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REVIEW

The role of phytohormones in plant stress: too much or too little water

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INTRODUCTION

Due to their sessile nature higher plants cannot avoid adverse environmental conditions. Plants encounter shifts in environmental conditions, such as flooding, drought, temperature extremes and physical stress, during their entire life cycle. In order to optimize survival and reproduction, plants must acclimatize to these environmental changes (Bradshaw 1965). The resistance of plants to stress can be subdivided into three strategies: (i) escape (a dormant phase of the life cycle survives a stressful period, e.g. in annual species dormant seeds survive harsh seasons); (ii) avoidance (e.g. waterlogging-induced oxygen deficiency of root tips can be relieved on a tissue level by internal

aeration via shoot and root aerenchyma); and (iii) true tolerance at biochemical level (e.g. a set of anaerobic stress proteins is synthesized in response to anoxic conditions to enable anaerobic ATP generation) (Jones & Jones 1989).

Phytohormones, especially abscisic acid (ABA) and ethylene, play a predominant role in the conversion of stressful environmental signals into changes in plant gene expression. For example, ABA is involved in the desiccation tolerance of established plants and seeds. Ethylene, in concert with auxin, gibberellins and ABA, plays an important role in enhanced shoot elongation in response to submergence, as observed in many semi-aquatic plant species (Voesenek *et al.* 1992). Both ABA and ethylene are involved in the change of plant gene expression upon wounding, resulting in the production of a set of proteins involved in both wound healing and prevention of subsequent pathogen attacks. Evidence accumulates for the existence of at least two different wound stimulus transduction pathways, one acting via ABA and one via ethylene (Sánchez-Serrano *et al.* 1991).

Recent evidence suggests that there is a basic physiological framework involved in the regulation of plant responses to environmental stress. Various stresses such as nutrient stress, insufficient water supply and flooding result in declines in growth rate and in the rate of acquisition of all resources (Chapin 1991). This is supposedly triggered by hormonal changes such as increased levels of ABA and decreased concentrations of cytokinins (Chapin *et al.* 1988). Although not mentioned explicitly in the work of Chapin (1991) we suppose that ethylene too might play a crucial role in controlling growth rates of plants under stress (see also Kieber *et al.* 1993). An upsurge in ethylene production is observed in response to a wide variety of environmental stresses, whereas a decrease in growth rate triggered by enhanced ethylene levels has been described for many plant species.

From an agronomical point of view, mankind has a long history of attention to the relationship between stressful environmental factors and plant resistance (Levitt 1941). Beyond the level of applied biology, stress-induced responses provide a unique model to study physiological and molecular mechanisms in plants. On a higher integration level, stress may determine temporal and spatial patterns in populations and communities through genetic differentiation and phenotypic plasticity of individual plants (Kuiper 1990).

This review describes the role of phytohormones in plant stress. Stress responses have been studied at various organizational levels, ranging from the whole plant to the genes. We will focus primarily on the role of the hormones ethylene and ABA in relation to water stress. Changes in concentrations and sensitivities to both ethylene and ABA, as well as changes in gene expression in response to plant stress will be discussed. Although it is clear that ABA is active during flooding (Jackson 1991a) and ethylene plays a role during prolonged exposure to drought (Wright 1978), only ethylene is discussed in relation to stress conditions caused by too much water, whereas ABA, the dominant hormone under conditions with too little water, will be discussed during drought.

ETHYLENE AND PLANT STRESS

Ethylene biosynthesis and action

The amino acid L-methionine serves as the primary precursor of ethylene in higher plants. Two intermediates in the conversion sequence from methionine to ethylene

are, in order of formation: S-adenosyl methionine (SAM or AdoMet) and 1-aminocyclopropane-1-carboxylic acid (ACC). SAM provides the mechanism for recycling the CH_3S group to methionine. This regeneration pathway allows high rates of ethylene production despite the low levels of methionine in many plant tissues (Yang & Hoffman 1984). The conversion of SAM to ACC is the rate-limiting step in ethylene biosynthesis and thus the most important controlling point in ethylene production (Sisler & Yang 1984; Yang & Hoffman 1984; Kende *et al.* 1985; Kende 1993). Gasing plant tissues with pure nitrogen completely blocks ethylene production (Hansson 1942) and leads to accumulation of ACC. On return to air ACC is rapidly converted to ethylene, indicating that the conversion of ACC to ethylene requires oxygen (Adams & Yang 1979).

The biochemical conversion of L-methionine to ethylene in higher plants includes three enzymes: methionine adenosyltransferase, ACC-synthase and ethylene-forming enzyme (EFE), now more correctly termed ACC-oxidase. Methionine adenosyltransferase, common among living organisms and not unique to higher plants, catalyses the conversion of L-methionine to SAM. The biosynthesis of ethylene can be inhibited by uncouplers of oxidative phosphorylation (Murr & Yang 1975). Burg (1973) proposed that ethylene biosynthesis requires a high energy step. It is concluded that most probably methionine is 'activated' with the aid of ATP before it is converted to SAM. The existence of an enzyme in a cell-free extract of tomato fruit capable of converting SAM into ACC was first demonstrated by Boller *et al.* (1979). This enzyme, ACC-synthase, unique to higher plants, requires pyridoxal phosphate as a co-factor and utilizes SAM specifically as substrate (Yu *et al.* 1979). Increases in ACC-synthase activity are usually due to *de novo* synthesis of the enzyme (Yoshii & Imaseki 1982; Acaster & Kende 1983). One of the most important characteristics of ACC-synthase in terms of regulating ethylene biosynthesis is its rapid turnover (Kende & Boller 1981; Yoshii & Imaseki 1982; Imaseki *et al.* 1982).

The activity of ACC-synthase and thus ethylene production can be induced by various chemical and environmental stimuli. Well known and widely described are physical wounding and auxin application (Yang & Hoffman 1984). ACC-synthase activity can also be induced during certain developmental processes, e.g. fruit ripening (Abeles 1973). Nakagawa *et al.* (1988) reported that the antibody to wound-induced ACC-synthase purified from mesocarp of winter squash (*Cucurbita maxima*) recognized ACC-synthase from wounded hypocotyls of winter squash and from wounded pericarp of tomato. However, auxin-induced ACC-synthase from hypocotyls of winter squash was not recognized. These results indicate the existence of two immunochemically different isozymes of ACC-synthase which are induced by two different stimuli. Although the enzymological properties of both isozymes are very similar (Nakagawa *et al.* 1988) a monoclonal antibody against ACC-synthase from ripe apple fruits failed to recognize the enzyme from ripe tomato, ripe avocado fruit or auxin-treated mungbean hypocotyls, but could immunoprecipitate the pear fruit enzyme (Dong *et al.* 1991). Recently, several genes have been identified for ACC-synthase in tomato. These genes have been shown to be differentially induced by various stimuli such as fruit ripening, mechanical wounding, auxin and flooding (Yip *et al.* 1992; Olson *et al.* 1993). In rice, two ACC-synthase genes come to expression in response to anaerobiosis; one specific for shoots (*OS-ACS1*) and another specific for roots (*OS-ACS3*) (Zarembinski & Theologis 1993). A recent study on the temporal and spatial expression of a specific ACC-synthase gene (ACS1) in *Arabidopsis* revealed that expression was generally

confined to young tissue and that it was strongly correlated with lateral root formation (Rodrigues-Pousada *et al.* 1993). It can be concluded that ACC-synthase is encoded by a multigene family and that the various genes are differentially expressed in time and space in response to developmental, environmental and hormonal factors.

In the last few years significant progress has been made in the purification and characterization of ACC-oxidase, the last enzyme in the ethylene biosynthetic pathway responsible for the conversion of ACC to ethylene. Yang & Hoffman (1984) have already suggested that the enzyme might be an ACC hydroxylase. A very important step forward was made when Hamilton *et al.* (1990, 1991) identified a gene (pTOM13) in tomato that encodes a polypeptide component of ACC-oxidase. The amino acid sequence of the pTOM13 polypeptide showed close homology with that of flavanone-3-hydroxylase. By applying extraction and assaying procedures used in flavanone-3-hydroxylase analyses, Ververidis & John (1991) and Fernández-Maculet & Yang (1992) recovered *in vitro* ACC-oxidase activity in melon and apple fruit. The enzyme requires ascorbate, Fe^{2+} and HCO_3^-/CO_2 for full *in vitro* activity (Smith *et al.* 1992; Smith & John 1993). Work of Bouzayen *et al.* (1990) indicates that there are most probably two sites of ACC to ethylene conversion, an external site at the plasma membrane operating with apoplastic ACC and an internal site accessible to intracellular ACC. However, Rombaldi *et al.* (1993) and Latché *et al.* (1993) revealed with immunocytolocalization techniques, that ACC-oxidase of ripe tomato fruits was mainly located in the cell wall space. No ACC-oxidase was detected at the tonoplast and the plasma membrane, previously considered as bearing ACC-oxidase activity.

Binding to receptor sites probably determines the mode of action of ethylene. It brings about some essential change in the receptor protein, dissociates from the receptor and diffuses away unchanged. The first papers on ethylene binding in tobacco leaves and *Phaseolus vulgaris* were published in the late 70s (Sisler 1979; Jerie *et al.* 1979; Bengochea *et al.* 1980a,b). Since then binding, measured with the ^{14}C-ethylene displacement assay (Sisler 1979), has been reported for many plant tissues and species (for review see Sisler 1991). So far there exist at least two, but probably more, types of ethylene-binding proteins (EBPs), which have identical affinities and specificities for ethylene but different rate constants of association and dissociation (Sanders *et al.* 1989, 1990, 1991a; Sisler 1990).

An important step forward in characterizing the action of ethylene was the discovery of ethylene-insensitive mutants of *Arabidopsis thaliana*. One such mutant showed an 82% reduction of ethylene binding compared to wild-type plants, indicating an impaired receptor function (Bleecker *et al.* 1988). Similarly, the *eti* series of mutants lack a number of responses to ethylene such as inhibition of cell elongation, promotion of seed germination, enhancement of peroxidase activity and acceleration of leaf senescence, indicating that ethylene binding may be necessary for ethylene responses to occur (Harpham *et al.* 1991; Sanders *et al.* 1991b). However, an underestimation of binding capacity in ethylene-insensitive mutants can occur due to the relatively high endogenous ethylene production rates in these mutants (Sanders *et al.* 1989, 1991b; Hall *et al.* 1993). Consequently, binding data of mutants should be treated with caution. Despite these problems, mutants are an important tool for the elucidation of the perception and transduction mechanisms for ethylene (Guzman & Ecker 1990). Recently, many ethylene mutants have been described for *Arabidopsis* (Guzman & Ecker 1990; Van der Straeten *et al.* 1993; Kieber *et al.* 1993; Theologis 1993). The gene products lacking in these mutants have specific roles in the ethylene signal transduction chain (Kieber *et al.*

1993). A gene (ETR1) responsible for an early step in the ethylene signal transduction pathway in *Arabidopsis*, possibly an ethylene receptor, was recently cloned and sequenced. The sequence of the carboxyl-terminal half of the ETR1 protein is similar in sequence as a prokaryotic family of signal transducers (Chang *et al.* 1993).

Stress ethylene

An upsurge in ethylene production can be observed in response to a wide variety of environmental stresses, such as mechanical stress, toxic chemicals, radiation, temperature extremes, flooding, drought, wounding and pathogen infection (Abeles 1973; Hyodo 1991). Ethylene production induced by mechanical stress, phytotoxic chemicals, wounding and pathogens will be discussed in general to underline the broad character of the ethylene–plant stress relationship. Ethylene in relation to flood-induced plant stress will be discussed extensively (see below).

Various mechanical stresses encountered by plants induce ethylene production (Hyodo 1991). These may range from bending by wind (Telewski & Jaffe 1986), root or shoot tips growing against an obstruction (Goeschl *et al.* 1966; Moss *et al.* 1988; Sarquis *et al.* 1991, 1992; Schwarzbach *et al.* 1992), stroking and rubbing plant parts (Hiraki & Ota 1975; Biro & Jaffe 1984) or just touching them (Braam 1992). Jaffe (1973) called this phenomenon 'thigmomorphogenesis'. Mechanical stress can induce characteristic morphological changes in both roots and shoots by inhibition of elongation and promotion of radial growth (Jaffe *et al.* 1985; Moss *et al.* 1988; Sarquis *et al.* 1991). These changes in morphology can be mimicked by exposing non-stressed plants to ethylene (Moss *et al.* 1988; Sarquis *et al.* 1991). This led to the hypothesis that ethylene action might be causally related to changes in patterns of plant growth under impeded conditions (Moss *et al.* 1988). The stimulated ethylene production of both roots and shoots of etiolated maize seedlings upon pressure-simulated impedance is regulated at both the ACC-synthase and ACC-oxidase level (Sarquis *et al.* 1992).

The shorter and thicker phenotypes induced by environmental stimuli such as wind are thought to be of adaptive significance. These plants are less easily damaged by the wind (Biddington 1986). Experiments by Braam & Davis (1990) indicate that mechanical stimulations, such as rain, wind, wounding and touch, result in the rapid and strong expression of five genes, so called touch (TCH) genes, in *Arabidopsis*. Three of these genes encode calmodulin-related proteins suggesting that calcium ions play an important role in the cellular responses to mechanical stimulation. Ethylene exposure alone, however, is not sufficient to induce fully the expression of all the TCH genes. Therefore, ethylene is probably not the primary response to mechanical stimulation which induces expression of TCH genes (Braam 1992).

High bulk densities in the soil profile restrict root penetration and thus the volume that can be explored for water and nutrients (Blom 1978). Radial expansion of roots under physical impedance may be of adaptive significance, since it can create fissures ahead of the root, allowing root tip penetration (Moss *et al.* 1988). Although maize roots produce more ethylene when mechanically impeded and exogenous ethylene simulates closely the effect of impedance, Moss and co-workers (1988) came to the conclusion that ethylene is not the cause of the morphological response of roots to physical impedance. This conclusion is based on the following arguments: (i) a lack of correlation between the kinetics of both increased ethylene evolution and changes in growth patterns; and (ii) the inability of ethylene action (norbornadiene) and ethylene synthesis (AVG) inhibitors to overcome the effects of impedance, although in the latter

case ethylene production was strongly reduced to levels below those of unimpeded roots. Sarquis *et al.* (1991) simulated mechanical impedance upon maize roots and shoot by pressurizing the growth medium. In contrast to the work of Moss *et al.* (1988), they observed that the kinetics of ethylene production and morphological changes are consistent with a regulatory role for ethylene in the response towards mechanical impedance. Additionally, AVG added simultaneously with silver ions, restored shoot and root growth to 84 and 90% of unimpeded values, respectively. This discrepancy in results may be caused by the different methods used to simulate mechanical impedance and from the always somewhat problematic use of inhibitors since they may not have access to the intended target and because side-effects may occur. Ethylene mutants offer new possibilities to study the role of ethylene in mechanical impedance. This was demonstrated clearly in a paper describing the inability of germinating seedlings of ethylene-insensitive *Arabidopsis* mutants to penetrate a light covering of sand (4 mm). One of the mutants even failed to push through 2 mm of sand (Harpham *et al.* 1991). These results suggest that shoot emergence through mechanically-impeding layers seems to depend on the action of ethylene; its role presumably being to strengthen the shoot by enhancing radial expansion.

Various phytotoxic chemicals induce the production of 'stress' ethylene. These chemicals include metals, inorganic compounds, pollutants and herbicides/pesticides (Yang & Hoffman 1984; Hyodo 1991; Abeles *et al.* 1992). The impact of various individual components is extensively reviewed by Abeles *et al.* (1992). There is evidence that phytotoxic chemicals such as silver ions increase ethylene production via an increase in ACC synthase activity (Atta-Aly *et al.* 1987). Recently, Theologis (1993) suggested that silver ions might interfere with the ethylene receptor. This interference is interpreted by the cell as an absence of ethylene, which in turn may lead to a positive feedback and thus an ethylene overproduction. Gora & Clijsters (1989) suggest an increase of malonyltransferase, the enzyme which catalyses the conjugation of ACC to 1-malonyl-ACC, upon exposure of *Phaseolus vulgaris* seedlings to phytotoxic levels of copper and zinc. At relatively low concentrations, both copper and zinc enhanced ACC-oxidase activity. However, this effect disappeared at higher concentrations. In addition to the methionine-SAM-ACC pathway of ethylene synthesis there is evidence that ethylene generated in response to copper and/or zinc application is from a free radical-mediated pathway (Mattoo *et al.* 1985; Gora & Clijsters 1989).

Ethylene and metal ions destabilize membranes and stimulate peroxidase activity. Vangronsveld *et al.* (1993) hypothesized that ethylene might play a role in the signal transduction chain from metal to a series of responses. However, kinetic experiments (Weckx *et al.* 1993) revealed that the increase of ethylene production occurred 48 h after zinc application, too late to explain membrane and enzyme changes. Additionally, ethylene was unable to mimic the effect of metals on the isoperoxidase pattern (Vangronsveld *et al.* 1993). In conclusion, responses to heavy metals are unlikely to be mediated by ethylene.

Both mechanical wounding and pathogen infection increase ethylene production (Abeles *et al.* 1992; Boller 1991). These stresses are discussed together since at least a part of the pathogen infection involves structural damage and can be interpreted as wounding by a biological agent. Wounding-induced ethylene production, in common with all stress-induced upsurges in the production of this growth regulator, has a very typical time course (Fig. 1). Two vegetative *Rumex acetosella* plants, placed in a cuvette in line with a flow-through system and a laser-driven photoacoustic ethylene detector

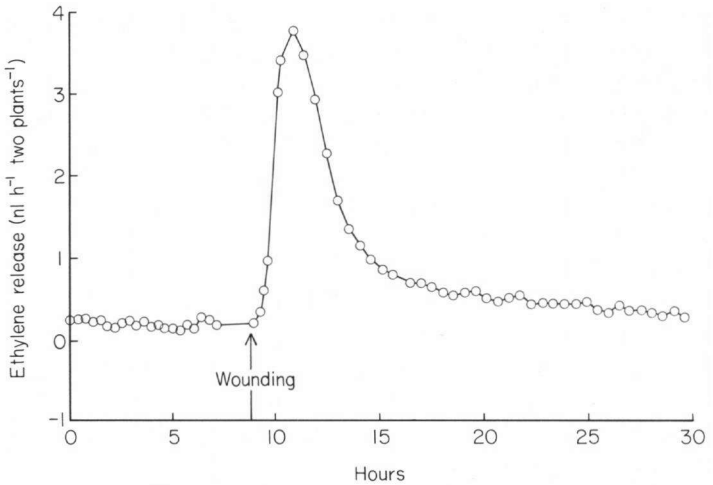

Fig. 1. Ethylene production (nl h^{-1}) of two mechanically wounded vegetative *Rumex acetosella* plants measured with a laser-driven photoacoustic ethylene detector (Voesenek *et al.* 1992).

(Voesenek *et al.* 1992), were mechanically wounded with a glass rod without opening the cuvette. After a lag-phase of 35 min this treatment led to a 20-fold increase in ethylene production, followed by an exponential decline to reach pre-treatment levels after 10–15 h. Both lag-phase and amount of production increase are very similar to results obtained with various other species and types of tissue (Saltveit & Dilley 1978; Jackson & Campbell 1976; Konze & Kwiatkowski 1981). Increased ethylene production in response to wounding is explained by increased activity of ACC-synthase (Yu & Yang 1980; Boller & Kende 1980; Hoffman & Yang 1982; Hyodo *et al.* 1985; Sitrit *et al.* 1987) and ACC oxidase (Hyodo & Nishino 1981; Hoffman & Yang 1982; Hyodo *et al.* 1985; Sitrit *et al.* 1987). This raises an important question: What induces the key enzyme ACC-synthase in wounded tissue? It is known that there exist several ACC-synthase genes which can come to expression upon different stimuli. Wounding has been found to activate a different ACC-synthase gene than auxin, even within the same plant (Nakagawa *et al.* 1988; Nakajima *et al.* 1990; Yip *et al.* 1992). It is thought that some sort of 'stress-indicator' controls the expression of ACC-synthase genes (Abeles *et al.* 1992). At present, the identity of this stress-indicator is not known. However, there is evidence that oligosaccharides are formed in response to mechanical stress in the internodes of bean (Takahashi & Jaffe 1984). These oligosaccharides can induce ethylene synthesis via enhanced ACC-synthase activity (Chappell *et al.* 1984). The role of oligosaccharides in the signalling mechanism that regulate the expression of plant genes was recently reviewed by Ryan & Farmer (1991).

Stimulation of ethylene biosynthesis is an early event in many plant–pathogen interactions (Boller 1991). Various types of pathogen, such as viruses, bacteria, fungi and nematodes, can cause stress ethylene production (Ecker & Davis 1987; Abeles *et al.* 1992). As in wounding, pathogen-induced stress ethylene is probably the result of increased ACC-synthase activity (De Laat & Van Loon 1982; Ecker & Davies 1987; Spanu & Boller 1989). De Laat & Van Loon (1983) showed that ACC-oxidase activity also increased in virus-infected tobacco leaves. Ethylene production in response to viral, bacterial and fungal infections is closely correlated with lesion development (Abeles *et al.* 1992) with ACC-synthase having its highest activity in the necrotizing area. The

additional ACC this produces diffuses into the surrounding tissue and presumably cause an upsurge in ethylene production there (Spanu & Boller 1989).

Enhanced ethylene synthesis in response to wounding and pathogen attack is often interpreted as a signal for the plant to enhance or activate its repair mechanisms against wounding and defences against pathogen infection (Stahmann *et al.* 1966; Pegg 1976; Yang & Pratt 1978; Boller 1982; Ecker & Davis 1987). If ethylene indeed plays an important role in signal transduction during wound repair, it would be expected that increases of wound-treated proteins and their mRNAs are prevented when wounded tissue is treated with ethylene action inhibitors or inhibitors of its biosynthesis. However, work by Henstrand & Handa (1989) demonstrated that less than 15% of wounding-induced gene expression is effected by the presence of 2,5-norbornadiene or silver thiosulphate, two ethylene action inhibitors. This strongly suggests that wound-induced changes in gene expression are not regulated by ethylene alone.

Activities of chitinase and β-1,3-glucanase increase strongly in many plant pathogen interactions (Moore & Stone 1972; Pegg 1977; Netzer *et al.* 1979; Pegg & Young 1981). It is thought that these two enzymes may act as defences against pathogenic fungi since both chitin and β-1,3-glucans are major components of fungal cell walls (Bartnicki-Garcia 1968). Plants treated with exogenous ethylene also show a large increase of chitinase and β-1,3-glucanase (Abeles *et al.* 1970; Boller *et al.* 1983; Boller 1991).

ETHYLENE AND FLOODING-INDUCED PLANT STRESS

A dramatic change in endogenous gas composition can be observed in all plant tissues when flooded by water (Stünzi & Kende 1989). In general, oxygen concentrations in plant tissues fall rapidly, whereas carbon dioxide and ethylene levels quickly increase (Raskin & Kende 1984a). The deprivation of oxygen is the first important factor limiting growth and survival of terrestrial plants in habitats with frequent floods (ap Rees *et al.* 1987). The immediate effect of oxygen deficiency is a reduction in ATP supply due to the complete inhibition of the oxidative phosphorylation under anaerobic conditions (Crawford 1992; Drew 1990). Additionally the normal pattern of aerobic protein synthesis is repressed, whereas the expression of a set of approximately 20 so-called anaerobic stress proteins (ASPs) is induced (Walker *et al.* 1987; Sachs 1991). Several ASPs have been identified and nearly all of them are glycolytic enzymes (Kennedy *et al.* 1992). It is obvious that a lack of oxygen directs cell metabolism to a metabolic route which generates ATP without oxygen. Oxidation of NADH by such a route is a prerequisite for the continuation of the glycolysis. In most plant tissues this is predominantly realized via ethanolic fermentation (Drew 1990). This brings us to the second important constraint in frequently flooded environments: due to the inefficient utilization of carbon assimilates during ethanolic fermentation, many tissues suffer from carbohydrate starvation (Brändle & Crawford 1987; Setter *et al.* 1987). It is therefore not surprising that species that can survive extended periods of anoxia and live in the most anaerobic habitats, also have large carbohydrate reserves (Brändle 1991).

Plants can adapt on a metabolic level to flooding-induced strains. These adaptations are extensively discussed in earlier reviews (Bertani & Reggiani 1991; Voesenek *et al.* 1992). This section focuses mainly on the role of ethylene in adaptations to avoid oxygen deficiency in plant cells: adventitious root formation, aerenchyma development and shoot elongation.

Table 1. Dry weights ($n=3$; ± SE) of adventitious roots of *Rumex* species developed during an 8-day period of waterlogging. Species are ranked according to their distribution in a flooding gradient near the river Rhine in The Netherlands

Habitat	Species	Dry weight of adventitious roots (g)
Dry	*R. thyrsiflorus*	0·01 ± 0
↓	*R. acetosa*	0·08 ± 0·04
	R. obtusifolius	0·13 ± 0·01
	R. conglomeratus	0·15 ± 0·02
	R. crispus	0·21 ± 0·06
Wet	*R. palustris*	0·47 ± 0·07

Adventitious root formation

Partial submergence or waterlogging induces initiation and/or outgrowth of adventitious root primordia in many plant species; monocotyledons as well as dicotyledons (Kawase 1974; Jackson & Drew 1984; Jackson 1985). Flood-induced adventitious roots of herbaceous plants are usually visible within a few days (McNamara & Mitchell 1990; Blom *et al.* 1994). In general, adventitious roots may arise from pre-formed primordia or from *de novo* initiation (Kozlowski 1992). Literature concerning adventitious root formation is dominated by work on cuttings in the horticultural context. In most cuttings, *de novo* regeneration of meristematic regions is a prerequisite for adventitious root formation (Liu & Reid 1992). Work on flood-induced adventitious roots in whole plant systems and consequently the type of primordia (preformed or *de novo*) involved, is scarce.

Adventitious roots are assumed to replace the old root system under conditions of disfunction of this system and thus increase survival of plants under partially flooded conditions (Blom *et al.* 1990; Kozlowski 1992). Adventitious roots are therefore associated with important functions such as uptake of water and nutrients, source of certain plant hormones (gibberellins and cytokinins) and sink for shoot-borne assimilates (Jackson & Drew 1984). The following arguments for the physiological importance of adventitious roots can be formulated.

(1) The formation of adventitious roots is correlated with the flooding resistance of plant species (Laan *et al.* 1989a; references in Kozlowski 1992; Blom *et al.* 1990, 1993). This is illustrated with an example of six *Rumex* species differentially distributed in Dutch river flood plains (Table 1). Species that naturally occur on seldomly flooded dykes and river levees, such as *Rumex thyrsiflorus* and *Rumex acetosa* produce only a limited biomass of adventitious roots. Frequently flooded species like *Rumex palustris* produce, in the same time-span, huge amounts of new roots.
(2) Pruning of adventitious roots reduces survival rates of flooded plants (Jackson & Palmer 1981).
(3) Flood-induced adventitious roots enhance the uptake of water and nutrients in flood resistant plants (Sena Gomes & Kozlowski 1980).
(4) Radial oxygen loss from adventitious roots creates a thin layer of oxidized substrate in an otherwise reduced environment. There is convincing evidence that in this oxidized layer nitrifying bacteria can remain active, providing the plant with nitrate. *Rumex palustris* is characterized by large amounts of porous adventitious roots which develop

in response to soil flooding. Nitrate availability for this plant is indicated by the high nitrate reductase activity in the leaves. *Rumex thyrsiflorus*, a species that develops few if any adventitious roots, does not aerate the soil resulting in a repressed nitrification capacity (Engelaar 1994; Blom *et al.* 1994).
(5) Due to the high porosity and radial oxygen loss of adventitious roots in flood-resistant *Rumex* species these roots play a role in the oxidation of reduced soil borne toxins (Laan *et al.* 1989b; Ernst 1990; Laanbroek 1990).

Overall there is evidence that flood-induced, newly developed aerenchymatous roots take over from the old roots in a physiological sense. It is important for adventitious roots to develop quickly upon flooding since oxygen levels can decline rapidly in old roots and thus interfere with their normal function (Laan *et al.* 1991).

Evidence for the role of ethylene in controlling adventitious root formation is rather contradictory (Yamamoto & Kozlowski 1987; Jackson 1991b). Ethylene is, however, a very reliable indicator of flooded conditions (Ridge 1987); a role in flood-induced adventitious root formation is therefore not unlikely. Under flooded conditions ethylene can occur at elevated levels in plant tissues. The primary cause of enhanced ethylene levels in tissues is entrapment by surrounding water. Ethylene has a diffusion rate which is approximately 10 000 times slower in water than in air (Musgrave *et al.* 1972). This physical mechanism can lead to endogenous ethylene concentrations in shoots of *Rumex* plants of 4–5 nl ml^{-1}, which are approximately 100 times higher than the concentration under non-submerged conditions (Voesenek *et al.* 1993). In addition to entrapment, some plant tissues produce more ethylene under sub-ambient partial pressures of oxygen (Jackson 1982; Jackson *et al.* 1984; Raskin & Kende 1984a; Brailsford *et al.* 1993). This increase in production is probably related to a low-oxygen induced enhancement of ACC-synthase activity (Cohen & Kende 1987). The third cause of increased ethylene levels is related to the fact that ACC easily moves into the xylem. Accumulated ACC in anaerobic roots can move to the shoot of the plant. Better aeration of the root–shoot junction leads to conversion of this ACC to ethylene (Bradford & Yang 1980; Wang & Arteca 1992; Jackson 1993). However, some movement of ethylene itself from root to shoot via aerenchyma channels or dissolved in the transpiration stream cannot be ruled out (Jackson & Campbell 1975; Zeroni *et al.* 1977).

Many studies concerning the role of ethylene in flood-induced adventitious root formation describe a relation between enhanced levels of ethylene and adventitious root formation (Zimmerman & Hitchcock 1933; Tang & Kozlowski 1984a,b; Bleecker *et al.* 1987). Others report that ethylene is not the major controlling factor in adventitious root formation (Michener 1935; Batten & Mullins, 1978; Wample & Reid 1979; Yamamoto & Kozlowski 1987). In these papers most conclusions are based on correlative evidence. Specific ethylene production and/or activity inhibitors and a test on their specificity were not included. Recent work of Liu & Reid (1992), however, showed a specific role for ethylene in adventitious root formation. With elegant experimentation they showed that auxin is the primary controller of adventitious root formation, but that it was ethylene involved in increasing the tissue sensitivity towards auxin. This conclusion is based on the following results.

(1) ACC and the ethylene-releasing compound ethephon stimulated adventitious root formation in sunflower hypocotyls.
(2) The promotive effects depended on the presence of cotyledons and the apical bud (the main sources of auxin) or the presence of exogenously applied auxin.

(3) ACC enhanced the rooting response of hypocotyls to exogenous auxin and decreased the inhibition of rooting by auxin transport inhibitors. Silver ions (ethylene action inhibitor) reduced the rooting response of hypocotyls to exogenous auxin and increased the inhibition of rooting by auxin transport inhibitors.

Based on these results, the work of Wample & Reid (1979), Yamamoto & Kozlowski (1987) and that of our own group (Blom *et al.* 1994), the evidence points to the following hypothesis of flood-induced adventitious root formation: flooding inhibits the basipetal auxin transport in flooded roots. This results in an accumulation of auxin at the basal part of the shoot. Enhanced ethylene levels increase the sensitivity of the tissue to auxin and thus stimulate adventitious root formation. There is evidence that accumulation of auxin in pea cuttings is prevented by conjugation of excessive auxin with aspartic acid. Removal of the shoot apex in these cuttings caused a reduction of the auxin level and resulted in almost complete inhibition of adventitious root formation (Nordström & Eliasson 1991). It can be concluded that enhanced levels of auxin are not necessary, although a certain minimum level is required. This observation can easily be combined with the suggested role of ethylene in changing the sensitivity to auxin.

In contrast to the literature concerning adventitious root formation in cuttings, only a very limited number of papers deal with flood-induced adventitious root formation. More attention should be paid to whether root primordia are preformed or *de novo* developed during flooding. Different processes are very probably involved in the initiation of root primordia and their elongation. In addition, more research should concentrate on the timing of hormone action. De Klerk & Ter Brugge (1992) showed recently that the timing of indolebutyric-acid (IBA) application during the rooting treatment of microcuttings of *Malus* had a significant effect: rooting was strongly decreased when the application was postponed. The use of antisense transgenic plants (English *et al.* 1993) and ethylene-deficient and -insensitive mutants will be important in unravelling the role of ethylene in the process of flood-induced adventitious root formation.

Aerenchyma development

Overwet soil conditions induce, in certain plant species, a series of interconnected gas-filled cavities. This so-called aerenchyma 'tissue' may develop in existing plant organs such as roots, rhizomes, stems, petioles and leaves, or may develop simultaneously with the elongation of, e.g. adventitious roots (Jackson 1989). Aerenchyma can develop through separation of cells (schizogenous aerenchyma) or through the lysis of cells (lysigenous aerenchyma) (Jackson & Drew 1984). Type of aerenchyma, amount of tissue porosity and the specific anatomy of the tissue before and after aerenchyma development strongly varies among families and genera of the plant kingdom (see Justin & Armstrong 1987; Crawford 1989). The work of Justin & Armstrong (1987) demonstrated that the cell configuration before aerenchyma development is strongly related to the amount of aerenchyma, expressed as fractional root porosity (FRP), formed upon flooding. Hexagonal cell packings are correlated to overall low FRP, whereas cubic packing often resulted in high FRP. These authors even suggested that, during evolution, soil wetness exerted a selective pressure favouring the cubic configuration.

In many other plants from wet habitats aerenchyma is constitutive and is formed as an integral part of plant growth and development. These plants are pre-adapted to soil

inundation (Jackson 1989), since aerenchyma decreases convection and diffusion resistances enabling gas ventilation to provide roots with respiratory oxygen, detoxifies chemically reduced ions via the process of radial oxygen loss (ROL) and increases nitrate uptake under anaerobic soil conditions through nitrification in the plant's rhizosphere (see above: *Adventitious root formation*). Evidence, ranging from purely correlative to highly causal, underlines this role of aerenchyma in plant survival under oxygen deficient soil conditions.

(1) There is a strong correlation between habitat preference of plants and the fractional root porosity of those species (Smirnoff & Crawford 1983; Justin & Armstrong 1987; Laan *et al.* 1989a; Smits *et al.* 1990; Voesenek *et al.* 1992).
(2) It is widely assumed that aerenchyma improves the oxygen status of roots. Based on theoretical modelling, Armstrong (1979) came to the conclusion that an increase in root porosity indeed enhanced the apical oxygen concentration. Experimental evidence for the process of internal aeration came from work of Laan and co-workers (1990) on *Rumex* plants. They showed that the oxygen depletion rate of a well stirred nutrient solution was highest when the root porosity was low. It was thought for many years that internal aeration mainly occurs through simple diffusion. More recently, it has been recognized that pressure gradients can develop in many wetland plants. These gradients induce a mass flow of gases in the aerenchyma, thus improving the oxygen status of roots and rhizomes significantly (Dacey 1980; Armstrong & Armstrong 1991; Sorell 1991; Grosse & Meyer 1992). This process can lead to flow rates in the aerenchyma tissue of *Victoria amazonica* up to $5\,l\,h^{-1}$ (Gross *et al.* 1991).
(3) Flood-induced chemical changes in the soil such as the reduction of manganese, iron and sulphate, produce potentially toxic soil components (Mendelssohn *et al.* 1981; Ponnamperuma 1984). High levels of ROL induce detoxification through precipitation of manganese (hydr)oxide, ferric hydroxide and iron sulphide (see references in Ernst 1990).

The mechanism of aerenchyma development and the role of ethylene has been extensively studied in the lysigenous type of aerenchyma which develops in the cortex of flooded maize roots. The collapse of cells in the cortex of maize roots can be mimicked by exogenous application of ethylene ($5\,\mu l\,l^{-1}$) (Drew *et al.* 1979). According to these authors, the mechanism of entrapment is important for enhancing internal ethylene levels (see also Kawase 1972; Musgrave *et al.* 1972; Konings & Jackson 1979; Voesenek *et al.* 1993). Addition of silver ions at low non-toxic concentrations inhibited aerenchyma development (Drew *et al.* 1981). Inhibitors of the ethylene biosynthesis, such as cobalt ions and aminooxyacetic acid (AOA), also prevented cavity formation (Konings 1982). Jackson and co-workers (1985) discovered that sub-ambient partial pressures of oxygen (3–12·5 kPa) also induced a substantial aerenchyma development. This stimulation of aerenchyma development was associated with a faster ethylene production of excised apical root segments in stoppered serum vials with low oxygen concentrations. The importance of ethylene in aerenchyma development under low partial pressures of oxygen was demonstrated with the addition of AVG, which halted the formation of gas-space in the cortex. The specificity of AVG in this system was tested by applying ACC, which as expected overcame the inhibition by AVG (Jackson *et al.* 1985). However, it cannot be excluded that during head space analysis of excised tissue enclosed in serum vials, artefacts may be introduced due to wounding, gravitropic disorientation and changes in gas composition. To avoid these problems ethylene

production of intact primary roots of *Zea mays* in response to sub-ambient partial pressures of oxygen was measured with the aid of a flow-through system in line with a laser-driven intracavity photoacoustic detector (Harren *et al.* 1990; Voesenek *et al.* 1992; Brailsford *et al.* 1993). Exposing one individual intact primary root to sequentially 21% oxygen, a sub-ambient concentration (1–12·5%) and 21% oxygen verified that low levels of oxygen can stimulate ethylene production (Brailsford *et al.* 1993). The increase in ethylene formation is associated with increased levels of ACC (Atwell *et al.* 1988) and with an enhanced activity of ACC-synthase (Morgan *et al.* 1993). No change was observed in the activity of ACC-oxidase (Atwell *et al.* 1988). Hypoxia and ethylene-induced aerenchyma formation is correlated with a strong increase in cellulase activity in the apical 10 mm of maize roots (Morgan *et al.* 1993). It was Kawase (1979) who reported the same phenomenon for *Helianthus annuus* stems. On the other hand, there is evidence that cortex cells collapse without apparent breakdown of cell walls in the process of aerenchyma development (Konings & Lambers 1991). These authors and Campbell & Drew (1983) suggest that disfunction of the tonoplast is probably the first step in the chain of events leading to cell collapse and aerenchyma development.

Evidence accumulates that the lysigenous formation of aerenchyma also is influenced by the nutrient status of roots. Deficiencies of N and P also induce aerenchyma formation in maize roots (Konings & Verschuren 1980), whereas low nutrient levels, as well as flooding, increase aerenchyma in roots of *Nardus stricta* (Smirnoff & Crawford 1983). Drew and co-workers (1989) demonstrated for *Zea mays* that N or P starvation depressed the rate of ethylene synthesis in adventitious roots. This decrease in ethylene production upon nutrient starvation was accompanied by decreases in ACC and MACC content and declines of ACC-synthase activity and ACC-oxidase activity. Although the ethylene production rates in N starved roots of maize were lower than in control roots, both silver ions and AVG were still able to prevent the formation of aerenchyma and the rise in cellulase activity, indicating that these processes still worked via ethylene. He *et al.* (1992) finally demonstrated that nutrient stress not only decreased the levels of ethylene production, but that it also increased the sensitivity to ethylene.

Shoot elongation

In response to submergence, shoot organs of certain aquatic and semi-aquatic plant species are able to increase their growth rate dramatically. This phenomenon has been called depth accommodation (Ridge 1987) and is observed in leaves (Voesenek & Blom 1989), petioles (Funke & Bartels 1937; Musgrave & Walters 1973, 1974; Voesenek *et al.* 1990), internodes (Musgrave *et al.* 1972; Samarakoon & Norton 1981; Métraux & Kende 1983; Van der Sman *et al.* 1991), coleoptiles (Ku *et al.* 1970) and mesocotyls (Suge 1971). Deep-water rice is a good example of a plant with an enormous capacity to elongate upon submergence. Growth rates of 250 mm per 24 h have been recorded for certain ecotypes in Bangladesh. In the rainy season this may eventually lead to a total plant height of nearly 7 m (Vergara *et al.* 1976).

With respect to shoot elongation under water, two categories of growth can be distinguished: (i) shoot growth without oxygen; and (ii) stimulated elongation in the presence of sub-ambient partial pressures of oxygen. Shoot elongation in complete absence of oxygen is thus far only described for the rice coleoptile (Taylor 1942), the mesocotyl of *Echinochloa oryzoides*, a serious weed of rice paddy fields (VanderZee & Kennedy 1981; Pearce & Jackson 1991), the leaves of *Schoenoplectus, Scirpus, Typha*

and *Potamogeton filiformis* (Barclay & Crawford 1982) and for the internodes of germinating tubers of *Potamogeton pectinatus* (Summers & Jackson 1993).

Ridge (1987) compared the time taken to reach the water surface (or maximum length) with the total height relative to air-grown controls for submerged shoots growing in the presence of low oxygen concentrations. She came to the conclusion that the species fall into two main subgroups: those showing a large and rapid response (*Nymphoides peltata, Ranunculus sceleratus* and *Regnellidium diphyllum*) and those showing a small, delayed growth response (*Ranunculus repens, Caltha palustris, Oenanthe fistulosa, Oenanthe crocata, Geum rivale, Ranunculus ligua, Ranunculus flammula, Alisma plantago-aquatica, Apium nodiflorum*). Ridge (1987) suggested that these two response types are associated with habitat preferences of the species involved. It was proposed that the latter group, in contrast to the former group, typically grows as emergents in shallow water, in marshes, or in areas subjected to brief shallow floods. The total elongation response of shoot organs upon submergence also depends on the following.

(1) *Stage of development of the elongating organ.* The leaf age determines the elongation capacity of *Nymphoides peltata* petioles. Leaves with an intermediate age (laminae newly expanded at the water surface) showed the highest elongation capacity, whereas both immature and fully mature leaves demonstrated a smaller elongation response (Ridge 1992). Comparable results were also observed in the semi-aquatic plant *Rumex palustris* (Voesenek & Blom 1989).

(2) *Stage of the life cycle.* The rosette, bolting and flowering stage of *Rumex palustris* strongly differ in petiole and internode elongation in response to submergence. The rosette stage was characterized by a rapid extension of petioles. The elongation of this organ was much less in the bolting stage, although a strong growth enhancement was observed in the internodes. In the flowering stage, elongation of both petioles and internodes was negligible (Van der Sman *et al.* 1991; Voesenek *et al.* 1992). Keith and co-workers (1986) reported that the marked difference in shoot elongation between deep-water rice and non-deep-water varieties is primarily determined by the timing of three growth stages: the rosette stage with no internodal growth, the bolting phase in which internode growth can be stimulated by submergence and the reproductive phase which sets a limit to the final size of the plant. In deep-water varieties the phase of internode elongation is lengthened due to the photoperiodically controlled flowering. However, in many non deep-water rice cultivars flowering is not under photoperiodic control, but occurs at a pre-determined stage of plant development.

(3) *Organ history.* Air-grown petioles of *Nymphoides peltata* quickly lose ability to respond to submergence. However, petioles developed in water show a degree of neoteny, i.e. they retain a substantial elongation capacity for considerable periods (Ridge 1992).

The stimulated shoot elongation is functionally related to the opportunity to reach better illuminated and aerated zones close to the water surface or preferentially above it. Due to improved access to both oxygen and carbon dioxide, aerobic respiration and photosynthesis will benefit. Additionally, shoot emergence will allow wind- or insect-mediated pollination (Jackson 1985). Since aerobic root respiration in flooded plants depends not only on the restoration of contact between the shoot and the atmosphere, but also on the porosity of shoot and root tissue, a strong positive correlation is expected between the ability to elongate shoot organs and the aerenchyma content

of these species. A literature survey revealed that the shoot elongation capacity of both aquatic and terrestrial plants was always accompanied by high root porosities (Voesenek *et al.* 1992).

Only a limited amount of research has been published concerning the mechanisms behind the fascinating process of shoot elongation without any trace of oxygen. The growth of anaerobic rice coleoptiles cannot be enhanced by exogenous ethylene, auxin or gibberellin (Satler & Kende 1985; Pegoraro *et al.* 1988; Horton 1991). ABA even inhibited elongation in anaerobic rice coleoptiles (Horton 1991). Despite the inability of anoxically grown rice coleoptiles to respond to added ethylene, they retain ethylene binding sites under anaerobic conditions (Sanders *et al.* 1990). Carbon dioxide produced by anaerobically growing rice coleoptiles has no influence on the anoxia-induced elongation (Horton 1991). Recently, Reggiani *et al.* (1989) demonstrated an absolute requirement for putrescine during anaerobic elongation of rice coleoptiles. This result was obtained with inhibitors of the polyamine biosynthesis, specificity in this system being tested by simultaneous addition of inhibitors and putrescine.

The first report on the possible involvement of ethylene in the growth promotion of shoot organs came from Ku and co-workers (1970) who worked with elongating rice coleoptiles. This group showed that exogenously applied ethylene stimulated coleoptile growth; maximum growth occurred when sub-ambient partial pressures of oxygen were added simultaneously with $10\,\mu l\,l^{-1}$ ethylene. They also provided evidence for the growth-stimulating effect of elevated (>0·5%) carbon dioxide levels. It is of great importance to place this finding in its perspective: this was the first time ethylene was seen as a growth-stimulating hormone, rather than a growth inhibitor or inducer of abscission, ripening and senescence (Ku *et al.* 1970; Osborne 1984). Ku *et al.* (1970) were the first to suggest the possibility of ethylene entrapment in submerged plant tissue: 'It can also be imagined that submergence would reduce diffusion of endogenous ethylene from the coleoptile, the result being to increase the effectiveness of the gas in stimulating growth'. It was not until 1972 that the first measurements of endogenous ethylene concentrations in submerged plant tissue were published. Working with *Callitriche platycarpa*, Musgrave *et al.* (1972) showed that oxygen-rich bubbles, given off by submerged shoots, contained ethylene at an average concentration of $1{\cdot}19 \pm 0{\cdot}24\ nl\ ml^{-1}$. Exogenously applied ethylene stimulated internode elongation in this plant. From here on ethylene involvement was demonstrated in many plant species belonging to many genera (Table 2). However, it should be kept in mind that there are plant organs such as the coleoptiles of *Echinochloa oryzoides* which do elongate in response to submergence, but in which ethylene is unable to stimulate shoot elongation (Pearce & Jackson 1991).

The first papers on ethylene-mediated shoot elongation suggested that ethylene is produced continuously in plant tissues. It accumulates in the intercellular air spaces due to its low diffusion rate in water; the resulting high levels of ethylene directly or indirectly stimulate cell extension. As soon as the water surface is penetrated, a rapid escape of entrapped ethylene is presumed to occur, followed by a decrease of cell extension. Subsequently, the lower equilibrium concentration of ethylene is in agreement with the lower growth rate. This entrapment story has stood the test of time, but more details on ethylene production levels during submergence, quantification of ethylene escape in response to desubmergence and desubmergence-induced enhanced ethylene production levels have become available during the last 10 years of research on this subject. Métraux & Kende (1983), working on submergence-induced internode

Table 2. Plant species and organ in which ethylene is involved in stimulated shoot elongation under water

Plant species	Organ	References
Apium nodiflorum	Petiole	Ridge 1987
Callitriche platycarpa	Internode	Musgrave *et al* 1972
Caltha palustris	Petiole	Ridge 1987
Epilobium hirsutum	Internode	Ridge 1987
Geum rivale	Petiole	Ridge 1987
Hydrocharis morsus-ranae	Petiole	Cookson & Osborne 1978
Nasturtium officinale	Internode	Cookson 1976 Schwegler & Brändle 1991
Nymphoides peltata	Petiole	Ridge & Amarasinghe 1984
Oenanthe crocata	Petiole	Ridge 1987
Oenanthe fistulosa	Petiole	Ridge 1987
Oryza sativa	Coleoptile	Ku *et al.* 1970
Oryza sativa	Internode	Métraux & Kende 1983
Plantago major	Petiole	Ridge 1987
Potamogeton distinctus	Stem	Suge & Kusanagi 1975
Ranunculus flammula	Petiole	Ridge 1987
Ranunculus lingua	Petiole	Ridge 1987
Ranunculus repens	Petiole	Ridge 1987
Ranunculus sceleratus	Petiole	Musgrave & Walters 1973
Ranunculus pygmaeus	Petiole	Horton 1992
Regnellidium diphyllum	Petiole	Musgrave & Walters 1974
Rumex crispus	Petiole	Voesenek & Blom 1989
Rumex maritimus	Internode	Van der Sman *et al.* 1991
Rumex palustris	Petiole	Voesenek & Blom 1989
Sagittaria pygmaea	Internode	Suge & Kusanagi 1975

elongation in deep-water rice, discovered that internodes from partially submerged plants had a much higher capacity to produce ethylene than internodes from controls. This enhanced synthesis of ethylene is triggered by low levels of oxygen (3%) (Kende *et al.* 1984; Raskin & Kende 1984a) acting to enhance ACC-synthase activity (Cohen & Kende 1987). This work gives no insight into the kinetics of enhancement of ethylene synthesis. It is therefore difficult to interpret the physiological significance of the increased production rate compared to the physical process of entrapment. In *Rumex palustris*, a wetland species, low levels of oxygen induce an increase in ethylene production, but only after a delay of 16–24 h (Voesenek, unpublished data). During total submergence ethylene had by this time already accumulated up to growth saturating concentrations of 3·4–4·4 $\mu l\, l^{-1}$ (Voesenek *et al.* 1993).

Rapid escape of accumulated ethylene upon water surface protrusion, as suggested by Musgrave *et al.* (1972), was tested experimentally in a recent study on the kinetics of ethylene release in desubmerged shoots of *Rumex palustris* (Voesenek *et al.* 1993). We demonstrated with the aid of photoacoustic spectroscopy that 90% of the ethylene entrapped during 24 h of submergence escaped within 1 min. Voesenek *et al.* (1993) observed that *Rumex* plants exhibit two desubmergence-induced peaks in ethylene release. The second post-submergence peak (maximum after 3–4 h) did not appear when *Rumex palustris* was desubmerged in a N_2 atmosphere. It was concluded that this peak represents ethylene biosynthesis after the submergence period. Preliminary evidence

Table 3. Ethylene production (n=8–10; ± SE) of *Rumex* species in response to 24 h of submergence and control conditions. After de-submergence entrapped ethylene was allowed to escape during a 10 min exposure to the atmosphere. Hereafter shoots were placed in stoppered serum vials for a period of 2 h. Species are ranked according to their distribution in a flooding gradient

Habitat	Species	Ethylene production (nl h^{-1} g DW^{-1})	
		Control	Submerged
Dry	*R. thyrsiflorus*	34·5 ± 6·4	14·0 ± 1·2
	R. acetosa	23·6 ± 3·7	11·6 ± 1·7
	R. obtusifolius	10·6 ± 1·8	25·0 ± 4·5
	R. conglomeratus	7·5 ± 0·8	56·5 ± 6·6
	R. crispus	7·4 ± 1·4	88·9 ± 5·4
	R. palustris	14·6 ± 2·1	159·3 ± 21·4
Wet	*R. maritimus*	25·5 ± 4·7	221·1 ± 18·6

indicates that this increase can only partly be explained by the conversion of accumulated ACC to ethylene; most of the ethylene production coming from ACC synthesized *de novo*. These flood-induced peaks in ethylene production can be observed predominantly in the wetland *Rumex* species (Table 3). There was a positive correlation between the post-flood ethylene production rate and the habitat type of the *Rumex* species, with *Rumex maritimus* and *Rumex palustris* which occur in frequently flooded river floodplains having the highest post-flood ethylene production. The following functions for high post-flood ethylene production levels are suggested.

(1) The post-submergence production increase may be interpreted as an adaptive mechanism to ensure that not only leaf tips, but a substantial part of the shoot will rapidly protrude the water surface. It has been demonstrated that this improves both survival (Voesenek *et al.* 1992) and reproduction (Van der Sman *et al.* 1991) in wetland *Rumex* species.
(2) Ethylene may play a role in the shoot growth and survival of shoots towards oxidative stress, induced by the switch from hypoxic/anoxic to normoxic conditions. Pearce *et al.* (1992) suggest that post-anoxia ethylene may help protect seedlings of *Oryza sativa* and *Echinochloa oryzoides* from oxidative stress due to re-oxygenation. When anaerobic tissue is aerated this may lead to the generation of hydrogen peroxide, which can give rise to the formation of highly reactive singlet oxygen molecules and hydroxyl radicals that can both induce damage to membranes. Ethylene may enhance ascorbate peroxidase activity, which increases the resistance of cells towards the formation of hydrogen peroxide (Mehlhorn 1990).

Besides ethylene, other hormones seem to play a crucial role in enhanced shoot elongation in response to submergence. The involvement of gibberellin (GA_3) in internode elongation of *Callitriche stagnalis* was demonstrated for the first time by McComb (1965). It was proposed that submergence enhances gibberellin production or sensitivity. An absolute requirement for gibberellin before internode tissue of *Callitriche platycarpa* will respond positively to ethylene was found by Musgrave *et al.* (1972). Moreover, it was demonstrated that ethylene sensitized internode tissue to gibberellin,

thus supporting McComb's original view. The same phenomenon was described later for submergence-induced petiole growth in *Ranunculus sceleratus* (Musgrave & Walters 1973) and in the elongation response of internodes of deep-water rice (Raskin & Kende 1984b; Khan *et al.* 1987). Submergence also increased the endogenous gibberellin level in deep-water rice as measured with the bioassay using a gibberellin-deficient dwarf rice Tan-ginbozu (Suge 1985) and measured with GC-MS (Hoffmann-Benning & Kende 1992). The gibberellin-induced internode elongation in deep-water rice is based initially on cell elongation in the intercalary meristem. It has been suggested that these increased cell sizes stimulate cell divisions; the resulting cells being more competent to respond to gibberellin and therefore growing larger than control cells (Sauter & Kende 1992).

Cookson & Osborne (1978) described that shoot elongation of *Hydrocharis morsus-ranae, Regnellidium diphyllum* and *Ranunculus sceleratus* can be stimulated by treatments with ethylene and auxin. The effects were additive and it was suggested that these two hormones may have separate mechanisms of stimulating cell elongation (Cookson & Osborne 1979). The growth enhancement of *Regnellidium diphyllum* by ethylene is dependent upon a supply of auxin. When the endogenous source was removed by deblading, no ethylene-induced petiole elongation was observed. Exogenous addition of auxin, however, restored the ethylene response (Walters & Osborne 1979). Auxin-dependent petiole growth in response to submergence is also observed in *Ranunculus sceleratus* (Horton & Samarakoon 1982). Preliminary results suggest strongly that submergence- and ethylene-induced petiole elongation in *Rumex palustris* acts independently of auxin (Blom *et al.* 1994).

Abscisic acid (ABA) can inhibit internode elongation in deep-water rice (Khan *et al.* 1987), coleoptile growth of *Oryza sativa* seedlings (Horton 1991) and petiole elongation in *Ranunculus sceleratus* (Smulders & Horton 1991). Moreover, Hoffmann-Benning & Kende (1992) showed that within 3 h, both submergence and exogenous ethylene application led to a 75% reduction of the endogenous ABA concentration in the intercalary meristems of deep-water rice. These authors therefore postulated that rapid internode elongation in deep-water rice may result from a changed balance between a growth stimulator (gibberellin) and a growth inhibitor (ABA).

ABSCISIC ACID AND DROUGHT STRESS

Without water, plant life is not possible. If plants are deprived of water, the net movement of water outward across the plasma membrane dehydrates the cells and the water-based chemistry that sustains the cell ceases. Structural integrity of membranes, proteins and nucleic acids all depend upon the unique bonding properties of water. Plants exposed to water shortage undergo a series of physiological, biochemical as well as molecular changes. These changes include increase and reallocation of abscisic acid (ABA) (Zeevaart & Creelman 1988), the closure of stomata, decrease in photosynthesis and alterations in gene expression. Because intracellular solute accumulation (sugars, potassium ions and/or certain proteins) to maintain turgor pressure is of limited capacity, protracted water deficiency causes wilting and eventual death. However, spores of micro-organisms and plant seeds can survive periods of severe desiccation. Moreover, during the maturation of seeds, the acquisition of desiccation tolerance is part of the normal developmental programme. The onset of germination disturbs that tolerance and the emerging seedling rapidly loses the ability to survive desiccation. Although the tolerance to dehydration in the majority of higher plants is restricted to

mature seeds, there is a small group of plants (the so-called resurrection plants) in which all vegetative organs can withstand desiccation and regain full function again upon rehydration. One resurrection plant, *Craterostigma plantagineum*, can withstand a water loss greater than 90% for long periods and yet recover after rewatering within 24 h (Bartels *et al.* 1990). Triggered by a certain sequence of metabolic events, cell structures become protected against decreased water activity and solute accumulation, enabling the cells to survive desiccation and subsequent rehydration.

The responses of living organisms to other types of abiotic stress than water stress show similarities over a wide evolutionary distance. For example, biochemical adaptations at cellular level are strongly conserved in evolution for heatshock response (Vierling 1991). There is also indication that the response to drought or water stress response is similar for different species. Dehydrins are proteins which generally accumulate in plants in response to cellular dehydration, regardless of whether dehydration is induced during seed development, low temperature or by osmotic stress. These proteins have an osmotically inducible immunologically related counterpart in cyanobacteria (Close & Lammers 1993), suggesting a conservation on protein structure level and perhaps also in function.

Involvement of ABA in drought stress

The phytohormone ABA influences the physiology, growth and development of the plant in a variety of ways. Apart from its involvement in the regulation of seed development and maturation, ABA also plays a central role in stress responses by enhancing adaptations to salt, high osmoticum (Close *et al.* 1989), wounding (Peña-Cortés *et al.* 1989), cold and drought (Henson 1984).

Plant responses to wounding include wound healing, reinforcement of the cell wall by deposition of callose, glycoproteins, production of phytoalexines and proteinase inhibitors (Bowles 1990). Exogenous ABA application has been shown to induce defence proteins, such as proteinase inhibitor (Peña-Cortés *et al.* 1989). Also endogenous ABA concentration rises upon wounding. In addition, ABA-deficient plants do not accumulate proteinase inhibitor mRNA after wounding (Hildmann *et al.* 1992), suggesting an important role for ABA in the defence response.

The consequences of water stress (the main topic of this section) for higher plants can be observed because plants protect themselves. Short-term drought induces stomatal closure. This rapid and sensitive acclimation to the environment serves to minimize the impact of the stress. However desiccation, the most extreme form of water stress, is a specialized condition and needs another type of acclimation, such as long-term closing of stomata, limited growth to reduce leaf area or leaf abscision and rapid flowering. A successful survival strategy must in some way integrate external signals and induce molecular-, metabolic- and physiological events. Only with the combined approach of various experimental disciplines, an understanding of events triggering adaptive responses to environmental stress in plants will be possible.

Attention was focused on the role of ABA in drought stress physiology, because unwatered plants show substantially enhanced ABA content (Wright & Hiron 1969) or redistribution of existing ABA (Hartung & Davies 1991). Also, detached leaves that are subjected to water deficit rapidly accumulate ABA within 30 min after applying the stress (Cohen & Bray 1990). Moreover, ABA application reduces water loss by restricting leaf conductance (Trejo *et al.* 1993).

A difficulty in assessing the role of ABA in the response of plants to water deficit is the variety of the responses. For example, experiments with ABA-deficient mutants have elucidated the relation between the capacity to synthesize ABA and the responses of stomata. These mutants wilt readily and appear to be unable to increase ABA biosynthesis in response to dehydration. As a consequence, the concentration of ABA available at the site of action in the apoplast surrounding the guard cell does not increase and these mutants do not close their stomata. It is clear that stomatal responses to drought are mediated by ABA synthesis and/or ABA release from storage pools, to the apoplast where it can be carried by the transpiration stream towards the guard cells. Subsequently guard cells somehow, perhaps via ABA receptors, recognize and respond to the ABA.

ABA synthesis takes place in the cytosol. The ABA biosynthesis occurs through the carotenoid biosynthetic pathway (Parry & Horgan 1991). Cleavage of carotenoids produces xanthophylls of which one, xanthoxin, can be converted rapidly into ABA. Physiological evidence for this pathway of ABA biosynthesis comes from corn mutants, deficient in carotenoid synthesis, of which many are also viviparous and contain very little ABA (Neill *et al.* 1986). In addition, ABA-deficient mutants of *Arabidopsis thaliana* appear to be impaired in the ability to convert zeaxanthin; resulting in greatly reduced levels of those xanthophylls, which are potential ABA precursors (Duckham *et al.* 1991; Rock & Zeevaart 1991). A decrease of specific xanthophylls has been observed concomitant with the synthesis of ABA in response to water stress in etiolated *Phaseolus vulgaris* leaves (Li & Walton 1990), or in soil-grown roots of *Lycopersicon esculentum* (Parry *et al.* 1992).

ABA synthesized in the cytosol accumulates mainly in chloroplasts. This accumulation is a result of stromal alkalinization. Because ABA is a weak acid, which can penetrate biomembranes only in protonated form (HABA), changes of pH values will cause an ABA redistribution. Moreover, under osmotic-, drought- and possibly cold stress, proton motive-forces decrease and this leads to compartmental pH shifts (Slovik & Hartung 1992). As a result water stress-induced redistribution of ABA amongst different leaf cell types (mesophyll, guard cells, phloem cells) and their compartments (cell wall, cytosol, chloroplast stroma) occurs. This process is fast and intensive enough to induce stomatal closure (Slovik & Hartung 1991). Apart from ABA release from leaf mesophyll cells, there is also evidence that water conditions in the soil influence stomatal closure (Blackman & Davies 1985). This suggests that root-produced ABA can be carried to the leaves as a messenger to stomata (Zhang *et al.* 1987; Tardieu *et al.* 1992).

During normal development ABA mediates in embryo maturation during late seed development. Maturation involves various morphogenetic and biochemical changes, including the programming of embryo dormancy, to prevent precocious germination and desiccation tolerance. The highest concentration of ABA in the embryo occurs in many seeds at a time when their dry weight is increasing. Genetic analyses implicate a role for ABA in the control of dormancy (McCarty *et al.* 1989; Koornneef *et al.* 1984). In addition, certain ABA-responsive genes, which are expressed during late embryogenesis may play a part in a developmental programme leading to desiccation tolerance (Bartels *et al.* 1988; Dure *et al.* 1989).

Mutational analysis to identify ABA-responsive pathways

The application of ABA has been used to identify responses to ABA in isolated systems, but application alone cannot demonstrate the *in vivo* role of ABA during stress. With the

help of mutants deficient in ABA, the role of endogenous ABA in plant responses can be demonstrated. For instance in tomato, the ABA-deficient *flacca* mutant exists which is blocked in the biosynthesis pathway. This mutant is impaired in the last step of the convertion from xanthin to ABA, resulting in the accumulation of an ABA-alcohol (Taylor *et al.* 1988). This *flacca* mutant, which is leaky and able to synthesize a limited amount of ABA, shows no accumulation of drought-induced polypeptides, which are present in detached drought-stressed wild type leaves. Application of ABA to *flacca* leaves results in the synthesis of these polypeptides (Bray 1988). Besides an ABA-deficient mutant (Koornneef *et al.* 1982; Karssen *et al.* 1983), also ABA-insensitive mutants have been described in *Arabidopsis thaliana* (Koornneef *et al.* 1984). These mutants are pleiotropic; they are impaired in seed dormancy, regulation of water status, growth and are resistant to levels of ABA toxic to the wild type. All insensitive mutants contain a normal or increased content of ABA and their phenotype cannot be restored by application of ABA, suggesting a defect in some early step in ABA signal transduction. The availability of ABA-insensitive mutants represents a unique approach for the identification of elements of the abscisic acid-response pathway. For instance, the use of ABA mutants has established an ABA requirement for the induction of dormancy during seed development, to prevent precocious germination. The importance of embryonic ABA in seed development was proven in an experiment in which fertilization of the ABA-deficient mutant with wild type pollen restricted ABA to only the zygotic tissues of developing seeds and thereby inhibited precocious germination (Karssen *et al.* 1983). The reciprocal cross indicated that maternal ABA concentration rises half-way seed development and resembles exogenously applied ABA which cannot induce dormancy in ABA-deficient seeds. Physiological studies of ABA mutants of *Arabidopsis thaliana* also have indicated that products of the distinct *ABI* loci act in at least two overlapping response pathways, because the responses of the mutants are not the same for the known ABA-inducible responses (Finkelstein & Somerville 1990). *Abi*-1 and *abi*-2 mutants showed reduced sensitivity to ABA for inhibition of seedling growth and induction of proline accumulation upon drought stress, while in contrast *abi*-3 (specifically impaired in seed development) had wild type phenotype to proline accumulation and was less responsive to seedling growth inhibition.

Drought stress elicits multiple changes in the physiology and metabolism of the plant, including alterations in gene expression. The most apparent changes in the pattern of gene expression in response to water deficit, is the accumulation of a number of newly synthesized mRNAs and polypeptides (reviewed by Bray 1991). Therefore, part of recent research concerning the working mechanism of ABA in relation to plant stress responses has been focused on the expression of ABA-regulated genes, since most hormone responses upon stress are thought to be directly or indirectly connected with alteration of gene expression in plant tissue. The remaining part of this section concentrates primarily on the molecular view of water stress. The genes involved and their regulation and possible function will be discussed. This approach, starting from genes, can help to understand the mechanism of water stress tolerance and perhaps can answer the question of how water stress tolerance can be controlled.

Molecular aspects of water stress

ABA-regulated gene expression during desiccation. Genes under ABA-control have been isolated from different plant species (Skriver & Mundy 1990). A common technique for the isolation of ABA-induced genes is differential screening of a cDNA library,

constructed from ABA-treated tissue. This procedure has identified numerous cDNA clones, that are specifically expressed under drought conditions. Moreover, they share considerable homology with a unique set of genes predominantly expressed in seeds during the later developmental stages, coinciding with high concentrations of endogenous ABA and the beginning of the desiccation phase. Depending mainly on the way they have been isolated, the genes have been named either RAB (Responsive to ABA) or LEA (Late Embryogenesis Abundant) genes (Galau *et al.* 1986; Mundy & Chua 1988). LEAs are a diverse group consisting of non-storage seed proteins accumulating in late embryogenesis, which are transcriptionally regulated and can be induced to accumulate earlier in embryogenesis in excised embryos incubated in exogenous ABA (Galau *et al.* 1986). Some, but not all of the LEA gene families can be induced to higher levels of expression in non seed organs by ABA and/or water, or salt or cold stress as well, perhaps reflecting the importance of the water status during the latter stresses.

To understand the role of these genes and the possible function of their accumulated gene products in osmotic stress, desiccation tolerance and normal development, these genes are being studied as tools to develop molecular models of ABA action. It is necessary to understand the expression characteristics of these genes: the timing of mRNA accumulation, expression in response to other stresses and the tissues in which these mRNAs accumulate.

To understand how ABA regulates specific gene expression, it is desirable to characterize *cis*-acting abscisic acid responsive DNA elements (ABREs) in the promoters of ABA-regulated genes. Thus far, transient gene expression assays as well as transgenic plants have been used to delineate hormone responsive elements. Fusion of sequences from the 5′ upstream promoter regions to CAT (chloramphenicol acetyltransferase) or GUS (β-glucuronidase) reporter genes, as well as chimaeric promoters assayed for hormone responsiveness have clearly delineated ABREs of which 5′ACGTGGC3′ forms the core sequence. In this way ABREs have been identified for the RAB16 gene in rice (Mundy *et al.* 1990) and in the Em gene of wheat (Marcotte *et al.* 1988). Analysis of transgenic tobacco plants, transformed with part of the rice RAB promoter fused to the GUS reporter gene, shows that the rice promoter can specifically induce GUS activity in developing tobacco embryos. The GUS activity closely correlates with the endogenous ABA hormone levels during seed development. However, no activity was found in vegetative tissue and activity also could not be induced in immature embryos by ABA treatment, suggesting that the promoter used only confers developmentally regulated expression (Yamaguchi-Shinozaki *et al.* 1990). Similar expression patterns have been observed for wheat Em-GUS gene fusions in tobacco transgenics (Marcotte *et al.* 1989). These promoter elements have been conserved between various known ABA-responsive genes and genes expressed during late seed development. The motifs are part of sequences with which so called *trans*-acting nuclear protein factors interact. Gel retardation and DNAse I footprint experiments have shown specific binding to ABRE sequences of nuclear factors, extracted from either roots and shoots of rice seedlings or mature wheat embryos (Mundy *et al.* 1990; Guiltinan *et al.* 1990). Guiltinan *et al.* (1990) have screened a cDNA expression library, made from wheat embryos to clone a gene encoding a protein capable of binding an ABRE probe. This way a protein (EmBP-1) was identified, which specifically interacts with an ABRE present in the wheat Em promoter. EmBP-1 resembles transcription factors found in plants and animals, since the protein contains conserved basic and leucine zipper domains, common in DNA binding factors. Further characterization of these binding factors will in turn provide

tools which can then be used to dissect earlier steps in the pathway of ABA-induced gene expression.

Another clue comes from the well characterized mutants with reduced sensitivity to ABA found in maize (*viviparous*-1, *VP*1 locus) and *Arabidopsis* (*abi*-3). Both genes VP1 and ABI3 have been cloned by respectively transposon tagging (McCarty *et al.* 1989) and positional cloning (Giraudat *et al.* 1992). These clones provide valuable tools for the molecular dissection of the ABA signal perception/transduction cascades and for the investigation of the physiological role and the functional properties of the respective proteins. The ABI3 protein contains a putative nuclear targeting signal and has several features previously described (Cress & Triezenberg 1991) in activation domains of transcription regulators. These observations suggest that the ABI3 protein participates in ABA-regulated gene expression during seed development. Sequence similarities exist between the ABI3 and the VP1 protein (McCarty *et al.* 1991), although the VP1 protein is in addition probably also involved in seed anthocyanin pathway (Hattori *et al.* 1992).

Drought-regulated gene expression. From the above described data it seems that, in plants, water deficit triggers the production of ABA, which in turn induces the expression of various genes. However, it has become apparent that ABA is not the only regulator involved in the control of changes in gene expression which occur in response to water deficit. It has been observed that some water deficit-induced changes in gene expression could not be mimicked by ABA application (Guerrero *et al.* 1990). Several genes have been isolated after differential screening of cDNA libraries made from drought-stressed or osmo-stressed material (Mundy & Chua 1988; Bartels *et al.* 1988). In this way, besides ABA-inducible drought-related genes also ABA-independent drought-induced genes have been isolated, e.g. Yamaguchi-Shinozaki *et al.* (1992) have isolated nine cDNAs, named RD (Responsive to Desiccation) from *Arabidopsis thaliana* that are induced by the effects of water deficit. Three of those cDNAs are not ABA-inducible. This indicates the existence of an ABA-independent as well as ABA-responsive signal transduction cascades between the initial signal of water stress and the expression of particular desiccation-related genes. The possible physiological importance of ABA-independent desiccation-responsive genes is illustrated by experiments using the ABA-deficient and ABA-insensitive double mutant (*aba*-1, *abi*3-1) of *Arabidopsis thaliana* (Koornneef *et al.* 1989). In this mutant no ABA is detectable. The seeds are viable, but desiccation-intolerant. The seeds fail to desiccate in the maturation phase, remain green throughout development and germinate precociously under high humidity conditions. However, desiccation tolerance can be induced by slow drying of immature seeds (Ooms & Van der Veen, personal communication); suggesting that either ABA-responsive or RD proteins would be sufficient to acquire full desiccation tolerance.

Signal transduction mechanisms involved in desiccation

Another goal of studies on gene expression in response to ABA and water stress is to understand how plants sense osmotic changes in their environment and how they transduce this signal to produce changes in specific gene expression. To date, the number of components in mediating ABA perception, as well as the components in transducing the appropriate signal are only beginning to be defined.

Usually signalling pathways consist of a series of secondary messengers and proteins, including receptors, binding proteins, kinases and regulatory proteins. Thus far, analysis has revealed that signalling proteins show enormous heterogeneity and

there are various interactions between different signalling systems (Bush 1993). In this respect the relation between desiccation stress and other stresses is relevant, since ABA is involved in responses to a diversity of environmental stresses. It has been demonstrated that several ABA genes expressed during drought are also expressed to a certain level during other environmental stresses, such as salt stress, low temperature or wounding. These responses of an ABA gene to a stress other than drought might be due to signals acting at different points of a partially shared signalling network from sensing to response. Current desiccation stress signal transduction studies suggest a primary turgor response in reaction to stress, followed by the induction of ABA-responsive and other genes (Olsen *et al.* 1992). The induction cascades could be mediated through a number of steps by secondary messengers such as pH and Ca^{2+} (Napier *et al.* 1989; Schroeder & Hedrich 1989; Van der Veen *et al.* 1992). Even ABA might be regarded as a secondary messenger mediating water deficit signals and the physiological responses (Trewavas & Jones 1991). Changes in cytosolic Ca^{2+} promote the formation of Ca^{2+} complexes with target proteins, such as calmodulines, that, in turn, may regulate an activity of the effector protein that determines cell response (Poovaiah & Reddy 1993). Although the action of these secondary messenger signals is often transient, the prolonged physiological response is thought to be due to sustained activation of, for instance, Ca^{2+}-dependent protein kinases (Harper *et al.* 1991) or phosphatases (Klauss & Jeblick 1991). Thus far, the importance of Ca^{2+} as a major regulator in signal transduction has been reported in response to various external stimuli as hormones or light (Gilroy *et al.* 1990, Chae *et al.* 1990) in various cellular processes. The use of Ca^{2+}-sensitive fluorescent indicators (Read *et al.* 1992) allows direct measurement of changes in intracellular concentrations in correlation with environmental stimuli and specific gene expression. Ca^{2+} influx from internal stores has been demonstrated in stomatal guard cells (MacRobbie 1989). Also ABA-induced elevation of guard cell cytosolic Ca^{2+} precedes stomatal closure (McAinsh *et al.* 1990). However, it is not yet clear, whether this Ca^{2+} influx is a primary event in the ABA response, although the ABA response is sensitive to external Ca^{2+}. As suggested above, detailed analysis of ABA mutants, in particular the multiple classes of ABA-responsive mutants from *Arabidopsis thaliana* can help to elucidate the nature, diversity as well as the specificity of ABA signalling cascades.

Possible functions of ABA-inducible proteins in desiccation and development

The precise biological function of most of the above discussed genes in late embryogenesis and in response to environmental stress remains unknown and preliminary studies of their temporal and spatial patterns of expression yield few clues (Olsen *et al.* 1992). At present, the connection between ABA effects during embryogenesis and in vegetative tissues in reaction to stress is not clear. The functions might relate to a defensive reaction to the stresses themselves, perhaps mediated through ABA. This lack of function is mainly caused by the limitation of differential screening procedures used to isolate the majority of known ABA-regulated genes. Determination of the DNA and the deduced amino acid sequence has led to the realization that several are homologous and conserved among species. The high relative abundance of these proteins argue against a role as regulatory proteins or as mediators in signal transduction.

Some LEA/RAB proteins are unusually high in glycine and lysine residues, extremely hydrophilic, readily soluble in aqueous buffers and resistant to boiling, suggesting that

these proteins have no enzymatic or structural function in the cell (Dure *et al.* 1989). A role as water-binding protein has been suggested (McCubbin *et al.* 1985; Roberts *et al.* 1993), since hydrophilic amorphous proteins could contain more hydration water and thus preventing desiccating seed from losing all its water. Thus far, experimental evidence concerning levels and distribution exists for only two types of LEA proteins. In cotton, both are present in high molar concentrations prior to seed desiccation. Moreover, these proteins are evenly present in the cytosol of all cell types in the embryo (Roberts *et al.* 1993).

It has been suggested that LEA proteins may be involved in protein and/or membrane protection against desiccation damage. One of them belongs to the LEA D7 type family, which is principally characterized by a tandemly repeated 11-mer amino acid motif, consisting of apolar residues and charged or amide residues at specific positions, and which structurally most probably exists as an amphiphilic α helix. A possible function for this structure may be that of an ion carrier to prevent precipitation or crystallization of ions inside the cells of tissues subjected to desiccation by ion sequestration (Dure 1993). Information available for a possible role as ion or water transport protein to facilitate the movement of solutes or water across membranes to minimize desiccation damage is available for some dehydration-inducible gene products, like RD28 from *Arabidopsis thaliana*, 7a from pea and nod26 from soybean (Sandal & Marcker 1988; Guerrero *et al.* 1990; Yamaguchi-Shinosaki *et al.* 1992). Their deduced amino acid sequences contain several transmembrane domains and a major intrinsic protein motif, present in many transmembrane channel proteins. However, these two genes are not ABA-inducible and their subcellular location remains to be determined.

Are other components/mechanisms involved in protection against dehydration?

Another line of reasoning suggests the importance of soluble carbohydrates and prolines in protection against desiccation. From model studies (Crowe *et al.* 1984, 1992) evidence has come that carbohydrates can fulfill a role in membrane protection during dehydration. For instance in the resurrection plants, besides many changes in gene expression, one of the striking observations is the change in soluble carbohydrate composition (Bianchi *et al.* 1991). In fully hydrated leaves 90% of the soluble carbohydrates is 2-octulose. However, during desiccation octulose disappears and sucrose becomes the dominant sugar. Whether there is any direct correlation to ABA remains to be seen. In developing soybean seeds, excised axes of immature seeds that were gradually dried (induction of desiccation tolerance) accumulated sucrose and stachyose, while axes held at high humidity (no induction of desiccation tolerance), did not (Blackman *et al.* 1992). On the other hand, comparison of the composition and accumulation of soluble carbohydrates between *Arabidopsis thaliana aba*-1, *abi*3-1 double mutant seeds (desiccation-intolerant) and seeds treated with an ABA analog to make them desiccation-tolerant shows almost no differences, suggesting no essential role for carbohydrates in the acquisition of desiccation tolerance (Ooms *et al.* 1994).

Prolines can accumulate after hyperosmotic stresses up to 20% of the free amino acid pool. Although the precise role of prolines during stress and in relation to ABA is not yet understood, several adaptive roles as osmoprotectants have been proposed and accumulation of this amino acid may be part of a general adaptation to adverse environmental conditions (Delauney & Verma 1993; Xin & Li 1993).

CONCLUDING REMARKS

The goal of studies on the relation between stress and phytohormones in plants is to elucidate the chain of events induced by external stress which leads to enhanced hormone production and/or sensitivity, followed by hormone-induced gene expression and ultimately to a functional physiological response. It is evident that this goal has not yet been reached. However, molecular biological techniques and the availability of mutants show great promise for studying the signal-transduction pathways related to plant stress. Substantial progress has been made recently in unravelling the signal transduction pathway of ethylene action.

This review has shown that in several cases there are serious doubts about the primary role of phytohormones in stress-induced gene expression, e.g. with respect to the role of ethylene in wound-induced gene expression, the expression of so-called touch genes, the contradictory results of ethylene-induced changes in root morphology upon physical impedance and in adventitious root formation. In addition, drought stress induces changes in gene expression either dependent or perhaps also independent of ABA. Most studies, however, are focused on the role of one hormone. In order to enhance progress, more effort should be directed into multi-hormone research. A good example of such an approach is the unravelling of the concerted action of oxygen, carbon dioxide, ethylene, gibberellin and/or auxin and ABA in submergence-induced shoot elongation in several plants from semi-aquatic habitats, e.g. deep-water rice and *Rumex* species.

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