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Review

Some cellular and molecular properties of abscisic acid: its particular involvement in growing plant roots

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Abstract. Several characteristics of the plant hormone abscisic acid (ABA) are critically discussed, more or less directly, in relation to the extension of root cells. A few topics have been selected: some biochemical characteristics of ABA (chemical structure, metabolism), inhibiting-β complex, inhibiting regulators from root caps, endogenous ABA in growing roots (ABA gradients, microsurgical experiments, light effects), applied ABA on elongating roots, ABA and indol-3yl acetic acid (IAA) interactions (root growth, proton extrusion, hormone transport, auxin herbicides), ABA effect on the root cell cycle, ABA and drought cells of elongating roots [water deficit conditions, IAA and jasmonic acid (JA) as 'stress hormones' other than ABA, gene expression].

Key words. Abscisic acid; auxins; cell cycle; cell extension; desiccation; drought cells; gene expression; growth inhibition; plant hormones; hormone interactions; jasmonates; light action on growth; root elongation; root gravireaction; stress hormones; water deficit.

Introduction

Abscisic acid (ABA) is one of the more recent plant hormones to be discovered [1]. The first papers related to ABA action on plants show its effects on tuber, bud and seed dormancy, fruit and leaf abscission, stomatal closure and cell senescence [2–4]. During the last few years there have been substantial advances in several fields of ABA research. Thus, in the present review, only a few topics, essentially related to the extension of root cells, will be considered. Most of the publications related to ABA before 1983 are listed in the book edited by F. T. Addicott [2]. More articles related to ABA effects on the elongation of root cells are cited in a few reviews [5–7].

Biochemical characteristics of ABA

The chemical structure of ABA and its analogues has been described in several articles [8–10]. In position 1′ ABA (fig. 1A) has an asymmetric carbon and consequently possesses optical activity which can be used for quantitative analyses. Only the $S-(+)$ enantiomorph is present in plant cells. Most of the endogenous ABA is the 2-*cis*,4-*trans* isomer. The 2-*trans*,4-*trans* form (B) occurs in small amounts. Conventionally, $S(+)$-2-*cis*,4-*trans*-abscisic acid is simply called abscisic acid, initially designed by the names abscisin and dormin, according to the first properties of this hormone reported [11]. Phaseic acid (C), dihydrophaseic acid (D) and 4′-desoxy-abscisic acid (E) are three metabolites of ABA [12].

The metabolism (biosynthesis, biodegradation) of ABA has been the subject of many papers [13–15]. The anabolism of ABA could be considered via a series of C15 intermediates to farnesyl pyrophosphate or a few other terpenoids. On the other hand, the carotenoid violaxanthin can be cleared to give (−)-xanthoxin

(fig. 1F), another plant growth inhibitor [16] and ABA. It has been reported that ABA can be produced in avocado fruits fed by tritiated mevalonate [17]. Cell-free extracts from coloured fruits of *Citrus sinensis* transformed mevalonate, isopentenyl pyrophosphate and all-*trans*-β-carotene into ABA. This reaction was stimulated by molybdate and inhibited by the oxidized form of the flavin adenine dinucleotide (FAD). Gel electrophoresis of the enzyme extract revealed the presence of a 53-kDa protein with peroxidase activity characteristic of a cytochrome P-450 [18]. With a similar material and using the gas-chromatography-mass-spectrometry (GC-MS) [19, 20], it has been reported [21] that β-carotene is formed from either farnesyl or geranyl pyrophosphates. Thereafter, β-carotene levels declined; it gives xanthophylles and ABA precursors. Labelled violaxanthin and two neoxanthins were used as substrates to confirm the metabolism interrelationship between carotenoids and ABA. 9′-*cis*-Neoxanthin is the immediate carotenoid precursor to ABA.

It is of great interest to study the genes involved in ABA biosynthesis [22, 23]. In the viviparous mutants of maize, the primary effect of the genetic lesions seems to be to impair one of the early steps in carotenoid anabolism [24]. This has been considered as evidence that ABA is formed from carotenoids. In some tomato mutants (*flaca* and *sitiens*), the ABA aldehyde can be considered as a precursor of ABA. Cell-free extracts from these mutants contrasted with those from wild-type in being unable to form ABA from such a precursor [25]. Similar observations have been reported with mutants of diploid tobacco [26]. A mutant of barley has a mildly wilty phenotype, a low level of ABA and produces low amounts of ABA in reaction to water stress (see below). This mutant was found to lack the capacity to oxidize ABA-aldehyde [27]. The low level of some ABA precursors (violaxanthin acid 9′-*cis*-neoxanthin) appear to be sufficient to explain the ABA deficiency in some bean plants [28] and *Arabidopsis* mutants [29].

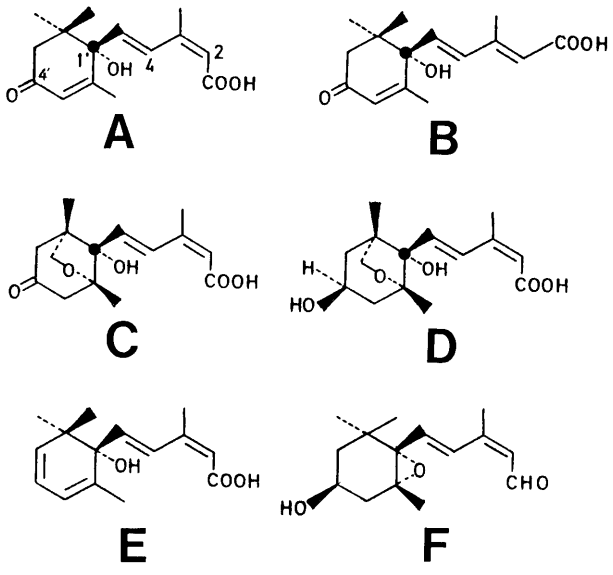

Figure 1. Abscisic acid and related compounds. (*A*) *S*(+)-ABA. (*B*) *trans*-ABA. (*C*) Phaseic acid. (*D*) Dihydrophaseic acid. (*E*) A^1-desoxy-ABA. (*F*) (−)-xanthoxin. Adapted from ref. 9.

The study of ABA biosynthesis has benefited from a new approach through molecular biology. Two directions of research are worth mentioning [23, 30, 31]. Several proteins are formed de novo under desiccation (see below). Some of these are also formed when ABA is applied to well-watered tissues. These proteins can be partially sequenced. The gene expression must precede drought-induced ABA accumulation. The messenger RNAs (mRNAs) transcribed from genes controlling the ABA synthesis would be more abundant in desiccated cells. They are likely to be represented in complementary DNA (cDNA) libraries related to water-stressed tissues [32]. On the other hand, transposable genetic elements may cause a mutant when they are inserted into active genes. This 'transposon-tagging' technique offers a means of cloning independent of prior knowledge of the gene product [33]. Moreover, several ABA-deficient mutants have been obtained using conventional mutagens [23].

Some interesting findings about the catabolism of ABA have been published. For instance, it is clear that light may act on ABA (see below), which is transformed in its 2-*trans*(*E*) isomer. This compound is almost inactive as a growth inhibitor [34]. The oxidation of (+)-ABA in phaseic acid could be due to a monooxygenase [35]. Numerous experiments have been conducted with plants fed with [^{14}C]-ABA. Some sugar conjugates were found as ABA glucose ester and ABA glucose [9, 13]. ABA turnover was first reported by measuring the rate at which this hormone was destroyed in bean leaves [36]. More recently, as an example, apical parts of intact maize root were immersed in buffer with or without (±)*cis-trans* [G-^{3}H]-ABA. GC-MS and liquid scintillation [19, 37, 39] were used to determine endogenous ABA, the amount of ABA taken up and that which had been metabolized. After 60 min the uptake of applied ABA was found to be eight times the level of the endogenous ABA. ABA turnover was about 160% for the apical zone of roots and about 75% for the differentiated portion of the root [40].

A final example related to the metabolism of ABA that summarizes an important biological problem is tuber dormancy, one of the first experiments described in which ABA was strongly implicated [2–4]. Potato tuber dormancy corresponds to a physiological state in which the inhibition of bud growth is due to hormonal con-

trol. Once dormancy is interrupted, sprouting proceeds under suitable environmental conditions [41].

The sprouting regulation avoids precocious senescence of tubers, loss of nutritional quality and disease progression [42]. Growth and storage conditions (such as temperature and light) influence dormancy duration [43], which is regulated by several hormones, including gibberellins [44] and cytokinins [41]. ABA has classically been implicated in potato tuber dormancy as the main component of the inhibiting-β complex (see below) [42, 45]. However, applied ABA can stimulate the growth of buds whose dormancy is terminated [46]. The implication of ABA in tuber dormancy is still questionable, because many investigations were affected by the restricted reliability of the experimental procedures, for example the application of exogenous ABA [47]. Recent and convincing experiments have been carried out on ABA and related metabolites of tubers (potato cv. Monabisa) [48]. Three tuber parts (eyes, subeye tissues and pith) were analysed during the final period of tuber growth until sprouting at 3 and 23 °C. ABA content rises in eyes and subeye tissues as sprouting approaches. Dihydrophaseic acid is the main ABA metabolite, while phaseic acid was not detected, probably because of its transformation into dihydrophaseic acid. The ABA glucose ester level is low except in eyes before harvest.

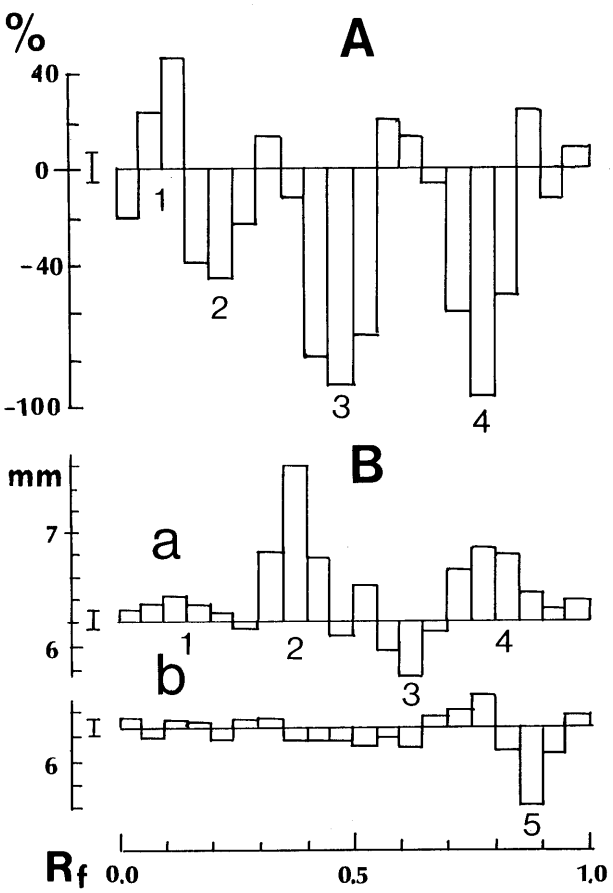

Figure 2. Extracts analysed by paper chromatography and subsequently bioassayed. Activity of the biotest in function of the Rf. (*A*) The acidic methanol/ethylacetate fraction obtained from a lentil root extract chromatographed on Whatman no. 1 paper with isopropanol-ammonia-water 8:1:1 as solvent. Biotest: lentil root segments the elongation of which being measured after 12 h. Data: growth in %. 1. Accelerator α. 2. Inhibitor I (?). 3. IAA. 4. Inhibitor β. Adapted from refs 50, 51. (*B*) The acidic (a) and neutral (b) ether fractions obtained from a pea seedling extract chromatographed on Schleicher and Schüll 2043 paper with isopropanol-ammonia-water 80:5:15 as solvent. Biotest: oat coleoptile segments the elongation of which being measured after 24 h. Data: length in mm. 1. Accelerator (?). 2. IAA. 3. Inhibitor β. 4. Accelerator (?). 5. Xanthoxin. Adapted from ref. 52.

Inhibiting-β complex

Inhibiting-β complex was first mentioned in a classic paper [49] which described the use of paper chromatography combined with bioassays to characterize the nature and activity of natural compounds regulating cell expansion. These growth inhibitors were detected in many roots and other plant organs [2, 5]. Figure 2 shows two 'histograms' comparing the chromatographic separation of these active substances with IAA and other growth factors [50–52]. The chemical structure of the inhibiting-β complex was subsequently reported to vary with the source of plant material. However, for a large list of these sources, it has been found that the activity of this complex could largely, if not entirely, be ascribed to ABA [53].

Inhibiting regulators from the root cap

Many papers have reported experiments showing that several regulators are produced in the root cap. They move basipetally in the extending zone of growing roots where they inhibit cell elongation. It has also been pointed out that these compounds are implicated in the regulation of positive root gravitropism. These articles have been systematically reviewed [5, 6, 54–57].

After the first experiment related the decapping of maize roots to prevention of the gravitropic response [58], it became accepted that gravisensitivity is located in the cap cells. Therefore, experiments have largely centred on the cap supposed to be the source of hormones regulating gravireaction [5–7]. Consistent results have come from experiments based on microsurgical operations. Data from the first two papers [59, 60] describing these experiments are presented in figure 3. Removal of one half of the cap caused the root to bend markedly towards the side on which the remaining half-cap is located. The cap cells must thus be the

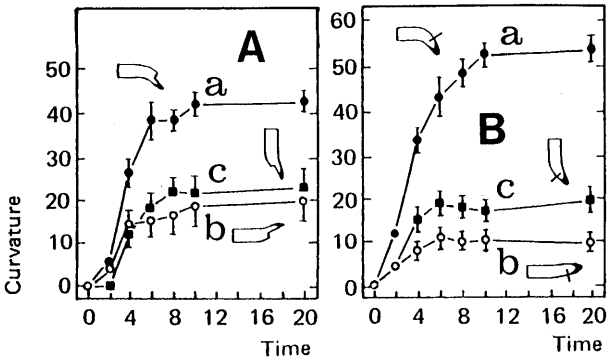

Figure 3. Kinetics (with time in hours) of curvature (in degrees) of maize roots having the half-tip removed (*A*) or a small piece of mica inserted inside the root tip (*B*). (*A*) a. Root horizontal: half tip lowermost; b. root horizontal: half tip uppermost; c. root vertical. (*B*) a. Root horizontal: barrier uppermost; b. root horizontal: barrier lowermost; c. root vertical. Adapted from refs 59, 60.

source of growth inhibitors. Although surgical trauma could be responsible for the bending induced by removal of one half of the cap, this possibility was eliminated by the fact that replacing the half-cap immediately after its removal prevented any curvature [61]. Inserting a barrier unilaterally and transversally into a slit on one side of the root tip just behind the apex caused curvature away from the mica, indicating that the reduction of inhibitor transport on one side gave a response similar to removal of the half-cap on that side. Formation and release of growth inhibitors in the cap cells have been critically summarized in two reviews [62, 63]. Only the results of three series of experiments [64–66] will be given in figure 4 and briefly commented on. As can be seen (fig. 4A), removal of the cap significantly enhanced root growth, but only during the first 3 h after decapping. This confirms [64] that the cap cells produce growth-inhibiting regulators that move from the tip to the base of the root and act in its extending zone. The gravicurvature of horizontal roots is reported in relation to time in figure 4B. When intact roots showed strong bending, decapped roots continued growing horizontally for a maximum of 11 h, after which gravitropism reappeared. When the cap was replaced [65] with a hydrophilous film between cap and apex, the gravibending resumed after a recovery time of about 5 h. In contrast, the presence of a lipophilous film (oleate) cancelled the gravireactivity. Consequently, the growth inhibitors have to be considered as water-soluble compounds. As shown in figure 4C, root segments placed horizontally developed strong bending after 6 h. The caps were then removed [66] and replaced on vertical decapped roots covering completely (b) or

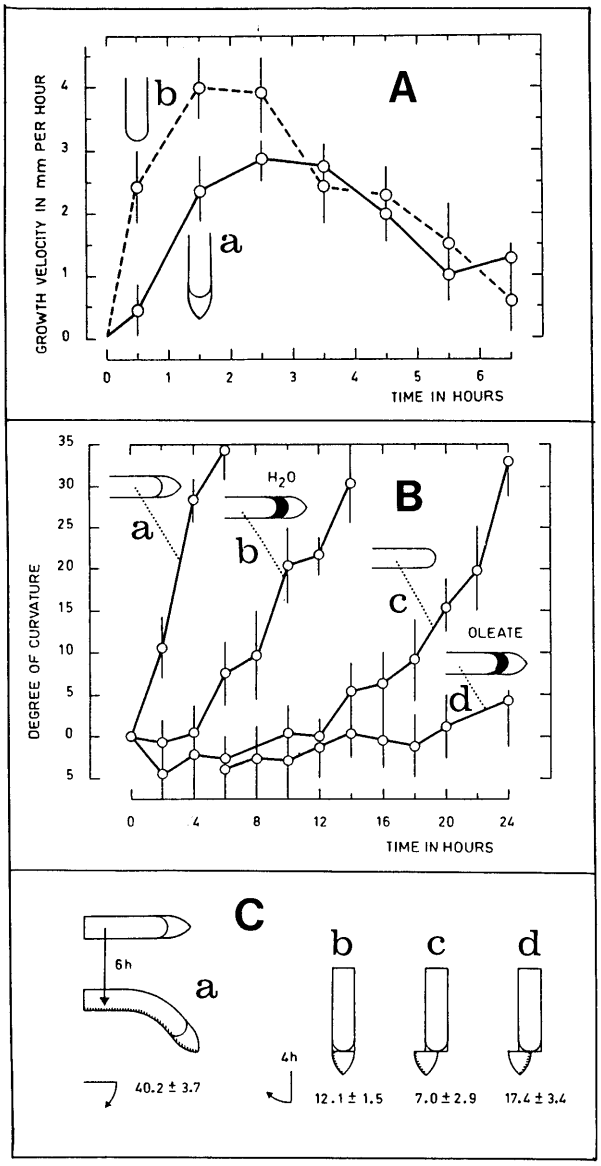

Figure 4. Growth (*A*) and graviresponsiveness (*B*, *C*) of primary roots of *Zea mays* cv.: Kelvedon in relation to the growth inhibitors produced by their cap cells. (*A*) Growth velocities (mm h^{-1}) of intact (a) and decapped (b) vertical roots as a function of time (h) [64]. (*B*) Gravibending (degrees) of horizontal intact (a), decapped (c), and decapped with cap replaced and H_2O (b) or oleate (d) between cap boundary and cap cells [65]. (*C*) Gravibending (degrees) of intact roots kept horizontally during 6 h (a) and decapitated roots after 4 h (caps having been removed and replaced [zero time] by caps from 6-h gravistimulated roots [66]). Adapted from refs 64–66.

half-recovering (c, d) the apical cut surface of these segments. After 4 h a curvature occurred that was stronger for b and d than for c. This indicates that the lower half of the cap from gravistimulated roots produced inhibitors in larger amounts than the upper half.

Endogenous ABA in growing roots

Publication of the first paper on ABA quantification (in pea roots) [67] was followed by many reports identifying (GC-MS) ABA content in roots [9, 19, 68, 20]. These reports have been exhaustively reviewed in ref. 5. We will cite some of these papers before presenting more recent data. One observation [69] worth noting at the outset is that ABA level in the roots of sugar maple depend on seasonal rhythm (fig. 5).

The first ABA gradients (detected by GC-MS) in growing roots were obtained for maize [9]. In the cap, the level was found to be 36.1 μg of ABA per kg of f.m. and 66.5 in the apex; the values were lower for the 10 mm below. GC-MS using negative chemical ionization [68] is a technique sensitive enough to quantify ABA on a single maize root [70]. Data showed that the more ABA, the slower the growth; but a wide range of individual values was obtained, indicating that this hormone is not only correlated to growth rate. This technique was also used to detect ABA in root protoplasts; the highest levels are found in protoplast from the cap and apex. The presence of ABA conjugates was reported only in protoplasts from the elongating and differentiating root parts [71]. Another more rapid technique has been used to measure changes in ABA content especially in response to environmental stress, that is the method of immunoassays utilizing monoclonal antibodies to ABA [72, 73]. With this technique, for instance, a significant increase in ABA content for maize roots during desiccation stress (see below) was reported [74]: after 7 h, ABA levels were about 0.65 μmol of ABA per 30 stressed roots and 0.15 for control roots.

Since the microsurgical experiments (see above), ABA quantification has been carried out on freshly decapped roots [5, 6, 63]. In *Pisum* roots, ABA and two xanthoxins (fig. 6) showed a decline of their level after decapita tion [75, 76]. In roots of *Phaseolus vulgaris*, the removal of the cap causes a significant decrease in ABA content (from 1.65 μg/g. of fresh mass (f.m.) to 0.215) [77]. Another kind of research will be discussed later on (see root cell cycle), but we will present some data here. The optimum DNA level was reported in the apex (quiescent centre, meristem) of maize roots. It significantly increased after removal of the cap. When roots were decapped and ABA-loaded agar blocks were immediately placed on the apical cut section, the level of DNA strongly decreased. This can be considered to be indirect proof that ABA is produced by the cap cells [78]. In another paper [79], similar confirmation can be suggested in terms of elongation regulation. It was reported that (i) slowly growing roots had long caps, whereas rapidly growing roots had short caps, and (ii) the production of growth inhibitors (such as ABA) – tested by the bending of half-decapped roots – is significantly higher for roots having longer caps than for those with shorter ones.

A classic article reported that illumination of roots enhanced their gravireaction [80]. This was confirmed

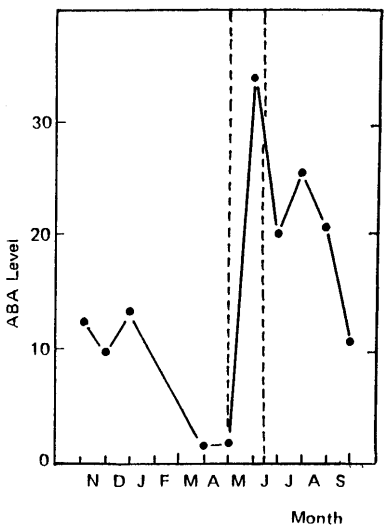

Figure 5. Seasonal rhythm of the ABA level (in μg/g. of d.m.) in roots of *Acer saccharum*. d.m., dry mass. (Data from November [N] to September [S].) Adapted from ref. 69.

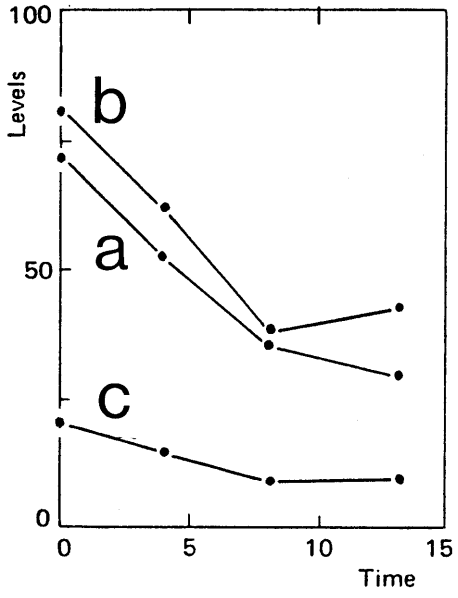

Figure 6. The effect of removal of 3-mm tips on the subsequent levels [in μg per g. of f.m. measured after a given time (h) after decapitation] of ABA (a), *trans,trans*-xanthoxin (b) and *cis,trans*-xanthoxin (c) in 3-cm subapical segments of *P. sativum* roots. f.m., fresh mass. Adapted from refs 75, 76.

for certain maize cultivars [81, 82]. If ABA is the 'gravitropic hormone' – as demonstrated in microsurgical experiments (see above) and, in particular, in which tips of light-sensitive roots have been exchanged with tips from light-insensitive roots [83–86] – one would expect to observe a rise in ABA level in illuminated light-sensitive roots. In fact, after thin layer chromatography (TLC) purification and a root growth assay, little difference in the ABA content between light- and dark-grown maize (cv. LG11) roots was reported [87]. However, using very sensitive GC-MS estimations with deuterated ABA as an internal standard, significant increases in the ABA content in light-grown roots of the same cultivar were found. Thus, for vertical roots the level in darkness was found to be 0.54 ± 0.03 μg ABA/g. of d.m. and in light 0.73 ± 0.09, and for horizontal roots, respectively, 0.57 ± 0.01 and 0.69 ± 0.03 [88]. More recently, again for maize roots (cv. LG11), the increase of the ABA level [89] after light treatment, both in the apical part and the elongating zone, has been confirmed (table 1). In the case of aerial and illuminated epiphytic orchids (*Azanda*, *Vanda*), ABA content was analysed using a monoclonal antibody-based immunoassay [72, 73]. In *Azanda* ABA levels vary with root position along the stem, with time and with growth stages. Both in *Azanda* and *Vanda* it is higher in root tips than elsewhere, and in *Azanda* higher in flowering plants than in nonflowering plants [90].

Applied ABA on elongating roots

A considerable number of articles, with various aims in view, have been published in relation to the measurement of the effects of applied ABA on root extension. One review paper [5] computed a table of the values for inhibition of root elongation by ABA reported by several authors. Early papers considered only the reduction of root growth after ABA application. For *Lactuca sativa*, 38–41 μM induced an inhibition of about 30% [91]. For *Lens culinaris* an inhibition of 50% was reported for 3.8 μM and of 30% for 0.38 μM [92]. For *Lycopersicon esculentum* cultured in vitro, 0.1 μM of ABA caused growth inhibition of about 50% [93]. A large range of sensitivities between cultivars of maize has been reported. The cv. LG11 appears to be the most sensitive to ABA [94]. There seems to be no correlation between these sensitivities and ABA content in these cultivars [37]. The same material will be used for testing the effect of ABA on gravitropism (see above). Primary maize roots were pretreated, then kept vertically with a droplet of buffer solution containing ABA (at different concentrations) applied on the root tip. Apical segments were placed horizontally in both light and darkness, and downward bending was measured. Curvature was greater in the light than in the dark. ABA significantly enhanced gravireaction in both light and darkness [95]. The droplet can be replaced by a resin bead, which is much more advantageous [96]. As can be seen in table 2, beads (diameter 0.45 ± 0.05 mm) loaded with [2-^{14}C]-ABA are placed on the cap of 15 ± 3 mm maize roots for 2 h. The radioactivity from the labelled ABA is tested for the bead (before its application) and for the root. This method gives clear information about the uptake and accumulation of ABA in roots.

Some articles reported a stimulating effect on root elongation by growth-inhibiting ABA. *P. sativum* root growth was stimulated (29%) at 1 μM ABA [97]. Roots of soybean cultured in vitro on White's medium (pH 4.8) showed significant growth stimulation (18.5% for cv. Waseshizoge and 28.4% for cv. Fiskeby V) at an ABA concentration of 0.04 μM [98]. The elongation of the tip of maize roots cultured in vitro, lower in darkness than in light, was enhanced by 0.1 μM ABA [99]. Apical root segments were treated for 1 h by immersion in a buffer (pH 6.0) ABA (at different concentrations) solution, then placed for 5 h in the dark/humid air. ABA at 10^{-8} M enhanced root growth [100].

Table 1. Endogenous ABA content (in μg ± SE per 20 segments) in the apical part and the elongation zone of *Zea mays* (cv. LG11) [38].

Groups of roots tested	ABA/20 segments			
	apical part*		elongation zone†	
	μg	%	μg	%
Control	0.71 ± 0.08	100	1.18 ± 0.19	100
2 h light	0.88 ± 0.15	124	1.66 ± 0.25	141
4 h light	1.17 ± 0.24	165	2.14 ± 0.36	181

Data are means of five classes from each group. *0.0–2.5 mm. †2.5–5.0 mm both counted from the tip. Twenty micrograms of hexadeuterated (±) ABA [19] were added at the beginning of the extraction ($CHCl_3$). GC-MS analyses were carried out. The detection of ABA-Me was performed with NC1 using ammonia as reactant gas. Quantifications were calculated using the stable isotope-internal standard dilution method and the selected ion-monitoring programme of the GC-MS [89].

Table 2. Fixation of [2-^{14}C]-ABA (925 MBq mmol^{-1}) by resin beads placed 2 h on the cap of primary roots of *Zea mays* (cv. LG11) and radioactivity recovered by these roots [96].

Resin used	Labelled ABA fixed per bead (B)		Radioactivity per root (R)	Efficiency* E
	Bq	μmol	Bq	%
Amberlite IRA 400	216.7 ± 6.7	0.234 ± 0.007	1.5 ± 0.3	0.7
Dowex WGR 44	56.7 ± 3.3	0.061 ± 0.003	10.8 ± 1.3	19.0

*$E = (R/B) \cdot 10^2$.

ABA and indol-3yl-acetic acid (IAA) interactions

Very few experiments have been conducted on the interactions of ABA with other hormones (i.e. IAA) in the change of root growth [5, 7, 57, 63]. One of the first papers [92] to be published was on the elongation of 3-mm apical segments of lentil grown in buffer solution containing 1% sucrose, using ABA (at 0.38 μM) and IAA (at several concentrations). The data given in figure 7 point to several conclusions. ABA acts as an IAA 'antagonist' when IAA is applied at low concentrations (from 0.01 to 0.04 μg/10 ml) or at higher levels (from 0.09 to 2 μg/10 ml) and as an IAA 'synergist' for intermediate doses.

Fusicoccin, a fungal toxin which mimics most of the IAA effects on cell growth [101] and stimulates proton extrusion in elongating maize roots [102] may control similar mechanisms in which IAA is implicated [103, 104]. Some experiments have been done based on the use of 10-mm apical segments of maize roots and the study of the effects of removing the cap. The application of ABA (5 μM) in agar block to the capless tip and fusicoccin (10 μM) in filter paper to the basal cut surface indicates that ABA brings the growth rate back to that of the intact segments. Clearly ABA eliminates the enhancement of the effect of cap removal by fusicoccin [105].

The effect of IAA on ABA movement was analysed in 10-mm apical segments of primary maize (cv. Kelvedon 33) with their tip (0.6 mm) removed. They were placed horizontally, and bending was measured after 7 h in the light [106]. ABA (1 μM), in agar block, asymmetrically placed on the apical cut section, caused downward curvatures when the block was on the lower half of the cut surface. Replacement of ABA by IAA (1 μM) in the block caused no response, but IAA applied to the base of the segment increased the response to the ABA. This can be considered as indirect proof that ABA is formed in the cap cells and moves basipetally [6].

It has been shown that the auxin content – analysed by GC-MS [68] – of the root segments of maize (cv. LG11) was lower in comparison to roots maintained in humid air. This decrease (exodiffusion) in the IAA level was significantly enhanced in ABA treated roots [107].

The effects of ABA and IAA applied basically (buffered filter paper placed on the basal cut end) and apically (buffered droplet on the tip) to root segments of two maize varieties (Orla 264 and Anjou 210) have been studied [108]. Briefly, it can be said that ABA has no significant effect when applied at the base of the segment, whereas it inhibits elongation in apical treatment. IAA induced growth inhibition that is stronger when it is applied on the basal than on the apical end of the root segments. However, IAA at 10^{-8} M induced growth stimulation when applied on the basal cut. These data could be explained in terms of comparative transport of both ABA and IAA, that of ABA being only basipetal and that of IAA being acropetal but preferentially basipetal.

In a final paper concerning this topic, the growth effects of both applied and endogenous ABA and IAA were compared [109]. At first, treatments were carried out

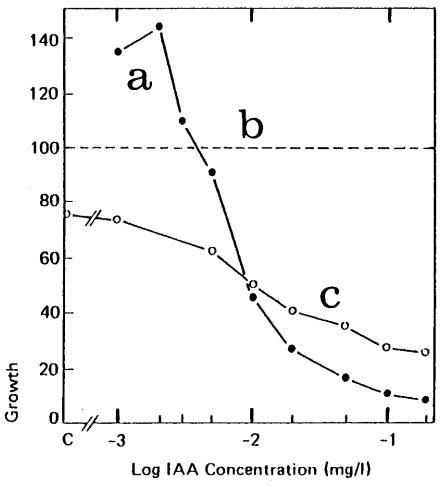

Figure 7. Effect of IAA (at several concentrations) with or without ABA (at 0.38 μM) on the elongation (growth in % of the control) of 3-mm apical segments of *Lens culinaris* roots incubated 16 h in buffered (pH 4.8) solution containing 1% sucrose (darkness). a, IAA (as a function of concentration); b, control (no IAA, no ABA); c, IAA + ABA (0.1 mg/l). Adapted from ref. 92.

(1-h immersion in buffered solution) on intact 10-mm maize roots attached to their caryopsis and maintained (4 h) vertically in humid air. It has been reported that the slow-growing roots were all inhibited by applied hormones at any concentrations used, whereas elongation of fast-growing roots was promoted by the two hormones at low concentrations. This was confirmed by using 12-mm roots treated 30 min in buffered solution containing ABA or IAA (10^{-8}–10^{-9} M) and after washing kept 6 h (darkness) [109]. As can be seen (fig. 8A), the effect of IAA on elongation is stronger than that of ABA. But both hormones, at 10^{-8} M, induced growth stimulation. The ABA effect was previously reported for apical segments of the same variety of maize treated with ABA (10^{-8} M) and kept 6 h in humid air [107]. In another set of observations [109], the root population was divided into growth rate classes in order to qualify the endogenous ABA and IAA, quantified by GC-MS, in the elongating zone (2.5–5.0 mm from the tip). As reported in figure 8B, a clear relation between the level of these hormones and the elongation rate can be discerned. Both for ABA and IAA, the higher the level, the lower the growth rate. In the elongation zone of fast-growing roots, the level was almost identical for the two hormones, but in the slow-growing roots more IAA was found in the extending zone, and the ABA level was dramatically increased compared with that of fast-elongating roots.

In relation to IAA, a few points about the effects on ABA of some auxin herbicides deserve mention. Synthetic compounds having auxin activity have been among the most successful herbicides [110]. It has been shown that the induction of ABA accumulation is a common effect of these types of herbicides, and it appears to be implicated in their growth inhibition of susceptible plants [111]. Other herbicides, for instance trifluralin, have also been well studied. Trifluralin is a selective preemergent herbicide that alters plant growth essentially by targeting roots. It inhibits elongation and stops mitosis in premetaphase [112]. Maize roots are sensitive to micromolar doses [113]. One of the primary modes of action of trifluralin is prevention of the formation of microtubules [114], and it is well established [115] that ABA may modify the synthesis and arrangement of microtubules. It is generally accepted that dinitroanilines act in a way similar to antimicrotubular drugs [116]. Recently, it was reported that ABA levels (GC-MS analyses) rapidly increase after trifluralin application, whereas IAA content changes slightly a few hours later. This action is more marked in fast-growing roots than in slow-growing roots [117]. Maize roots are sensitive to trifluralin (at 5 μM), since their growth is slowed down and their diameter is strongly increased, whereas pea roots are not affected at this concentration.

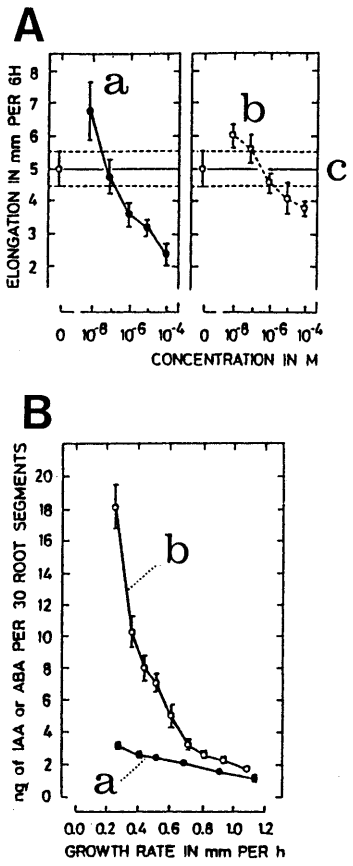

Figure 8. Elongation (*A*) and ABA and IAA content (*B*) of primary roots of maize (cv. LG11). (*A*) Roots pretreated 30 min in buffered (pH 6.0) solution containing IAA (a) or ABA (b) at different concentrations or not containing these hormones (c). Roots of 12 mm were partly (the distal 10 mm) immersed in vertical position. Data are the mean of five experiments, each with 40 ± 5 roots. (*B*) Changes in IAA or ABA level as a function of the elongation rate during an 8-h period. The levels are given for the elongating zone (2.5–5.0 mm). Adapted from ref. 109.

^{14}C-Trifluralin uptake was higher in maize than in pea roots, but only in the elongating zone. The ABA level is about 10 times higher in control pea than in maize roots. Moreover, applied trifluralin induced an increase in ABA content in maize roots [118]. We note that detached spikes from *Triticum aestivum* cultivars that produce caryopses with high (Brevor) or low dormancy (Greer) levels were cultured from anthesis in basal media and amended with fluridone or fluridone and ABA. Effects of these two compounds on kernel development and dormancy were assessed by measuring fresh and dry mass of embryos and caryopses, and ABA content (by enzyme-linked immunoassays) in embryos and caryopses which accumulated very little ABA. Dry matter and ABA decreased in caryopses, whereas embryos

continued to accumulate both. Fluridone and ABA induced higher dormancy levels in Brevor than in Greer [119].

ABA effect on the root cell cycle

Cell division and extension play an essential role in root growth. Progression through the cell cycle is regulated at the G1/S and G2/M boundaries [120] by protein kinases [121] associated with regulatory proteins (cyclins) [122]. A recent article linking molecular and physiological mechanisms controlling plant cell division has shown cell-cycle stage – specific and hormone – inducible cyclin expression [123]. Using roots of maize (cv. LG11), cap removal accelerates the entry of nuclei into the DNA synthetic phase of the mitotic cycle and enhances the rate of cell proliferation in the quiescent centre. ABA diminished these effects, but did not suppress them. Thus, ABA cannot wholly substitute for the presence of a cap. One of the primary effects of applied ABA is to retard cell enlargement, which may in turn affect the rate of cell division; endogenous ABA may act similarly [124]. Root immersion in ABA solution increased the proportion of nuclei in S phase (replication) to the detriment of these in the high polyploidy classes (4C, 8C). ABA reduced or rather suppressed the S-phase nuclei.

In an attempt to detect the control points in the cell cycle of the root apical meristem of *Zea mays* (cv. LG11), quiescent centre cells were stimulated to synthesize DNA and to enter mitosis [125] either by decapping or by immersing intact roots in a buffered solution. Microdensitometric and flow-cytometric data show that, upon immersion, the G2 phase of intact roots was shortened. However, when 50 μM ABA was added to the immersion buffer, parameters of the cell cycle were restored to those characteristic of intact roots held in moist air. On the other hand, decapping of roots shortened the G1 phase in the quiescent centre; ABA reversed this effect [126].

ABA and droughted cells of growing roots

During the last few years evidence has accumulated that desiccation stress alters the level of some phytohormones. ABA has the most studied in this regard, and findings have been reported in many papers [15, 30, 127] that water deficit induces an increase of ABA levels in tissues. Classical experiments have been carried out in the leaves [128], as well as the stomatal closure, thus preserving water. As can be seen in figure 9, a reduction of leaf turgor induces (for the wilted cell) a rapid and strong increase in ABA levels. Upon rehydration (stress relief), ABA content returns to the prestressed level within 5 h. Similar results have been reported for the whole leaves of *Vicia faba* [129].

Many papers on roots have been published [6, 50, 130], specifically concerning water deficit conditions, osmotic potential, and elongation of root cells and their ABA levels. Desiccation may influence growth via effects on several parameters, such as hydraulic conductivity of tissues [131], osmotic characteristics of the cell [130, 132, 133] and rheological properties of the cell wall [134]. Although it is well established that ABA is synthesized in roots [37, 88, 109], it was not until comparatively recently that an essential role was postulated for root-sourced ABA in the control (as a signal) of desiccated plants [30, 130, 135]. It has been suggested that ABA produced in large quantities by dehydrated roots and subsequently transported to the shoots provides a sensitive indication of the degree of soil drying [136]. The findings of several experiments will serve to clarify [130]. Under water stress conditions induced by mannitol solutions (0–0.66 M) applied to the apical 12 mm of intact roots of maize (cv. LG11), growth inhibition, a decrease in the osmotic potential of cell sap and a significant accumulation of ABA (quantified by GC-MS [68]) were observed. When the roots were placed in a humid atmosphere after desiccation, the growth rate increased again, even if elongation had been totally inhibited. The effect of water stress on the ABA level was studied for three parts of the root. The greatest increase in ABA (about 10-fold) was obtained in the

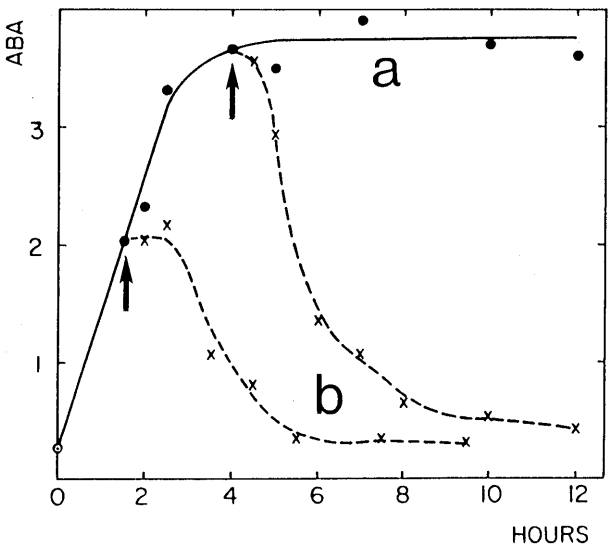

Figure 9. Accumulation of ABA (10^3 ng per g. of f.m.) in wilted leaves (a) of *Xanthium strumarium* and decrease in ABA level following relief of stress by submerging the water-stressed leaves (b) in distilled water for 5 min. Adapted from ref. 128.

elongating zone. But ABA is not the only plant hormone to react under the cell water deficit. Results related to IAA as a 'stress hormone' are scarce and often conflicting [137]. In flowers, desiccation slightly increases the level of conjugated IAA but has no effect on the level of free IAA [138]. To our knowledge, IAA levels in water-stressed roots (treated by mannitol solutions: 0–0.66 M) has been reported only recently [137]. With increasing stress, a decrease in growth, correlated with an increased IAA level (quantified by GC-MS), was obtained. The largest increase in IAA (about 2.7-fold) was found in the apical 5 mm of the maize root (corresponding to an osmotic potential of −1.39 MPa). Contradictory reports appear in the literature regarding the effect of applied ABA on water status. The use of mutants (see below) helped to solve a few questions. For instance, the ABA effect was approached by comparing two tomato genotypes (*Ailsa Cornig*: wild-type, Wt, and the ABA-deficient mutant: *notabilis*, N) [133]. Differences in growth and water relations might be expected due to lower ABA levels in N compared with Wt [139].

Another plant hormone, jasmonic acid, also reacts in water-stressed root cells. This compound and its methyl ester are now considered to be putative hormones for several reasons: their wide occurrence in plant cells, their activities in multiple aspects at low levels and their interactions with other plant hormones [140–142]. A comparative study of the effect of drought-stressed maize roots has been done on the ABA, IAA and jasmonate (JA) content [72–74]. From the data reported in figure 10, it can be concluded that water stress induced an enhancement in the levels of all the hormones tested. The largest increase was found for ABA at 5 h, for IAA at 3 h and for JA at 1 h and at 13 h. IAA content was rapidly decreased, and after 5 h was found to be lower than that of the control.

Cells subjected to water deficit thus rapidly accumulate ABA. Regulation of gene expression by ABA during drought stress may also come into play [31, 143]. A few aspects of this problem will be briefly discussed. At the outset, alterations in gene expression in response to water stress must occur. The most important response to desiccation, with respect to gene expression, is the formation of new polypeptides and mRNAs [144]. Four aspects of polypeptide synthesis can be retained in relation to water deficit: unaltered, decreased, increased and transiently enhanced [145]. Because its level is elevated during imposed water deficit, ABA is a good candidate for regulating change in gene expression [146]. Single-gene mutants with decreased ABA levels have been used to show the role of ABA in controlling gene expression [147]. It has been demonstrated, for instance, that the induction of a specific set of genes is the consequence of enhanced ABA levels during imposed water stress [148].

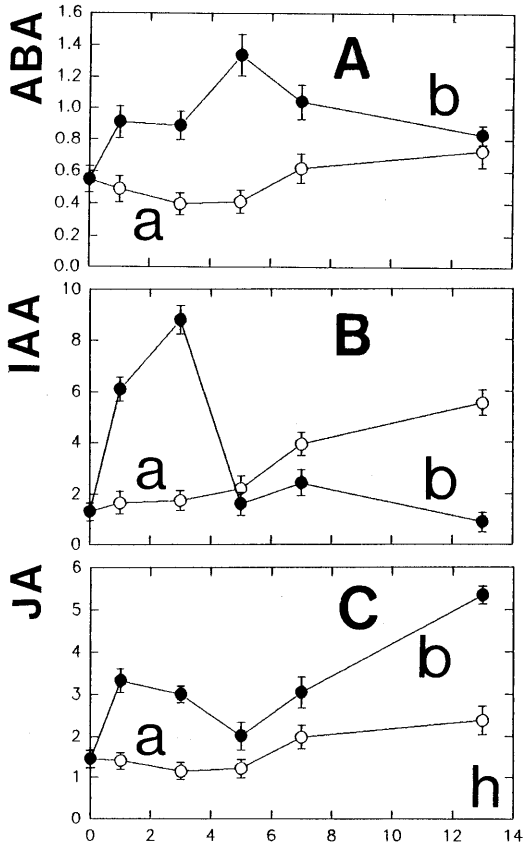

Figure 10. Changes in endogenous hormones (nmol per g. of d.m.) of maize (cv. *Zhengsan* 3) roots with time (h). Desiccation stress was obtained by immersing roots (3 h) in a solution of polyethylene glycol (−1.03 MPa). Hormone levels [(*A*) ABA, (*B*) IAA and (*C*) jasmonate], quantified by immunoassays [72, 73], were determined a few hours after the water stress treatment: a, control; b, drought-stressed roots. Adapted from ref. 74.

It is also essential to understand the characteristics of the expression of these genes in order to describe the action of ABA during water deficit. In detached tomato leaves subjected to desiccation, ABA-regulated mRNAs accumulate rapidly [147]. The pattern of accumulation parallels the increase in ABA levels. For instance, transcripts of pLE4 could be formed in these leaves within 1 h of water deficit when ABA content had increased 2-fold. After 16 h it had increased 5.5-fold, and the transcript content had increased 27-fold. But in addition to the genes which require ABA for expression, the transcript content of several other ABA-regulated genes induced by desiccation are also correlated with changes in ABA content [31]. It has been found that several genes expressed during water deficit in vegetative tissues are also expressed during desiccation of

seeds. During development their ABA levels increase with the increase in fresh mass; they decrease as the seeds begin to desiccate [148]. A set of genes was first identified in cotton [149]. Some of these are homologous to those of vegetative tissues. For instance, the cotton gene D-113 is similar to gene pLE25 expressed in drought-induced tomato tissues [150].

ABA-associated signal transduction and ion channels

The specialized physiology and structure of stomatal guard cells make them an attractive choice for studying several bioprocesses (e.g. water exchanges, some ABA effects) [151]. ABA is imported into leaves under water deficit conditions (see above). It induces loss of K^+ salt from the guard cells, with a decrease in turgor and finally the closure of stomatal pore. The signal transduction mechanisms by which ABA causes changes in some ion fluxes are well established [152]. Guard cells have enabled significant electrobiological investigations of ion channels [153]. It seems clear now that the events leading from the ABA stimulus to stomatal closure conjoin the activities of all the major ion currents at the plasmalemma to effect K^+, Ca^{2+} and Cl^- loss from the guard cells [154, 155]. The tonoplast of the guard cells includes two different cation channels carrying K^+ [156] and an anion channel with high selectivity for Cl^- over K^+ and dependent on protein phosphorylation for activity [157]. These and other ion channels are likely to prove crucial targets under ABA control, but their contributions still remain to be demonstrated [155]. It is clear that the effect of ABA and IAA – as regulators of stomatal aperture – on ion channels is mediated by second messengers (e.g. pH, Ca^{2+}). The two hormones induce the increase of the Ca^{2+} content. Phosphorylation is another essential factor in cellular signalling. The *abi1* gene (see below), which encodes a 2c-type protein phosphatase, has been shown to be a key parameter of ABA-dependent plasmalemma voltage and is also an important component of signalling and ion channel control [158]. Experimental values support a role for the *abi1* protein phosphatase and protein kinase elements in gating K^+ channel sensitivity to pH and ABA. Variations in the Ca^{2+} level are sensitive to pH. Each of these signals modulates and is influenced by the activity of several ion channels [155].

Mutants at *aba* and *abi* loci

The study of these mutants has provided new information about ABA action [31, 143]. *aba* mutants strongly show reduced seed dormancy and lack the normal light requirement for germination, but they all respond to ABA treatment [159]. The *aba1* mutant showed no cold acclimation [160], or a reduced one [161] when compared to wild-type. ABA applied (20 °C) on *aba1* plants resulted in cold acclimation [160]. It can be concluded from these observations that a low level of ABA in the mutants results in an impaired ability to develop cold tolerance.

Mutants at the three *abi* loci also show a strong reduction in seed dormancy and may wilt more rapidly under water stress than normal plants. They are potentially altered in a 'component' that recognizes ABA, such as a receptor [159]. Several bioreactions associated with ABA (e.g. dormancy, drought, prolin accumulation) are affected in the *abi* mutants [161, 162]. Since mutants at each of the *abi* loci may retain some responses to ABA, they are not truly insensitive. The *abi3* mutant, for instance, affects a protein [163] that has significant similarity to the product of the *vp1* locusin maize, which acts as an ABA-regulated transcriptional activator during seed germination [164]. Mutants *abi1*, *abi2* and *abi3* show cold acclimation similar to the wild-type [161]. The ABA responses may not be necessary for cold tolerance, and the apparent insensitivity of these mutants to some ABA effects does not extend to other possible effects of ABA [159]. It must be noted, on the other hand, that the mRNA level significantly increased after cold treatment (4 °C) of wild-type *aba* and *abi* mutants. ABA applied at 20 °C enhanced mRNA content except in the *abi1* mutant [159, 162].

Concluding remarks

In this review article, we have focused on only a few problems related to ABA properties. Certain comments can be drawn from this report with regard to possible advances in some fields of ABA research. The level of ABA in cells is related to its anabolism, catabolism, import and export. ABA action on root growth depends on both the ABA content and the sensitivity of tissues to ABA, which is related – as for other plant hormones – to the number of available receptors and their affinity to bind ABA. In this context several questions remain unanswered: the chemical nature of receptors and their relative specificity and the location in the cell of both ABA and its receptors... Following ABA fixation on receptors, several events (signal transduction) appear to change gene expression, leading to new physiological states. The response to cell dehydration – induced ABA accumulation – is compatible with a 'turgor-sensing mechanism'. When using ABA-deficient mutants, some data have indicated that the increase in ABA level is a prerequisite for induction of drought-stressed genes. The changes in gene expression that occur during water deficit in cells indicate a possible action of some ABA-regulated gene products. Such reactions could exist also

in other processs in which ABA is implicated. This is an alternative perspective of research concerning the molecular characteristics of this plant hormone. "Understanding the action of ABA becomes, in some ways, as difficult and perhaps as revealing as understanding the control of plant development itself" [47, p. 184].

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