least one developmental process in one moss, *Funaria hygrometrica.* Since exogenous salicylate shows a concentration-dependent effect on bud formation, and as this effect is both temporally and kinetically distinct from the action of cytokinin, the hormone that triggers bud formation, *Funaria* must possess a signal perception and transduction pathway for salicylate. Because there is no reason to suspect this responsiveness is unique to *Furnaria*, the basal position of mosses in land plant phytogeny suggests that the ability to use salicylate as a developmental signal is ancient and not an innovation associated with the apparent complexity of morphology in vascular plants.

ACKNOWLEDGMENTS

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