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# Abscisic Acid Mediates Drought-Induced Developmental Change in the Roots of *Helianthus annuus* L.

by

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## ABSTRACT

Drought elicits a response at the root apex. This response is characterized by cell elongation and vacuolation nearer the apex and a concomitant decrease in the rate of cell division, which result in a decrease in the size of the meristematic zone. After 72 hours, the meristematic zone begins to recover in size. These changes at the apex are reflected by changes in the rate of root elongation, which is briefly promoted followed by a period of virtually complete inhibition. After 72 hours, there is some recovery in root elongation.

Cell division in the root meristem declines within 6 hours of the initiation of drought; the cell cycle appears to be arrested in $G_1$. At about 72 hours after the imposition of drought, the cells of the meristem begin to cycle again, although the meristematic region is much reduced in size. The recovery in cell division is accompanied by activation of the quiescent centre.

There is evidence that these changes are a direct response by the meristem to a decrease in external water potential, and are not the consequence of loss of turgor or a lack of photosynthate. Exogenously applied abscisic acid (ABA) elicits the same pattern of response. It is suggested that ABA mediates these changes at the meristem. The endogenous levels of ABA increase in the root apices of droughted plants. This increase does depend on transport from the shoot. Excised root apices (apical 3 mm) produce large amounts of ABA when dessicated.

Drought stress and ABA treatment initially promote photosynthate transport to the root. After about 12 hours of treatment, they inhibit transport .

Drought stress and ABA treatment promote a transitory decrease in osmotic potential, which results in a burst of increased turgor during the early stages of treatment. This burst of turgor is contemporary with the period of promoted root elongation.

The writer suggests that drought-induced ABA in the root apex blocks DNA synthesis and promotes the differentiation of cells in the proximal regions of the meristem. This results in an apical meristem which is reduced in size and activity. The quiescent centre then activates and the meristem resumes its activities, but at a rate which is consistent with the limitations imposed by drought.

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DEDICATION

To Mr. P.R. Baskerville, with gratitude and affection

and to

C.A. Robertson, *In Memoriam*

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Although the timing of events differs slightly, the general depression of DNA synthesis and advanced vacuolation in the root apex, followed by recovery in DNA synthesis in the cells

## LIST OF ABBREVIATIONS

ABA.....abscisic acid

ABA-Me.....abscisic acid methyl ester

$a_w$.....thermodynamic activity of water

DNA.....deoxyribonucleic acid

dw.....dry weight

d6ABA.....hexadueterated cis, trans abscisic acid

GC.....gas chromatography

GC-MS.....combined gas chromatography-mass spectrometry

GC-MS-SIM.....gas chromatography-mass spectrometry-selected ion monitoring

HPLC.....high performance liquid chromatography

P.....probability

PAS.....periodic acid Schiff's reaction

$\Psi$.....water potential

$\Psi_p$.....pressure or turgor potential

$\Psi_\pi$.....osmotic potential

QC.....quiescent centre

Rt.....retention time

R : S.....root to shoot biomass ratio

TBO.....toluidine blue O

$u_w$.....chemical potential of water

# CHAPTER I: GENERAL INTRODUCTION

The effect of environmental stress upon a plant is the result of two interacting processes: the stress itself, and the response of the plant to that stress. A plant, if it is to survive, must possess the means with which to respond to its environment. Response implies agency on behalf of the plant; it is the expression of inherent genetic characters of the plant under the influence of particular environmental conditions. In this way, response is distinct from passive changes (requiring no agency) that result directly from environmental conditions. A response then may be defined as a change which is not the immediate consequence of external factors. Those responses which confer fitness to the plant in adverse conditions may be termed adaptive, and those which allow a plant to grow or at least survive when water is in limited supply are adaptations to drought. Some adaptations are permanently expressed, whereas adaptive responses are expressed only under certain stimuli.

Sussex (1974) observes that a plant, because of its sessile habit, cannot avoid unfavourable environments, but that it can alter its developmental pattern by growth changes in the meristems. He concludes, "thus the meristems are the plant's means of responding to its external environment, and the response made by plants is a developmental response" (Sussex, 1974). If Sussex's argument holds, then the adaptive responses of plant roots should reveal themselves through physiological or structural events in the root meristems.

In this study, the writer reports on investigations into the effects of drought on root development in sunflower, Helianthus annuus L.. These investigations concentrate on changes at the root apical meristem. The arguments will focus on the thesis: abscisic acid mediates drought-induced developmental change in the roots of Helianthus annuus L.. What follows in the remainder of this section is background information to these inquiries and a justification for their presentation.

**Drought**

Drought is a vague term, meaning lack of rainfall or a prolonged period of dry weather, Swindale and Bidinger (1981) list several alternate definitions; but its usage has an advantage over that of water stress which may include osmotic (salt) stress, and in some cases (Levitt, 1980), flooding. The writer, in this study, uses the term drought to describe a water deficit, and the term water stress when discussing the work of others who have used osmotica to lower water potential. Stress is used to describe any factor that disturbs the normal functioning of the plant (Kramer, 1980). Levitt (1980) has attempted more rigorous definitions, but they are in many ways too restrictive for this treatise.

However restricted the definition, drought is not a simple stress. The uptake of inorganic ions is a water-mediated process, and when the supply of water is limited, so too are supplies of nutrients (Pitman, 1981). Stomatal closure in response to severe or prolonged drought is common to most terrestrial plants; this leads to reduced photosynthesis and deficiencies in fixed carbon. These secondary stresses contribute to the overall stress facing the plant. However, the immediate stress of water deficit is in itself formidable.

Plants require water for several processes. In a plant cell, macromolecules must be hydrated to function; biochemical reactions require water either as a direct participant or as a solvent (Borowitzka, 1981). The maintenance of turgor and volume, and hence, of cell expansion depends on water (Borowitzka, 1981, Westgate and Boyer, 1985).

Differences in chemical potential provide the driving force for water flow in plant cells and tissues (Dainty, 1976). The chemical potential of water, $u_w$, is a measure of the molar Gibbs free energy relative to that of pure free water (Dainty, 1976). Chemical potential is defined by the following equation:

$$u_w = u_w^* + RT\ln(a_w) + V_w P \qquad \text{(eqn. 1)}$$

where $u_w^*$ is the chemical potential of pure free water at one atmosphere pressure and at the same temperature as the system under consideration, R is the gas constant, T is the temperature in K, $a_w$ is the thermodynamic activity of the water in the system, $V_w$ is the partial molar volume and P is pressure (Borowitzka, 1981).

A comparison of the chemical potentials for two adjoining systems provides an indication of the direction of water flow. For convenience, chemical potential is often expressed in units of pressure, as water potential ($\Psi$).

$$\Psi = \frac{u_w - u_w^*}{V}$$

or by rearrangement of equation 1:

$$\Psi = \frac{u_w - u_w^*}{V_w} = \frac{RT\ln(a_w)}{V_w} + P \qquad \text{(eqn. 2)}$$

The term, $RT\ln(a_w) / V_w$, represents osmotic potential, $\Psi_\pi$, (Borowitzka, 1981). Equation 2 is usually written as:

$$\Psi = \Psi_\pi + \Psi_p \qquad \text{(eqn. 3)}$$

where $\Psi_p$ is pressure potential or turgor. Another component, matric potential, a surface tension factor, is often included in this equation. Matric potential is important in the soil, but is insignificant for intracellular plant systems (Hsiao, 1976, Dainty, 1976).

Solutes affect $\Psi$ by depressing the thermodynamic activity of water, $a_w$, defined as:

$$a_w = y_w \cdot n_w \qquad \text{(eqn. 4)}$$

where $y_w$ is the activity coefficient and $n_w$ is the mole fraction of water (Borowitzka, 1981). Solutes depress $a_w$ according to the following equation:

$$\ln(a_w) = \frac{(-18.016)\, n \cdot m \cdot \Phi}{1{,}000} \qquad \text{(eqn. 5)}$$

where n is the number of solute particles produced by solvation, m is the molality of the solute, and Ø is the molal osmotic coefficient (Borowitzka, 1981). It follows then, from equation 2, that the value of $\Psi$ will fall as $a_w$ falls.

The cell membrane is differentially permeable; it allows for the free flow of water but not solutes (Borowitzka, 1981). Water deficits lower the thermodynamic activity and, thereby, the water potential of the water in the environment . This could lead to water flow from the plant to the environment and subsequently to the dehydration of plant cells.

Plant cells prevent this by regulating internal osmotic potential, $\Psi_\pi$, to maintain a favourable balance in water potential with their external environment. This process is called osmoregulation or osmotic adjustment. In a growing plant, the cells thereof are continuously regulating their $\Psi_\pi$ to allow for growth even when external water potentials remain steady. Cell enlargement proceeds when a demand for water is created by extension of the cell walls under the action of turgor, $\Psi_p$, and water is supplied by gradients in water potential (Boyer, 1968, Westgate and Boyer, 1985). If elongation is to continue, solutes must be added to the cell sap as water enters, in order to maintain the osmotic forces necessary to drive elongation and to supply the metabolites for wall synthesis (Westgate and Boyer, 1985). The terms osmotic adjustment and osmoregulation are often used interchangeably. Osmotic adjustment will be used herein to distinguish the situation in which the cells of a plant osmoregulate in response to a drop in external $\Psi$. Turner and Jones (1980) define osmotic adjustment as the lowering of osmotic potential arising from the net accumulation of solutes in response to water deficit; they further qualify their definition by adding that osmotic adjustment refers only to the active accumulation and not the passive concentration of solutes.

**Root Development**

Primary development in angiosperms proceeds from the apices of the plant axes. Groups of actively dividing cells at the apices function by continuously repeating the developmental pattern first established in the embryo, a progression often referred to as permanent embryogeny (Steeves and Sussex, 1972). In longitudinal section, the root apical meristem appears as a group of densely cytoplasmic cells near the apex, but subterminal to a protective tissue called the root cap. Growth at the root apical meristem is bidirectional; that is, the apical meristem displaces cells both acropetally and basipetally to the root cap and the root body, respectively. Basipetally displaced cells are seemingly fated by position. Three distinct regions, the protoderm, ground meristem, and procambium, together called the primary meristems, produce cells of the epidermis, cortex, and stele.

The term apical meristem is not used consistently in the literature. It is used to describe both the region and the continuously unfolding developmental process that occurs there. In the latter sense, the term is not substantive, but conceptual. Any attempt to define the apical meristem must consider the dynamics of root growth.

Cell division at the apical meristem proceeds in such a way that certain cells remain with the meristem, while others pass through the meristem to become the mature tissues of the root. The cells remaining with the meristem were termed initials, and the cells passing through termed derivatives (Esau, 1977). The derivatives, however, remain meristematic (actively dividing) for some time and are at first indistinguishable in morphology from the initials. This hampers attempts to strictly define the apical meristem. A general definition would include the initials and their meristematic derivatives. This definition does not allow for detailed analysis of the apical meristem. A distinction between the apical meristem and the primary meristems is often used (Esau, 1977), but is imprecise. The pattern of cell division in the apical meristem is such that files of cells in the primary meristems can be

traced back to a few layers of cells at and around the stelar pole. Hanstein (See Esau, 1977) called these layers histogens, and for many years they were thought to be the initials.

Clowes in a series of experiments (Clowes, 1954, 1958, 1961, see Clowes, 1967, and 1975 for reviews) demonstrated that the cells at and contiguous to the stelar pole were quiescent, i.e. not actively dividing. This region which Clowes called the quiescent centre (QC) occurs in the roots of all normally growing angiosperms (Clowes, 1975, 1984), and is always located at the pole of the stelar and cortical complexes of cells adjacent to the initials of the central part of the root cap (Clowes, 1975). This is precisely the site of the proposed initials for the primary meristems. The concept of initials had to be re-examined to account for Clowes's findings. Clowes (1975) pointed out that the cells of the QC are not inherently quiescent; he argued that the QC acts as a reservoir of cells relatively immune from perturbations which damage the cycling cells (Clowes,1975). Regeneration of the root after damage would seem to be one function of the QC. Feldman and Torrey (1976) demonstrated that the organised pattern of the root was latent within the QC, for when isolated and cultured, the QC of the Zea mays root reproduced the whole root without reversion to callus tissue. Treatments which halt cycling in the meristem induce the activation of the QC, and the root recovers its growth by the repopulation of the meristem with cells derived from the QC; this has been observed in roots recovering from X-radiation (Clowes, 1970), cold dormancy (Barlow and Rathfelder, 1985, Clowes and Stewart, 1967), and carbohydrate deprivation (Webster and Langenauer, 1973). Clowes (1975) predicted that small environmental changes would affect activity in the QC.

The discovery of the QC has led to new conceptual models of the root apex. Barlow (1976) viewed the QC as a reservoir of cells of indeterminate reproductive life span, which he called founder cells. Surrounding the QC are actively dividing cells of determinate reproductive lifespan that serve as initials. The initials divide a limited number of times producing derivatives, and then are displaced by a cell from the infrequently dividing population of the founder cells in the QC. Feldman and Torrey (1975) noted a

relationship between the size of the QC and the complexity of the vascular system in cultured Zea roots. From this work, they proposed that a meristematic zone above the QC, which they called the proximal meristem, functions as the immediate source of all new cells in the root, except those of the root cap. They argued that the proximal meristem fluctuated in size and position in conjunction with the QC, and was the site of regulation of the complexity of the vascular system. Expanding upon these ideas, they advanced a model of the root apex (Torrey and Feldman, 1977) which features a QC flanked by two dividing faces, the proximal and distal meristems. The distal meristem produces the cells of root cap and is, more or less, limited in size. The proximal meristem is not distinct: it is "composed of a relatively wide band of cells arranged in arcs, on the proximal face of the QC, averaging perhaps eight or more cell layers and varying in depth during changing activities of the root meristem" (Torrey and Feldman, 1977). They argue that the maintenance of a steady-state QC separating the distal and proximal meristems in a rapidly elongating root is the "expression of the multiple activities of the cellular components, resulting in the physiological homeostasis which produces the genetic characteristics of the root. Drastic alterations in this balanced state will effect a change in activities and in turn, in structure, until a new balance has been achieved. The new state is usually initiated when the cells of the QC begin active division" (Torrey and Feldman, 1977).

The value of Torrey's and Feldman's model is that it relates form and function to growth conditions, thus providing a firmer basis for physiological investigations into root development.

## Terminology

The terminology used hereafter is explained in Figure 1. Apical meristem or meristematic zone, unless qualified, will refer to all the densely cytoplasmic meristematic (capable of actively dividing) cells in the apex. The terms ground meristem, procambium, and protoderm are used to indicate regions fated by position as described above, but are not used to distinguish derivatives from initials or from the proximal meristem as described by Torrey and Feldman (1977).

Two basic types of meristems are recognized: closed and open. Closed meristems have discrete caps; open meristems show apparent interchange between ground meristem and cap and are characterized by vertical files of cells called a columella extending from the stelar pole into the root cap (Clowes, 1981, see Figure 1). Sunflower roots show both patterns and may shift from one form to the other (Armstrong and Heimsch, 1976). In sunflower, the QC is limited to cells at the stelar pole, although the adjacent ground meristem cells go out of cycle transiently (Clowes, 1981).

Figure 1

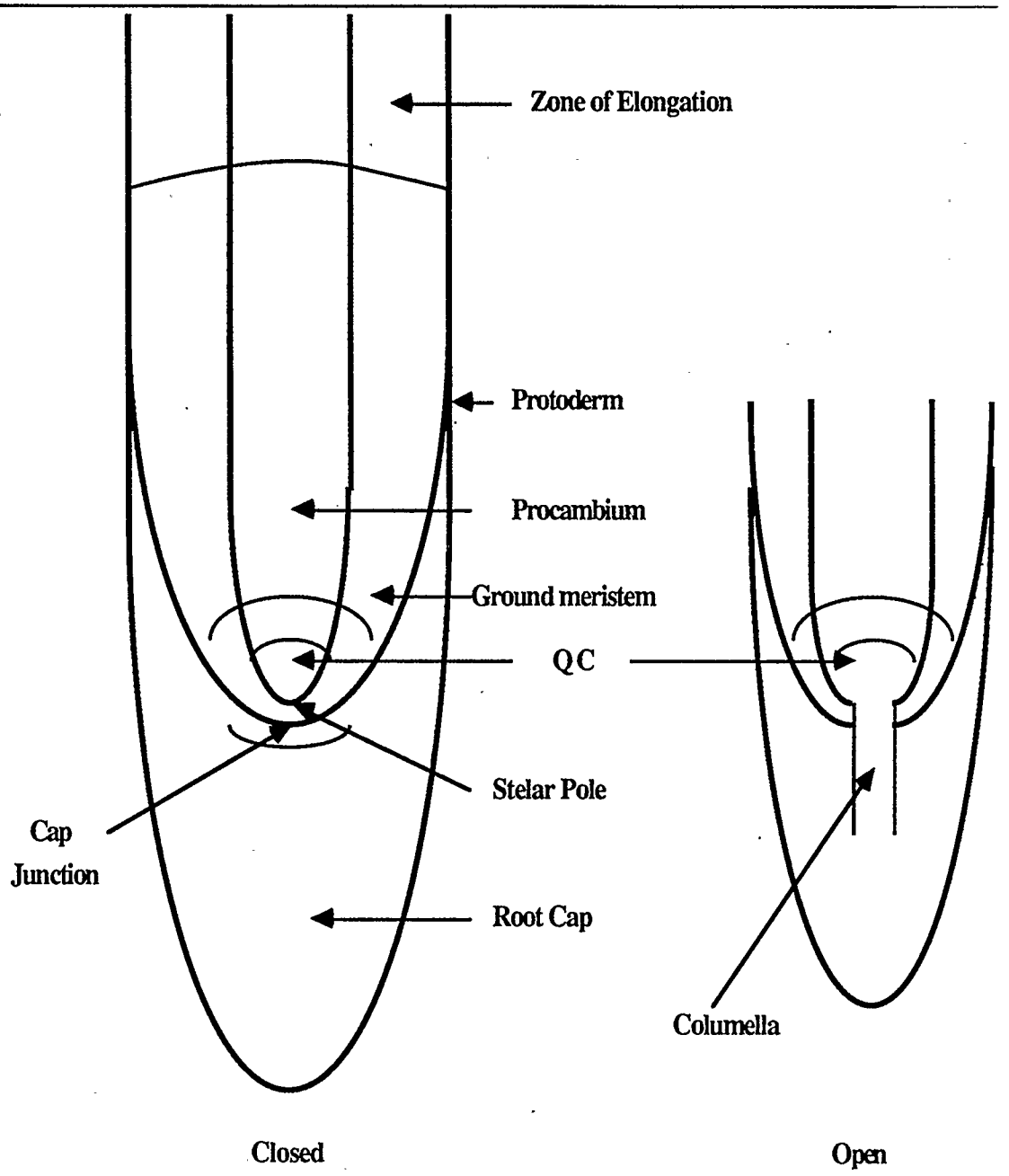


Figure 1. Diagramatic representations of the root apex of sunflower, showing both open and closed meristems. The unlabelled arched lines roughly indicate the positions of the proximal (variable in size) and distal meristems (Torrey and Feldman, 1977). QC, quiescent centre.

**The Effects of Drought on Root Growth and Development**

Inhibition of root growth at low soil $\Psi$ has been reported in several species: white clover (Stevenson and Laidlaw, 1985), blue grama (Briske and Wison, 1980), tall fescue (King and Bush, 1985), black walnut (Kuhns, et al., 1985), barley (Prasad, et al., 1982), corn (Westgate and Boyer, 1985). Undoubtedly, severe or prolonged drought would inhibit root growth. However, there are reports of absolute increases in root elongation in response to lower $\Psi$ (Hsiao and Acevedo, 1974, Jupp and Newman, 1987, Sharp and Davies, 1979, Watts, et al., 1981). It is difficult to compare these different studies, since many variables, such as the rate and magnitude of stress, would contribute to the seemingly contradictory results. Species variation is certainly a factor. Molyneux and Davies (1983) compared root growth under the same soil drying conditions in three species of pasture grasses. They observed an inhibition of root growth in Phleum pratense, but a promotion of root growth in Dactylis glomerata, a drought adapted species.

There are, however, indications that plants facing drought act to preferentially maintain root growth over shoot growth. Root to shoot biomass ratios may increase in response to drought (Bradford and Hsiao, 1982, Cutler and Rains, 1977, Hubick, et al., 1986, Meyer and Boyer, 1981, Sharp and Davies, 1979, Westgate and Boyer, 1985). Hsiao and Acevedo (1974) and Sharp and Davies (1979) attributed continued root growth at low $\Psi$ to a high capacity for solute accumulation and osmotic adjustment in roots. Westgate and Boyer (1985) disputed this hypothsis and demonstrated that roots of corn, while not osmotically adjusting any more than the leaves, were able to maintain a favorable gradient in $\Psi$ with the vascular system

**Drought, ABA, and Plant Development**

Abscisic acid was first regarded as a general growth inhibitor which affects growth in all plant parts (Milborrow, 1974), but it has since been shown to regulate many physiological and developmental processes including: seed development and seed dormancy (Dure, 1975, Robichaud and Sussex, 1986), assimilate partitioning (King and Patrick, 1982), bud dormancy (Wareing and Phillips, 1983, Benzioni and Dunstone, 1985), and leaf abscission (Addicott, 1983). It may even play a part in animal physiology (Huddart, et al., 1986).

Abscisic acid is widely recognized as an agent of stomatal closure (see Mansfield and Davies, 1981, and Rashke, 1979, review). However, plants produce ABA in great excess of what is required for stomatal closure (Milborrow, 1981). Endogenous levels of ABA rise in the roots of water-stressed (drought or osmotic stress) plants (Hubick, et al., 1986, Lachno, 1984, Milborrow and Robinson, 1973) where clearly it can have no stomatal function. Moreover, increases in the root do not depend on transport from the shoot (Rivier, et al., 1983, Walton, et al., 1976). These observations suggest that ABA may have other roles to play in the adaptation of plants to drought.

Several investigations have centred around changes in the uptake and transport of water and solutes. Glinka (1977, 1980) reported that ABA increased conductance of water and ions in excised sunflower roots, which he argued would decrease the $\Psi$ gradient and facilitate water movement into cells. Karmocker and Van Steveninck (1979a) noted that ABA stimulated ion transports in excised barley roots, but inhibited them in intact roots. Fiscus (1981) reported that ABA decreased hydraulic conductance but promoted ion flux; he suggested that, despite lowering the conductance, ABA may still increase water transport because of the increased ion flux. Karmocker and Van Steveninck (1979b) reported that ABA increased the contents of total sugar and of reducing sugar in the roots of Phaseolus. This accumulation was shoot dependent, since excised roots when treated with ABA did not show these increases. They suggested that ABA stimulated transport of sugar

from shoot to root. Watts, et al. (1981), Biddington and Dearman (1982), and Hubick (1983) have all reported that ABA increases root to shoot biomass ratios.

Watts, et al. (1981) found that the effects of exogenously applied ABA substituted for the effects of drought in many plant responses. They applied the findings of Karmocker and Van Steveninck (1979b) and Sharp and Davies (1979) and suggested that the accumulation of ABA in the roots of droughted plants could result in the preferential accumulation of solutes by the roots. They argued that changes such as increased root to shoot biomass ratios in droughted plants may be the result of ABA in the roots, and that the influence of water stress on root and shoot growth may be mediated to some extent through changes in the endogenous levels of ABA.

Several authors (Bradford and Hsiao, 1982, Davies, et al., 1980, Davies, et al., 1982, Davies and Mansfield, 1983, Jones, 1978, 1983, Jones, et al., 1987, Watts, et al., 1981) have suggested that ABA acts as a general drought stress signal which co-ordinates stomatal closure with ion, sugar and water transports; these factors then act together to maintain water balance and thus contribute to the adaptation of plants to drought stress. The dramatic effect of ABA on stomatal closure and its importance to water relations has, the writer suggests, unduly influenced these authors. Their arguments are all concerned with water relations in one way or another, i.e.the accumulation and distribution of water and osmotica. They largely ignore the body of literature which establishes ABA as a regulator of growth and development. While changes in transports would be important, it seems to the writer that the major influence of a large accumulation of a potent growth regulator like ABA would be directly at the sites of growth and development, i.e. the meristems. Changes in transports could simply reflect the changing demands of the meristems for ions and sugar. However, Aspinall substantially disagrees with the writer's view (see Aspinall, 1980).

There is evidence that ABA has a developmental role in the shoots of droughted plants (Quarrie, 1984). Quarrie and Jones (1977) found that exogenous application of

ABA induced morphological changes similar to those induced by drought in the ears of wheat. Zeng and King (1986) and Morgan and King (1984) attribute decreases in seed set in droughted wheat plants with increases in ABA in the spike. King and Evans (1977) demonstrated that the inhibition of flowering of Lolium temelentem by drought stress was due to an increase in apical ABA.

Research into gravitropic curvature has implicated ABA in the regulation of root growth directly at the root apex. Abscisic acid has been variously reported to inhibit or stimulate root growth (see discussions in Barlow and Pilet, 1984, and Pilet and Chanson, 1981). These inconsistencies probably stem from differences in treatment and the use of excised roots versus whole plants. Its effects are concentration dependent; in lower concentration ABA may promote growth (Pilet and Rebeaud, 1983). It was noted above that drought, depending on its severity, may inhibit or promote root growth. In sunflower, ABA at $10^{-5}$ M strongly inhibits both root and shoot growth (Hubick, 1983).

Abscisic acid is thought to be one of the inhibitors which is produced in the root cap and which moves basipetally into the root proper, where it effects downward bending of the root (Feldman, 1985, Wilkins, 1984). Its presence in the root cap (Rivier, et al., 1977) and its movement into the root proper following stimulation (Feldman, et al., 1985, Pilet, 1977, Wilkins and Wain, 1974) have been reported. However, there are reports that ABA is not required for gravitropic curvature (Feldman and Sun, 1986, Moore and Smith, 1984, 1985). Nevertheless, its endogenous presence in the root apex and its effects on root growth are relevant to this discussion.

Abscisic acid is intimately associated with drought responses, as a regulator of stomatal closure, of water and ion movement, of assimilate partitioning, and of changes in root to shoot biomass ratios. Studies of gravitropic curvature have established the presence of ABA in the root apex and its capacity to alter root development. In addition, ABA effects drought-like developmental changes in the shoots of some plants. The writer proposes that ABA has a direct role in mediating root development in droughted plants, and

is not limited to co-ordinating water and solute transport with stomatal closue. The writer reports on a series of experiments which define some drought-induced developmental responses in roots and explore the possible involvement of ABA: Chapter II describes the growth and apical anatomy of roots from droughted and ABA-treated sunflower plants; Chapter III reports on the the effects of drought and ABA treatment on the activity of the root apical meristem; Chapter IV reports on the endogenous levels of ABA in the root apices and explores the source of drought-induced ABA in the root apex; Chapter V reports on sugar transports to the root and specifically to the root meristem; Chapter VI reports on water relations in the root apex and further defines the response of the root to drought; Chapter VII discusses the results of the experimental work in terms of root development and its adaptations to drought.

The experiments were designed to support or reject the thesis: Abscisic acid mediates drought-induced developmental change in the roots of Helianthus annuus L.

# CHAPTER II : GROWTH AND APICAL ANATOMY

## INTRODUCTION

While drought stress is generally inhibitory to plant growth, its effects on roots and and root growth are seemingly complex and highly varied. Root to shoot biomass ratios may increase (Meyer and Boyer, 1981, Sharp and Davies, 1979), which suggests that root growth is preferentially maintained over shoot growth. Mild stress can actually lead to an absolute increase in root elongation (Hsiao & Acevedo, 1974, Sharp & Davies, 1979, Watts, et al., 1981). Morphological features, such as root depth and root density, may change in some plants when exposed to drought (Jupp and Newman, 1987, Taylor, 1980). If Sussex's arguments are correct (Sussex, 1974), drought should trigger a developmental response, and its effects should be revealed at the apical meristem of the root. The first part of this study describes the effects of drought stress and of exogenously applied ABA on the growth and apical anatomy of sunflower roots.

The method of stressing becomes an important consideration here. Different methods of applying a particular stress may activate different adaptive responses (Van Steveninck and Van Steveninck, 1983). The writer has attempted to simulate the gradual increase in drought stress that a plant would encounter in drying soil. Since drought is not a simple stress (see Chapter I), attempts were made to eliminate as many complicating factors as possible. Excess sugar was added as a supplement to one set of plants to eliminate the effects of reduced carbohydrate supply. An aeroponics system was used to eliminate the problem of increased mechanical resistance in drying soil.

## METHODS AND MATERIALS

### Plant Material

Sunflower (Helianthus annuus L. cv. Russian Giant) achenes were germinated in Terra Green (a baked clay material). Seven days after planting, seedlings were transplanted into aeroponics chambers where they were allowed to acclimate for 3 days before treatments were begun. The aeroponics chambers were constructed of Plexiglas according to the design of Hubick, et al. (1982). The plants were illuminated (ca.300 μEinsteins $m^{-2}$ $s^{-1}$ PAR at the top of the plants) by Sylvania Gro-Lux lamps (F72T12-GRO-WS-VHO), for 16 hours each day at 25° C, followed by a dark period of 8 hours at 16° C. Relative humidity of the atmosphere was 28% ± 2% (range).

The plants were maintained by thirty liters of nutrient solution (0.75 strength Hoagland solution, Hoagland & Arnon, 1938), which was misted onto the plant roots by means of a motor driven spinner. The roots of control (well-watered) plants were continuously misted for the duration of the experiment.

### Drought regime

Drought was imposed by withholding water for timed intervals. On one of the aeroponics chambers, a one hour time clock was attached to the electric motor which drove the spinner. For the first 24 hours of droughting, the plants were given a 60 second burst of watering every 30 minutes; thereafter the plants were given alternately a 60 second burst or a 30 second burst every 30 minutes. Leaf water potential, $\Psi$, as an indication of water stress was monitored by the dewpoint method with Wescor C-52 chambers attached to a Wescor HR-33T Dew Point microvoltmeter (Wescor Inc., Logan, UT). Stomatal diffusive resistance was measured with a Licor porometer.

**ABA treatment**

Abscisic acid was introduced to the root systems of ten-day-old plants through the nutrient solution. A known amount of synthetic ABA (cis-trans, ± ABA, Sigma Grade IV) was dissolved in 10 ml of absolute ethanol. This was diluted to 15 liters in nutrient solution to make up final concentrations of $10^{-5}$ M, $10^{-6}$ M, $10^{-7}$ M, $10^{-8}$ M, or $10^{-9}$ M ABA. The nutrient solution in one of the chambers was then replaced with ABA solution.

Solutions in the control and drought-treatment chambers were changed every 48 hours; in the ABA treatment chambers, solutions were changed every 24 hours.

**Growth measurements**

Root elongation was measured by rule. Root and shoot dry weights were measured after the tissue had been frozen in liquid $N_2$ and then dried <u>in vacuo</u> at - 50° C for 3 days.

**Anatomical studies**

Root tips (apical 3 mm segments) were cut and then fixed for 12 hours in a solution of 4% gluteraldehyde in 0.025 M phosphate buffer, pH 6.8. The root tips were then dehydrated in an ethanol series, embedded in JB4, a glycol methacrylate resin (Polyscience Inc.), and sectioned longitudinally at 2 μm thickness with glass knives. The sections were mounted on glass slides, stained by the periodic acid-Schiffs reaction and counterstained with toluidine blue O (Feder & O'Brien, 1968). Median sections were selected and examined. An eyepiece micrometer was used for microscopic measurements of cell size and of the size of the meristematic zone.

**Sucrose treatments**

Well-watered, droughted, and ABA-treated ($10^{-5}$ M) plants were fed sucrose exogenously through a leaf flap (Walbot, 1971). Longitudinal cuts were made parallel to and on either side of the midrib of one of the plumules. A cut transverse to the midrib at the distal end of the leaf (about 1 cm from the tip) completed the flap. All cuts were made under water. The leaf flap, about 3 cm long and 1 cm wide, was then placed in a 25 ml scintilation vial filled with a solution of 0.3 M sucrose. A strip of parafilm was stretched over the leaf flap to hold it in the vial. The plant with the vial in place was then returned to its chamber. In preliminary tests, it was found that radiolabelled sucrose, added as a trace to the sucrose solution, readily transported to the root. A second set of plants in which the leaf flaps were placed in vials filled with water served as controls for this experiment. Sucrose was continuously supplied during these experiments.

**RESULTS**

The drought regime produced a steady decline in leaf $\Psi$ over the seven day period of the experiment (see Figure 2). In drought studies, it is important that the rate of stress as well as the magnitude of the stress be considered. This drought regime was chosen to effect a gradual increase in stress and to avoid a severe initial shock to the plant. Although the stress retarded growth and some necrosis occurred (after 72 hours), the plants were able to adjust to the stress and did not wilt; living tissue remained turgid.

Figure 2.

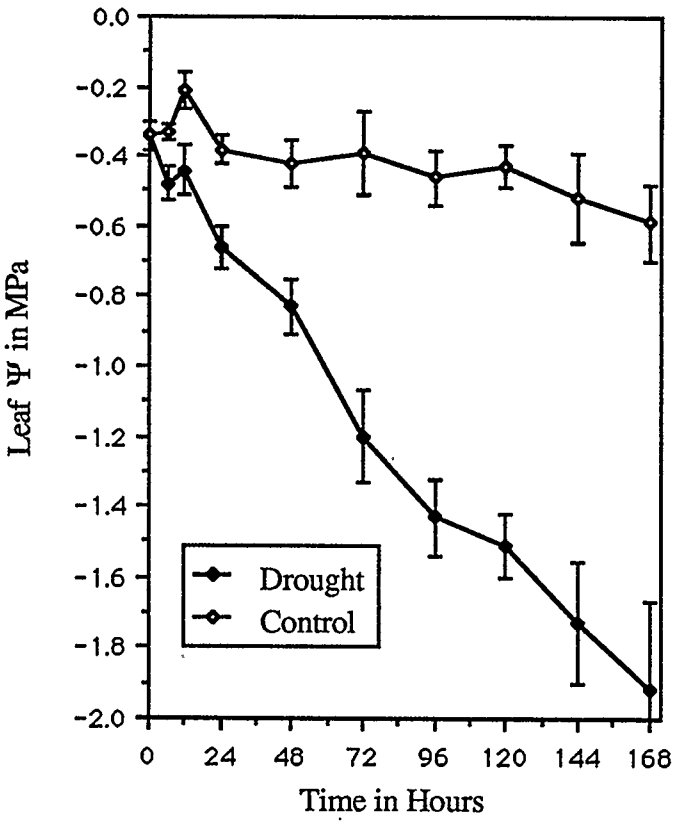


Figure 2. shows leaf water potential ( Ψ ) in Megapascals (MPa) for control (well-watered) and droughted sunflower plants over the course of seven days. The plants were ten days old at the beginning of the experiment. Error bars indicate standard deviations; n = 6 at each sample point.

Drought stress reduced growth in both shoots and roots. Figures 3 and 4 show the elongation of main roots in control (well-watered), droughted and ABA-treated ($10^{-5}$ M) plants. In the control plants, root elongation remained nearly constant at a rate of about 3.5 cm day $^{-1}$. After 6 hours of drought, root elongation virtually stopped. After 72 hours, there was a small resumption in elongation. Root necrosis had become a complicating factor by this time, however. Figure 4 includes data from necrotic roots (they were scored as having zero increase, even though the roots actually shrank in length following necrosis). The slight upward trend in the growth curve after 72 hours reflects resumed growth in surviving roots. The superimposed line in Figure 4 represents elongation in surviving roots only. Necrosis in roots is not easily identified, until well after the fact. For this reason, it is difficult to estimate the number of surviving roots. A conservative estimate would be that fewer than 25% of the roots survived 120 hours of drought (leaf $\Psi \approx -1.5$ MPa) and that fewer than 20% survived 168 hours of drought (leaf $\Psi \approx -1.9$ MPa).

By the time treatments were begun (ten-day-old plants), the root system had become fibrous. At this stage, elongated primary laterals had much the same morphology and growth rate as the main root: they elongated at the same rate, and they had the same apical dimensions (e.g. width, number of files of cells). They also responded to drought in the same way. If these long roots (main root and elongated primary laterals) are treated as a distinct subpopulation within the larger population of the whole root system, an interesting pattern emerges. In the root system of droughted plants, most of the long roots died between 72 and 120 hours. In each plant, however, a few long roots survived. These seem to have adjusted to the stress and begun to grow again, albeit at a much reduced rate relative to controls.

Figure 3.

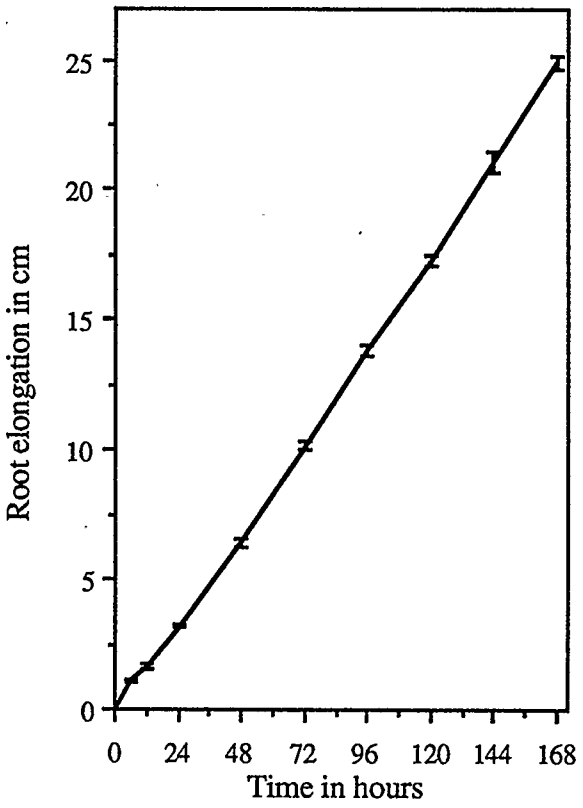


Figure 3. The rate of elongation in cm of main roots for control (well-watered) sunflower plants over the course of seven days. At the beginning of the experiment (time 0), the plants were ten days old; the average root length at time 0 was 17.4 cm. Error bars indicate standard errors; n = 27.

Figure 4.

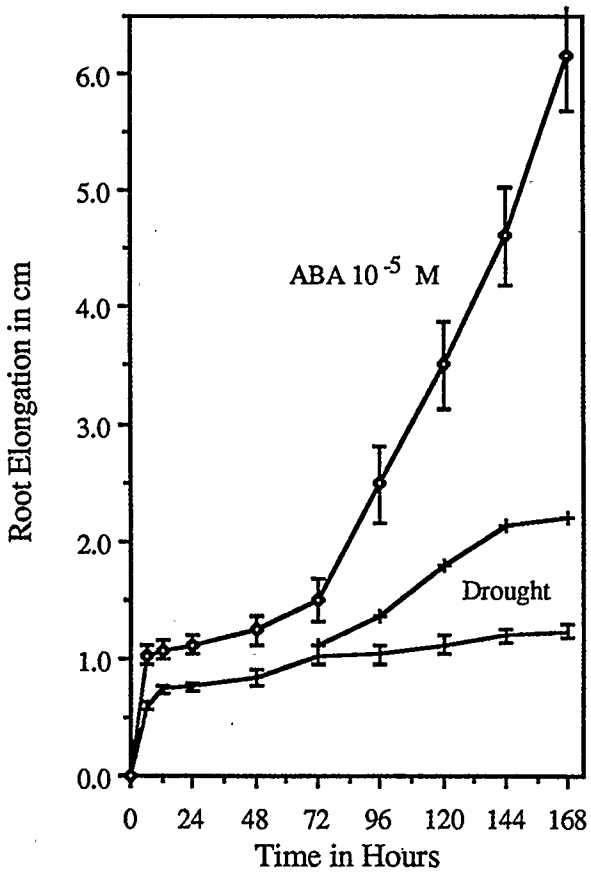


Figure 4. The rate of elongation in cm of main roots for droughted and ABA-treated ($10^{-5}$ M) sunflower plants over the course of seven days. At the beginning of the experiment (time 0), the plants were ten days old; the average root length at time 0 was 17.6 cm (droughted) and 17.7 cm (ABA). Error bars indicate standard errors; n = 27. The second line for droughted plants shows elongation in surviving roots only.

Short lateral roots behaved much differently. They were more susceptible to necrosis; few of these roots survived the stress. The emergence of new laterals ceased early in the drought regime, probably within the first 6 hours. Occasionally, in the later stages of the regime (after 120 hours), new laterals in the proximal region of the root, and adventitious roots from the base of the hypocotyl did emerge. When droughted plants were restored to favourable water conditions (full watering) after 72, 120 or 168 hours of droughting, the root system recovered. Most of the recovery depended on the addition of new roots; however, the surviving long roots began to grow vigorously again within 48 hours.

Treatment with ABA inhibited root elongation (see Figure 4). At a concentration of $10^{-5}$ M, elongation in ABA-treated plants roughly matched elongation in control plants for the first six hours; thereafter, elongation in the ABA-treated plants slowed dramatically. Elongation resumed after 72 hours, but at a much reduced rate relative to controls. There was evidence of a dose response to treatment with ABA, there was less inhibition of root elongation at a concentration of $10^{-6}$ M, and still less at $10^{-7}$ M (see Figure 5). With $10^{-8}$ M ABA concentration, there was only slight inhibition of elongation, and with $10^{-9}$ M, there was no measurable difference from control plants (data not shown). At no concentration was there an increase, relative to control plants, in root elongation over the course of the experiment. However, at concentrations of $10^{-6}$ M and $10^{-7}$ M, ABA treatment yielded a slight increase in elongation over the first 6 hours.

Figure 5.

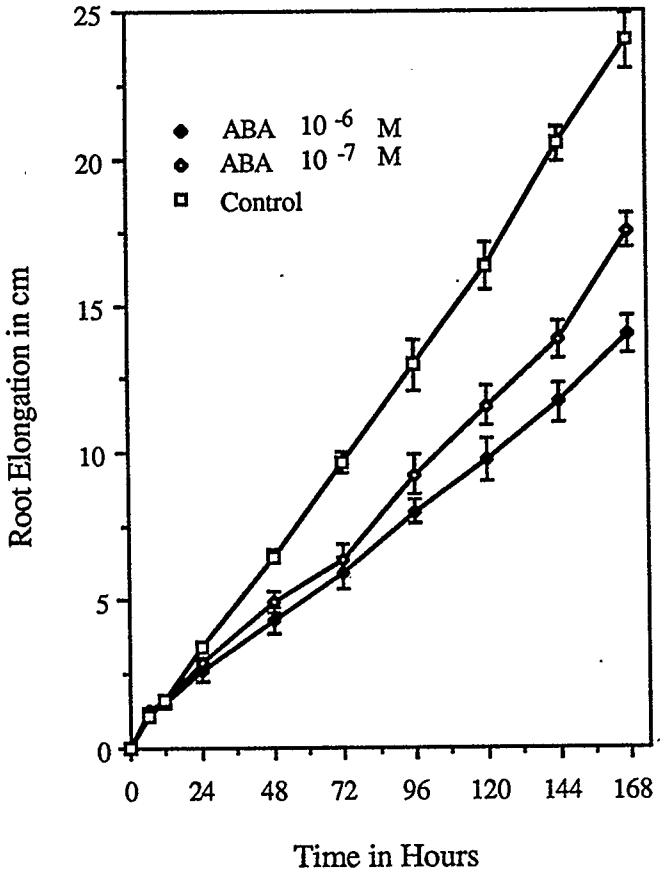


Figure 5. Rate of root elongation $10^{-6}$ M and $10^{-7}$ M ABA-treated plants. A repeat of well-watered (control) measurements are also shown. Error bars indicate standard errors; n = 9.

As in droughted plants, primary lateral roots responded to ABA-treatment in much the same way as the main root. The emergence of new roots was greatly inhibited by ABA treatment until late in the experiment, after 120 hours. Some necrosis did occur at a concentration of $10^{-5}$ M ABA. Again the number of necrotic roots was difficult to estimate, but certainly greater than 90% of the long roots survived the treatment. This percentage was probably somewhat lower in short laterals. Necrosis occurred at about 48 hours after the beginning of the experiment. Roots which were still viable at 72 hours generally survived the treatment. No root necrosis was evident in plants treated with lower concentrations of ABA.

The overall morphology of the root systems in both droughted and ABA-treated plants differed from that in control plants; the roots were shorter and fewer in number, resulting in root systems which were smaller and less dense.

Shoot growth was also greatly inhibited by drought and treatment with ABA. In both cases, the rate of leaf expansion declined sharply in the early stages of the experiment. What little growth did occur in the shoot was primarily from new primordia. In droughted plants, necrosis in the leaves began at about 72 hours. Necrosis was not a factor in the shoots of ABA-treated plants. When ABA was removed from the system, the older leaves began to grow again.

Not surprisingly, these general reductions in growth were reflected by dry weight measurements. The accumulation of dry weight in the roots and shoots of both droughted and ABA-treated plants was much below that in control plants. There was evidence, however, that root growth was less affected than shoot growth. Root to shoot biomass (R : S) ratios increased in treated plants. In droughted plants, the R : S ratio reached a maximum at 72 hours and then declined back to below control levels. This decline may have been because of root necrosis. In ABA-treated plants ($10^{-5}$ M), the R : S ratio increased and remained high through 120 hours, the last sample time point. Table 1 shows the R : S ratios with 95% confidence limits for control, droughted and $10^{-5}$ M ABA-treated

plants. The data was transformed for analysis by the arcsine transformation (Zar, 1984, chapter 14). This transformation yields confidence limits which differ slightly from one another, and so both the lower ($L_1$) and upper ($L_2$) confidence limits are shown.

Table 1.

| Control | 12 hours | 24 hours | 48 hours | 72 hours | 96 hours | 120 hours |
|---|---|---|---|---|---|---|
| x | 0.222 | 0.210 | 0.238 | 0.253 | 0.249 | 0.259 |
| $L_1$ | 0.201 | 0.203 | 0.225 | 0.229 | 0.232 | 0.225 |
| $L_2$ | 0.244 | 0.218 | 0.252 | 0.278 | 0.267 | 0.293 |
| Drought | | | | | | |
| x | 0.240 | 0.275 | 0.333 | 0.297 | 0.243 | 0.208 |
| $L_1$ | 0.218 | 0.237 | 0.292 | 0.278 | 0.213 | 0.179 |
| $L_2$ | 0.262 | 0.313 | 0.370 | 0.314 | 0.273 | 0.238 |
| ABA $10^{-5}$M | | | | | | |
| x | 0.250 | 0.297 | 0.336 | 0.319 | 0.344 | 0.362 |
| $L_1$ | 0.237 | 0.250 | 0.313 | 0.308 | 0.330 | 0.331 |
| $L_2$ | 0.262 | 0.344 | 0.359 | 0.331 | 0.357 | 0.394 |

Table 1. Root : Shoot biomass ratios for control, droughted, and $10^{-5}$ M ABA-treated over the first 120 hours of treatment. The values are the average, x , of six plants at each sample point. The data was analysed by the arcsine transformation; lower ($L_1$) and upper ($L_2$) 95% confidence limits are shown.

**Anatomical studies**

A histological study was conducted to see if the changes in growth were reflected in the anatomy of the root apex.

The most striking effect of drought and ABA-treatment was a reduction in the size of the zone of densely cytoplasmic cells at root apex (see Plates 1 and 2). This zone, tentatively described as the meristematic zone, was measured as the distance from the cap junction to the first fully vacuolated cells of the procambium and ground meristem (see Table 2). In the roots of control plants, these distances remain nearly constant at about 1600 μm (procambium) and about 1350 μm (ground meristem). These distances declined to minimums of 562 μm (procambium) and 434 μm (ground meristem) at 72 hours in droughted plants, and to 520 μm (procambium) and 445 μm (ground meristem) in ABA-treated ($10^{-5}$ M) plants. After reaching a minimum, the meristem began to recover in size. This recovery roughly coincided with the resumption of root elongation. The decline in the size of the meristematic zone is the result of cell elongation and differentiation in the proximal regions of the apical meristem (see Plates 1 and 2). Vacuolation has advanced distally into cells, which by position are cytoplasmic in control plants. These cells have seemingly differentiated in droughted and ABA-treated plants. Table 2 shows cell lengths at 500 μm and 1000 μm from the cap junction. The measurements are the average cell lengths, ± standard error, from the number (n) of median secions examined at each time point.

Plate 1.

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Median longitudinal sections of root apices from a well-watered (control) plant, 1a, at 96 hours (i.e. 14 days old) and a $10^{-5}$ M ABA-treated plant, 1b, at 24 hours after the beginning of treatment. Note the advanced (nearer the apex) vacuolation (arrow head) and cell elongation (arrow) in 1b, and the resultant decrease in the size of the meristematic zone. X 180.

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Plate 1.

Plate 2.

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Median longitudinal sections of root apices from droughted plants at 48 hours, 2a, and 72 hours, 2b, after the beginning of treatment. Note the advanced (nearer the apex) vacuolation (arrow head) and cell elongation (arrow) in 2a, and the resultant decrease in the size of the meristematic zone (compare plate 1a). By 72 hours, 2b, the meristematic zone has reached a minimum. x180.

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Plate 2.

Table 2.

| | | | Meristem Size | | Cell Length | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Procambium | Ground Meristem | Procambium | | Ground Meristem | |
| Hour | | n | | | 500 µm | 1000 µm | 500 µm | 1000 µm |
| 6 | C | 5 | 1653 ± 104 | 1452 ± 91 | 46 ± 4 | 102 ± 10 | 17 ± 1 | 25 ± 2 |
| | Dr | 5 | 1157 ± 22 | 950 ± 55 | 62 ± 4 | 155 ± 19 | 18 ± 1 | 32 ± 3 |
| | ABA | 10 | 977 ± 31 | 755 ± 23 | 72 ± 5 | 176 ± 18 | 20 ± 1 | 64 ± 4 |
| 12 | C | 5 | 1496 ± 59 | 1290 ± 58 | 48 ± 1 | 89 ± 13 | 19 ± 2 | 28 ± 3 |
| | Dr | 9 | 999 ± 28 | 868 ± 19 | 87 ± 7 | 161 ± 15 | 21 ± 1 | 38 ± 2 |
| | ABA | 8 | 776 ± 31 | 573 ± 22 | 67 ± 3 | 163 ± 8 | 26 ± 3 | 56 ± 6 |
| 24 | C | 6 | 1501 ± 71 | 1262 ± 62 | 49 ± 5 | 97 ± 14 | 18 ± 2 | 24 ± 2 |
| | Dr | 9 | 734 ± 35 | 693 ± 29 | 101 ± 5 | 262 ± 24 | 22 ± 1 | 76 ± 6 |
| | ABA | 7 | 561 ± 29 | 414 ± 39 | 96 ± 8 | 300 ± 17 | 30 ± 2 | 91 ± 11 |
| 48 | C | 12 | 1603 ± 49 | 1305 ± 42 | 51 ± 6 | 107 ± 9 | 19 ± 1 | 27 ± 3 |
| | Dr | 13 | 692 ± 15 | 549 ± 23 | 101 ± 4 | 304 ± 14 | 44 ± 5 | 87 ± 5 |
| | ABA | 10 | 520 ± 29 | 445 ± 16 | 131 ± 13 | 332 ± 18 | 47 ± 7 | 117 ± 11 |
| 72 | C | 8 | 1584 ± 69 | 1396 ± 70 | 61 ± 6 | 111 ± 10 | 18 ± 1 | 23 ± 2 |
| | Dr | 9 | 562 ± 30 | 434 ± 33 | 91 ± 7 | 299 ± 21 | 38 ± 4 | 84 ± 9 |
| | ABA | 7 | 654 ± 41 | 579 ± 47 | 78 ± 6 | 283 ± 11 | 27 ± 4 | 74 ± 8 |
| 96 | C | 6 | 1666 ± 107 | 1387 ± 95 | 44 ± 4 | 78 ± 4 | 17 ± 1 | 18 ± 1 |
| | Dr | 7 | 612 ± 35 | 493 ± 42 | 81 ± 5 | 277 ± 9 | 40 ± 5 | 107 ± 9 |
| | ABA | 8 | 666 ± 34 | 569 ± 22 | 99 ± 9 | 287 ± 17 | 29 ± 2 | 99 ± 8 |
| 120 | C | 7 | 1694 ± 59 | 1467 ± 52 | 51 ± 4 | 87 ± 7 | 17 ± 1 | 23 ± 1 |
| | Dr | 9 | 768 ± 85 | 693 ± 50 | 99 ± 10 | 247 ± 11 | 32 ± 5 | 80 ± 6 |
| | ABA | 7 | 764 ± 77 | 712 ± 71 | 93 ± 18 | 263 ± 24 | 23 ± 4 | 76 ± 12 |
| 144 | C | 6 | 1748 ± 104 | 1434 ± 97 | 42 ± 2 | 92 ± 20 | 18 ± 1 | 25 ± 4 |
| | Dr | 9 | 876 ± 70 | 768 ± 63 | 69 ± 4 | 252 ± 15 | 24 ± 2 | 80 ± 6 |
| | ABA | 10 | 830 ± 24 | 698 ± 22 | 99 ± 12 | 301 ± 16 | 23 ± 1 | 97 ± 7 |
| 168 | C | 8 | 1659 ± 45 | 1411 ± 78 | 47 ± 2 | 92 ± 6 | 18 ± 1 | 21 ± 1 |
| | Dr | 8 | 904 ± 124 | 816 ± 128 | 72 ± 5 | 274 ± 10 | 23 ± 2 | 70 ± 12 |
| | ABA | 11 | 810 ± 57 | 670 ± 16 | 110 ± 15 | 308 ± 16 | 23 ± 2 | 83 ± 8 |

Table 2. Meristem size and Cell length. The size of the densely cytoplasmic zone of cells (meristematic zone) was measured as the distance from the cap junction to the first fully vacuolated cells. Length of cells at 500 µm and 1000 µm proximal to the cap junction in control (C), droughted (Dr), and $10^{-5}$ M ABA-treated, (ABA) plants. Values are the average of the number, n, of median sections examined at each time point ± standard error.

Abscisic acid-treatment at concentrations of $10^{-6}$ M and $10^{-7}$ M ABA had much the same effect on the meristem at 6 and 24 hours as did $10^{-5}$ M ABA (see Table 3). However, recovery in the size of the cytoplasmic region was evident by 48 hours, somewhat earlier than in $10^{-5}$ M treatments.

Table 3.

| | | Meristem Size | | Cell Length | | | |
|---|---|---|---|---|---|---|---|
| | | Procambium | Ground Meristem | Procambium | | Ground Meristem | |
| ABA $10^{-6}$ M | | | | 500 µm | 1000 µm | 500 µm | 1000 µm |
| Hour | n | | | | | | |
| 6 | 4 | 955 ± 68 | 853 ± 77 | 83 ± 19 | 285 ± 71 | 23 ± 2 | 67 ± 10 |
| 24 | 4 | 688 ± 53 | 598 ± 57 | 139 ± 25 | 304 ± 18 | 37 ± 13 | 126 ± 10 |
| 48 | 5 | 1078 ± 78 | 922 ± 53 | 83 ± 5 | 253 ± 28 | 19 ± 2 | 43 ± 6 |
| ABA $10^{-7}$ M | | | | | | | |
| 6 | 5 | 1010 ± 93 | 940 ± 89 | 91 ± 12 | 292 ± 23 | 18 ± 1 | 528 ± 13 |
| 24 | 4 | 710 ± 36 | 653 ± 25 | 122 ± 15 | 367 ± 44 | 27 ± 1 | 151 ± 10 |
| 48 | 4 | 1050 ± 44 | 980 ± 64 | 93 ± 13 | 260 ± 38 | 23 ± 1 | 49 ± 7 |

Table 3. The effects of $10^{-6}$ M and $10^{-7}$ M ABA on the size of the densely cytoplasmic zone of cells and the length of cells at 500 µm and 1000 µm proximal to the cap junction. The values are the average of the number, n, of median sections examined at each time point ± standard error.

Root apices from droughted and ABA-treated plants produced fewer periclinal cell divisions in the distal regions of the meristem. Median sections from plants that were

treated for 72 and 96 hours had fewer files of cells in the ground meristem. Periclinal cell division is expressed by the ratio of files of cells in the ground meristem (100 to 500 μm from the cap junction) to the number of files in the expanding cortex. Table 4 shows these ratios. The numbers are the average of six sections for each treatment. In control plants, this ratio was about 1:4, whereas, in treated plants, this ratio declined to near unity. Statistics are from one-way analysis of variance.

Table 4.

| | no of files in cortex/ no of files in the ground meristem |
|---|---|
| Control | 1.391 |
| ABA | 1.000 |
| Water stress | 1.030 |

Table 4. The effects of $10^{-5}$ M ABA and drought stress on periclinal divisions in the ground meristem in plants that have been treated for 120 hours or longer. Values are expressed as the ratio of the number of cell files in the elongating cortex over the number of files in the ground meristem at 100 μm from the cap junction. Six root apices were examined for each sample. Statistics are from one-way analysis of variance; $P < 0.001$.

**Exogenous sucrose treatments**

Stomatal closure, as measured by stomatal diffusive resistance, is a complicating factor in both the droughted and ABA-treated plants. Figure 6 shows diffusive resistance as a measure of stomatal closure in control, droughted, and $10^{-5}$ M ABA-treated plants. Drought did not increase diffusive resistance during the first 6 hours of treatment, but by

24 hours, drought had significantly increased resistance. Abscisic acid at $10^{-5}$ M concentration rapidly closed stomata. In both droughted and ABA-treated plants, stomata began to open again in the latter stages of treatment. At lower concentrations, ABA did not have such a dramatic effect; at $10^{-6}$ M, concentration diffusive increased noticeably, but at $10^{-7}$ M, ABA had no effect (see Figure 7).

A lack of photosynthate in the root would affect growth and development (Feldman and Torrey, 1975, Van't Hof, 1966, Webster and Lagenhauer, 1973). During the early stages of treatment, the addition of sucrose did little to alleviate the inhibition of root elongation, but, after 72 hours in droughted plants and 48 hours in ABA-treated plants, it did have an appreciable effect (see Figure 8). Relative to well-watered (control) plants, however, root elongation was still greatly inhibited in the sucrose-supplemented plants.

The remarkable effect of exogenously supplied sucrose, however, was in the maintenance of the root systems in droughted plants. Root necrosis declined sharply compared to drought alone; new lateral roots emerged and elongated. Root to shoot biomass ratios in the sucrose-treated plants increased compared to drought alone at 72 hours, and remained high relative to control plants at 120 hours (see Table 5). The root system in droughted plants took on a brownish appearance, probably because of a build up of phenolic compounds, in the later stages of the experiment. Sucrose supplements greatly reduced this effect.

In ABA-treated plants, sucrose supplements promoted an increase in the length and number of short lateral roots, relative to ABA treatment alone. Root to shoot biomass ratios, already high relative to control plants, remained high, but did not increase with the addition of sucrose (see Table 5).

Figure 6. Stomatal diffusive resistance (s $cm^{-1}$) in control, droughted and $10^{-5}$ M ABA-treated plants over 168 hours of treatment. Error bars indicate standard errors; n = 6.

Figure 7. Stomatal diffusive resistance (s $cm^{-1}$) in $10^{-6}$ M and $10^{-7}$ M ABA-treated plants over 168 hours of treatment. Error bars indicate standard errors; n = 6.

Figure 6.

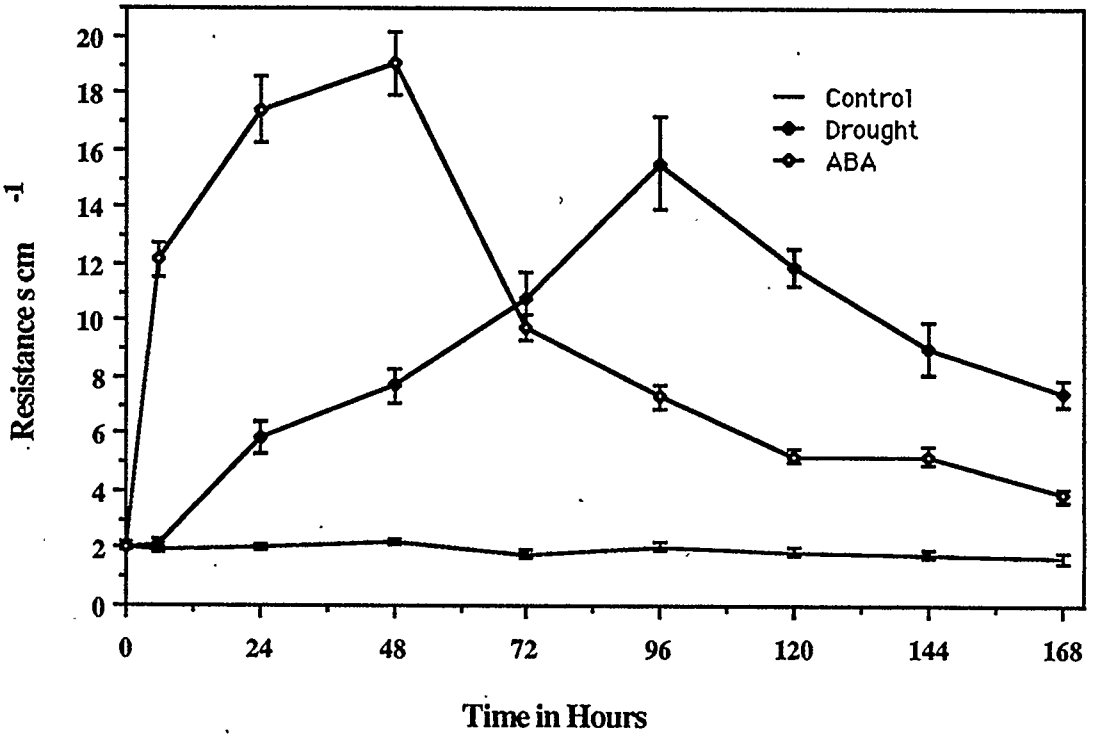


Figure 7.

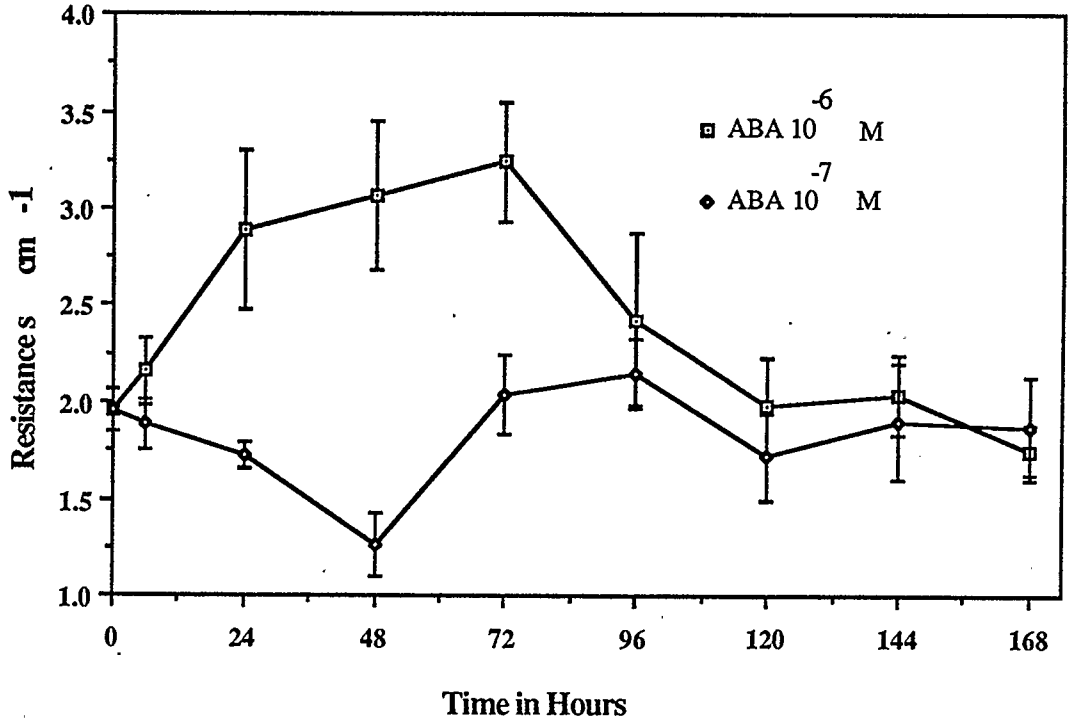

Figure 8.

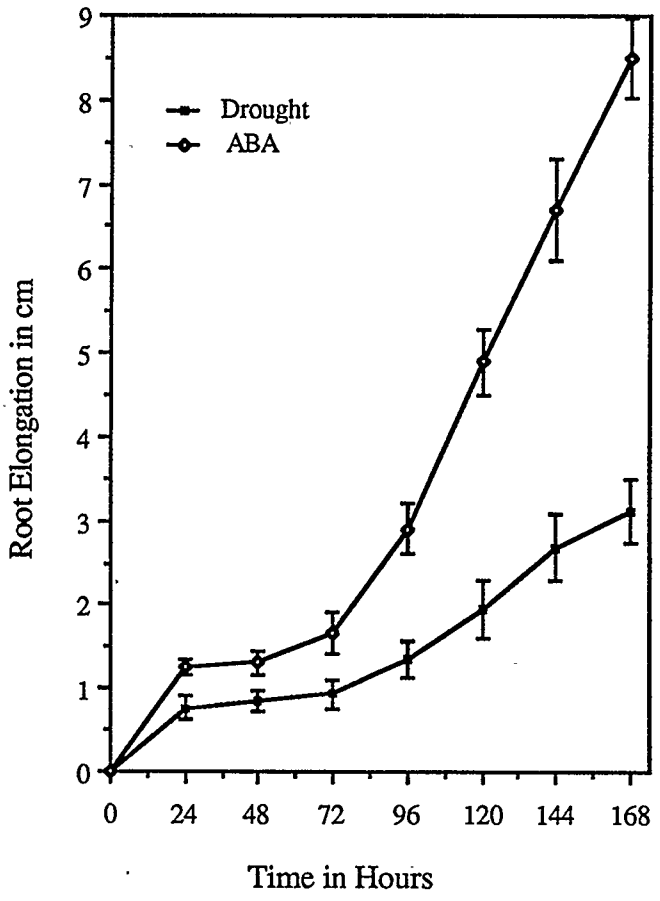


Figure 8. Root elongation in sucrose-supplemented plants. Sucrose in a 0.3 M solution was exogenously supplied through leaf flaps. This figure shows the effects of sucrose supplements on root elongation in droughted and ABA-treated plants. Exogenous sucrose-treatment had no effect on control plants. Error bars indicate standard errors; n = 9.

Table 5.

| | | 24 hours | 72 hours | 120 hours |
|---|---|---|---|---|
| Control | | | | |
| | x | 0.230 | 0.231 | 0.241 |
| | $L_1$ | 0.209 | 0.213 | 0.219 |
| | $L_2$ | 0.253 | 0.249 | 0.264 |
| Drought | | | | |
| | x | 0.270 | 0.374 | 0.307 |
| | $L_1$ | 0.252 | 0.316 | 0.291 |
| | $L_2$ | 0.290 | 0.434 | 0.322 |
| ABA $10^{-5}$M | | | | |
| | x | 0.284 | 0.301 | 0.334 |
| | $L_1$ | 0.262 | 0.276 | 0.300 |
| | $L_2$ | 0.306 | 0.327 | 0.371 |

Table 5. Root : Shoot biomass ratios of control, droughted and ABA-treated plants that had been treated with exogenous sucrose through leaf flaps. Statistics are as in Table 1; n = 6 at each sample point.

Table 6.

| | | Meristem Size | | Cell Length | | | |
|---|---|---|---|---|---|---|---|
| | | Procambium | Ground Meristem | Procambium | | Ground Meristem | |
| Drought Hour | n | | | 500 µm | 1000 µm | 500 µm | 1000 µm |
| 24 | 4 | 688 ± 29 | 623 ± 28 | 93 ± 7 | 329 ± 27 | 31 ± 4 | 116 ± 16 |
| 48 | 4 | 653 ± 61 | 540 ± 58 | 101 ± 11 | 416 ± 20 | 40 ± 10 | 162 ± 35 |
| 72 | 5 | 621 ± 93 | 523 ± 110 | 134 ± 24 | 318 ± 34 | 54 ± 13 | 100 ± 9 |
| ABA $10^{-5}$ M | | | | | | | |
| 48 | 3 | 610 ± 47 | 537 ± 35 | 117 ± 26 | 373 ± 39 | 40 ± 12 | 151 ± 15 |
| 72 | 5 | 590 ± 71 | 508 ± 58 | 148 ± 44 | 354 ± 23 | 51 ± 13 | 145 ± 10 |

Table 6. Meristem size and Cell length in the roots of sucrose-supplemented plants. The size of the densely cytoplasmic zone of cells (meristematic zone) was measured as the distance from the cap junction to the first fully vacuolated cells. Cell length at 500 µm and 1000 µm from the cap junction was measured in droughted (Dr), and $10^{-5}$ M ABA-treated (ABA) plants which had been fed 0.3 M sucrose through leaf flaps. The values are the average of the number, n, of median sections examined at each time point ± standard error.

Exogenous sucrose did not noticeably affect growth in well-watered plants; neither root elongation nor R : S biomass ratios were affected. When water was used in place of sucrose in the vials, there was no effect on droughted or ABA-treated plants.

Shoot growth in droughted and ABA-treated plants was not affected by the addition of sucrose. In droughted plants, the leaves were stunted and necrosis occurred at about 72 hours. The result was rather curious: the plants had stunted necrotic shoot systems and relatively healthy and dense root systems.

When the apical anatomy of the long roots was examined, it was found that the sucrose supplements did not suppress drought or ABA-induced changes at the meristem. The size of the meristem decreased as in plants treated with drought or ABA alone. If there was any effect, it was that cell length in droughted and ABA-treated plants increased even more with the addition of sucrose. This would suggest that sucrose supplements supported cell elongation, but did not increase the number of cells being added to the system, and that the increase in root length with the addition of sucrose was because of increased cell elongation.

The secondary stress of reduced photosynthate supplies did not appear to have been a factor in the initial response of the root to drought and ABA-treatment. Three lines of evidence support this claim. First, the changes in the meristem had begun before stomata had closed to any appreciable extent in droughted and $10^{-7}$ M ABA-treated plants. Second, the exogenous supply of sucrose, although it had an effect on root morphology, did not suppress drought and ABA-induced changes at the meristem. Third, cell elongation, a process which requires a ready supply of organic solutes, continued.

The exogenous supply of sucrose did support elongation in the later stages of treatment. Its effects become noticable during the period of resumed growth.

## DISCUSSION

Drought stress reduced root growth. This was the direct result of drought (i.e. lack of water), since external mechanical resistance is not a factor in aeroponically grown plants. Anatomical changes at the root apex were evident within 6 hours of the imposition of drought. These changes did not result from a lack of fixed carbon.

Root elongation virtually stopped in the period from 6 to 72 hours. What little root growth did occur would be largely accounted for by cell elongation. Cells, which by position were cytoplasmic at the beginning of the drought treatment, vacuolated and seemingly differentiated as the size of the meristematic region shrunk.

Other studies have noted a positive correlation between growth rate and the distance from the cap junction at which cells differentiate and mature (Kurth, et al., 1986, Torrey, 1953, Salim and Oryem-Origa, 1981, and Wilcox, 1954). These observations in droughted roots are of particular consequence, however. Two conclusions can be drawn from these results: 1. the apical meristem actively responds to drought, and 2. the response must include a decrease in cell division. These points are dealt with in turn.

The immediate stress imposed by drought is a reduction in water potential, $\Psi$, external to the plant. In this case, it was a concentration change immediately adjacent to the root that led to a water deficit. A lack of response to this stress would initiate a loss of water to the external environment and a subsequent loss of turgor. Turgor was not lost, since cell elongation, a turgor driven process, continued. The only way in which this could occur would be through osmotic adjustment, an energy requiring process. Part of the response then must be the input of energy to drive cell elongation.

The differentiation of cells in the proximal regions of the meristem and the resultant decrease in the size of the meristem would reduce the number of cycling cells. This would perhaps in itself reduce the rate at which cells are added to the root. But the rate of root elongation was such that the rate of passage of cells into the zone of elongation must have

slowed. Therefore, the cycle times of the cells which had remained meristematic must have increased, and the rate of cell division decreased.

Conversely, loss of turgor cannot account for reduced cell division. Hsiao and colleagues (Hsiao, 1973, Hsiao, et al., 1976) suggested that the sensitivity of cell division to reduced $\Psi$ is an indirect effect of the stress, which restricts meristematic cells from enlarging to a size minimal for the commencement of division, eg. a loss of turgor. However, cells in the proximal regions of the meristem did not lose turgor; yet they stopped dividing and started to elongate. The cells of the proximal regions of the meristem then must receive a signal to differentiate and stop dividing. It could be argued that cell age may be a factor in this response, but even so the rates of root and cell elongation are such that these cells could not have fulfilled their potential for division and then elongated. Some signal altered their development, and that signal was not loss of turgor. A reduction in the rate of cell division then is not an immediate consequence of, but rather an active response to drought.

It appears then that the apical meristem responds directly to an external stimulus, lower $\Psi$, and not to growth limiting factors. A key point here is that the response seemingly preceeds the time when the effects of stress become limiting. The root still has the ability to take up water as evidenced by cell elongation; carbohydrate supply was probably not limiting in the early stages of drought, and even when it was supplied exogenously, did not suppress the response. A response such as this could be regarded as a survival mechanism. The meristem reduces its size and activity in response to an external stimulus, lower $\Psi$. It thereby lowers its demands for water, nutrients, and energy against a time when these resources might become limiting. It appears that the root meristem did not lack resources in the early stages of drought, but had utilized its available resources to a different purpose.

If this response aids in survival, the question becomes: why do some some roots survive while others perish? A clue to the answer may lie in the sucrose supplement

experiments. It is of interest that necrosis occured in some roots at about the same time as resumed growth and recovery of the size of the meristem began in others. Reduced carbohydrate supply may be a factor at this stage; and it is perhaps only those meristems which receive a sufficient supply of carbohydrate that survive. The fact that exogenous supplies of sucrose alleviate root necrosis supports this view. Apical meristems exercise a considerable degree of autonomy (Torrey and Feldman, 1977). Their responses to drought may be expected to differ, not only from other parts of the plant, but from one another. Surviving roots then may be those with greater sink strengthts for carbohydrate. Moreover, when one meristem dies its reserves of carbohydrate could be re-allocated to surviving roots. This would suggest that the meristems are in competition with one another, and that a root meristem survives at the expense of others. This argument could be applied to explain why short laterals are more susceptible to drought than the long roots.

The writer suggests that the root apical meristem responds directly to drought, and that its response is largely independent of other processes within the plant. The basis of this response is a reduction in the size of the meristematic region and a concomitant decrease in the rate at which cells are added to the root. For reasons given above, these changes cannot be passive. There must be some mediatory signal which dictates a change in the developmental pattern. The exogenous application of ABA elicits the same pattern of change in the root apex. Previous studies have shown that ABA levels rise in the roots of droughted plants (see Hubick, et al.,1986). Taken together, these observations suggest that ABA may act as mediator of drought-induced developmental change at the root apex.

For each of the observed changes in the roots of droughted plants, an argument can be advanced to support the thesis that ABA acts as a mediator. Such arguments, however, stem from comparison and can only show correlation. A more profitable exercise is to set up a series of testable hypotheses, which can either disprove or support the thesis.

If ABA does indeed mediate drought-induced changes in the root apical meristem, then the following conditions should obtain:

1. The effect of ABA must express itself in the events of the cell cycle.

2. Drought must increase endogenous ABA levels in the tissue of interest, the root apex.

3. ABA must effect some shift in differentiation to signal the cells at the proximal regions of the meristem to elongate and stop dividing.

4. ABA must effect these changes in a manner consistent with the drought response.

# CHAPTER III : MITOTIC ACTIVITY

In Chapter II, it was argued that, because cell elongation continued even though the rate of root elongation declined, the rate at which cells are added to the root must decrease in droughted and ABA-treated plants.

Earlier reports (Barlow and Pilet, 1984, Nagl, 1972) have shown that ABA can affect mitotic activity in the root apical meristem. This section reports the effects of drought and exogenously applied ABA on two parameters of the cell cycle: the labelling index (the percentage of cells in S-phase) and the mitotic index (percentage of cells in mitosis). These parameters give an indication of the activity of the meristem. A full study of the cell cycle (a measure of cell doubling times) would have been preferable for this study, but such studies generally require prolonged periods of hydration which would have obviated the drought treatment.

## MATERIALS AND METHODS

Plant materials, growth conditions, drought and ABA treatments were as reported in Chapter II.

### Labelling Experiment

Root tips were cut on moist paper towels and placed immediately in a sterile solution (10 $\mu$Ci ml$^{-1}$) of [$^3$H] methyl-Thymidine ( ICN; specific activity 74 Ci mmol$^{-1}$) in 0.75 strength Hoagland solution for 1 hour. The root tips were then washed in three rinses of sterilized Hoagland solution, and fixed in 4% gluteraldehyde in 0.025 M phosphate buffer, pH 6.8. They were then dehydrated in an ethanol series, embedded in Historesin (LKB), and sectioned longitudinally at 4 $\mu$m thickness. The sections were mounted on

glass slides. The slides were dipped in a nuclear track emulsion (Kodak, NTB2) and exposed in darkness for 21 days at 4° C. The slides were then developed as autoradiographs. The autoradiographs were stained by the periodic acid-Schiff's (PAS) reaction and counterstained with Toluidine blue O (PAS-TBO) (Feder and O'Brien, 1968). The acid hydrolysis step for the PAS reaction was done before autoradiography to avoid chemographic effects (Rogers, 1979, p. 142). Autoradiographs were made from the roots of droughted, $10^{-5}$ M ABA-treated, and control plants over the seven day period of the experiment. A second set of autoradiographs were made from root tips of plants treated with $10^{-6}$ M and $10^{-7}$ M ABA at 6, 24, and 48 hours after treatment. Autoradiographs were also made from sucrose-supplemented plants at 48 and 72 hours after treatment. Sucrose treatment was by the leaf flap method as in Chapter II.

Median sections were selected and examined. Labelling index was scored as the percentage of nuclei which exposed the emulsion. Mitotic index was scored as the percentage of nuclei in mitosis. The labelling and mitotic indices were taken from the same set of plants.

**RESULTS**

A cursory examination of the autoradiographs revealed the same anatomical changes as noted in Chapter II. It was also evident that the changes induced by drought and ABA differed from one part of the meristem to another. For that reason the meristem was arbitrarily divided into regions; labelling and mitotic indices were then scored separately for each region.

Table 7 presents labelling indices from each of the three primary meristems of root apices from control, droughted and $10^{-5}$ M ABA-treated plants at distances of 0-100, 100-500, 500-1000,and 1000-1500 μm from the cap junction.

Table 8 shows mitotic indices from the autoradiographs; the meristem was divided into the same regions as in Table 7. The PAS-TBO stain used for the autoradiographs was not the best for scoring mitotic index. However, the writer wished to correlate mitotic activity with the histological changes noted in Chapter II; this stain was better for that purpose than Feulgen's stain. A comparison of the autoradiographs with root tips stained by Fuelgen's stain indicated a slight underestimation of mitotic index (< 0.5 %) with the PAS-TBO stain. Early prophase figues were particularly difficult to identify in the autoradiographs.

Table 7. Labelling indices from control (C), droughted (Dr), and $10^{-5}$ M ABA-treated (ABA) roots from 6 to 168 hours after the beginning of treatment. Labelling index values were calculated as the mean percentage of cells in S-phase, ± 95% confidence limits, in specified regions of the meristem, from number of sections (n) examined at each sample point.

Table 7.

| Hour | | n | Procambium Section 0-100 | 100-500 | 500-1000 | 1000-1500 | Ground Meristem 0-100 | 100-500 | 500-1000 | 1000-1500 | Protoderm 0-100 | 100-500 | 500-1000 | 1000-1500 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | C | 5 | 28 ± 4 | 45 ± 11 | 12 ± 4 | 2 ± 3 | 30 ± 7 | 56 ± 11 | 51 ± 8 | 5 ± 2 | 43 ± 5 | 36 ± 5 | 27 ± 6 | 0 ± 0 |
| | Dr | 4 | 8 ± 4 | 17 ± 3 | 2 ± 2 | 0 ± 0 | 9 ± 3 | 30 ± 2 | 12 ± 3 | 0 ± 0 | 26 ± 17 | 16 ± 8 | 7 ± 2 | 0 ± 0 |
| | ABA | 6 | 12 ± 3 | 26 ± 7 | 2 ± 3 | 0 ± 0 | 13 ± 9 | 34 ± 11 | 6 ± 6 | 1 ± 1 | 29 ± 10 | 23 ± 12 | 0 ± 0 | 0 ± 0 |
| 12 | C | 4 | 23 ± 4 | 38 ± 7 | 18 ± 4 | 7 ± 4 | 44 ± 12 | 52 ± 6 | 54 ± 8 | 8 ± 2 | 49 ± 4 | 40 ± 8 | 18 ± 6 | 0 ± 0 |
| | Dr | 4 | 4 ± 3 | 11 ± 6 | 3 ± 5 | 0 ± 0 | 4 ± 5 | 12 ± 11 | 4 ± 6 | 0 ± 0 | 11 ± 5 | 11 ± 11 | 0 ± 0 | 0 ± 0 |
| | ABA | 4 | 3 ± 4 | 4 ± 5 | 0 ± 0 | 0 ± 0 | 3 ± 3 | 6 ± 8 | 2 ± 3 | 0 ± 0 | 13 ± 10 | 8 ± 8 | 1 ± 3 | 0 ± 0 |
| 24 | C | 4 | 20 ± 5 | 41 ± 8 | 12 ± 3 | 5 ± 4 | 36 ± 10 | 56 ± 8 | 51 ± 10 | 6 ± 3 | 42 ± 6 | 36 ± 7 | 21 ± 4 | 1 ± 3 |
| | Dr | 5 | 12 ± 5 | 18 ± 7 | 2 ± 2 | 0 ± 0 | 15 ± 8 | 15 ± 10 | 4 ± 5 | 0 ± 0 | 20 ± 6 | 13 ± 5 | 2 ± 3 | 0 ± 0 |
| | ABA | 5 | 2 ± 3 | 3 ± 4 | 0 ± 0 | 0 ± 0 | 7 ± 14 | 6 ± 12 | 0 ± 0 | 0 ± 0 | 5 ± 8 | 4 ± 10 | 0 ± 0 | 0 ± 0 |
| 48 | C | 4 | 22 ± 8 | 40 ± 5 | 13 ± 4 | 0 ± 0 | 39 ± 5 | 59 ± 6 | 48 ± 8 | 3 ± 2 | 48 ± 10 | 42 ± 12 | 22 ± 9 | 1 ± 2 |
| | Dr | 5 | 11 ± 3 | 12 ± 5 | 0 ± 0 | 0 ± 0 | 10 ± 5 | 18 ± 7 | 3 ± 3 | 0 ± 0 | 15 ± 10 | 9 ± 5 | 0 ± 0 | 0 ± 0 |
| | ABA | 7 | 22 ± 10 | 29 ± 4 | 1 ± 2 | 0 ± 0 | 20 ± 4 | 28 ± 5 | 1 ± 2 | 0 ± 0 | 30 ± 14 | 29 ± 13 | 1 ± 2 | 0 ± 0 |
| 72 | C | 4 | 22 ± 8 | 49 ± 8 | 19 ± 10 | 3 ± 5 | 36 ± 10 | 60 ± 4 | 55 ± 3 | 8 ± 6 | 46 ± 13 | 55 ± 9 | 26 ± 14 | 2 ± 2 |
| | Dr | 4 | 24 ± 10 | 24 ± 9 | 0 ± 0 | 0 ± 0 | 25 ± 8 | 22 ± 9 | 0 ± 0 | 0 ± 0 | 21 ± 4 | 13 ± 11 | 0 ± 0 | 0 ± 0 |
| | ABA | 5 | 20 ± 6 | 38 ± 5 | 1 ± 1 | 0 ± 0 | 18 ± 7 | 32 ± 6 | 8 ± 9 | 0 ± 0 | 32 ± 10 | 28 ± 6 | 2 ± 5 | 0 ± 0 |
| 96 | C | 6 | 24 ± 4 | 48 ± 8 | 18 ± 6 | 7 ± 4 | 42 ± 7 | 62 ± 9 | 58 ± 5 | 8 ± 2 | 47 ± 6 | 42 ± 3 | 20 ± 10 | 4 ± 2 |
| | Dr | 4 | 19 ± 10 | 19 ± 8 | 7 ± 6 | 0 ± 0 | 28 ± 8 | 27 ± 7 | 10 ± 4 | 0 ± 0 | 32 ± 3 | 32 ± 17 | 5 ± 9 | 0 ± 0 |
| | ABA | 4 | 22 ± 8 | 34 ± 18 | 0 ± 0 | 0 ± 0 | 25 ± 12 | 43 ± 5 | 4 ± 3 | 0 ± 0 | 27 ± 13 | 30 ± 18 | 0 ± 0 | 0 ± 0 |
| 120 | C | 3 | 26 ± 7 | 45 ± 8 | 11 ± 3 | 3 ± 4 | 40 ± 1 | 56 ± 6 | 48 ± 4 | 5 ± 3 | 51 ± 9 | 57 ± 8 | 29 ± 7 | 2 ± 4 |
| | Dr | 3 | 18 ± 2 | 39 ± 5 | 12 ± 3 | 0 ± 0 | 36 ± 8 | 40 ± 4 | 18 ± 7 | 0 ± 0 | 41 ± 1 | 34 ± 5 | 0 ± 0 | 0 ± 0 |
| | ABA | 3 | 29 ± 6 | 42 ± 5 | 1 ± 1 | 0 ± 0 | 15 ± 6 | 43 ± 3 | 12 ± 2 | 0 ± 0 | 41 ± 4 | 46 ± 7 | 7 ± 8 | 0 ± 0 |
| 144 | C | 5 | 20 ± 3 | 49 ± 11 | 15 ± 4 | 5 ± 4 | 35 ± 8 | 56 ± 6 | 50 ± 8 | 12 ± 6 | 44 ± 5 | 46 ± 11 | 25 ± 11 | 3 ± 3 |
| | Dr | 5 | 17 ± 7 | 31 ± 6 | 10 ± 7 | 0 ± 0 | 19 ± 7 | 42 ± 5 | 19 ± 4 | 0 ± 0 | 30 ± 10 | 47 ± 9 | 0 ± 0 | 0 ± 0 |
| | ABA | 4 | 23 ± 9 | 35 ± 5 | 1 ± 2 | 0 ± 0 | 33 ± 7 | 47 ± 5 | 4 ± 4 | 0 ± 0 | 47 ± 14 | 47 ± 11 | 0 ± 0 | 0 ± 0 |
| 168 | C | 4 | 25 ± 5 | 42 ± 4 | 15 ± 5 | 5 ± 4 | 35 ± 4 | 62 ± 8 | 55 ± 9 | 12 ± 4 | 48 ± 8 | 44 ± 3 | 26 ± 7 | 4 ± 3 |
| | Dr | 4 | 25 ± 5 | 34 ± 5 | 0 ± 0 | 0 ± 0 | 23 ± 6 | 38 ± 2 | 10 ± 4 | 0 ± 0 | 36 ± 10 | 34 ± 5 | 0 ± 0 | 0 ± 0 |
| | ABA | 6 | 21 ± 6 | 35 ± 6 | 0 ± 0 | 0 ± 0 | 32 ± 6 | 43 ± 6 | 3 ± 4 | 0 ± 0 | 44 ± 8 | 40 ± 11 | 0 ± 0 | 0 ± 0 |

Table 8. Mitotic indices from control (C), droughted (Dr), and $10^{-5}$ M ABA-treated (ABA) roots from 6 to 168 hours after the beginning of treatment. Mitotic index values were calculated as the mean percentage of cells in mitosis, ± 95% confidence limits, in specified regions of the meristem, from n number of sections examined at each sample point.

Table 8.

| Hour | | n | Section | Procambium 0-100 | Procambium 100-500 | Procambium 500-1000 | Procambium 1000-1500 | Ground Meristem 0-100 | Ground Meristem 100-500 | Ground Meristem 500-1000 | Ground Meristem 1000-1500 | Protoderm 0-100 | Protoderm 100-500 | Protoderm 500-1000 | Protoderm 1000-1500 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | C | 5 | | 4±3 | 10±2 | 12±5 | 4±2 | 8±3 | 9±2 | 9±2 | 8±5 | 11±9 | 8±2 | 10±3 | 2±3 |
| | Dr | 4 | | 5±1 | 9±3 | 9±7 | 3±3 | 5±4 | 8±2 | 8±3 | 1±2 | 7±3 | 9±4 | 5±4 | 0±0 |
| | ABA | 6 | | 8±9 | 6±2 | 2±3 | 0±0 | 4±1 | 5±2 | 4±2 | 0±0 | 2±4 | 4±3 | 0±0 | 0±0 |
| 12 | C | 4 | | 4±2 | 10±3 | 11±2 | 5±4 | 8±3 | 9±2 | 9±2 | 7±4 | 11±5 | 9±4 | 9±3 | 2±3 |
| | Dr | 4 | | 5±2 | 8±2 | 3±5 | 0+0 | 5±3 | 6±2 | 5±2 | 0±0 | 8±3 | 7±2 | 1±3 | 0±0 |
| | ABA | 4 | | 10±6 | 7±3 | 1±1 | 0±0 | 7±2 | 6±1 | 3±2 | 0±0 | 5±6 | 6±3 | 0±0 | 0±0 |
| 24 | C | 4 | | 5±3 | 13±2 | 11±6 | 4±3 | 10±4 | 10±2 | 10±2 | 9±4 | 10±5 | 8±2 | 12±5 | 2±4 |
| | Dr | 5 | | 2±4 | 1±2 | 2±4 | 0±0 | 4±3 | 2±2 | 2±1 | 0±0 | 3±1 | 2±1 | 0±0 | 0±0 |
| | ABA | 5 | | 0±1 | 1±1 | 0±0 | 0±0 | 1±1 | 1±1 | 0±0 | 0±0 | 1±3 | 1±1 | 0±0 | 0±0 |
| 48 | C | 4 | | 6±2 | 13±2 | 11±1 | 6±5 | 9±5 | 8±2 | 9±2 | 8±4 | 10±4 | 9±4 | 9≠3 | 1±3 |
| | Dr | 5 | | 4±3 | 2±2 | 0±0 | 0±0 | 3±1 | 2±1 | 1±1 | 0±0 | 1±3 | 1±1 | 0±0 | 0±0 |
| | ABA | 4 | | 6±3 | 5±3 | 1±2 | 0±0 | 4±2 | 5±2 | 2±2 | 0±0 | 4±4 | 3±3 | 0±0 | 0±0 |
| 72 | C | 4 | | 6±3 | 13±3 | 12±3 | 3±3 | 13±5 | 9±1 | 9±2 | 9±4 | 12±8 | 7±2 | 9±4 | 3±4 |
| | Dr | 4 | | 2±8 | 2±5 | 0±0 | 0±0 | 3±6 | 1±4 | 0±3 | 0±0 | 4±7 | 2±6 | 0±0 | 0±0 |
| | ABA | 5 | | 6±4 | 7±4 | 0±0 | 0±0 | 5±1 | 5±2 | 3±2 | 0±0 | 5±5 | 7±3 | 0±0 | 0±0 |
| 96 | C | 6 | | 5±2 | 11±1 | 10±2 | 5±3 | 9±3 | 8±2 | 9±1 | 7±4 | 11±3 | 8±2 | 9±3 | 4±4 |
| | Dr | 4 | | 8±3 | 5±2 | 0±0 | 0±0 | 6±2 | 4±2 | 3±2 | 0±0 | 7±5 | 6±2 | 0±0 | 0±0 |
| | ABA | 4 | | 8±2 | 7±1 | 0±0 | 0±0 | 7±2 | 7±2 | 3±3 | 0±0 | 7±6 | 7±2 | 0±0 | 0±0 |
| 120 | C | 3 | | 7±3 | 10±4 | 9±4 | 3±3 | 10±5 | 8±2 | 8±0 | 7±2 | 6±6 | 8±5 | 10±2 | 5±5 |
| | Dr | 3 | | 8±4 | 6±2 | 2±4 | 0±0 | 6±1 | 5±1 | 4±0 | 0±0 | 6±6 | 5±2 | 0±0 | 0±0 |
| | ABA | 3 | | 7±2 | 8±2 | 0±0 | 0±0 | 7±3 | 7±2 | 4±2 | 0±0 | 12±8 | 6±1 | 6±6 | 0±0 |
| 144 | C | 5 | | 5±3 | 11±3 | 10±4 | 5±4 | 10±3 | 8±1 | 9±3 | 9±4 | 8±6 | 9±2 | 9±3 | 4±3 |
| | Dr | 5 | | 5±3 | 5±2 | 3±3 | 0±0 | 7±4 | 5±2 | 2±1 | 0±0 | 7±7 | 5±4 | 0±0 | 0±0 |
| | ABA | 4 | | 6±3 | 5±4 | 2±4 | 0±0 | 6±1 | 7±1 | 4±3 | 0±0 | 7±2 | 7±2 | 0±0 | 0±0 |
| 168 | C | 4 | | 6±2 | 11±3 | 13±6 | 5±2 | 9±3 | 9±2 | 8±3 | 8±3 | 9±3 | 10±2 | 8±2 | 4±5 |
| | Dr | 4 | | 6±3 | 5±2 | 0±0 | 0±0 | 5±2 | 5±1 | 0±0 | 0±0 | 7±4 | 3±3 | 0±0 | 0±0 |
| | ABA | 6 | | 9±3 | 9±2 | 0±0 | 0±0 | 5±2 | 7±1 | 1±1 | 0±0 | 7±2 | 5±2 | 0±0 | 0±0 |

In control plants, the greatest greatest amount of labelling occurred in the distances 100-500 and 500-1000 μm from the cap junction. The ground meristem was the most actively sythesizing and dividing tissue. Labelling above about 1200 μm was sparse in all three of the primary meristems. The appearance of mitotic figures extended to the interface of the cytoplasic region and the zone of elongation. Their appearance beyond 1300 μm from the cap junction, however, was sporadic. Plate 3 shows autoradiographs from longitudinal sections of root apices from well-watered (control) plants at 24 hours (3a) and 168 hours (3b) after the beginning of the observation period. These roots were growing rapidly and show a definite quiescent centre at the stelar pole (3c).

Drought caused a general reduction in labelling throughout the densely cytoplasmic region. Initially the proximal regions of the meristem were most affected as the zone of cytoplasmic cells declined in size, but by 12 hours the stress had reduced labelling in all regions of the meristem (see Table 7) At about 72 hours, the labelling index began to recover in the regions 0-500 μm from the cap junction. This was about the same time as recovery in the size of the meristem began (Chapter II). Plate 4 consists of a series of autoradiographs showing the effects of drought on DNA synthesis at 6 hours (4a), 24 hours (4b), and 72 hours (4c). Plate 4c shows the root beginning to recover its capacity for DNA synthesis in the now much reduced meristem. Recovery of DNA synthesis is accompanied by activation of the QC (Plate 4d). After 72 hours, the regions of active labelling coincide roughly with the densely cytoplasmic region. The labelling index then holds at about 50-70% of control values in these regions for the duration of the experiment. There was some difference according to tissue type; the region of the procambium nearest the stelar pole was the first to recover.

In droughted plants, the mitotic index fell within 6 hours of treatment. This was most evident in the regions greater than 1000 μm from the cap junction (see Table 8). The decline in mitotic index was more gradual in the regions less than 1000 μm from the cap junction, but by 24 hours, there was general depression of mitosis throughout the

meristem. By 96 hours, there was a significant recovery in the frequency of mitotic figures in the regions within 500 μm of the cap junction. After this time, mitotic indices remained at about 40-60% of control values in the cytoplasmic regions of the meristem.

Treatment with $10^{-5}$ M ABA produces the same pattern of change as drought. A sudden initial decline in labelling in the regions greater than 1000 μm from the cap junction was followed by a general decline throughout the meristem (see Table 7). The period of recovery came sooner than in droughted plants, at about 48 hours, but again the area of active labelling, following recovery, roughly coincided with the densely cytoplasmic regions. Plate 5. consists of a series of autoradiographs showing the effects of ABA on DNA synthesis at 12 hours (5a), 48 hours (5b), and 72 hours (5c). The roots had begun to recover the capacity for DNA synthesis by 48 and 72 hours. Plate 5c shows activation of the QC. The QC, once activated, stayed active for the duration of the experiment; plate 5d shows a root with an actively labelling QC at 168 hours.

Mitotic indices reached a minimum at 24 hours in $10^{-5}$ M ABA-treated plants. The pattern was much the same as for droughted plants: however, the initial decline was somewhat sharper, and the recovery earlier than in droughted plants; by 48 hours there was a noticeable reappearance of mitotic figures in the cytoplasmic regions.

Plate 3.

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Autoradiographs from median longitudinal sections of roots from well-watered plants at 24 hours (i.e. 11 days old), 3a, and at 168 hours (i.e. 17 days old), 3b. Note labelled nuclei extending through the meristematic region in 3a and 3b. Note absence of labelled nuclei near stelar pole, arrow heads; X 125.

Plate 3c high magnification showing the location of the quiescent centre (QC) in an autoradiograph from a well-watered plant at 144 hours ((i.e. 16 days old). Note absence of labelled nuclei at stelar pole. X 560.

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Plate 3.

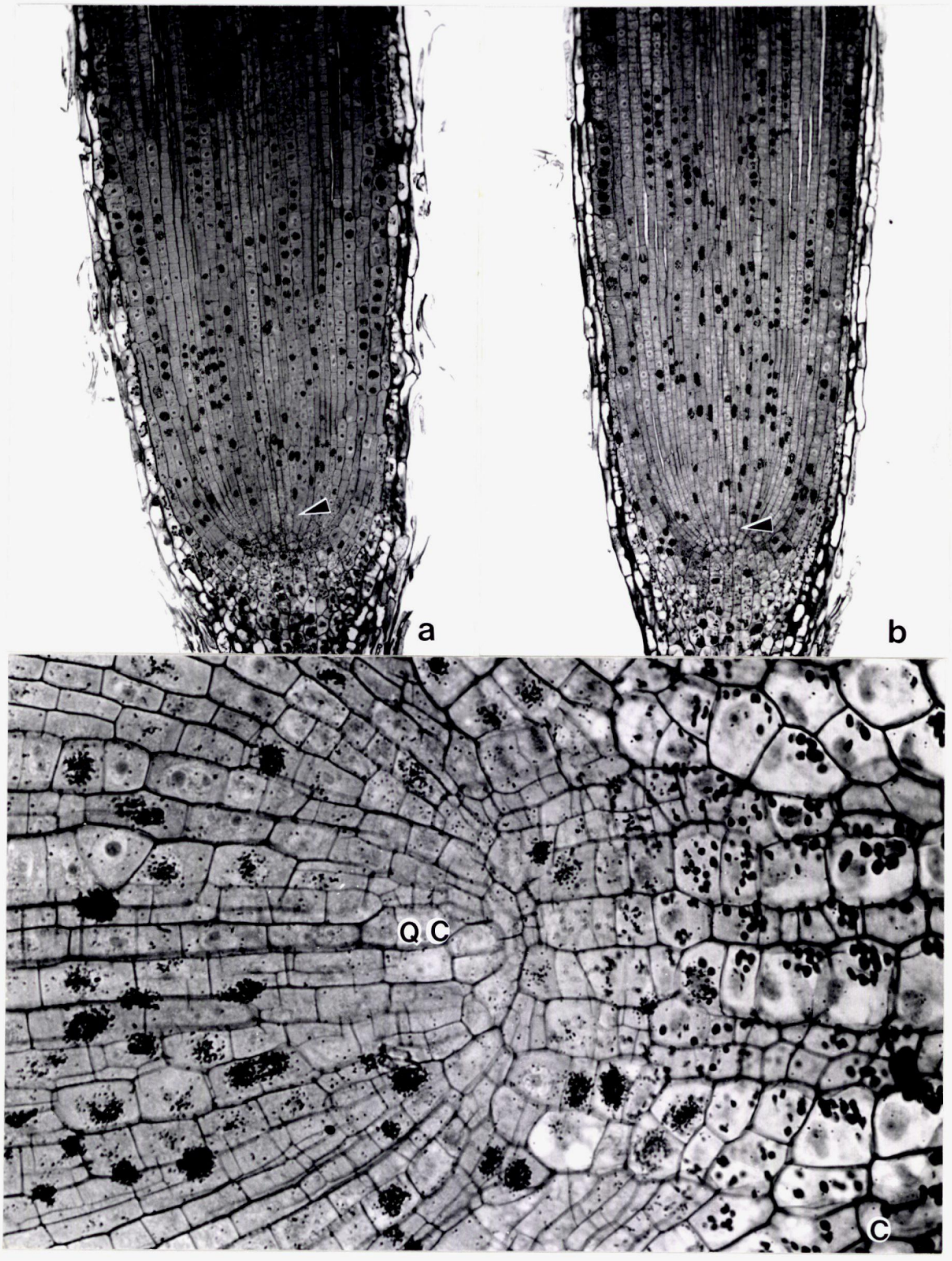

Plate 4.

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Autoradiographs from longitudinal sections of the roots of plants which had been droughted for 6 hours, 4a, 24 hours, 4b, and 72 hours, 4c and 4d.

Plate 4a: Autoradiograph showing vacuolation nearer the apex, relative to control treatment, (3a) and the absence of labelled nuclei in the vacuolating cells. Note the general reduction of labelled nuclei throughout the meristematic region. x 125.

Plate 4b: Autoradiograph showing advanced (nearer the apex) vacuolation; labelled nuclei appear only infrequently in the meristematic zone. The pinched appearance of the apex occurs occasionally in the roots of droughted plants, refer to Chapter I x 125.

Plate 4c: Autoradiograph of root beginning to show recovery in DNA synthesis after 72 hours of treatment. The meristematic region (densely cytoplasmic cells) has been greatly reduced relative to controls, but more labelled nuclei appear in the meristematic region, relative to 24 hour treatment. x 125.

Plate 4d: High magnification of the autoradiograph shown in 4c. Note the appearance of labelled nuclei and mitotic figures (arrow) at the stelar pole, indicating activation of the QC. x 420.

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Plate 4.

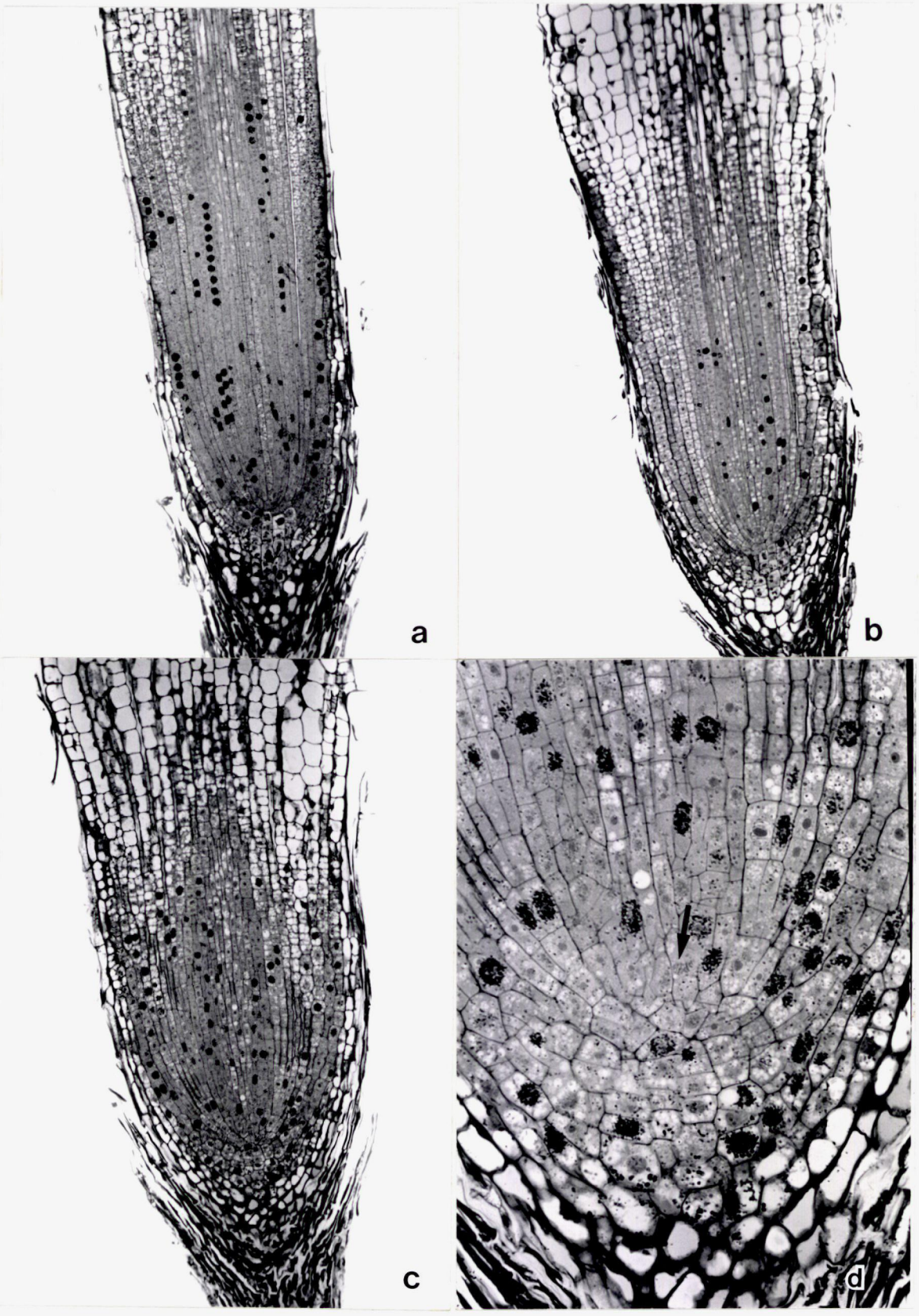

Plate 5

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Autoradiographs from longitudinal sections of the roots of plants which had been treated with $10^{-5}$ M ABA for 12 hours, 5a, 48 hours, 5b, and 72 hours, 5c and 5d.

Plate 5a: Autoradiograph showing only very infrequent occurrence of labelled nuclei after 12 hours of treatment. x 125.

Plate 5b. Autoradiograph showing advanced vacuolation in the apex after 48 hours of treatment; note recovery (relative to 12 hour treatment) in DNA synthesis in the cells remaining meristematic. x 125

Plate 5c: Autoradiograph showing larger cytoplasmic zone and recovery of DNA synthesis within that zone. Note the appearance of labelled nucleus at the stelar pole, indicating activation of the QC. Some nuclei in the QC are in prophase. x 125

Plate 5d: High magnification of the autoradiograph of root after 168 hours of treatment. Note the labelled nuclei (arrow) in the stelar pole, indicating that the QC once activated, remains active through the treatment period. x 420

Although the timing of events differs slightly, the general depression of DNA synthesis and advanced vacuolation in the root apex, followed by recovery in DNA synthesis in the cells remaining meristematic, indicates the same pattern of change as was observed in the roots of droughted plants in Plate 4.

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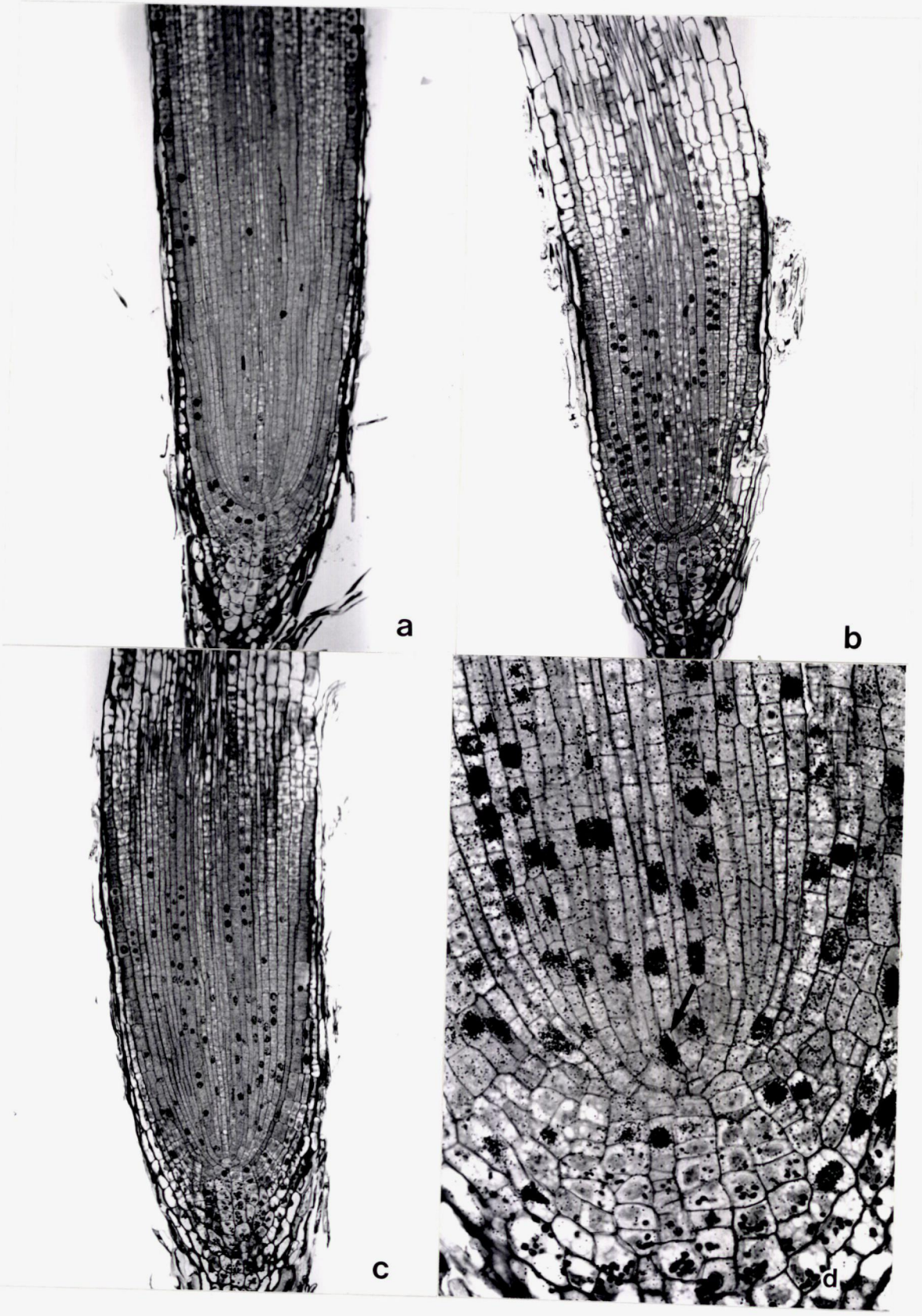
Plate 5.



At concentrations of $10^{-6}$ M and $10^{-7}$ M, labelling above 1000 μm regions fell off sharply as these cells vacuolated. The effects of ABA at these concentrations were not as severe in the regions which remained cytoplasmic (see Table 9). Again the region of active labelling coincided with the region of densely cytoplasmic cells.

Treatment with ABA at concentrations of $10^{-6}$ M and $10^{-7}$ M produced a significant decline in the mitotic index in the vacuolating regions. As with labelling, the depression of mitosis in the regions which remain cytoplasmic was not as severe as in plants treated with $10^{-5}$ M ABA.

Table 9.

| Labelling Index | | | Procambium | | | | Ground Meristem | | | | Protoderm | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hour | n | Section | 0-100 | 100-500 | 500-1000 | 1000-1500 | 0-100 | 100-500 | 500-1000 | 1000-1500 | 0-100 | 100-500 | 500-1000 | 1000-1500 |
| ABA $10^{-6}$ M | | | | | | | | | | | | | | |
| 6 | 4 | | 14 ± 5 | 28 ± 3 | 2 ± 2 | 0 ± 0 | 15 ± 7 | 39 ± 10 | 14 ± 13 | 0 ± 0 | 32 ± 3 | 36 ± 12 | 12 ± 17 | 0 ± 0 |
| 24 | 4 | | 17 ± 8 | 38 ± 10 | 0 ± 0 | 0 ± 0 | 19 ± 12 | 38 ± 10 | 2 ± 3 | 0 ± 0 | 35 ± 19 | 22 ± 15 | 0 ± 0 | 0 ± 0 |
| 48 | 5 | | 15 ± 3 | 28 ± 11 | 1 ± 1 | 0 ± 0 | 17 ± 7 | 45 ± 9 | 12 ± 6 | 0 ± 0 | 30 ± 8 | 38 ± 5 | 5 ± 5 | 0 ± 0 |
| ABA $10^{-7}$ M | | | | | | | | | | | | | | |
| 6 | 5 | | 22 ± 6 | 28 ± 8 | 2 ± 2 | 0 ± 0 | 21 ± 3 | 46 ± 6 | 5 ± 4 | 0 ± 0 | 34 ± 15 | 38 ± 9 | 4 ± 6 | 0 ± 0 |
| 24 | 3 | | 17 ± 9 | 25 ± 13 | 0 ± 0 | 0 ± 0 | 29 ± 6 | 36 ± 9 | 0 ± 0 | 0 ± 0 | 45 ± 2 | 44 ± 3 | 0 ± 0 | 0 ± 0 |
| 48 | 4 | | 14 ± 7 | 44 ± 9 | 0 ± 0 | 0 ± 0 | 22 ± 5 | 46 ± 6 | 32 ± 8 | 0 ± 0 | 49 ± 13 | 48 ± 5 | 24 ± 6 | 0 ± 0 |

| Mitotic Index | | | Procambium | | | | Ground Meristem | | | | Protoderm | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hour | n | Section | 0-100 | 100-500 | 500-1000 | 1000-1500 | 0-100 | 100-500 | 500-1000 | 1000-1500 | 0-100 | 100-500 | 500-1000 | 1000-1500 |
| ABA $10^{-6}$ M | | | | | | | | | | | | | | |
| 6 | 4 | | 6 ± 3 | 6 ± 2 | 2 ± 2 | 0 ± 0 | 5 ± 1 | 6 ± 1 | 6 ± 2 | 0 ± 0 | 7 ± 6 | 4 ± 4 | 2 ± 3 | 0 ± 0 |
| 24 | 4 | | 4 ± 3 | 5 ± 1 | 1 ± 1 | 0 ± 0 | 5 ± 2 | 4 ± 1 | 1 ± 1 | 0 ± 0 | 4 ± 4 | 7 ± 2 | 0 ± 0 | 0 ± 0 |
| 48 | 5 | | 4 ± 2 | 6 ± 2 | 2 ± 2 | 0 ± 0 | 4 ± 2 | 5 ± 1 | 4 ± 2 | 0 ± 0 | 3 ± 4 | 5 ± 2 | 1 ± 2 | 0 ± 0 |
| ABA $10^{-7}$ M | | | | | | | | | | | | | | |
| 6 | 5 | | 6 ± 5 | 7 ± 5 | 1 ± 2 | 0 ± 0 | 4 ± 1 | 6 ± 3 | 4 ± 3 | 0 ± 0 | 9 ± 3 | 7 ± 2 | 3 ± 1 | 0 ± 0 |
| 24 | 5 | | 3 ± 1 | 3 ± 2 | 0 ± 0 | 0 ± 0 | 2 ± 3 | 4 ± 2 | 1 ± 1 | 0 ± 0 | 6 ± 2 | 2 ± 2 | 0 ± 0 | 0 ± 0 |
| 48 | 4 | | 6 ± 2 | 3 ± 3 | 0 ± 0 | 0 ± 0 | 4 ± 1 | 5 ± 3 | 2 ± 4 | 0 ± 0 | 6 ± 2 | 6 ± 3 | 0 ± 0 | 0 ± 0 |

Table 9. Labelling and Mitotic indices in the procambium, ground meristem and protoderm at measured distances from the cap junction of the roots of plants treated with $10^{-6}$ M and $10^{-7}$ M ABA. Measurements taken at 6, 24, and 48 hours after the beginning of treatment; n = number of sections examined at each sample point; statistics as in Table 7.

When these data are taken together, the following observations hold in both droughted and ABA-treated plants. Cells in the proximal regions of the root meristem elongate and go out of cycle. In the distal regions of the meristem, labelling and mitotic indices decline to a minimum and then begin to recover to a level at which they remain for the duration of the experiment. The decline in labelling index appears to be sharper than the decline in mitotic index. This indicates that cells which are in $G_2$ and at the beginning of treatment continue through mitosis and that the cell cycle is arrested in $G_1$. It appears then that drought and ABA-treatment both depress cell division by preventing cells from entering S-phase, the period of DNA synthesis. Clowes and Stewart (1967) argued that meristematic cells rest in $G_1$ during cold-induced dormancy in the long roots of Zea mays.

The recovery of activity is most rapid and most nearly complete in the procambium near the stelar pole. Indeed, there is evidence of an absolute increase in labelling index and mitotic index in this region, relative to control values. In sunflower roots, the quiescent centre QC is confined to the stelar pole (Clowes, 1981). This suggests that the QC may activate in droughted and ABA-treated plants. An examination of autoradiographs from the root apices of control plants, revealed a QC which began at the stelar pole and extended about 40 μm proximally in the procambium. The QC began to activate at about 24 hours in droughted and 48 hours in ABA-treated plants (see Table 10) There is considerable variation between samples, but this was largely a function of the small population of cells in the QC. A difference of 1 labelled cell per section translates into a large difference in percent. There was an early rise in the mitotic index, but not in labelling index, with ABA-treatment. This would suggest that the cells of the QC may have been in $G_2$ prior to treatment. If so, then the situation in the QC of sunflower differs from that in Zea where the cells of the QC rest in $G_1$ (Clowes and Stewart, 1967). This observation may be an anomoly, since it is not evident in droughted plants. If the cells of the QC were in $G_2$, then upon activation, regardless of cause, they would have to pass through mitosis before

reaching S-phase. Alternatively, the initial rise in mitotic index in the QC may have been missed in droughted plants, and the observation for ABA-treated plants accurate.

Table 10.

| | Control | | Drought | | ABA | |
|---|---|---|---|---|---|---|
| hours | labelling index | mitotic index | labelling index | mitotic index | labelling index | mitotic index |
| 6 | 0 ± 0 | 1.3 ± 2.8 | 1.9 ± 3.9 | 0 ± 0 | 3.0 ± 4.6 | 2.9 ± 4.6 |
| 12 | 2.7 ± 3.7 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 1.7 ± 3.4 | 3.4 ± 4.0 |
| 24 | 1.7 ± 3.4 | 1.6 ± 3.4 | 13.7 ± 11.1 | 5.1 ± 4.7 | 1.3 ± 2.8 | 0 ± 0 |
| 48 | 3.8 ± 5.4 | 1.7 ± 3.3 | 7.1 ± 4.1 | 2.3 ± 3.1 | 14.8 ± 8.7 | 5.8 ± 4.0 |
| 72 | 2.9 ± 3.5 | 0 ± 0 | 20.0 ± 7.6 | 7.1 ± 1.1 | 12.3 ± 3.6 | 7.4 ± 6.1 |
| 96 | 0.9 ± 1.9 | 0 ± 0 | 16.5 ± 9.1 | 7.4 ± 4.9 | 9.1 ± 7.2 | 5.4 ± 3.6 |
| 120 | 3.9 ± 4.3 | 1.4 ± 3.8 | 9.4 ± 4.3 | 8.5 ± 2.5 | 15.0 ± 5.9 | 7.5 ± 0.7 |
| 144 | 2.0 ± 4.5 | 0 ± 0 | 9.2 ± 3.8 | 5.0 ± 2.9 | 5.0 ± 3.4 | 6.8 ± 3.6 |
| 168 | 1.9 ± 1.2 | 1.2 ± 2.7 | 13.1 ± 10.3 | 9.7 ± 5.1 | 7.2 ± 6.1 | 9.6 ± 1.0 |

Table 10 shows the labelling and mitotic indices in the quiescent centre of control, droughted $10^{-5}$ M ABA-treated plants over 168 hours of treatment. Number of sections, n, at each time point as in Table 7. Statistics as in Table 7.

The exogenous addition of sucrose did not overcome drought and ABA-induced inhibitions in mitotic activity at the meristem. Table 11 shows labelling and mitotic indices, at 48 and 72 hours, of droughted and ABA-treated plants to which sucrose had been supplied through a leaf flap. Plate 6a shows a sucrose-supplemented root which had been droughted for 48 hours. The meristem is much reduced in size relative to controls and labelled nuclei appear infrequently. Plate 6b shows a sucrose-supplemented root which was treated with $10^{-5}$ M ABA for 72 hours (compare with Plate 4c). The size of meristem is reduced relative to controls. This root was beginning to recover its capacity for DNA synthesis in the lower regions of the meristem (compare with Plate 5c). Sucrose supplements prevented necrosis in droughted roots and increased root to shoot biomass ratios (Chapter II), but they had little or no effect on the response at the meristem to drought or ABA.

Table 11.

| Labelling Index | | | Procambium | | | | Ground Meristem | | | | Protoderm | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hour | n | Section | 0-100 | 100-500 | 500-1000 | 1000-1500 | 0-100 | 100-500 | 500-1000 | 1000-1500 | 0-100 | 100-500 | 500-1000 | 1000-1500 |
| 48 Dr | 4 | | 10 ± 7 | 11 ± 12 | 0 ± 0 | 0 ± 0 | 12 ± 8 | 15 ± 7 | 4 ± 4 | 0 ± 0 | 18 ± 10 | 13 ± 9 | 0 ± 0 | 0 ± 0 |
| | | | 16 ± 8 | 25 ± 6 | 0 ± 0 | 0 ± 0 | 17 ± 6 | 26 ± 11 | 3 ± 4 | 0 ± 0 | 22 ± 8 | 31 ± 16 | 0 ± 0 | 0 ± 0 |
| ABA | 5 | | | | | | | | | | | | | |
| 72 Dr | 4 | | 11 ± 9 | 14 ± 13 | 0 ± 0 | 0 ± 0 | 14 ± 11 | 8 ± 7 | 0 ± 0 | 0 ± 0 | 27 ± 9 | 14 ± 10 | 0 ± 0 | 0 ± 0 |
| | | | 19 ± 5 | 23 ± 5 | 0 ± 0 | 0 ± 0 | 19 ± 7 | 27 ± 3 | 0 ± 0 | 0 ± 0 | 36 ± 5 | 24 ± 5 | 0 ± 0 | 0 ± 0 |
| ABA | 3 | | | | | | | | | | | | | |

| Mitotic Index | | | Procambium | | | | Ground Meristem | | | | Protoderm | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hour | n | Section | 0-100 | 100-500 | 500-1000 | 1000-1500 | 0-100 | 100-500 | 500-1000 | 1000-1500 | 0-100 | 100-500 | 500-1000 | 1000-1500 |
| 48 Dr | 4 | | 2 ± 2 | 2 ± 2 | 0 ± 0 | 0 ± 0 | 1 ± 2 | 1 ± 0 | 0 ± 0 | 0 ± 0 | 2 ± 3 | 1 ± 2 | 0 ± 0 | 0 ± 0 |
| | | | 6 ± 3 | 3 ± 2 | 1 ± 1 | 0 ± 0 | 3 ± 3 | 3 ± 1 | 1 ± 2 | 0 ± 0 | 3 ± 1 | 3 ± 2 | 0 ± 0 | 0 ± 0 |
| ABA | 5 | | | | | | | | | | | | | |
| 72 Dr | 4 | | 3 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 2 ± 2 | 1 ± 2 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 1 ± 3 | 0 ± 0 | 0 ± 0 |
| ABA | 3 | | 3 ± 1 | 2 ± 0 | 0 ± 0 | 0 ± 0 | 2 ± 1 | 1 ± 0 | 0 ± 0 | 0 ± 0 | 6 ± 5 | 0 ± 0 | 0 ± 0 | 0 ± 0 |

Table 11. Labelling and Mitotic indices in the procambium, ground meristem and protoderm at measured distances from the cap junction. Plants were supplemented with 0.3 M sucrose and either droughted (Dr) or treated with 10-5 M ABA (ABA). Measurements taken at 48 and 72 hours after the beginning of treatment; n = number of sections examined at each sample point; statistics as in Table 7.

Plate 6.

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Autoradiographs of roots from droughted, 6a, and ABA-treated, 6b, plants which had been given a supplement of sucrose.

Plate 6a: Autoradiograph of root from droughted plant after 48 hours of droughting with sucrose supplement. The sucrose supplement does not suppress drought-induced changes in the root apex; note infrequent occurrence of labelled nuclei and advanced vacuolation. x 125.

Plate 6b: Autoradiograph of root from $10^{-5}$ M ABA-treated plant after 48 hours of treatment with sucrose supplement. The sucrose supplement does not suppress ABA-induced changes in the root apex; note infrequent occurrence of labelled nuclei (relative to controls) and advanced vacuolation. x 125.

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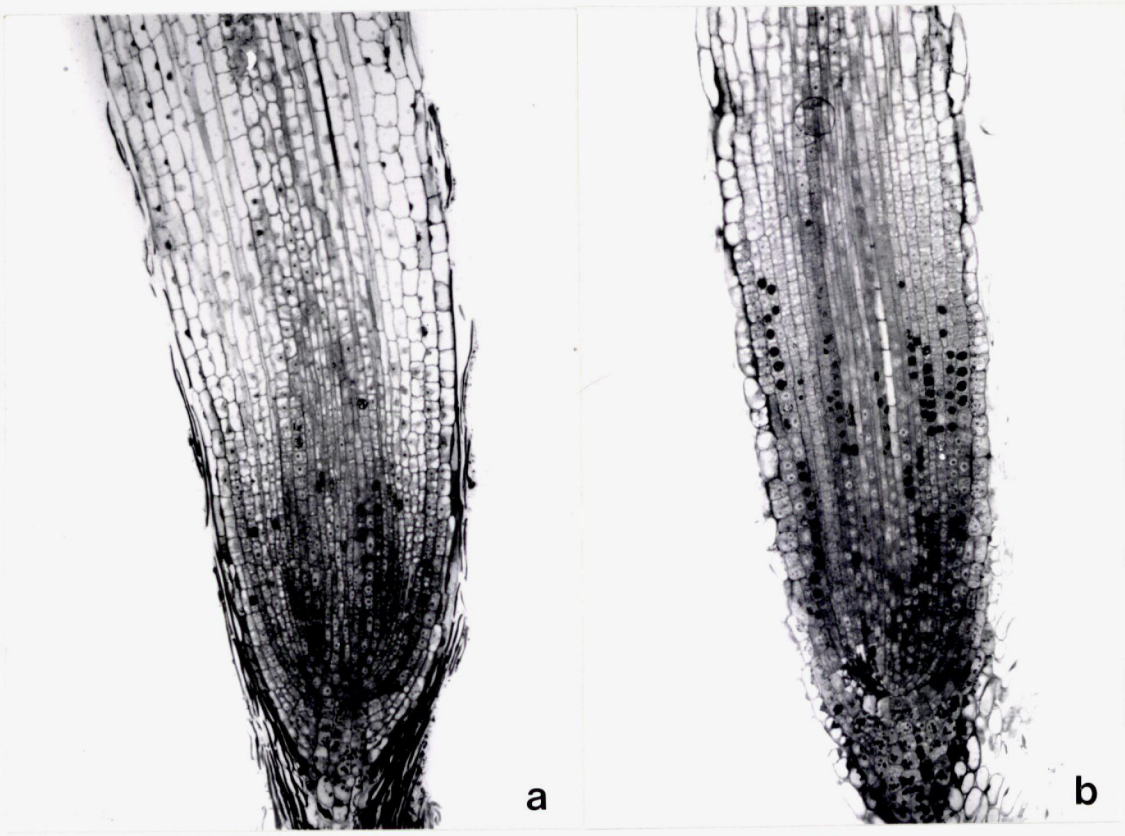
Plate 6.



## DISCUSSION

The autoradiographs show the same anatomical changes noted in Chapter II; vacuolation and cell elongation advanced distally into the meristem. Furthermore, as predicted in Chapter II, there was a general decline in mitotic activity in the region of the meristem which remained cytoplasmic. Of the two parameters of growth, cell elongation and the addition of new cells, it seems that it is the latter which is most affected by drought and ABA. This observation applies only to the meristem; final cell sizes in the zone of elongation and maturation were not measured, and they may well have been smaller in the treated plants. This would seem likely since root elongation rates are very low.

Sucrose supplements, although they had an effect on root morphology and helped to maintain the number of viable meristems (see Chapter II), did not support mitotic activity in the apical meristems. The changes in the apical meristem to drought do not result from loss of turgor (see Chapter II) or a reduction in carbohydrate supplies. Rather, it seems that these changes proceed in direct response to a drop in water potential external to the root.

These studies further elucidate the drought-induced response at the meristem. Moreover, they reveal a mechanism by which ABA could mediate part of that response.

In many ways, the initial response of sunflower apices is analogous to dormancy in cold-treated Zea mays roots (Barlow and Rathfelder, 1985, Clowes and Stewart, 1967). Mitotic activity in the meristem virtually stops; recovery is accompanied by activation of the QC. The difference is that recovery in droughted plants proceeds before the alleviation of stress. In droughted plants it would seem that dormancy-like conditions are imposed temporarily by a direct signal from the apical meristem.

The model of Torrey and Feldman (1977) may apply here. They argue that the relationship of the various components of the meristem in a rapidly elongating root results

in "the physiological homeostasis which produces the genetic characteristics of that root. Drastic alterations in this balanced state will effect a change in the activities and, in turn, in structure, until a new balance has been achieved. The new state is usually initiated when the cells of the QC begin active division" (Torrey and Feldman, 1977).

The apical meristem undergoes modifications in response to drought, which result in a reduction in its size and a general depression of its mitotic activities. Upon activation of the QC, the meristem emerges from a dormancy-like period as a smaller, less active entity which has lower demands for resources. The result is an apical meristem whose activities are in line with the physiological conditions imposed by drought.

Abscisic acid affects the cell cycle, probably by arresting meristematic cells in $G_1$, and subsequently initiates mitotic activity in the QC. This effect could certainly account for reduced mitotic activity in the root meristems of droughted plants; it may also account for reduction in the size of the meristematic region. The writer suggests that a meristematic cell may have to go through a certain number of divisions before it can differentiate. This number is something less than its full potential for division. Halting DNA synthesis for a period of time may be enough to trigger differentiation in cells that have passed through a critical number of divisions. If so, then it is tempting to identify those cells proximal to the QC which remain cytoplasmic with the proximal meristem of Torrey's and Feldman's model.

The direct effect of ABA on the cell cycle then identifies a mechanism by which this hormone could mediate drought-induced developmental change at the meristem. The fact that ABA levels increase in the roots of sunflower in response to drought (Hubick, et al., 1986) lends credibility to this statement.

# CHAPTER IV : ENDOGENOUS ABA

## INTRODUCTION

The observations that ABA levels increase in the roots of water-stressed plants (Hubick, 1983, Hubick, et al., 1986, Lachno, 1984, Milborrow and Robinson, 1973) and that this increase does depend on transport from the shoot (Rivier, et al., 1983, Walton, et al., 1976) suggest that ABA may have other roles to play, apart from its function as agent of stomatal closure, in the adaptation of plants to drought stress. The writer argues that one of the roles of ABA is to mediate developmental change directly at the root apical meristem. If so, then drought must induce an increase in endogenous ABA levels in the regions of the meristem, since the writer argues, the meristem responds directly to an external drop in $\Psi$ and not to secondary signals such as loss of turgor or reduced carbohydrate supplies.

In this chapter, the writer reports on ABA levels in the root apices of well-watered and droughted plants. Endogenous ABA levels were quantified by a highly sensitive and selective technique, selected ion monitoring gas chromatography-mass spectrometry (GC-MS-SIM), using hexadueterated cis, trans ABA as an internal standard.

## MATERIALS AND METHODS

The plant material, growth conditions and drought regime are as in Chapter II. Leaf $\Psi$ was measured at each sample point as indicator of stress.

### Harvest

In the first experiment (trial 1), droughted and control samples (about 1000 root apices sample$^{-1}$) were harvested at 24, 72, and 168 hours after the initiation of drought.

The experiment was repeated (trial 2), since not enough material could be harvested at one time to provide for more than one sample. The 3-mm apical segments were cut from the main root and from elongated primary laterals. Short laterals were rejected. The root apices were cut on ice-cold plates and placed immediately into liquid $N_2$. All work was performed in reduced light. After freezing in liquid $N_2$, the samples were freeze dried and weighed. The dry weight of the samples ranged from 9 to 16 mg.

In a second experiment, 3-mm apical root segments were harvested from 11-day-old plants and allowed to dry in the air for 1 hour in darkness at room temperature, then frozen in liquid $N_2$ and treated as the samples from experiment 1, thereafter. Root apices were harvested in small batches that were timed separately. The individual small harvests were pooled to make up sample sizes of about 1000 root apices. The appropriate controls for this experiment are the 24-hour control samples. The experiment was repeated 2 more times from another harvest of plants. In the repeat experiments, the degree of stress on the root apices was monitored as water potential. Two methods were used: the dew point method had proven unreliable for measuring $\Psi$ in the root apices from droughted plants. For that reason, an isopiestic method, Chardakov's dye drop method (Chardokov, 1948), was used as a check on the accuracy of the dew point readings. In repeated tests, the dew point values fell within the upper and lower limits of $\Psi$ determined by Chardakov's method. For estimation of the $\Psi$ of the samples, batches of about 50 root apices were taken from the sample, after treatment, for dew point readings. Three readings were taken from each sample. Root apices from each sample were tested against sucrose solutions of known $\Psi$ (from -1.56 to -2.69 MPa) for determination of $\Psi$ by Chardakov's method; for each test, one ml of solution was placed in a sealed Pasteur pipet, to this about 50 root apices from the sample were added.

Two further control samples were done for experiment 2. Root apices were excised and then placed in 2 ml of bathing solution (0.75 strength Hoagland solution) for 1 hour.

The root apices and the solution in which they were kept hydrated were analyzed separately for ABA.

**Extraction Procedure**

The tissue was extracted 3 times in 13.3 ml of methanol:water:acetic acid (80:19:1 v/v) by grinding in a cold mortar and pestle. Approximately 150 ng of d6ABA (hexadeuterated cis, trans ABA) as an internal standard (d6 ABA was taken from a single dilution and the volume was kept constant) and 50,000 dpm of [$^3$H]ABA (Amersham 24 Ci mmol$^{-1}$) were added at the beginning of the extraction procedure. The 80% methanol filtrate (40 ml) was then passed through a preparatory column of $C_{18}$ material (Kosioka, et al., 1983) to remove nonpolar compounds. The column was then washed with an additional 20 ml of extraction solvent. The 60 ml of eluate was then taken to the aqueous phase in vacuo and partitioned 3 times against equal volumes of diethyl ether at pH 2.8. The organic phase containing the ABA was taken to dryness in vacuo, at 35° C, resuspended in absolute methanol, filtered through a 0.5 µm FH type Millipore filter, taken to dryness under a $N_2$ stream, and resuspended in 500 µl of 32.5% methanol:1% acetic acid.

The entire sample was then loaded onto a Waters reverse-phase $C_{18}$ µ-bondapak analytical column (4 mm x 30 cm). The HPLC conditions were: 32.5% methanol:1% acetic acid isocratic for 55 minutes, followed by a 5-minute gradient to absolute methanol and 20 minutes of absolute methanol at a flow rate of 1 ml min$^{-1}$. Under these conditions, the Rt for ABA was 44 to 46 minutes. The UV-absorbing substances in the extract were monitored in-line by UV absorbance at 254 nm. The internal standard of [$^3$H]ABA was monitored by a Berthold model LB503 HPLC radioactivity detector (Labserco Ltd. Oakville, Ontario, Canada) equipped with a homogeneous flow-through scintillant cell. The radioactive fractions were collected and taken to dryness under $N_2$, in a Reacti-vial,

then methylated in a few drops of methanol with 300 µl of cold ethereal diazomethane. The reaction mixture was taken to dryness under $N_2$, then resuspended in 500 µl of 32.5% methanol:1% acetic acid and again subjected to reverse-phase $C_{18}$HPLC under the same conditions noted above. The retention time for ABA-Me was 65.5 minutes. The radioactive ABA-Me fraction was collected, taken to dryness under $N_2$, and resuspended in 25 µl of methylene chloride.

**GC-MS-SIM Analysis**

For quantitative GC-SIM analysis, the writer used a method similar to that of Rivier and Pilet (1982). However, the writer's stock of d6ABA could not be weighed with confidence. Therefore, a constant amount, about 150 ng of d6ABA, was used in each extract. This was accomplished by making an approximate dilution from which a constant volume was taken for each extract. Endogenous ABA levels were quantified from a standard curve, which established the ratio of the base ion (m/z 194) peak height of the constant amount of d6ABA to the base ion (m/z 190) peak height of precisely known quantities of authentic ABA (Sigma grade IV). The following ratios of ABA and d6ABA were used for the standard curve: a constant amount of d6ABA and 50,000 dpm of [$^3$H]ABA were mixed with 0, 0.5, 2.5, 5.0. and 25.0 ng of ABA. Standards were handled in the same way as samples. Quantification of the samples was done by entering the ratio of m/z 190 to m/z 194 into the standard curve.

GC-MS conditions were: 50°C injector port; column temperature 50°C initial, programmed to increase by 20°C min$^{-1}$ to 240°C. The Rt for ABA-Me was 9.73 ± 0.05 (range) min. A Hewlett-Packard model 5790A series GC with a 15-m DB5 capillary column (Chromatographic Specialities Ltd.) connected to Hewlett-Packard 5970 MSD was used. The selected ion monitoring mode was used with the Autotune program: m/z 190

and m/z 194 were monitored, window size 0.1, ioning potential 1800 eV, dwell time 50 ms.

ABA was identified from the Rt of ABA and of ABA-Me on HPLC, and from the Rt of ABA-Me on the GC column. A full-spectrum analysis on the most active sample (72 hours drought) was also attempted. The following ion fragments, characteristic of ABA-Me (Rivier and Pilet, 1982), were found at the correct Rt: m/z 278, m/z 222, m/z 190, m/z 162, m/z 134, m/z 125, m/z 106, m/z 91.

The advantage of the internal standard technique is that it allows for the trace analysis of ABA from very small amounts of tissue (Rivier and Pilet, 1982). The fact that quantification is accomplished from a ratio greatly reduces error from calculating losses and from machine variability. Variability between samples was about ± 3% based on repeated injections of known standards.

## RESULTS

The standard curve yielded the following relationship: a = 0.0063; b = 0.0290; where:

$$y = \frac{\text{m/z 190 peak height}}{\text{m/z 194 peak height}}, \quad x = \text{ng ABA; when } y = ax + b.$$

The b term accounts for any contribution from d6ABA or from [$^3$H]ABA to the m/z 190 peak. The plot of this curve was linear through the concentrations tested (0 to 25 ng): r = 0.9986.

Endogenous ABA levels showed an increase with drought at all sample times in experiment 1 (Table 12). After 24 hours of drought, the increase was 6.4-fold in trial 1 and 3.1-fold in trial 2 relative to control values. After 72 hours, the increases were much greater: 19.3-fold in trial 1 and 24.6-fold in trial 2 relative to control values. After 168 hours, the increases relative to controls declined: 5.9-fold in trial 1 and 3.5-fold in trial 2. Overall, the trends were similar in trials 1 and 2: initially ABA levels increased rapidly with

drought, but then tended to level off or decline as stress continued and became more severe. Leaf $\Psi$, although not an accurate monitor of stress at the root tip, does give some indication of the increasing degree of drought stress encountered by the plant. At 24 hours, the sunflower seedlings showed few visible signs of stress. By 72 hours, the droughted plants were suffering noticeably: chlorosis of leaves was evident, and growth was obviously reduced relative to control plants. After 168 hours of drought, plants were severely stressed; some tissue necrosis had occurred in both root and shoot tissue. Care was taken to avoid harvesting necrotic tissue. The ABA levels in the root apices of control plants increased about 2- to 4-fold by 168 hours relative to earlier control samples. There is no obvious explanation for this, although by hour 168, the plants had begun to outgrow the capacity of the aeroponics chambers and were under mild stess, as indicated by leaf $\Psi$ (Table 12).

Table 12.

| Duration of Drought | | Trial 1 | | Trial 2 | |
|---|---|---|---|---|---|
| | | ABA | Leaf Ψ | ABA | Leaf Ψ |
| hours | | ng $g^{-1}$ dw[a] | MPa ± SD | ng $g^{-1}$ dw | MPa ± SD |
| 24 | Control | 65 | - 0.38 ± 0.08 | 92 | - 0.41 ± 0.08 |
| | Drought | 418 | - 0.76 ± 0.15 | 290 | - 0.71 ± 0.14 |
| 72 | Control | 106 | - 0.42 ± 0.12 | 57 | - 0.35 ± 0.06 |
| | Drought | 2048 | - 1.47 ± 0.18 | 1411 | - 1.34 ± 0.21 |
| 168 | Control | 275 | - 0.63 ± 0.08 | 231 | - 0.56 ± 0.10 |
| | Drought | 1630 | - 2.07 ± 0.24 | 799 | - 1.79 ± 0.30 |

Table 12. Levels of ABA in the 3-mm apical segment of sunflower roots, after 24, 72, and 168 hours of drought. Leaf Ψ is included as an indicator of the water stress to which the plants from each treatment were subjected. Three factor (treatment, time, trials) analysis of variance showed a significant difference between the roots of droughted and control plants, $p < 0.05$. No other factor was significant; [a]dw, dry weight

In the second experiment, where excised root apices were allowed to dessicate at room temperature, the increases relative to controls were remarkable: up to about 50 times control levels (Table 13). Control plants for this experiment are the same as for 24 hours in experiment 1. There was an appreciable disparity between absolute levels of ABA (4-fold) in the two trials of experiment 2. This was due, perhaps, to variability in the method of stressing. In the repeat trials (trials 3 and 4, Table 13), root tip $\Psi$ was monitored as an indicator of stress. In separate tests, the $\Psi$ of root apices dessicated for 1 hour at room temperature varied from -1.5 to -2.8 MPa. The $\Psi$ of the sample root apices varied considerably as did the ABA levels in the samples (Table 13). It would seem that variation in the levels of stress accounts at least partially for the disparity in absolute levels of ABA in the dessicated samples.

Table 13.

| Trial | Treatment | ABA | Root Tip Ψ | |
|---|---|---|---|---|
| | | | Dew Point (± SD) | Chardokov |
| | | ng $g^{-1}$ dw[a] | MPa | |
| 1 | Dessicated | 1225 | Not measured | Not measured |
| 2 | Dessicated | 4977 | Not measured | Not measured |
| 3 | Dessicated | 700 | - 1.79 ± 0.37 | - 1.56 > Ψ > - 2.11 |
| | Hydrated | 94 | 0.00 | Ψ > - 0.08 |
| | Effluxed into solution | 59 | N.A. | N.A. |
| 4 | Dessicated | 1654 | - 2.33 ± 0.41 | - 2.11 > Ψ > - 2.69 |
| | Hydrated | 104 | - 0.09 ± 0.05 | Ψ > - 0.08 |
| | Effluxed into solution | 81 | N.A. | N.A. |

Figure 13. Levels of ABA in 3-mm apical root segments which had been excised from 11-day-old seedlings and then dessicated or kept hydrated at room temperature for one hour. Trials 1 and 2 were taken from the same harvests as 24-hour controls shown in Table 12; trials 3 and 4 were taken from a separate harvest. The water potential of the excised root apices was measured by dew point psychrometry and Chardakov's method. The amount of ABA effluxed from hydrated controls into the bathing solution is also shown; it is expressed as ng effluxed $g^{-1}$ dry weight of root tip tissue. [a]dw, dry weight; N.A., not applicable.

Nevertheless, in all four trials, the trend was the same: ABA levels increased dramatically in the excised root apices subjected to rapid dessication. This experiment shows that the apical 3 mm possesses the capacity to produce ABA in large quantity and to produce it rapidly in response to drought. ABA levels in the control samples which had been excised and kept hydrated were in the same range as aeroponically grown control samples. This indicates that the dessication treatment, not excision, was responsible for the large increase in ABA levels in the dessicated root apices. The $\Psi$ of the hydrated control samples was near 0.00 MPa (Table 13). This compares with $0.11 \pm 0.04$ MPa for the root apices of aeroponically grown control plants. The bathing solution used to hydrate excised control root apices contained measurable amounts of ABA; this indicates some efflux of ABA from well-watered root apices. Efflux may be a means of keeping ABA levels low in well-watered roots.

The data from experiment 1 were tested by three factor analysis of variance comparing treatments (droughted versus control), hours of treatment, and trials. First-order interactions were not significant at the $p < 0.05$ level. Of the main effects, only treatment was significant at the $p < 0.05$ level. This indicates that the chief factor involved in the differences between readings was the drought treatment. No statistical analysis was possible for experiment 2.

## DISCUSSION

There are two noteworthy observations from these results: (a) levels of ABA in the root apices of sunflower increase with drought stress; and (b) the apical 3-mm segment of the root is capable of producing ABA in response to drought.

Watts, et al. (1981) have suggested that ABA mediates drought responses indirectly through its effect on solute transport. However, the fact that ABA levels rise dramatically

in the root apices suggests that, in sunflower at least, ABA may have a more direct effect on root development.

ABA has been variously reported to both promote and inhibit root growth. Most studies found ABA inhibitory to root growth and extension (for a full discussion, see Pilet and Chanson, 1981). The effects may be concentration related; in lower concentration, ABA may promote growth (Pilet and Rebeaud, 1983, Watts, et al., 1981).

In most cases, water stress reduces root growth (Ciamprova and Luxova, 1976, Hubick, 1983, Hubick, et al., 1986, Prasad, et al., 1982, Watts, et al., 1981), although mild stress can increase extension (Hsiao and Acevedo, 1974, Sharp and Davies, 1979, Watts, et al., 1981. As mentioned above, ABA inhibits root growth at higher concentrations, but may promote growth at lower concentrations. Controlling root growth through changes in the endogenous levels of ABA may provide the plant with a mechanism to balance the need for expansion of the root system in droughty soils with the restrictions placed upon it by reduced carbon supplies from the shoot. If so, then increasing ABA levels at the root apices could act as an adaptive mechanism which serves to optimize available reserves within a changing environment.

In the introduction, the writer argues that for ABA to be considered a mediator of the drought responses described in Chapters II and III, its endogenous levels must increase in the regions of the meristem. These findings meet that stipulation. If changes in ABA levels at the root apex do indeed serve to mediate drought-induced development change during periods of drought stress, then it would seem that the root apex has the capacity to perceive and respond directly to the stress without any requirement from the rest of the plant (Table 13).

# CHAPTER V : PHOTOSYNTHATE TRANSPORTS

## INTRODUCTION

In Chapter II, it was noted that root to shoot biomass ratios (R : S) increased in droughted and ABA-treated plants. Several researchers have noted that water stress (drought or osmotic stress) causes an increase in R : S, and have used R : S as an indication of the relative growth of roots and shoots and of carbon allocation in conditions where water is limiting (Kummerow, 1980, Westgate and Boyer, 1985). Other researchers (Biddington and Dearman 1982, Hubick, 1983) have reported that ABA causes an increase in R : S. This may be the result of ABA-directed transport of photosynthate. Karmocker and Van Steveninck (1979b) found that the application of ABA stimulated the transport of sugars from the shoot to the root in Phaseolus. Watts, et al. (1981) suggested that ABA accumulation in the roots of water-stressed plants could result in the preferential accumulation of solutes by the roots.

This chapter deals with the effects of drought and ABA on sucrose and photosynthate transport into the root. Transports to the whole root system and to the root apex were examined. The possibility that drought-induced ABA may affect sugar transport into the root tip is explored.

## MATERIALS AND METHODS

Growth conditions, the drought regime, and ABA treatment were as described in Chapter II.

**Labelling with [$^{14}$C] $CO_2$**

Two methods were used for monitoring sugar transports. In the first experiment, [$^{14}$C] $CO_2$ was used to label the products of photosynthesis. The transport of those products into the root was monitored over a 24-hour period. A small vial containing [$^{14}$C] sodium carbonate solution (Amersham, 55 mCi mmol$^{-1}$) was placed inside a clear plexiglas chamber, 0.8 L in volume. The entire shoot system was enclosed in the chamber and the chamber sealed. A plastic wrap (Stretch 'n Seal, Imperial Oil Ltd.) was used to seal the chamber around the base of the hypocotyl. The plants remained in the aeroponics chambers and were given continuous misting throughout the labelling period. Excess lactic acid was injected into the vials through a three-way valve to release [$^{14}$C] $CO_2$ from the sodium carbonate solution. Each plant was exposed to 1 μCi of [$^{14}$C] $CO_2$ for 1 hour. A small battery driven fan was installed inside the chamber to mix the air and break down the boundary layer around the leaf surface. Four plants were treated for each sample point. At the end of the 1-hour labelling period, the air in the plexiglas chamber was flushed through two KOH traps with pressurised air. The chambers were then removed from around the plant. Prior to flushing, five cc of the air from inside the chamber was collected to determine how much of the labelled $CO_2$ had been taken up by the plant. The sample was injected into 5 ml of Carbosorb, a $CO_2$ trapping agent, and the solution mixed with 10 ml of Permafluor (Packard) for scintillation counting. The plants were then droughted, treated with $10^{-5}$ M ABA, or given full watering. After measured periods of time, the plants were harvested; the root and shoot systems were separated and freeze-dried. The plant material was then oxidized on a Tricarb $CO_2$ oxidizer (Packard). Radioactivity from the plant tissue was determined following oxidation by scintillation counting.

In the second set of experiments, [U $^{14}$C]-labeled sucrose (ICN Pharmaceuticals, 560 mCi mmol$^{-1}$ ) was applied to one or both plumules. The radioactive sucrose was applied along the midrib of the leaf, 2.5 cm from the base of the petiole. The leaves were

lightly abraided with 300 grit carborundum paper at the point of application to facilitate better uptake of the sugar. In one experiment, 0.45 μCi of radioactive sucrose was applied in a 5 μl drop of 10% aqueous ethanol at different stages of treatment to one leaf. At harvest the plant was separated into several parts: epicotyl, hypocotyl, treated plumule, opposite leaf and root. The plant tissue was then freeze-dried, oxidized, and counted for radioactivity as above.

In another experiment, radioactive sucrose was applied to both plumules. Each plumule was supplied with 1 μCi of radioactive sucrose in the method described above. Apical 3-mm segments were harvested from the long roots at measured periods of time after treatment. Radioactivity was measured in the root apices following oxidation by the method described above.

## RESULTS

The results of [$^{14}C$] $CO_2$ labelling are shown in Figure 9. The values shown in Figure 9 have been corrected for differential uptake of the label and for transport during the labelling period. Uptake varied from 68% to 92% of the 1 μCi of labelled $CO_2$ to which each plant was exposed; values were extrapolated to complete uptake. Transport to the root during the labelling period was estimated at 104,302 dpm based on the average of four readings taken at hour 0, i.e. at the beginning of drought and ABA treatment. This number was subtracted from each value to correct for transport during the labelling period.

Overall, there was less of the label transported to the roots in droughted and ABA-treated plants. During the early stages of treatment, up to 6 hours, however, drought and ABA-treatment promoted movement of label into the root. These values may indicate a transitory period of directed transport into the root. However, the fact that transport of label during the one-hour labelling period was greater than transport during the first three

hours of treatment, suggests that treatment may have simply activated earlier the transport of label stored in the starch pools.

Figure 9.

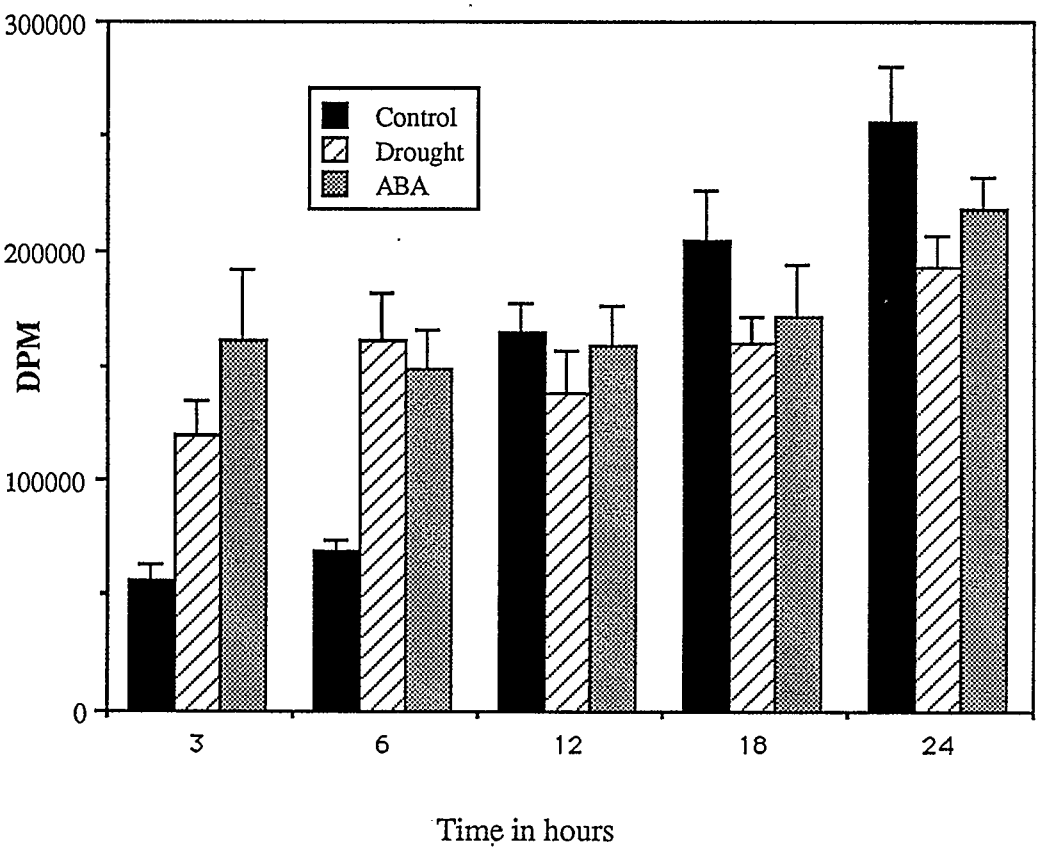



Figure 9. The amount of $^{14}C$ ( in disintegrations per minute, dpm) transported to the root in control, droughted and $10^{-5}$ M ABA-treated plants. Plants were labeled with 1 μCi of [$^{14}C$] $CO_2$ for 1 hour, then droughted, treated with $10^{-5}$ M ABA, or left untreated. The times shown are from the beginning of the drought or ABA treatment. The values are the average of three trials; error bars indicate standard deviations. The values were corrected to account for transport during the labelling period and for incomplete uptake of the label.

In the second experiment, the rate of sugar transport was examined. Three time points were chosen; 2 hours, 24 hours, and 48 hours after treatment. Labelled sucrose was applied as described above, and its rate of transport followed for 1 hour. Time points were 5, 10, 30, and 60 minutes after labelling. The results are shown in Figures 10-12. Each value is the average of four trials, ± standard error.

Drought and ABA-treatment increased the rate of transport to the root at 2 hours, but was inhibitory at 24 and 48 hours after treatment. It appears that the increase in labelled products in the root noted in the $CO_2$ labelling experiment in the early stages of treatment may have been an actual promotion of photosynthate transport in the root. This cannot be stated with certainty, since only labelled products, and not the entire transporting pool of photosynthate, were followed.

The decline in the rate of sugar transport at 24 and 48 hours was part of a general decline in rate of transport. More of the label remained in the treated leaf in droughted and ABA-treated plants compared to controls. If only exported sugar is considered, then a higher proportion goes to the root in droughted and ABA-treated plants than in controls. Figure 13 shows the label recovered from the root as a percentage of the label that was actually exported from the treated leaf.

Figures 10-12. The rate of transport of labelled carbon into the root in well-watered, droughted, and $10^{-5}$ M ABA-treated plants at 2 hours (Figure 10), 24 hours (Figure 11), and 48 hours (Figure 12). The times note the time after treatment at which the [$^{14}$C] sucrose was applied to the leaf. A 5 µl drop containing 1.0 x $10^{6}$ dpm of labelled sucrose was applied to one leaf in each plant. The values are in dpm, and are the averages, ± standard error, of four trials at each time point.

Figure 10.

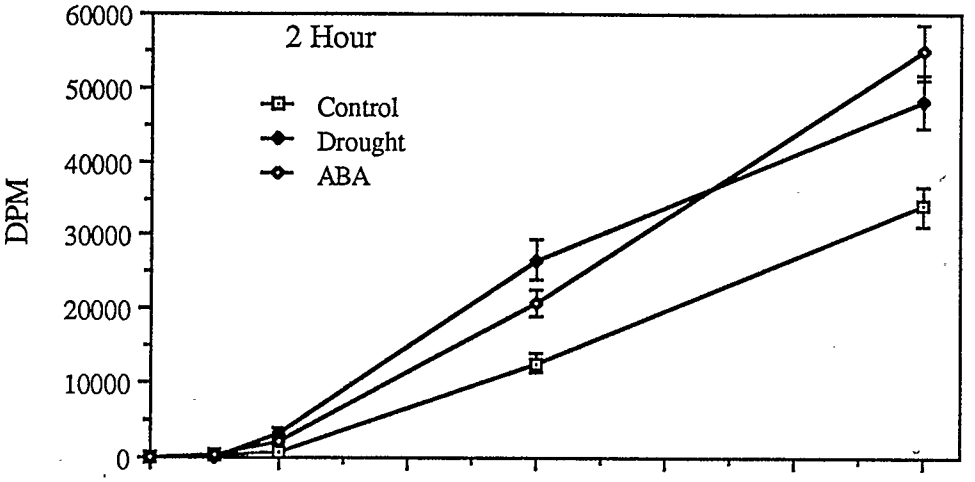



Figure11.

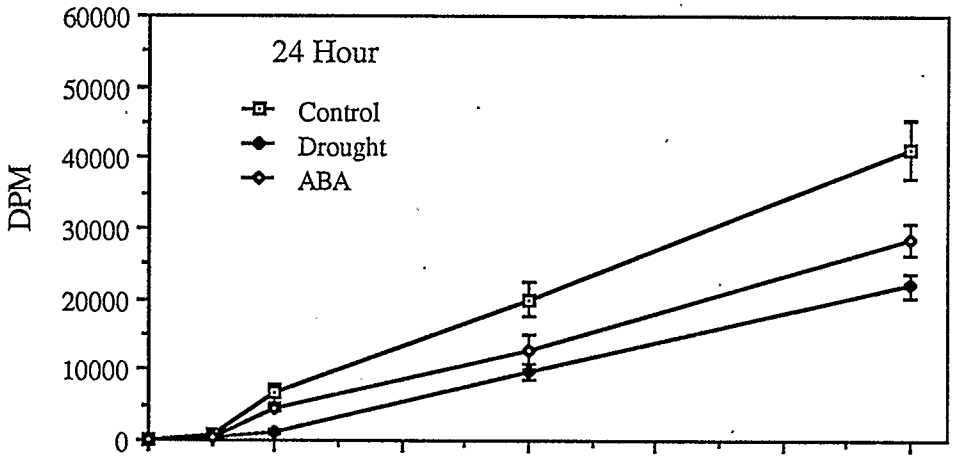



Figure 12.

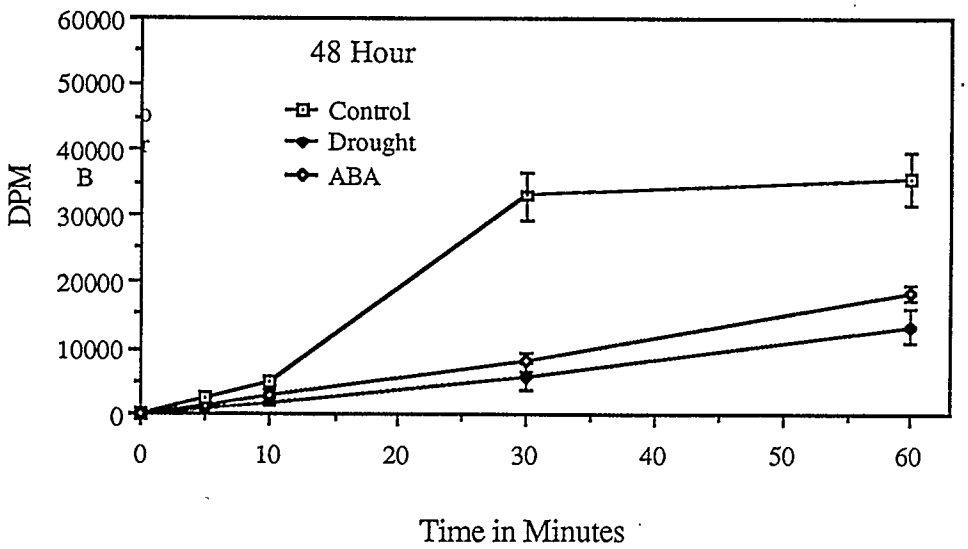


Figure 13.

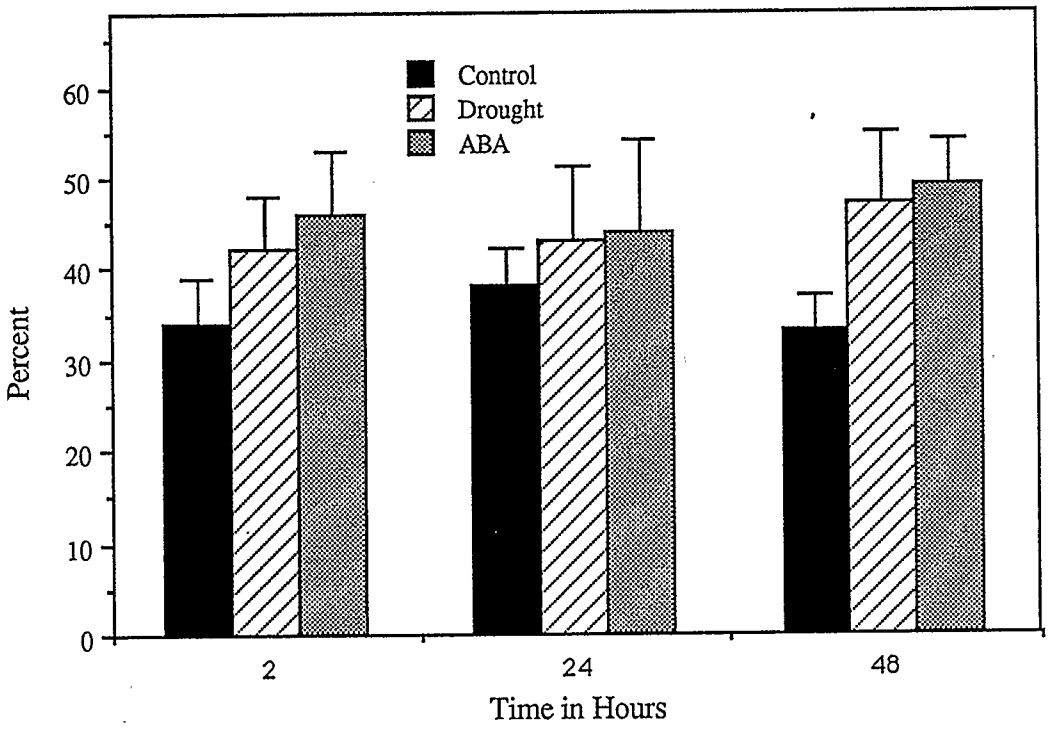



Figure 13. The amount of label recovered from the root expressed as a percentage of label exported from the treated leaf after one hour. Data is from the same plants as in Figures 11-13. Error bars indicate standard deviations, n = 4.

These studies indicated that ABA may be acting to stimulate the transport of photosynthate into the root. Directed transport of carbohydrate would seem a good mechanism for maintaining the root meristem. Another experiment, however, showed that drought and ABA inhibited transport into the apical 3 mm, during the first 12 hours of treatment (see Figure 14). The recovery of label fell off after 12 hours in control plants, but gradually increased in droughted and ABA-treated plants.

Figure 14.

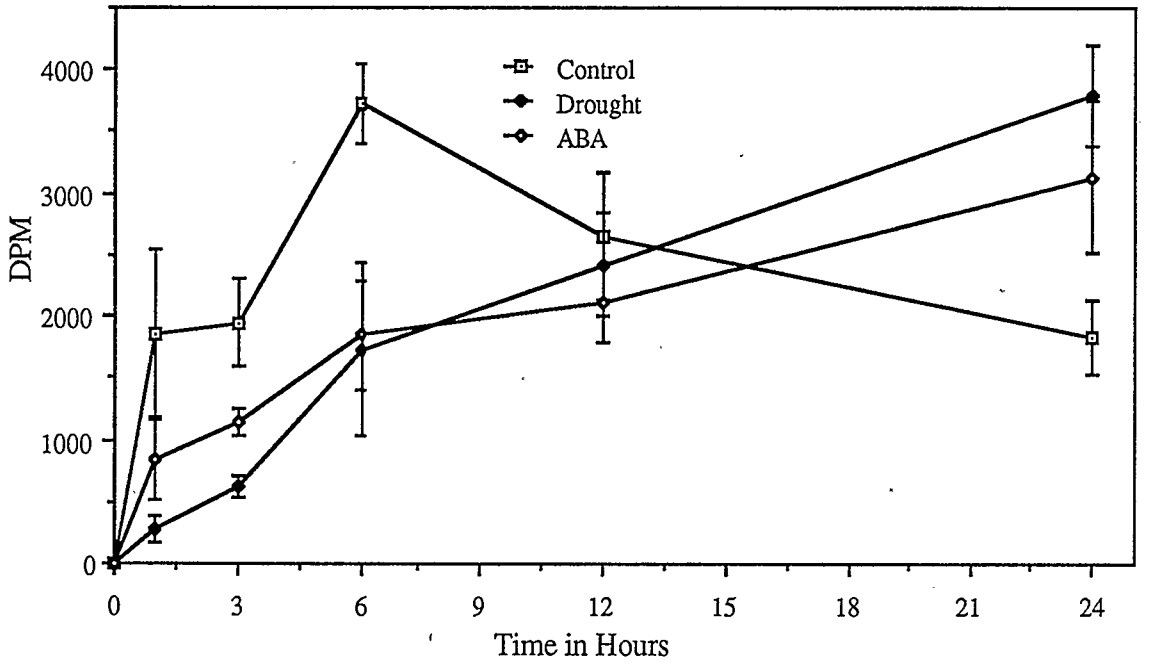



Figure 14. The amount of label recovered from root tips expressed as dpm root $tip^{-1}$ up to 24 hours after labelling each plumule with 1 μCi of [$^{14}$C] sucrose. Plants were labelled at the beginning of the drought and ABA-treatment period. Error bars indicate standard deviations; n = 3.

## DISCUSSION

The results of the sugar transport experiments are rather puzzling. Drought and ABA seem to promote the transport of sugar into the root, but inhibit its movement into the root apex. The fact that the root apices from control plants show less label after 24 hours may be explained by rapid growth in well-watered plants. Labelled carbon is not moving into the root apex as rapidly, because most of it has probably been incorporated into other tissues by this time. It may be that the sugar which is transported to the root in the early stages of drought or ABA-treatment is stored to be used later.

The best evidence for directed trransport to the root is from the exogenous sugar supplement experiments in Chapter II. There sugar had a marked effect on R : S in droughted plants and served to maintain the viability of meristems. It did not suppress the drought or ABA-induced response at the meristem, however. These experiments show that carbohydrate readily moves to the root during the initial stages of treatment. Other evidence (Chapter II) has shown that carbohydrate supplies are not limiting during the early stages of treatment, and even when it is supplied exogenously does not suppress the response of the meristem to drought or ABA. Although carbohydrate is available, the root apical meristem does not utilize it. The controlling factor here then, would seem to be the demands of the meristem rather than the supply of sugar available. Drought and ABA have slowed the activity of the meristem, thereby lessening its need for carbohydrate.

# CHAPTER VI : WATER RELATIONS

## INTRODUCTION

In Chapter II, it was argued that some process of osmotic adjustment must occur in the root apical meristem of droughted sunflower plants. The proximal derivatives of the meristem not only stopped cycling, as reported in Chapter II, but elongated in response to drought. Treatment with ABA produced the same pattern of response.

Water moves from regions of relatively high water potential, $\Psi$, to regions of relatively low water potential (Borowitzka, 1981). Cell enlargement proceeds when a demand for water is created by extension of the cell walls under the action of turgor, $\Psi_p$, and water is supplied by gradients in water potential (Boyer, 1968, Westgate and Boyer, 1985). If elongation is to continue, solutes must be added to the cell sap as water enters, in order to maintain the osmotic forces necessary to drive elongation, and to supply the metabolites for wall synthesis (Westgate and Boyer, 1985). Osmoregulation refers to the process by which plant cells regulate their osmotic potential, $\Psi_\pi$, to maintain a favourable balance in water potential with their external environment. In a growing plant, the cells thereof are continuously osmoregulating to allow for growth even when external water potentials remain steady. The terms osmotic adjustment and osmoregulation are often used interchangeably. Osmotic adjustment will be used herein to distinguish the situation in which the cells of a plant regulate their internal osmotic potential in response to a drop in external $\Psi$ (Turner and Jones, 1980).

Root elongation in droughted plants had slowed by 6 hours after treatment. Inhibition of growth was attributed largely to a decrease in DNA synthesis and cell division in the apical meristem. What growth that did occur was attributed primarily to cell elongation. In plants treated with $10^{-5}$ M ABA, root elongation had not declined noticeably by 6 hours, although the same changes in the apical meristem noted in droughted plants had

occurred. Moreover, in plants treated with lower concentrations of ABA, there was slight, but perhaps, insignificant increase (relative to well-watered plants) in elongation over the first 6 hours of treatment, followed by a period of inhibition. As in droughted plants, the growth that did occur in ABA-treated plants was attributed primarily to cell elongation. Drought-induced ABA in the apical meristems may be altering water relations to promote cell elongation as external $\Psi$ falls.

In this chapter, the writer examined the water relations of the root apex in droughted and ABA-treated plants. Root elongation was re-examined with respect to changes in water relations; root elongation during the first 6 hours of treatment was monitored more closely.

## MATERIALS AND METHODS

Growth conditions, plant material, drought and ABA treatment were as in Chapter I. Root elongation was measured by rule.

### Water Relations

Plants were removed from their aeroponics chambers; excess moisture was blotted from their root systems with paper towels. The plants were then placed on glass plates, and root apices (apical 3-mm segments) cut with a razor blade. The root apices were quickly collected and placed in Wescor C-52 chambers attached to a Wescor HR-33T Dew Point μvoltmeter (Wescor Inc., Logan, UT). Water potential was measured by thermocouple psychrometry in the dewpoint mode. Although thermocouple psychrometry methods are well established (Barrs, 1968, Boyer, 1968) and relatively simple to perform, the logistics of sampling root apices presented some problems. An accurate and stable reading required about 15 root apices. To cut, collect, and place this many root apices in the thermocouple chamber takes some time, during which changes in $\Psi$ may occur. Two

tests were designed to determine the accuracy of the water potential readings. In the first, Chardakov's dye drop method (Chardakov, 1948) was used to estimate the water potential. The advantage of this method is that tissue, once cut, can be placed immediately in the solution so that no drying occurs. The second test was to cut and collect root apices on wet paper towels. The root apices were left on the wet towels until 15 to 20 were collected. They were then blotted as above and placed on the glass plates. The rate at which $\Psi$ changed in the excised root apices was monitored by taking readings at measured intervals of time. It was found that $\Psi$ in excised root apices did not change within two minutes after exposure on glass plates. The same test was conducted on intact plants. They were removed from the aeroponics chambers, blotted dry, and allowed to dessicate on the glass plates. Root apices were then cut and their water potential measured. It was found that the water potential of root apices from intact plants did not change until five minutes after removal from the chambers.

Osmotic potential was measured in the thermocouple chambers following freezing of the tissue; turgor was calculated as the difference between $\Psi$ and $\Psi_\pi$ (Boyer, 1968). At the completion of $\Psi$ measurements, the tissue and the sample dish in which it was contained were removed from the thermocouple chambers. The sample dish containing the tissue was sealed with foil and plunged into liquid $N_2$ for five minutes. Upon removal from liquid $N_2$, the sample dish was allowed to return to room temperature; whereupon, the foil was removed and the dish returned to the thermocouple chambers for $\Psi_\pi$ measurements. Measurement of tissue after freezing yields osmotic potential; freezing ruptures membranes and thereby dissipates turgor factor in the equation $\Psi = \Psi_p + \Psi_\pi$. Turgor potential was calculated as the difference between $\Psi_\pi$ and $\Psi$ (Boyer, 1968).

**Excised Root Apices**

Osmotic adjustment can occur in two ways: by the import of external ions, or by the accumulation of organic solutes (Zimmerman, 1978). An accumulation of organic solutes can be effected by import from other plant parts or by the breakdown of larger molecules into their component parts; this increases $\Psi_\pi$ since it is the number of moles of solute and not the mass of the solute which primarily determines $\Psi_\pi$ (Borowizka, 1981). Both processes probably operate in most tissues; however, both require a source of fixed carbon, either as osmotica or as an energy source to drive active uptake of ions. In the last chapter, it was found that drought and ABA promoted a transitory increase in the import of fixed carbon to the root, but inhibited its movement into the root apex. This suggested that the root apex does not depend on the import of sugars for osmoregulation. To explore this idea further, the writer examined the response of excised root apices. Apical 3-mm segments from well-watered plants were cut on moist paper towels, and then were allowed to dessicate or were kept hydrated on filter papers saturated with either nutrient solution (0.75 strength Hoagland solution) or $10^{-5}$ M ABA solution. Two different ABA solutions were used: in one, ABA was dissolved in nutrient solution; in the other, ABA was dissolved in distilled water. Treatments were for 1 hour; the tissue was covered and left in in darkness during the treatment.

RESULTS

When root elongation was examined more closely, it was found that both drought and treatment with ABA caused a transitory promotion in root elongation (see Figure 15).

Figure 15.

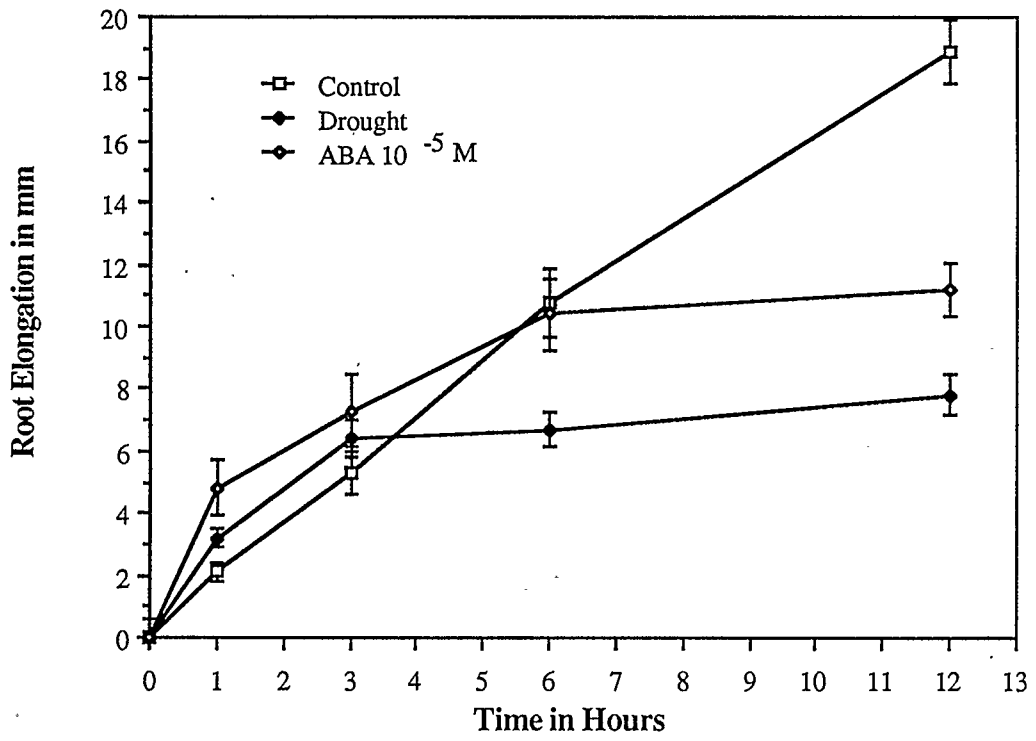



Figure 15. Rate of elongation of main roots in well-watered, droughted and $10^{-5}$ M ABA-treated plants over the first 12 hours of treatment. Error bars indicate standard deviations; n = 9.

There have been reports of absolute increases in root elongation in response to drought (Hsiao and Acevedo, 1974, Sharp and Davies, 1979, Watts, et al., 1981). Abscisic acid has been variously reported as an inhibitor and as a promoter of root growth. Mulkey, et al. (1983) reported an initial promotion of root elongation, followed by a period of inhibition and a subsequent recovery to 80% control rate in Zea mays. This closely follows the pattern observed in sunflower (see Chapter II). Explanations for these diverse reports may be found in the differential effects of both drought and ABA on cell division and cell elongation. In Chapter II, it was argued that some process of osmotic adustment must have occurred in the root apices of droughted plants. Figures 16, 17, and 18 show the water relations of control, droughted, and $10^{-5}$ M ABA-treated root apices.

The water relations in root apices of control plants did not change appreciably during the experiment. There was a noticeable increase in turgor at 12 hours. This seemed to be the result of a rise in $\Psi$ rather than an fall in $\Psi_\pi$ (see Figure 16). The 12-hour reading was taken during the dark period (see growth conditions) and the rise in $\Psi$ may have been the result of a general increase in plant water potential during dark periods when transpiration pull was minimum (compare leaf $\Psi$ at 12 hours, Figure 2, Chapter II).

Drought caused a transitory fall in $\Psi_\pi$ and a resultant increase in $\Psi_p$ over the first 6 hours of treatment. Turgor potential rose sharply in droughted plants, reaching a maximum at about 3 hours. By 6 hours, the burst of turgor had dissipated; thereafter, turgor remained at or slightly below control values. After 24 hours of drought, $\Psi$ fell as the stress became more severe. Turgor was not lost, however; even during the latter stages of drought treatment, the viable root apices maintained turgor (data not shown). The transitory rise in turgor was contemporary with the period of promotion in elongation (see Figure 17).

In ABA-treated plants, $\Psi$ and $\Psi_\pi$ fell sharply during the first 3 hours of treatment. The rise in turgor was not as great as in droughted plants. The roots of ABA-treated plants were growing rapidly at this time (see Figure 18), and turgor may have been dissipated by

cell expansion. In later stages of treatment, $\Psi$ gradually returned to control levels; $\Psi_\pi$ remained slightly lower and $\Psi_p$ slightly higher than in the root apices of control plants.

Figure 16.

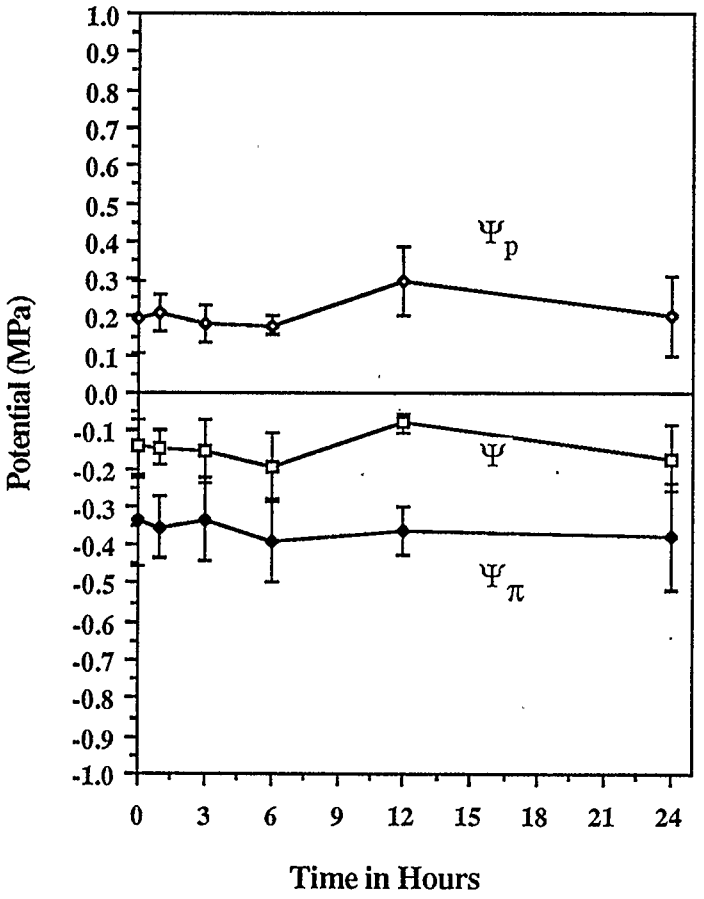



Figure 16. Water potential, $\Psi$, osmotic potential, $\Psi_\pi$, and turgor potential, $\Psi_p$, for root apices from well-watered plants. Error bars indicate standard deviations; n = 4.

Figure 17.

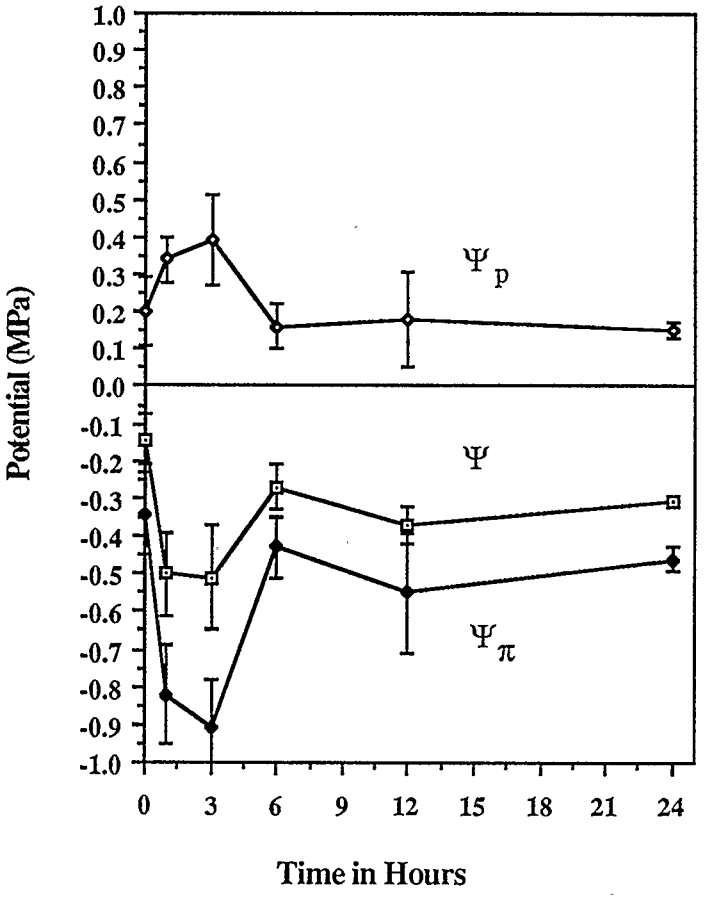



Figure 17. Water potential, $\Psi$, osmotic potential, $\Psi_\pi$, and turgor potential, $\Psi_p$, for root apices from droughted plants. Error bars indicate standard deviations; n = 4.

Figure 18.

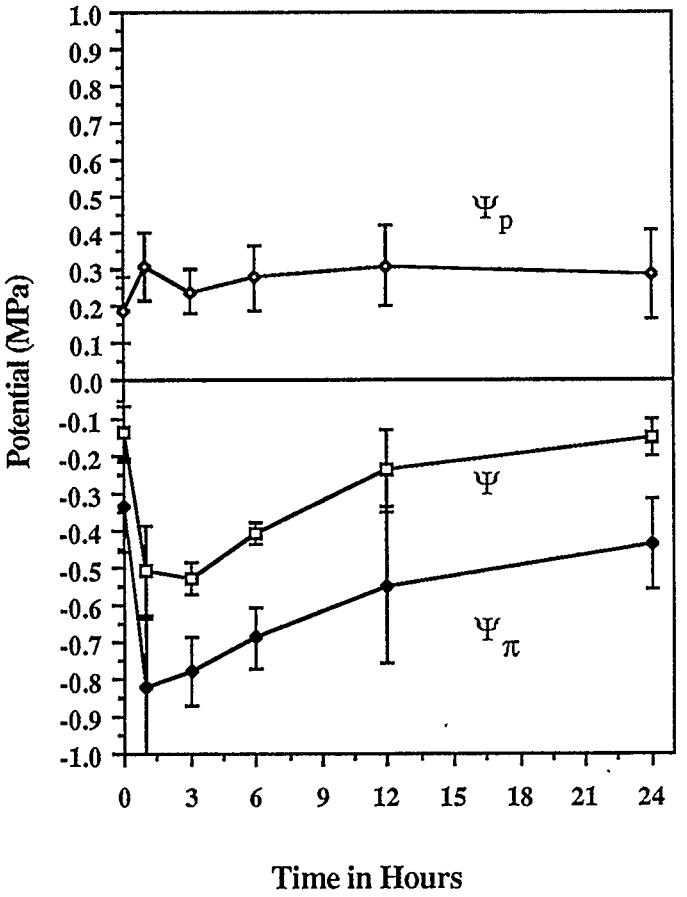



Figure 18. Water potential, $\Psi$, osmotic potential, $\Psi_\pi$, and turgor potential, $\Psi_p$, for root apices from $10^{-5}$ M ABA-treated plants. Error bars indicate standard deviations; n = 4.

## Water Relations in Excised Root Apices

Osmotic adjustment can occur in two ways: by the import of external ions, or by the accumulation of organic solutes (Zimmerman, 1978). An accumulation of organic solutes can be effected by import from other plant parts or by the breakdown of larger molecules into their component parts; this increases $\Psi_\pi$ since it is the number of moles of solute and not the mass of the solute which primarily determines $\Psi_\pi$ (Borowizka, 1981). Both processes probably operate in most tissues; however, both require a source of fixed carbon, either as osmotica or as an energy source to drive active uptake of ions. In the last chapter, it was found that drought and ABA could promote the import of fixed carbon to the root, but inhibited its movement into the root apex. This suggested that the root apex does not depend on the import of sugars for osmotic adjustment.

Figure 19 shows the water relations of root apices (3-mm apical segments) which were excised from well-watered plants and then treated for 1 hour by dessication or with ABA ($10^{-5}$ M) dissolved in either nutrient solution or distilled water. Control samples were kept hydrated in nutrient solution.

The osmotic adjustment process in root apices apparently did not depend on the rest of the plant. Dessicated root apices showed the ability to osmotically adjust to the very severe and rapid stress imposed by dessication. Moreover, the dessicated root apices showed an absolute increase in turgor. This is all the more remarkable when it is considered that the shoots of whole sunflower plants exposed to a dessication this severe would wilt. Neither does the ABA-response depend on transport from the rest of the plant. Turgor increased and $\Psi_\pi$ fell as in the root apices from whole plants. There was some indication that the response was greater when nutrient solution was used as the solvent for ABA (see Figure 19). This indicates that the uptake of external ions may be part of the osmotic adjustment process in sunflower root apices. Behl and Raschke (1986) and Behl

and Jeschke (1979) reported that ABA treatment promoted the accumulation of cations in barley roots.

Excision apparently had no effect on these readings, since the water relations in hydrated root apices did not change (relative to apices from intact plants) appreciably during the one-hour period.

Figure 19.

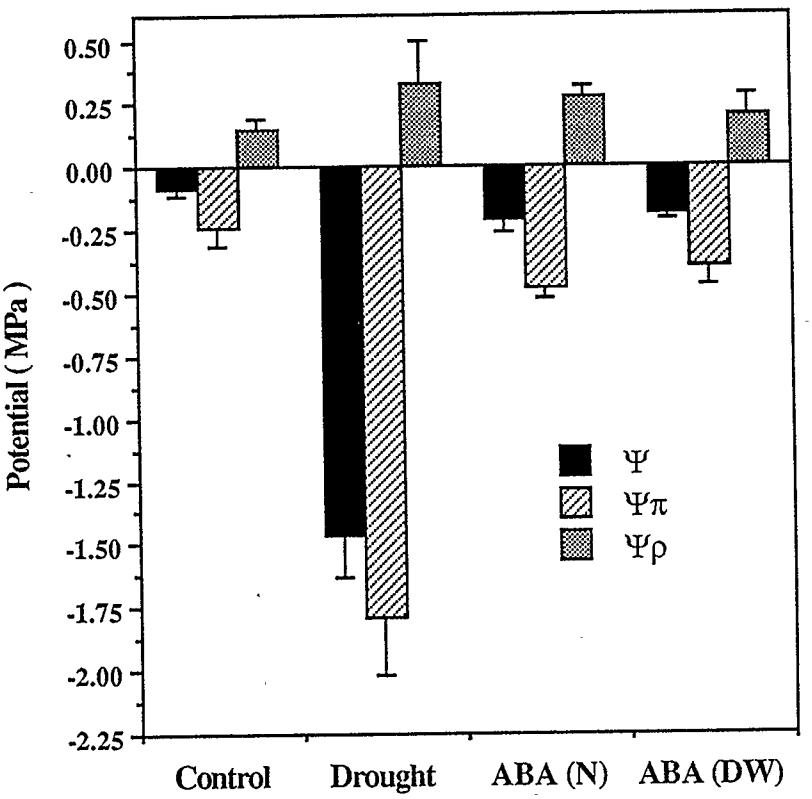



Figure 19. Water potential, $\Psi$, osmotic potential, $\Psi_\pi$, and turgor potential, $\Psi_p$, of root apices which had been excised and then placed on filter paper with nutrient solution (control), dessicated (droughted) in a covered petri dish, or placed on filter paper saturated with $10^{-5}$ M ABA dissolved in either nutrient solution (ABA (N)) or distilled water (ABA (DW)). Treatments were for 1 hour. Error bars indicate standard deviations; n = 4.

## DISCUSSION

Sunflower root apices demonstrate a high capacity for osmotic adjustment. In the early stages of drought, they adjust to produce an absolute increase in turgor, which corresponds in time to an absolute increase in root elongation relative to controls. Moreover, this response to drought has no requirement for transport of osmotica or energy from the rest of the plant, since it occurred in apices which had been excised and then treated.

In Chapter II, it was argued that if ABA was to be considered a mediator of drought-induced developmental change in the root, its action must account for the differentiation and elongation of cells in the proximal regions of the meristematic zone. The increase in turgor which ABA promotes certainly accounts for elongation, but it may also provide the signal for these cells to move out of cycle. Carmona and Cuadrado (1986), from studies of rapidly and slowly growing roots of Allium cepa, found evidence that indicates cell size is the key factor responsible for the passage of cells out of the meristematic zone. If so, then ABA could possibly effect the differentiation of cells by increasing their turgor beyond the threshhold limits of the wall, thus forcing them out of cycle by increasing their size beyond the point where they continue to divide. This suggestion is the opposite of what Hsiao (1973) and Hsiao, et al. (1976) suggest. They argue that the sensitivity of cell division to water stress may be an indirect effect of the stress, which prevents cells from attaining a size minimal for the commencement of division. However, their arguments did not allow for osmotic adjustment.

These studies confirm that turgor is not a limiting factor in the deactivation of mitotic activity. They also may explain why labelled carbon did not move into the root apex. Excised apices had sufficient energy and osmotica to adjust to a rapid and severe dessicatory stress. Despite a drop of about 1.6 MPa in $\Psi$ in only 1 hour, they adjusted and produced an absolute increase in turgor.

## CHAPTER VII : GENERAL DISCUSSION

Drought stress radically alters the primary development of sunflower roots. Vacuolation and differentiation of cells in the proximal regions of the meristem result in a meristem that is much reduced in size. Autoradiographic studies revealed that drought had inhibited DNA synthesis. The root meristem shifts from an active to an inactive state within a few hours of the imposition of drought. The state of inactivity is not imposed by growth limiting factors: experiments in Chapters II, III, and V eliminated carbohydrate shortages as a factor in the early stages of treatment, by which time the response has already progressed. A decrease in external water potential is not in itself a growth-limiting factor; loss of cell turgor is. However, the root apex maintained turgor by osmotic adjustment, and even increased turgor temporarily in response to drought.

The changes at the apex, the writer argues, are an adaptive response. Torrey and Feldman (1977) state that drastic alterations in the physiological homeostasis that exists in a rapidly growing root would effect changes in activity and in structure until a new balance has been achieved. The inactive period would appear to be a time of adjustment, during which the meristem is modified to better meet the conditions of drought. It is of interest that the response proceeds before the stress becomes limiting. It seems the root apex perceives a drop in external $\Psi$ as an early indication of unfavourable conditions to come.

A decrease in external water potential seemingly triggered an early distress signal. The nature of that signal is unknown. It is possible that, before osmotic adjustment occurred, a small initial loss of turgor (undetected by the writer) acted as a signal. The perception of the stress and the mediation of the response are probably separate events. There is a clear role for a mediating molecule. The response, as described in the text, represents a directed change in development. Abscisic acid meets the requirements of a mediating molecule. It elicits the same pattern of response as drought, and its endogenous levels increase in the apex in response to drought.

If this response is indeed an adaptive response, the question becomes: How does it serve the plant? Clowes and Stewart (1967), when discussing the role of the quiescent centre in root meristem recovery in corn, question the utility of preserving roots. Roots are ephemeral organs, easily replaced by the plant. With drought stress, there are putative advantages in maintaining some of the long roots; they could serve to tap water from unexplored parts of the soil. Clowes and Stewart (1967) suggest that the preservation of the meristem may not be of benefit to the plant so much as to the meristem itself. This raises the point of autonomy of meristems. The apical meristem does exercise a good deal of autonomy (see Torrey and Feldman, 1977). Some of the older literature (reviewed in White, 1979) described the plant as a collection of individual meristems. If indeed the meristems are each behaving individually, it would explain why some manage to adjust and survive while others perish. Furthermore, if ABA mediates the adaptive response, then perhaps the fitness of each meristem to survive drought depends on its ability to produce ABA.

In Chapter IV, the writer reported that excised apical 3-mm segments were capable of producing ABA in response to a drop in external water potential. The segments included the root cap, a tissue which has been identified as a source of ABA in gravitropically reacting plants (Wilkins and Wain, 1974, Feldman, 1985, Feldman, et al., 1985, Wilkins, 1984, Pilet, 1977). Drought stress may activate the same ABA-synthesizing process in the root cap as does gravitational disorientation. But if the mediating molecule of the response is produced in the root cap, then perhaps the cap is also the site where the drop in external potential is perceived. These ideas are highly speculative, but they suggest a role for the root cap in the adaptation of plants to drought and perhaps to other environmental stresses.

If ABA is involved in the drought response, how large is its role? The writer suggests that it is involved at least until the meristem begins to recover its activity. The activation of the QC which accompanies recovery may be a consequence of the depression of mitotic acitivity in the rest of the meristem. Generally, conditions which depress the

actively dividing cells will activate the QC (see Clowes, 1975, Feldman and Torrey, 1975), It is possible that the role of ABA extends only to the point of deactivating the meristem, and that recovery proceeds from the QC as a result of this deactivation.

The findings reported here may explain some of the contradictory evidence on the effects of drought and ABA on root growth. As mentioned in the introduction, drought and ABA have been variously reported to both inhibit and promote root elongation. Drought and ABA affect both parameters of growth, cell division and cell elongation. Initially, the two effects oppose one another; that is, the promotion of cell elongation acts to increase and the inhibition of cell division acts to decrease root growth. The effects are not of equal magnitude; in this study, the promotive effect on cell elongation predominated initially; after which, the inhibitory effect on cell division predominated. Most studies have not examined both parameters of growth. Reports of promotion or inhibition probably reflect which effect was dominant at the time of observation. Mulkey, et al. (1983) noted that ABA initially promoted root elongation in corn roots; this was followed by a period of inhibited growth which was followed, in turn, by a period of recovery. This is the same response observed here in Helianthus. They were probably observing the differential effects of ABA on cell division and cell elongation.

## CONCLUDING REMARKS

The writer has advanced the thesis: Abscisic acid mediates drought-induced developmental change in the roots of Helianthus annuus L.. In support of the thesis, the writer has shown the following: 1. drought and ABA elicit the same pattern of change at the meristem; 2. drought increases the endogenous levels of ABA in the root apex; and 3. both drought and ABA promote osmotic adjustment and inhibit DNA synthesis -- physiological changes which could account for the change in the development of sunflower roots. While these findings are not conclusive, they do support the thesis.

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