ESSAY REVIEW

Plants and hormones: an ecophysiological view on timing and plasticity

L. A. C. J. VOESENEK and C. W. P. M. BLOM
Department of Ecology, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

Summary

1 This paper demonstrates the role played by plant hormones in linking environmental signals with plant responses. It concentrates on two strategies for a sessile organism as a plant to cope with changing environmental conditions: life cycle timing and phenotypic adjustment.

2 The significance of abscisic acid and gibberellins for dormancy and germination, respectively, is discussed in relation to life cycle timing. Cytokinins are presented in relation to a possible role in carbon allocation. The gaseous plant hormone ethylene is discussed in relation to its involvement in wind- and water-induced changes in shoot growth.

3 Evidence for the role played by plant hormones in developmental processes and plastic responses comes from only a very few plant species. It will be a task for ecologists to come to a more generalized understanding of the involvement of plant hormones in ecological processes by applying the existing knowledge to a much wider range of species.

Keywords: life cycle timing, phenotypic plasticity, plant hormones

Introduction

Since most plants are sessile they have to cope with changing environmental conditions because escape by migration is not possible. Two strategies to mitigate this environmental variability are to restrict plant activity to the most favourable seasons or to attempt phenotypic adjustment on a rapid time scale. Precise timing of plant activities and the adaptive modification of a plant's phenotype can only be realized if plants perceive signals containing information about the plant's direct environment (signal perception). Within and between plant cells this information will then be passed on to a defined sequence of messenger molecules (transduction chain), ultimately leading to the induction of the required plant response. Plant hormones can play a key role as mediators in transduction chains and thus can be involved in the regulation of environment-induced plant responses such as timing and phenotypic plasticity.

Correspondence: L. A. C. J. Voesenek, Department of Ecology, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands. Fax: 080 652409. E-mail: RENSV@SCI.KUN.NL

The generally accepted five groups of phytohormones, i.e. auxins, gibberellins, cytokinins, abscisic acid and ethylene, play roles in many aspects of plant development. While these growth regulators are synthesized predominantly in certain organs, they can, in principle, be formed in all vegetative plant parts (Van Loon & Bruinsma 1992). The effects of plant hormones on development are pleiotropic and depend on the type of cell, tissue or organ involved, developmental stage and external environment signals (Bradford & Trewavas 1994). Many developmental processes are not regulated by one single hormone, but are under multiple hormonal control (Barendse & Peeters 1995). Plant hormones are thought to exert their regulatory role by affecting gene expression and/or membrane function (Ho & Hagen 1993). Hormonal control of development can be regulated by changes in concentration and/or by modifications in the sensitivity of tissue towards hormones (Trewavas & Cleland 1983).

Evolutionary adaptations to low-resource environments, characterized by changes in many plant traits, can occur through accumulation of many small mutations or by a few mutational events with large pleiotropic effects. Due to the pleiotropic nature of
the effects of plant hormones a few genetic changes in hormone physiology will result in a change in a broad range of seemingly unrelated plant traits (Chapin et al. 1993). In this respect reduced production of gibberellins, morphologically expressed as shortened height, causes a dramatic change in allocation of dry matter, photosynthesis and yield (references in Chapin et al. 1993).

The plant hormone abscisic acid (ABA) participates in the process of induced seed dormancy (Hilhorst & Karssen 1992). Here, ABA delays the moment of germination by ‘switching off’ the seed. Plant hormones may also control relatively rapid responses to changing environmental conditions, as with cytokinin-mediated changes in carbon allocation upon nitrogen stress (Fetene & Beck 1993). Moreover, plant hormones act as metabolically inexpensive (Sultan 1992), mechanistic links between perception of environmental signals and phenotypic responses (Trewavas 1986). In this case, meristematic activity is not switched on or off, but made more or less active. Plant hormone action thus creates a specific allocation pattern.

This paper aims to demonstrate the important role of plant hormones as intermediates between environmental signals and adaptive plant responses. We will focus here on two essential plant traits linking a sessile way of life with environmental stress, namely the timing of life cycle events and the regulation of phenotypic plasticity.

Life-cycle timing

Plant hormones are involved in the timing of events in the life cycle such as flowering and seed germination. The transition from vegetative growth to flowering is regulated to occur in favourable periods in the year. The induction of flowering is under multifactorial control involving both assimilates (e.g. sucrose) and phytohormones (e.g. cytokinins). The role of plant hormones as intermediates between environmental signals and flowering has recently been reviewed by Bernier et al. (1993); this section will therefore focus on dormancy mechanisms and the initiation of germination.

Primary dormancy may develop during seed ripening when the seeds are still attached to the mother plant; a state of secondary dormancy can be induced by prolonged environmental inhibition of germination. This last type of dormancy can be relieved and re-induced in a cyclic fashion unique to seeds (Derkx 1993). The regular variation in depth of dormancy is delayed until the following summer. During the course of this grassland succession a gradual shift in seasonal timing of germination was observed.

Another example of the importance of timing of germination is given by the composition of plant species in natural gradients of environmental harshness as found in river floodplains. Summer floods in Dutch river floodplains are erratic and can be classified as ‘catastrophes’. Winter floods, however, are more or less predictable in timing and duration. Rumex species can be found in floodplain gradients in well-defined zones ranging from rare to very frequently flooded. Rumex acetosa, a species from seldom flooded grasslands with predictable gap formation, is characterized by autumn germination and a transient seed bank. Two other Rumex species are characterized by delayed germination until next spring, a persistent type of seed bank and flood tolerant seeds. These species live in areas with harsh but predictable winter floods and unpredictable summer floods (Voesenek et al. 1992a; Voesenek & Blom 1992). By delaying germination, small seedlings avoid these winter floods.
Timing of germination is regulated by dormancy-inducing and releasing mechanisms and by mechanisms involved in the initiation of germination. In general these mechanisms are sensitive to external stimuli such as light, nitrate and temperature. The initiation of primary dormancy in *Arabidopsis thaliana* involves the action of ABA produced by the embryo itself (Karssen *et al.* 1983). Evidence obtained from a study with several wheat cultivars indicates that besides concentration, sensitivity of the seed towards ABA is also important for the initiation of primary dormancy (Walker-Simmons 1987). Even closely related species can have a substantial different ABA content and thus dormancy level: differences in embryo dormancy between *Acer pseudoplatanus* and *A. platanoides* were related to the timing of peaks of ABA content during seed development (Black 1991).

In some plant species, *e.g.* the mangrove *Rhizophora mangle*, germination occurs while the seeds are still attached to the mother plant. These viviparous seeds have a low sensitivity to ABA (Sussex 1975).

Little is known about the exact way in which ABA suppresses precocious germination and induces dormancy in seeds. ABA is involved in a change in the pattern of gene expression (Chandler & Robertson 1994); the production of several kinds of polypeptides is induced (Bray 1991), whereas genes for certain reserve-mobilizing enzymes are inhibited (Black 1991). During maturation, seeds dehydrate and develop desiccation tolerance. ABA-deficient mutants of *Arabidopsis thaliana* demonstrated that ABA plays a role in the induction of desiccation tolerance (Voesenek & Van der Veen 1994). However, ABA-independent pathways have also been described (Ooms *et al.* 1994).

ABA is probably only indirectly involved in the induction of secondary dormancy. Even ABA-deficient mutants of *Arabidopsis thaliana* can develop secondary dormancy with the appropriate signal (Hilhorst & Karssen 1992).

**Germina**

Termination of dormancy and initiation of germination are two distinct processes (Taylorson & Hendricks 1977). According to Vleeshouwers *et al.* (1995) the degree of dormancy as a seed characteristic is defined by the conditions needed to make the seed germinate. Germination is the reaction of a seed to the overlap between environmental conditions actually experienced and the dormancy-defined germination requirements.

Release of dormancy, is poorly understood as a mechanism (Hilhorst & Karssen 1992). It can, at least in *Arabidopsis thaliana*, be achieved without the synthesis of gibberellins (GAs), whereas induction of germination in both tomato and *Arabidopsis thaliana* strongly depends on the presence of some GA. Environmental factors that can break dormancy (e.g., stratification) sensitize seeds to GA (Karssen *et al.* 1987). In addition, endogenous GA can replace the need for environmental stimuli such as light or cold stratification (Karssen *et al.* 1989). ABA action during seed development of *Arabidopsis thaliana* determines the GA requirement during germination. High ABA levels during seed maturation result in a deep dormancy, consequently leading to an initiation of germination that can only be realized by high levels of GA (Karssen *et al.* 1987).

The impact of light on germination is controlled by the photo-reversible pigment phytochrome (Pons 1992). During darkness it is in an inactive form that is capable of absorbing red light (Pr). Red light exposure induces a quick conversion to an active, germination-stimulating form that can absorb far red light (Pfr). Recently a hypothetical model on receptor-regulated dormancy cycling in *Sisymbrium officinale* involving action of temperature, light, nitrate and the plant hormone gibberellin was developed (Hilhorst *et al.* 1995). The basis of the model is a temperature-dependent, and thus seasonal increase and decrease in the number of membrane-bound phytochrome receptors. This would mean that a seasonal pattern of seed sensitivity to light may exist. In *Sisymbrium officinale* a parallel pattern was observed in the sensitivity towards nitrate (Derkx 1993). It is hypothesized that nitrate activates the presumed phytochrome receptor (Hilhorst *et al.* 1995; Vleeshouwers *et al.* 1995). The GA requirement of seeds of *S. officinale* did not shift in parallel to the seasonal pattern of seed dormancy (Derkx 1993). Once established, the phytochrome-receptor complex presumably initiates synthesis of GAs and an increase in receptor sensitivity towards GAs. A signal from the GA-receptor complex ultimately leads to germination (Hilhorst *et al.* 1995).

An example of how environmental signals such as light and temperature can result in fine tuning via hormonal mediation of an ecological response, in this case germination of three *Rumex* species, is presented below. Figure 1 shows the effects of several light and temperature treatments on the dark germination of *R. acetosa*, *R. crispus* and *R. palustris*. Primary dormancy, as indicated by poor dark germination, differs substantially between the species. This mechanism, together with perianth-imposed primary dormancy, prevents autumn germination in both *R. crispus* and *R. palustris*. In this way dormancy prevents seedling establishment in periods favourable for germination, but unsuitable for seedling survival (Vleeshouwers *et al.* 1995). We suggest that different concentrations of and/or sensitivities to ABA during seed development may explain the variation in levels of primary dormancy in *Rumex*.

Germination in *R. acetosa* is relatively independent of the various light and temperature treatments, indicating that the phytochrome-receptor complex is not
the rate limiting step. This species will germinate if enough of the GA-receptor complex can be formed. According to the receptor-regulated dormancy model of Hilhorst (1990) it is to be expected that specific inhibitors of GA biosynthesis would antagonize the germination of R. acetosa. In both other Rumex species, the phytochrome-receptor complex probably limits the initiation of germination. Red light stimulates formation of the germination-stimulating form of phytochrome, whereas cold-stratification might stimulate the synthesis of phytochrome receptors (Hilhorst & Karssen 1992). Only one of these stimuli is required to initiate germination in R. crispus, whereas both treatments are essential to stimulate germination in the species with the most persistent seed bank, R. palustris.

When seeds germinate in the soil the shoot must penetrate through several millimetres or centimetres of soil to reach the soil surface. Action of the gaseous plant hormone ethylene facilitates shoot emergence through these soil layers. Mechanical resistance signals the shoot to produce more ethylene, possibly mediated by a mechanical strain-induced ACC-synthase gene (Botella et al. 1995). Ethylene strengthens the shoot by stimulating radial growth (Voeseck & Van der Veen 1994). Ethylene-insensitive Arabidopsis mutants failed to penetrate through a 4 mm covering of sand. One of the mutants even failed to push through 2 mm of sand. The importance of ethylene in this respect is highlighted by the fact that these mutations would be lethal under natural field conditions (Harpham et al. 1991).

**Phenotypic adjustment to adverse growth conditions**

**Carbon allocation**

Raunkiaer (1934) observed that the physiognomy of plants correlates with abiotic characteristics of habitats. The partitioning of biomass over roots, leaves and stems has a marked impact on maximal growth rates and the competitive ability of plant species (Tilman 1988; Van der Werf et al. 1993). Partitioning of biomass is an aspect of natural plant development. In this respect inherently fast-growing, nonwoody plants maximize shoot functioning, whereas slow-growers tend to maximize root functioning (Poorter & Remkes 1990). However, biomass partitioning can also change in response to environmental stimuli. In nutrient-poor soils, most of the photosynthate is allocated to roots, whereas transfer of plants to low light levels stimulates carbon allocation to shoots. These phenotypic plastic responses of plants are generally interpreted as mechanisms to compensate for the reduced levels of nutrients and light. In view of the importance of biomass partitioning in plant ecology (Kozlowski 1992) surprisingly little is known about the mechanistic control of this process (Van der Werf 1995). Here we will focus on the potential role of endogenous plant hormones, especially cytokinins and ABA, as regulators of the movement of photosynthate from sites of synthesis in leaf tissue (source) to the sites of net accumulation in shoot tissue and/or root tissue (sink).

Cytokinins are predominantly root-borne phytohormones that are distributed in the shoot tissue via the xylem stream (Jackson 1993); many aspects of synthesis, metabolism and activities are still unknown (Binns 1994). Root tips, as sites of cytokinin production, react rapidly upon alterations in mineral supply. Work with various plant species showed that a reduction in mineral supply to the root tips induced a decrease in internal cytokinin concentrations in roots, shoots and xylem sap (Kuiper et al. 1989 and references herein). The strongest reductions in cytokinin levels were induced by nitrogen, the effects of phosphate were less pronounced (Coleman et al. 1990). In Urtica dioica plants, grown in a range of nitrate concentrations that did not affect biomass pro-
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Production but significantly changed biomass partitioning, it was shown that low-nitrogen treated plants showed reduced levels of nitrogen and cytokinins only in the roots (Wagner & Beck 1993). A carbon (¹⁴C) export study using Urtica dioica showed that at medium and high nitrogen supplies most of the upper leaves in the stem exported carbon to the shoot apex, the lower leaves exported to the root. Upon exposure to low-nitrogen levels nearly all leaves on the stem exported to the root (Fetene et al. 1993). An increase of the root : shoot ratio of Plantago major spp. pleiot sperma resulting from diluted nutrient solutions could be retarded by application of benzyladenine, a synthetic cytokinin (Kuiper & Staal 1987). When a natural cytokinin (zeatin riboside) was supplied to a portion of de-tipped roots of Urtica dioica growing at optimal nitrogen supply, expanding and mature leaves substantially increased their rate of photosynthesis and intensity of carbon export and transport to the shoot (Fetene & Beck 1993). Cytokinins probably control the sink strength of the shoot apex, and thus the carbon partitioning between various sinks (Van der Werf 1995). More mechanistically this means that cytokinins stimulate photosynthesis and thus promote leaf growth leading to a relief of the sink limitation of photosynthesis (Fetene & Beck 1993). The increase of photosynthesis upon cytokinin feeding is strengthened by the observation that benzyladenine stimulates the transcription of genes coding for the small subunit of ribulose biphosphate carboxylase oxygenase (Rubisco), the single enzyme responsible for the chemical reduction of carbon dioxide in photosynthesis, and for the light-harvesting chlorophyll pigment (Ohya & Suzuki 1991). Another line of evidence for the possible role of cytokinins in carbon partitioning comes from transgenic plants overproducing cytokinins. These plants typically have underdeveloped root systems, an increased growth of axillary buds and a darker green colour related to assimilate unloading (Brenner 1987). In this way the site of application becomes a stronger sink. In a recent study with an ABA-deficient tomato mutant, Nagel et al. (1994) demonstrated that low levels of ABA resulted in a different pattern of biomass allocation. The mutant made less leaf area per unit biomass (lower specific leaf area) and invested relatively more biomass to the roots and stems compared to the wild type. However, addition of ABA to the roots did not raise the root weight ratio as expected from the ABA-sink strength hypothesis, but actually lowered the biomass allocation to roots to the level of the wild type. Nagel et al. (1994) concluded that the differences in biomass partitioning observed between ABA-deficient mutants and wild-types result from an effect of ABA on plant water relations. ABA probably has no direct influence on carbon allocation by shifts in sink strength.

Shoot plasticity

Plants are very plastic with respect to the morphology of their shoots (e.g. number and position of branches, length of petioles, internodes and stolons, leaf area). The pattern of growth determines the use of local space and thus the capture of resources (Trewavas 1986). The first example shows the impact of wind stress and competition for light on the shoot morphology via the action of the phytohormone ethylene.

Two populations of Stellaria longipes, an alpine and a prairie form, differ significantly in level of plasticity. The alpine tundra type has a short erect habit and an overall low amount of phenotypic plasticity, whereas the prairie population can be semi-dwarf or highly elongated depending on its temperature and wind environment, indicating a high level of phenotypic plasticity (Macdonald & Chinnappa 1988, 1989). The gas ethylene plays a major role in signalling environmental alterations such as stress (Voosen & Van der Veen 1994). The comparative eco-physiological study on two populations of Stellaria longipes resulted in observed differences in sensitivity and production levels of ethylene in response to wind stress. This indicates that ethylene may be a significant factor in the variation in phenotypic plasticity between both ecotypes (Emery et al. 1994a). For alpine tundra ecotypes, wind is an important selective force, whereas competition for light is the predominant force in prairie ecotypes (Emery et al. 1994b). Alpine tundra plants respond to wind with an inhibition of elongation growth. Applying either inhibitors of ethylene action or ethylene biosynthesis can restore normal growth rates in the presence of wind indicating that ethylene might be the principle mediator of this growth reduction (Emery et al. 1994a). Dwarfism in windy mountain habitats is functionally related to the development of a morphology that avoids future damage during wind blasting and thus loss of photosynthetic leaf area. Prairie ecotypes cannot afford dwarf growth because of competition for light. These plants have a completely different sensitivity to ethylene and respond to low levels of this gas with a slight stimulation of growth. Ethylene production levels in unstressed plants stimulate elongation as is demonstrated by the growth inhibition upon application of specific ethylene action or biosyn-
The second example concentrates on submergence-induced shoot elongation as a mechanism to reach better illuminated and aerated zones close to the water surface or preferentially above it (Voesenek et al. 1992b; Armstrong et al. 1994). This not only improves the resource capture (carbon dioxide, light) of leaves that emerge above the water surface, but can also be seen as a mechanism improving the resource supply (oxygen) of remote plant organs (roots). The hormonal regulation of enhanced shoot elongation has been intensely studied in the genus Rumex, typically characterized by a specific field distribution in floodplains. Rumex species from the most frequently flooded sites are characterized by a high degree of phenotypic plasticity with respect to both petiole and internode length. In these species submergence induces ethylene- and gibberellin-mediated growth enhancements. Seldom-flooded dry-land Rumex species are unable to accommodate the length of the shoot to prevailing water levels and thus show a low degree of plasticity with respect to this plant trait (Voesenek & Blom 1989; Van der Sman et al. 1993; Blom et al. 1994). Under frequently flooded field conditions enhanced shoot elongation is highly adaptive as demonstrated by increased biomass, increased survival rates and enhanced levels of seed production (Voesenek et al. 1992b). Under flooded conditions ethylene, which is produced continuously by nearly all plant tissues, accumulates in shoots of Rumex due to the slow rate of diffusion of gases in water (Voesenek et al. 1993). Preliminary results of work in our group indicate that in the wetland species R. palustris high levels of ethylene negatively regulate the gene expression of ACC-synthase, the rate limiting enzyme in ethylene biosynthesis. However, a submergence-induced decrease of endogenous oxygen levels stimulates the gene expression of ACC-synthase. This results in an overall mechanism with the ability to tune the endogenous ethylene concentration to allow enhanced shoot elongation relatively independent of environmental conditions (stagnant water; streaming water) with the aid of two gases, oxygen and ethylene. This fine-tuning mechanism is only poorly developed in dry-land Rumex species, such as R. acetosa, which are rarely flooded in their natural environment and therefore do not experience the selective force (frequent floods) to develop such a mechanism.

The adaptive value of an ethylene fine-tuning mechanism and the ecological significance in terms of field distribution in floodplains lies in continuation of shoot elongation independently of prevailing flood conditions. When a shoot of R. palustris is submerged in stagnant water, endogenous oxygen levels decline, whereas internal levels of ethylene increase due to entrapment by water. Low levels of oxygen stimulate ACC-synthase gene expression. However, production of ethylene will not increase because low levels of oxygen hamper optimal ACC-oxidase activity (the activity of this enzyme is obligately oxygen dependent). Under these conditions shoot elongation is maintained because physical entrapment of ethylene produced at low rates can still generate internal growth-saturating concentrations of ethylene (Voesenek et al. 1993). After several hours, production levels decline further due to down-regulation of ACC-synthase by the enhanced ethylene concentrations. Even under these conditions endogenous ethylene will probably still be enough to saturate petiole elongation. This view is further strengthened by the observation that, by this time, lower levels of ethylene are actually required to saturate petiole elongation. This enhanced sensitivity is brought about by low-oxygen conditions (Blom et al. 1994). This equilibrium can, however, dramatically be disturbed if stagnant water starts to flow rapidly. Due to the reduction of the thickness of the unstirred boundary layer around the R. palustris shoot, ethylene diffusion to the surrounding water is stimulated. At the same time more oxygen from the water will enter the plant. Due to the lowering of the endogenous ethylene concentration the ACC-synthase gene expression will increase. The accompanying higher oxygen concentration no longer limits ACC-oxidase activity and consequently more ethylene can be produced to compensate for the extra diffusive loss of ethylene caused by streaming. Thus, fast petiole growth is sustained even under rapidly flowing water conditions.

According to Smith (1990) such a gradient in adaptability, as observed in this fine-tuning mechanism in Rumex, reflects the extent and sophistication of control over synthesis and action of specific key proteins, in this case ACC-synthase.

Epilogue

This short review gives some examples of the regulating role plant hormones play in developmental processes and plastic responses. It also stresses the impact of these biochemical processes on plant survival and reproduction. However, many results come from only a few plant species (e.g. tomato, Arabidopsis). It will be a task for future ecologists to incorporate knowledge on hormone action into their research and to extend it to a much wider range of species. In this way a more generalized understanding of the involvement of plant hormones in ecological processes can be achieved. The work of Emery et al. (1994a) is a shining example in this context. Most of the physiological and molecular research on plant hormone synthesis, action and function is performed with crop plants. However, crop species represent only a very small proportion of the total flora and are predominantly selected for maximal yield. Thus, care
should be taken when extrapolating this research to wild plants.

Only rarely are analyses of hormone concentrations and/or sensitivities performed under realistic field conditions. Developments in molecular biology during the last decade and the increased sensitivity of other analytical techniques may allow more measurements under outside conditions in the future. In addition, it might be possible to transplant transgenic or mutated plants, having a changed level of hormone production and/or sensitivity, into controlled plots under natural environmental conditions. Together with increased knowledge on hormone-regulated genes this approach will give insight into the mechanisms underlying seed dormancy, germination, carbon allocation and stress-induced shoot elongation and more generally into the ecological significance of hormonally controlled mechanisms.

Slight changes in hormonal concentrations and/or tissue sensitivities may exert a wide range of simultaneous effects since each hormone can have several effects. This stresses the importance of hormones as controlling agents acting between environmental signals and plant responses. However, this may be an oversimplification. Many developmental processes and adaptive responses are not regulated by one single hormone (see Trewavas 1986; Barendse & Peeters 1995). Very often plant responses, such as shoot elongation and the formation of adventitious roots (Visser et al. 1995) in response to flooding, are tuned through complex networks of hormone concentrations and sensitivities and other molecules. Despite this complexity, the phenomenon of hormone-mediated plant responses gives the unique opportunity to relate life history theory, a core theory in ecology, to ecophysiology and population genetics. For example ethylene-induced shoot elongation can be studied at the level of mRNA. Genes of two essential enzymes in the biosynthesis of ethylene (ACC-synthase and ACC-oxidase) have been cloned and sequenced for several plant species, and cDNA probes are available to monitor variation in mRNA levels. This allows population research on the variation of important fitness related plant traits, such as enhanced shoot elongation, to be related to mRNA levels, thus linking molecular biology and ecophysiology to population genetics.

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