CURRENT CONCEPTS ON THE RELATIONSHIP BETWEEN ABSCISIC ACID AND LEAF WATER STRESS[1]

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ABSTRACT - A review of the available information on the relationship between ABA (abscisic acid) and leaf water stress is presented. Major subjects focused on are: leaf ABA content and water stress, possible mechanisms of ABA-induced stomatal closure, ABA effects as related to CO_2 and other plant hormones, ABA and proline accumulation, and ABA as related to the "after-effect" of water stress. Remarkable results and some contradictory data are commented on briefly. Finally, the extension of present knowledge is analyzed and suggestions for further work are made.

Index terms: ABA, stomata.

CONCEITOS CORRENTES SOBRE A RELAÇÃO ENTRE O ÁCIDO ABSCÍSICO E O DÉFICIT HÍDRICO FOLIAR

RESUMO - O presente trabalho constitui uma revisão bibliográfica sobre a relação entre o ácido abscísico (ABA) e o déficit hídrico foliar. Os principais assuntos enfocados são: teor foliar de ácido abscísico e déficit hídrico, possíveis mecanismos de indução de fechamento de estômatos pelo ácido abscísico, relação entre os efeitos do ácido abscísico e CO_2 e outros hormônios vegetais, ácido abscísico e acumulação de prolina, e relação entre o ácido abscísico e o "efeito posterior" do déficit hídrico. Comentam-se brevemente os resultados marcantes e alguns dados contraditórios. Analisa-se a extensão do atual conhecimento no assunto. Apresentam-se sugestões para futuros trabalhos.

Termos para indexação: estômatos.

INTRODUCTION

ABA (abscisic acid) is a growth inhibitor widely studied in plant growth and development, and recent reviews (Walton 1980, Milborrow 1981 and 1984) have covered various aspects of its biosynthesis, metabolism and function. It has been suggested that native ABA is derived from the 2-cis isomer of C_{15} aldehyde xanthoxin (Taylor & Burden 1972 and 1973), but most workers now believe it is ultimately synthesized from MVA (mevalonic acid) (Milborrow 1981 and Creelman & Zeevaart 1984) in plastids of plant cells. However, questions still remain about the site of ABA biosynthesis, and some results indicate that ABA is synthesized in the cytoplasm (Hartung et al. 1982), and is then stored in plastids, where it can be released during water stress (Walton 1980).

It is still unclear how the further metabolism of ABA is related to its activity as a growth regulator. There are suggestions that ABA is metabolized outside the chloroplasts (Milborrow 1979) and Hartung et al. (1980) hypothesize that the enzymes involved in ABA degradation occur in the cytoplasm. The major pathway of ABA metabolism is its conversion into PA (phaseic acid) and DPA (dihydrophaseic acid) (Murphy 1984). In addition, ABA is conjugated to ABA-GE (abscisic acid-B-D--glicose ester) (Milborrow 1981).

Several problems are recognized in studies of ABA. First, naturally occurring ABA is highly (+) in optical rotation, whereas the synthesized material commonly applied to tissues is (±) racemic (Milborrow 1984). Because differences are known to exist in the functions of the two forms (Milborrow 1984), applied forms of ABA should be clearly stated. Second, inherent problems are associated with the analysis of ABA levels in tissues. ABA content was originally assayed with biological inhibitor studies, but results sometimes differed substantially from those obtained with physical methods such as GLC. Lastly, it is recognized that ABA must be partially purified before attempts are made to analyze it with the more recently favored methods such as HPLC (high-performance liquid chromatography and GLC (gas liquid chromatography) (Milborrow 1984). A recent description of separation and

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quantification of ABA, PA and DPA by HPLC is presented by Mapelli & Rocchi (1983). Also, several RIA (radioimmunoassay) methods have been described for ABA quantification (Walton et al. 1979, Weiler 1979 and 1980), including the use of TLC (thin-layer chromatography) to test the distribution of immunoreactive material (Le Page-Degivry & Bullard 1984). RIA methods have been recently reviewed by Weiler (1984) and Hanke (1984).

ABA was first isolated as an abscission-accelerating substance in cotton fruit (Ohkuma et al. 1965) and also as an inhibitor of bud development in woody plants (Cornforth et al. 1965). Although subsequent work has shown that abscission in leaves is normally regulated by ethylene rather than ABA (Milborrow 1984), ABA is clearly involved in many plant developmental processes such as growth inhibition (Rehm & Cline 1973, Noggle 1983 and Pilet 1983) and promotion (Blumensfield & Gazit 1970, Milborrow 1974, McWha & Jackson 1976 and Bradford 1983), tissue permeability increase .(Glinka & Reinhold 1971), root geotropism (Kundu & Audus 1974), seed dormancy (Wareing & Sounders 1971 and Ackerson 1984) and germination (Karssen 1982), fruit ripening (Coombe & Hale 1973), and exogenously-induced fruit seedlessness (Jackson & Blundell 1966), plant cell cold hardiness (Chen & Gusta 1983). Inhibition of trap closure in Venus flytrap (Kondo & Yaguchi 1983) and counteractive effect on stress-induced decrease of root hydraulic conductance (Markhart et al. 1979, Fiscus 1981 and Markhart 1984). In addition, Raschke (1982) believes he has evidence that ABA acts as a photosynthesis inhibitor. There are also controversial information about ABA effects on flowering (Milborrow 1984) and correlative inhibition of bud development (Walton 1980). Finally, ABA is also known to have a major role in controlling responses of plants to water stress, and much of the current research and controversy is focused on this area.

Despite the extensive number of reports on this subject, studies on the role of ABA in plant metabolic adjustment to wilt and prevention of further water loss are still at a relatively early stage, mainly because there are many gaps in the knowledge of several aspects of ABA action (Walton 1980). In this review, I intend to provide a general picture of the relationships of ABA to leaf water stress and also to present thoughts on the available data.

LEAF ABA CONTENT AND WATER STRESS

Interest in the possible role of ABA in water stress responses of plants began when Little & Eidt (1968) observed that exogenous applications of ABA led to reduced transpiration rates in leaves of several woody plants. Their findings were extended to other plants by Mittelheuser & Stevenick (1969) and by Jones & Mansfield (1970). The role of ABA as a natural regulator of stomatal aperture was suggested by Wright (1969), who noted an increase in "β-inhibitor" in detached leaves of wheat following a period of stress, and by Wright & Hiron (1969), who identified this inhibitor as being ABA in brussel sprouts and also observed that increases in ABA levels during stress could be related to regaining of leaf turgidity. Subsequent work confirmed these results (Orton & Mansfield 1974, Uehara et al. 1975, Itai & Meidner 1978a and Mansfield & Davies 1981). Further evidence of the relation of ABA to stomatal regulation was derived from the Flacca mutant of tomato, which is unable to produce ABA nor closes stomata (Imber & Tal 1970, Tal & Imber 1970, Bradford 1983 and Bradford et al. 1983). This plant is constantly wilted and as a result it is stunted in growth. In addition to stomatal effects, stress-induced increases in ABA content are considered to be responsible for proline accumulation in some species (Aspinall et al. 1973 and Stewart 1980).

ABA accumulation in water-stressed leaves is accepted as a genetic character associated with drought tolerance (Larquè-Saavedra & Wain 1976, Quarrie & Jones 1979, Quarrie 1981 and Kirkham 1983), but many factors are known to affect the extent of this increase. Variable results have been obtained because of differences in leaf age (Quarrie & Henson 1981 and Cornish & Zeevaart 1983) and, in intact plants, younger leaves show higher capacity to accumulate ABA than older ones (Zeevaart & Boyer 1984). Increases in ABA levels are affected by environmental factors such as temperature (Wright & Hiron 1969) and light intensity (Bengtson et al. 1978, Rajagopal & Andersen 1980 and Henson 1983), and also by duration and intensity of water stress (Wright 1977, Pierce & Raschke 1980, Eze et al. 1981, Henson & Quarrie 1981, Pierce & Raschke 1981 and Henson 1982). Under stress, field-grown plants accumulate much less ABA than pot-grown ones (Henson et al. 1981 and 1984). Finally, leaf ABA level is also raised by warm air, waterlogging (Hiron & Wright 1973), salinity (Milborrow 1984), and chilling (Rikin et al. 1976 and 1979, Chen et al. 1983 and Markhart 1984).

Wright & Hiron (1969) observed that ABA levels increased detectably within 2 hours after the onset of wilting and rapidly decreased following rewatering. Similar rapid and reversible changes in ABA content of water stressed leaves have been subsequently reported (Hiron & Wright 1973, Bengtson et al. 1977, Dörffling et al. 1977, Ludlow et al. 1980, Zeevaart 1980 and Pierce & Raschke 1981). Overall, foliar levels of ABA frequently rise from about 20 ng/g initial fresh weight (1×10^{-7} M) to 500 ng/g (2×10^{-6} M) during wilting (Milborrow 1984). However, as shown on Table 1, intraspecific differences in ABA accumulation in response to water stress are also found. There is evidence that the degree of stress affects time to increase ABA to maximal values, since Loveys & Kriedemann (1973) observed that detached leaves of grapevines doubled ABA content in 15 minutes at a water potential of -1.5 MPa (megapascal), but in intact plants the increase was slower (about -1.0 MPa of water potential) up to 6 days, when ABA content had increased 44 fold.

Many papers suggest that ABA content increases suddenly when a threshold value of water potential is reached during water stress and that there are but small differences among several species in relation to the values of the threshold. Zabadal (1974) observed that ABA levels remained low in *Ambrosia artemisiifolia* and *Ambrosia trifida* leaves until water potential decreased to a value between -1.0 and -1.2 MPa. At this point, ABA started accumulating, and this accumulation was proportional to further decreases in water potential down to -2.4 MPa. However, the observed threshold represents just 0.1 MPa in water potential decrease from unstressed conditions, and values like this are associated with errors in thermocouple psychrometry so that the real value of water potential to cause ABA to increase in level remains to be established. Also, this ABA increase starts rapidly (less than 1 hour after stress imposition) and other factors might be regulating ABA synthesis prior to detectable changes in water potential. Under similar conditions, subsequent work has obtained the same kind of results, such as between -0.8 and -1.0 MPa in excised leaves of maize and sorghum (Beardsell & Cohen 1975). between -1.0 and -1.2 MPa in seedlings of Douglas-fir (Blake & Ferrel 1977), between -0.7 and -0.9 MPa in bean leaves (Walton et al. 1977 and Wright 1977) and between -1.0 and -1.2 MPa in fully and half-expanded leaves of *Xanthium strumarium* (Cornish & Zeevaart 1983).

Since the increase in ABA concentrations appears to be generally coincident with wilting onset (Milborrow 1981), it has been suggested that turgor pressure is the critical component of water potential for ABA accumulation (Pierce & Raschke 1978). Beardsell & Cohen (1975) observed the beginning of ABA raising in water potential values probably corresponding to zero turgor for maize and sorghum. Pierce & Raschke (1978) report that the beginning of ABA accumulation in fact occurred at zero leaf turgor for cocklebur. Further papers confirm this observation (Pierce & Raschke 1980 and 1981). However, although there is a proportionality between ABA levels and water potential during wilting, turgor pressure remains at zero while ABA content changes. Hence, turgor is unlikely to regulate ABA synthesis during stress. Also, Henson et al. (1984) noted very different levels of ABA at nearly the same value of turgor pressure in pearl millet stressed leaves. Other suggestion for the regulation of ABA synthesis under stress is given by Walton (1980), who believes that osmotic readjustment is the cause of poor correlation between ABA levels and water potential in field-grown wheat and good correlation in excised chamber-grown wheat leaves, since Davies & Lasko (1978) report that ABA levels in apple seedlings, which undergo osmotic readjustment, appear to correlate better with leaf turgor than with water potential. This hypothesis looks reasonable, since plant reservoirs outside the leaves could avoid the observation of a threshold phenomenon for ABA accumulation as noted to detached leaves. Other possibility is that, although ABA synthesis under stress conditions appears to be controlled by water potential as a whole, ABA translocation in intact plants in response to stress might trouble the observation of a well-defined threshold value. In fact, there is evidence that ABA recirculates in cocklebur plants, moving down the stem in the phloem and back up in the transpiration stream to the mature leaves (Zeevaart & Boyer 1984), yet ABA is present in sieve tube sap of *Lupinus albus* (Hoad 1978).

POSSIBLE MECHANISMS OF ABA-INDUCED STOMATAL CLOSURE

Applied ABA initiates stomatal closure either in detached or in attached leaves (Little & Eidt 1968 and Mittelheuser & Stevenick 1969) and several groups of researchers have reported extremely rapid response times. Cummins et al. (1971) and Itai & Meidner (1978a) observed stomatal closure within 10 minutes and Kriedemann et al. (1972) noted reductions in aperture can be detected within 1 minute following applications of ABA. These rapid response times have led some workers to suggest that exogenously added or endogenous ABA must somehow act to alter the distribution of osmotically important solutes of guard cells, and this could be by causing a reduction in K^+ concentration (Horton & Moran 1972 and Squire & Mansfield 1972), since potassium ions are reported as the major promoters of solute potential variations (Humble & Raschke 1971) because they migrate from the subsidiary cells into the guard cells when stomata open and return to subsidiary cells during closure (Raschke & Fellows 1971). However, it has been also suggested that ABA can act through an H^+ expulsion mechanism in the plasmalemma of the guard cells (Raschke 1975a) and Mansfield & Davies (1981) infer that the fast response of stomata to low doses of ABA supports this view.

TABLE 1. Results on the maximal accumulation of leaf ABA content following the imposition of water stress.

Species	ABA content unstressed	ABA content stressed	Unit	Stress duration (hours)	Type of stress	Reference
Ambrosia artemisiifolia L.	0.5	15	ng/g dry wt.	22	drought (desiccating environment)	Zabadal (1974)[1]
Ambrosia trifida L.	0.5	15	ng/g dry wt.	22	drought (desiccating environment)	Zabadal (1974)[1]
Gossypium hirsutum L.	0.2	5.6	ng/mg dry wt.	8.8	withholding of water until wilting of mature leaves	Pierce & Raschke (1980)[1]
Helianthus annus L.	30	65	% control	0.5-5	drought of detached leaves	Dörffling et al. (1977)[1]
Lycopersicon esculentum Mill.	68	524	µg/kg fresh wt.	96	waterlogging	Hiron & Wright (1973)
Menyanthes trifoliata L.	100	100	% control	0.5-5	drought of detached leaves	Dörffling et al. (1977)[1]
Oryza sativa L.	25	1,200	ng/g fresh wt.	4.2	drought of detached leaves + incubation in wet paper	Henson & Quarrie (1981)[1]
Pennisetum americanum (L.) Leeke	20-25	100-200	ng/g fresh wt.	1.2	drought of detached leaves	Henson (1981)
	30	370	ng/g fresh wt.	120	witholding of water	Henson et al. (1981)[1]
	20	220	ng/g fresh wt.	4.2	drought of detached leaves + incubation in wet paper	Henson & Quarrie (1981)[1]
Phaseolus vulgaris L.	40	301	µg/kg fresh wt.	1.5	warm air	Hiron & Wright (1973)
	30	190	µg/kg fresh wt.	96	waterlogging	Hiron & Wright (1973)
	240	1,300	ng/g fresh wt.	5	PEG 6000 (-0.3 MPa)	Walton et al. (1977)[1]
	0.2	9.8	ng/mg dry wt.	8	withholging of water until wilting of mature leaves	Pierce & Raschke (1980)[1]
Pisum sativum L.	100	275	% control	0.5-5	drought of detached leaves	Dörffling et al. (1977)[1]
	290	619	ng/plant	2	PEG 6000 (-0.3 MPa)	Rajagopal & Andersen (1980)
	182	369	ng/plant	6	PEG 6000 (-0.3 MPa)	Rajafopal & Andersen (1980)
Sorghum bicolor (L.) Moench	0.1-0.2	5	ng/cm^2	168	withholding of nutrient solution	Beardsell & Cohen (1975)
Spinacea oleracea L.	0.7	2.8	10^{-10} moles/g fresh wt.	6,480	cold stress - intact old leaves	Heilmann et al. (1980)
Triticum aestivum L.	31	252	µg/kg fresh wt.	96	waterlogging	Hiron & Wright (1973)
	28	139	ng/g fresh wt.	3	root cooling (-1/+1°C ion solution)	Bengtson et al. (1977)[1]
	33	130	ng/g fresh wt.	10	dry air on dark-grown detached leaf	Bengtson et al. (1978)[1]
	33	230	ng/g fresh wt.	20	dry air on dark-grown detached leaf	Bengtson et al. (1978)[1]
	20	650	ng/g fresh wt.	4.2	drought of detached leaves + incubation in wet paper	Henson & Quarrie (1981)[1]

TABLE 1. Continued.

Species	ABA content unstressed	ABA content stressed	Unit	Stress duration (hours)	Type of stress	Reference
Vicia faba L.	100	720	% control	0.5-5	drought of detached leaves	Dörffling et al. (1977)[1]
Vitis vinifera L.	0.15	0.27	mg/kg fres wt.	0.25	excising of leaves	Loveys & Kriedemann (1973)
	0.6	38.3	mg/kg fresh wt.	144	drought of intact plants	Loveys & Kreidemann (1973)[1]
Xanthium strumarium L.	3.8	11.8	ng/mg dry wt.	8.5	withholding of water until wilting of mature leaves	Pierce & Raschke (1980)[1]
	400	2,800	ng/g fresh wt.	31	stream of warm air (up to 30 min.)	Zeevaart (1980)[1]
	5.3	24.5	µg/g dry wt.	8	not stated	Creelman & Zeevaart (1984)
	0.2	2.9	µg/g fresh wt.	31	stream of warm air (up to 30 min.)	Zeevaart & Boyer (1984)
Zea mays L.	0.3-0.5	10.7	ng/cm^2	168	withholding of nutrient solution	Beardsell & Cohen (1975)

[1] Data are estimated from figures.

Other possibility is that ABA acts on metabolic processes needed for interconversions between starch and malate (Mansfield & Davies 1981). It has been hypothesized that the frequent starch loss from chloroplasts of guard cells during stomatal opening (Meidner & Mansfield 1968) is perhaps caused by its conversion into malate (Willmer & Rutter 1977 and Mansfield & Davies 1981). Consequently, the result of ABA action on stomata may be the inhibition of K^+ uptake and starch conversion (Mansfield & Jones 1971 and Mansfield & Davies 1981). However, Dittrich & Mayer (1983) observed that regular closure of stomata is a physical process due to leaking of osmotically relevant ions, whereas the action of ABA appears to be related to a protein-catalyzed process such as facilitated diffusion, which opens additional leaks within the membrane.

Most of the evidence leads to the possibility that the ABA required to close stomates is formed in the mesophyll at the onset of wilting and transferred, actively or by diffusion, to the guard cells (Milborrow 1981). The fact that a considerable amount of ABA in leaves is located in the mesophyll (Singh et al. 1979 and Weiler et al. 1982), especially in the chloroplasts (Heilmann et al. 1980), is an indication of this possibility. Also, there are suggestions that ABA translocation from chloroplasts into cytoplasm during darkness is caused by a reduction of the pH of stroma (Heilmann et al. 1980 and Cowan et al. 1982), and that ABA release depends only on intracellular pH gradients (Hartung et al. 1983).

Although the potential role of ABA as a regulator of stomatal aperture is supported by a variety of experiments, several studies have shown that changes in ABA content in leaf tissues are not always correlated with stomatal movements. Hsiao (1973) cites many papers which indicate that ABA level changes lagged behind stomata opening following the relief of stress, and Beardsell & Cohen (1975) noted that increases in stomatal resistance precede increases in ABA levels of stressed maize and sorghum leaf tissues. Also, Loveys (1977) found that no ABA was synthesized when broad bean epidermis was wilted. More recently, Raschke (1982) has assembled several reports that cast doubt on the existence of a simple, straight forward relation of leaf ABA to stomatal conductance.

The possibility of ABA compartmentation in leaf tissues can be presented for explaining the discrepancy. Since stomata normally comprise less than 5% of the cells found in the epidermis (Milborrow 1984), ABA levels in the mesophyll or even in the epidermis need not to reflect the level acting on the guard cells. This possibility was suggested in early studies by Walton & Sondheimer (1972) who found no relationship of ABA to growth rates of excised bean axis. Cummins (1973) suggests the existance of ABA compartmentation because he observed stomatal reopening in a short period of time after stopping additions of ABA to the transpiration stream. Raschke & Zeevaart (1976) suggest ABA compartmentation in leaves

of cocklebur as a reason for no correlation between ABA levels and stomatal conductance in plants under different environments. Actually, if there were ABA compartmentation in the presented time-course studies, and the ABA type related to stomatal closure changed rapidily, if the background were high, that type would not be detectable. Recently, Bray & Zeevaart (1983) observed an irreversible conjugation of ABA to ABA-GE in cocklebur which led to the finding that ABA-GE but not ABA is sequestered in the vacuole, where it is metabolically inaccessible.

The discussed points lead to the feeling that there are still some aspects to be clarified. It seems that crucial questions are the biosynthesis and fate of ABA as related to stomatal movements, the rapidity of stomatal closure as related to ABA levels, and ABA compartmentation in the leaf. Potentially, they can be resolved with newer techniques for "*in situ*" determinations of ABA. Weiler (1984) presents a significant approach on the methods that have been reported for quantitative analysis of ABA in plants RIA and ELISA (Enzyme-linked immunosorbent assay). Many limitations of methods used in the past are likely to be sources of error. While antisera were first raised against (R, S)-ABA coupled via C_1, it was later found that this resulted in the production of predominantly anti-(R)-ABA antibodies, rendering the assay of (S)--ABA rather imprecise, being this problem overcome by the use of (S)-ABA-immunogens. Nevertheless, the free and conjugated ABA, in turn, were also highly reactive with ABA-amides. To solve this problem, Weiler infers that it has been used ABA-C_4-tyrosylhydrazone-substituted immunogens, which leads to little or no interference from conjugates. Finally, the recent use of a mAB-RIA (monoclonal antibody) technique allows not to have any significant cross-reaction against compounds such as xanthoxin, PA, DPA, ABA-B-D-gluco pyranosyl ester, or 2-trans- and (R)-ABA. Also, it allows the determination of even traces of the physiologically active form of ABA in plant extracts. Conclusively, it seems that the use of mAB-RIA technique for the quantification of active ABA (2-cis(S)-ABA) in plant material could propitiate a clue for those questions. Major perspectives appear to be the determination of the actual ABA level needed to induce stomatal closure and, if it is the case, the real cellular level of ABA binding proteins required by the plant to close stomata.

ABA EFFECTS AS RELATED TO CO_2 AND OTHER PLANT HORMONES

In addition to light, stomatal aperture in unstressed plants is mediated by CO_2 levels and pH values, and some controversy exists regarding the possible relation of ABA to CO_2 (Zeiger 1983). Raschke (1975a) showed that ABA and CO_2 interact in regulating stomatal closure. However, Mansfield (1976) found that ABA and CO_2 influences on stomata are additive rather than interactive, and Itai & Meidner (1978b) infer that ABA and CO_2 do not interact because they probably act at different sites.

Although it has been observed that ABA-affected stomatal closure depends upon CO_2 presence (Raschke 1975b) and that intracellular CO_2 levels decrease after ABA is supplied to detached leaves (Cummins et al. 1971). Snaith & Mansfield (1982) noted that ABA dependency on CO_2 is cancelled by exogenous IAA (indoleacetic acid) at high concentrations. More recently, Eamus & Wilson (1984) also observed associated effects of ABA, IAA and CO_2, which were more remarkable under low temperature: ABA was found to have no effect upon stomatal closure in the presence of CO_2 when IAA was added. A last aspect on this issue is that, although not related to ABA, several natural and synthetic cytokinins have promoted stomatal opening in *Anthephora pubescens* (Jewer & Incoll 1980).

The reasons for the different conclusions on how ABA and CO_2 affect stomata still have to be established - Walton (1980) calls attention to such things as differences in experimental techniques and result interpretation. However, the determination of the sites of ABA and CO_2 action seems to be imperative. Complementarily, the influence of exogenous IAA upon ABA-CO_2 associated effects leads to the need of measurements of endogenous IAA in studies on stomatal movements and the search of interactive effects of the two plant hormones on stomatal behavior. Lastly, it is worth verifying whether ABA is related to other plant hormones that can modulate stomatal movements, such as cytokinins.

ABA AND PROLINE ACCUMULATION

During water deficit, many species change their content of free amino acids, being the increase in proline concentration the most remarkable response (Aspinall & Paleg 1981). Aspinall et al. (1973) observed ABA-induced increases in proline levels in barley and *Lolium temulentum*, and Stewart (1980) noted that proline levels began to rise following a treatment with ABA in cuts of barley leaves. This evidence, along with the observation of accumulation of both endogenous proline and ABA in leaves of dark-grown wheat seedlings during water stress (Bengtson et al. 1978), suggests that proline biosynthesis in response to stress is mediated by ABA. However, Aspinall & Paleg (1981) cite some studies in which tobacco plants showed increases in proline and ABA levels during water stress, but did not accumulate proline in response to applied ABA.

The evidence is not clear yet about what is causing the increase in proline levels during water deficit. The relevance of changes in ABA concentration or of any other plant hormone in those reported proline increases remains to be verified. Moreover, because there are molecular differences between natural and synthetic ABA, investigations on native ABA effects upon proline

accumulation rather than exogenous ABA applications, are necessary. In fact, if ABA plays a role in this phenomenon in leaves, it should begin to elevate before proline accumulates following water stress imposition, and a pattern like this was been observed in grape berries of vines subjected to salinity (Downton & Loveys 1978).

ABA AS RELATED TO THE AFTER-EFFECT OF WATER STRESS

In many species, a period of water stress also results in an "after-effect" on stomatal aperture, whereby stomata will not open fully for hours or even days after the relief of stress (Dörffling et al. 1977). Also, the recovering in turgor is much faster than the normalization of metabolic events (Bengtson et al. 1978). Allaway & Mansfield (1970) propose that this effect may be caused by an inhibitor of stomatal aperture, and it has been pointed out that the after-effect of water stress is due to a slow ABA level decline after the relief of stress (Willmer 1983). In fact, Hiron & Wright (1973) observed a maintainance of both high ABA level and leaf resistance in dwarf bean seedlings after rehydration. Dörffling et al. (1974) report a correlation between the delay in stomata opening and the slow decrease in ABA level following rehydration of pea seedlings. Further, Dörffling et al. (1977) noted a direct correlation of duration of after-effect to ABA level in pea, sunflower and broad bean plants, and concluded that the increase in ABA concentration is the primary cause of the phenomenon, also because applied ABA to stressed leaves transferred to water caused stomata to remain closed.

However, there are some disagreements on this viewpoint, since results have shown that leaf ABA and stomata recovery do not correlate well (Willmer 1983). Yet it has been noted in some studies that ABA concentration decrease too fast to be related to stomata recovery (Loveys & Kriedemann 1973 and Beardsell & Cohen 1975). Moreover, Bengtson et al. (1977) found no direct relationship between the after-effect and leaf ABA content, since rehydrated wheat plants showed a re-attaining of pre-stress ABA levels at the same time (3 hours) for three different durations of water stress and, hence, different ABA levels during stress. Finally, Henson (1981) observed, after the relief of water stress in pearl millet, that ABA content decreased within 3 hours in detached leaves, and within 8 hours in intact plants, but stomatal conductance increased shortly in both cases, suggesting that the prolonged inhibition of stomatal opening after rehydration is not entirely due to continued high levels of bulk leaf ABA.

Hypotheses have been made to explain how the after-effect of water stress could occur. Since the overall content of ABA accumulated during stress is much higher than the amount necessary to affect stomatal movements, Bengtson et al. (1977) infer that the existence of an indirect relationship between the "extra" ABA and the duration of the after-effect is possible. Dörffling et al. (1977), aiming at results obtained with broad beans, argue the following possibilities: the sensitivity of guard cells to ABA is reduced by prolonged stress or enhanced ABA level consequently rendering the threshold level relative to concentration and duration; the reduction of the level of ABA at its active site in guard cells may be accomplished by plant processes other than degradation; and ABA removal into storage sites where it cannot act on the stomata may be involved. Bengtson et al. (1978) argue that the after-effect might be linked to chlorophyll metabolism through ABA or some of its metabolites. Raschke (1982) presents the following possibilities: ABA removal from the vicinity of the receptor sites in the guard cells is slower than the one from the rest of the tissue; after-effect is a reflection of stress-induced enzyme degradation instead of a phenomenon related to ABA; and PA causes stomatal narrowing. Although PA has been reported as having virtually no growth-inhibition activity (Tinelli et al. 1973), the possibility that degradation products of ABA can play a role in the after-effect is supported by Milborrow (1981), since Kriedemann et al. (1975) observed an inhibitory effect of PA on photosynthesis and a recovery pattern of CO_2 uptake well behind the pattern of transpiration following rewatering of water-stressed grapevine leaves. Furthermore, a compartmentation phenomenon involving ABA or any of its metabolites in leaf tissues could explain the after-effect, since compartmentalized active ABA-like compounds could be available to maintain stomatal closure in spite of the relief of water stress. Finally, the investigation of differences among species as to the concentration of ABA-receptor molecules could lead to the finding of both ABA actual level needed to affect stomata and the causes of extra ABA synthesis.

CONCLUSIONS

1. There is direct evidence that leaf ABA accumulation is rapid and reversible following the onset of water stress and that cultural and environmental factors influence the pattern of ABA level increases. Also, it is clear that slight decreases in water potential lead to ABA accumulation and that there is a proportionality between this increase in ABA level and the decrease in water potential. However, the evidence for a water potential threshold is not convincing yet. Nor is the suggestion that turgor pressure is the critical component for increases in leaf ABA content.

2. ABA causes rapid stomatal closure, and the mechanisms of this action appear to be more extensive than a single induction of decreases in K^+ concentration or inhibition of starch conversion into malate in guard cells - a catalized process might be promoting membrane leakage. Besides, some results showing no correlation of ABA with stomatal movements appear to indicate that ABA effects upon stomata do not follow a simple and straight forward relationship. In fact, ABA effects are associated with CO_2 levels and affected by exogenous IAA. Also, some species show increases of both ABA and proline during stress, and one might be related to each other. In addition, the occurrence of ABA compartmentation in leaf tissues could contribute for the obtained conflicting data regarding ABA-regulated stomatal aperture and after-effect of water stress.

3. Some aspects are still unclear such as how water potential could regulate ABA accumulation, how to explain the fast stomatal closure as due to ABA, and whether the after-effect of water stress is a consequence or a cause of remaining high ABA levels in leaves.

4. Methodological limitations probably are causative of controversial data, and newer techniques such as mAB-RIA might provide adequate ABA analysis in time-course experiments.

5. Suggestions for further work are: the investigation of stomatal behavior as being regulated by more than one single factor such as ABA by examining whether ABA is related to endogenous IAA or any other plant hormone that can modulate stomatal aperture such as cytokinins, the verifying whether endogenous ABA induces increases in proline content, the determination of the role and fate of ABA metabolites in relation to water stress, and the search for ABA putative receptors and binding sites in leaf tissues.

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REFERENCES

ACKERSON, R.T. Abscisic acid and precocious germination in soybean. J. Exp. Bot., 35:414-21, 1984.

ALLAWAY, W.G. & MANSFIELD, T.A. Experiments and observations on the after-effect of wilting on stomata of *Rumex sanguineus*. Can. J. Bot., 48:513-21, 1970.

ASPINALL, D. & PALEG, L.G. Proline accumulation; physiological aspects. In: PALEG, L.G. & ASPINALL, D., eds. The physiology and biochemistry of drought in plants. San Francisco, Academic Press, 1981. p.205-41.

ASPINALL, D.; SINGH, T.N. & PALEG, L.G. Stress metabolism. V. Abscisic acid and nitrogen metabolism in barley and *Lolium temulentum* L. Aust. J. Biol. Sci., 26:319-27, 1973.

BEARDSELL, M.F. & COHEN, D. Relationships between leaf water status, abscisic acid levels, and stomatal resistance in maize and sorghum. Plant Physiol., 56:207-12, 1975.

BENGTSON, C.; FALK, S.O. & LARSSON, S. The after-effect of water stress on transpiration rate and changes in abscisic acid content of young wheat plants. Physiol. Plant., 41:149-54, 1977.

BENGTSON, C.; KLOCKARE, B.; KLOCKARE, R.; LARSSON, S. & SUNDQVIST, C. The after-effect of water stress on chlorophyll formation during greening and the levels of abscisic acid and proline in dark grown wheat seedlings. Physiol. Plant., 43:205-12, 1978.

BLAKE, J. & FERREL, W.K. The association between soil and xylem water potential, leaf resistance, and abscisic acid content in droughted seedlings of Douglas-fir (*Pseudotsuga menziessi*). Physiol. Plant., 39:106-9, 1977.

BLUMENSFIELD, A. & GAZIT, S. Interaction of kinetin and abscisic acid in the growth of soybean callus. Plant Physiol., 45:535-6, 1970.

BRADFORD, K.J. Water relations and growth of the flacca tomato mutant in relation to abscisic acid. Plant Physiol., 72:251-5, 1983.

BRADFORD, K.J.; SHARKEY, T.D. & FARQUHAR, G. D. Gas exchange, stomatal behavior, and σ^{13} values of the flacca tomato mutant in relation to abscisic acid. Plant Physiol., 72:245-50, 1983.

BRAY, E. & ZEEVAART, J.A.D. Cellular compartmentation of abscisic acid and its glucose ester. Plant Physiol., 71:103, 1983. Supplement.

CHEN, H.H. & GUSTA, L.V. Abscisic acid induced freezing resistance in cultured plant cells. Plant Physiol., 71:45, 1983. Supplement.

CHEN, H.H.; LI, P.H. & BRENNER, M.L. Involvement of abscisic acid in potato cold acclimation. Plant Physiol., 71:362-5, 1983.

COOMBE, B.G. & HALE, C.R. Hormone content of ripening grape berries and the effects of growth substances. Plant Physiol., 51:629-34, 1973.

CORNFORTH, J.W.; MILBORROW, B.V.; RYACK, G. & WAREING, P.F. Chemistry and physiology of 'Dormins' in sycamore; identity of sycamore 'Dormin' with abscisin II. Nature, 205:1269-70, 1965.

CORNISH, K. & ZEEVAART, J.A.D. Accumulation of abscisic acid in relation to water stress in *Xanthium strumarium* leaves of different ages. Plant Physiol., 71:103, 1983. Supplement.

COWAN, I.R.; RAVEN, J.A.; HARTUNG, W. & FARQUHAR, G.D. A possible role for abscisic acid in coupling stomatal conductance and photosynthetic carbon metabolism in leaves. Aust. J. Plant Physiol., 9:489-98, 1982.

CREELMAN, R.A. & ZEEVAART, J.A.D. Incorporation of oxygen into abscisic acid and phaseic acid from molecular oxygen. Plant Physiol., 75:166-9, 1984.

CUMMINS, W.R. The metabolism of abscisic acid in relation to its reversible action on stomata in leaves of *Hordeum vulgare* L. Planta, 114:159-67, 1973.

CUMMINS, W.R.; KENDE, H. & RASCHKE, K. Specificity and reversibility of the rapid stomatal response to abscisic acid. Planta, 99:347-51, 1971.

DAVIES, F.S. & LASKO, A.N. Water relations in apple seedlings; change in water potential components, abscisic acid levels and stomatal conductance under irrigated and nonirrigated conditions. J. Am. Soc. Hortic. Sci., 103:310-3, 1978.

DITTRICH, P. & MAYER, M. Action of ABA on stomata of *Commelina communis*. Plant Physiol., 71:104, 1983. Supplement.

DÖRFFLING, K.; SONKA, B. & TIETZ, D. Variation and metabolism of abscisic acid in pea seedlings during and after water stress. Planta, 121:57-66, 1974.

DÖRFFLING, K.; STREICH, J.; KRAUSE, W. & MUXFELDT, B. Abscisic acid and the after-effect of water stress on stomatal opening potential. Z. Pflanzenphysiol., 81:43-56, 1977.

DOWNTON, W.J.S. & LOVEYS, B.R. Compositional changes during grape berry development in relation to abscisic acid and salinity. Aust. J. Plant Physiol., 5:415-23, 1978.

EAMUS, D. & WILSON, J.M. A model for the interaction of low temperature, ABA, IAA and CO_2 in the control of stomatal behavior. J. Exp. Bot., 35:91-8, 1984.

EZE, J.M.O.; DUMBROFF, E.B. & THOMPSON, J.E. Effects of moisture stress and senescence on the synthesis of abscisic acid in the primary leaves of bean. Physiol. Plant., 51:418-22, 1981.

FISCUS, E.L. Effect of abscisic acid on the hydraulic conductance and the total ion transport through *Phaseolus* root system. Plant Physiol., 68:169-75, 1981.

GLINKA, Z. & REINHOLD, L. Abscisic acid raises the permeability of plant cells to water. Plant Physiol., 48:103-5, 1971.

HANKE, D. Antibodies to the rescue. Nature, 310: 272-3, 1984.

HARTUNG, W.; GIMMLER, H. & HEILMANN, B. The compartmentation of abscisic acid (ABA), of ABA-biosynthesis, ABA-metabolism and ABA--conjugation. In: WAREING, P.F., ed. Plant growth substances 1982. New York, Academic Press, 1982. p.325-33.

HARTUNG, W.; GIMMLER, H.; HEILMANN, B. & KAISER, G. The site of abscisic acid metabolism in mesophyll cells of *Spinacea oleracea*. Plant Sci. Lett., 18:359-64, 1980.

HARTUNG, W.; KAISER, W.M. & BURSCHKA, C. Release of abscisic acid from leaf strips under osmotic stress. Z. Pflanzenphysiol., 112:131-8, 1983.

HEILMANN, B.; HARTUNG, W. & GIMMLER, H. The distribution of abscisic acid between chloroplasts and cytoplasm of leaf cells and the permeability of the chloroplast envelop for abscisic acid. Z. Pflanzenphysiol., 97:67-8, 1980.

HENSON, I.E. Abscisic acid and after-effects of water stress in pearl millet (*Pennisetum americanum* (L.) Leeke). Plant Sci. Lett., 21:129-35, 1981.

HENSON, I.E. Abscisic acid and water relations of rice (*Oryza sativa* L.); sequential responses to water stress in the leaf. Ann. Bot., 50:9-24, 1982.

HENSON, I.E. Effects of light on water stress-induced accumulation of abscisic acid in leaves and seedling shoots of pearl millet (*Pennisetum americanum* (L.) Leeke). Z. Pflanzenphysiol., 112:257-68, 1983.

HENSON, I.E.; MAHALAKSHMI, V.; ALAGARSWAMY, G. & BIDINGER, F.R. Leaf abscisic acid content and recovery from water stress in pearl millet (*Pennisetum americanum* (L.)). J. Exp. Bot., 35:99-109, 1984.

HENSON, I.E.; MAHALAKSHMI, V.; BIDINGER, F.R. & ALAGARSWAMY, G. Stomatal response of pearl millet (*Pennisetum americanum* (L.) Leeke) genotypes, in relation to abscisic acid and water stress. J. Exp. Bot., 32:1211-21, 1981.

HENSON, I.E. & QUARRIE, S.A. Abscisic acid accumulation in detached cereal leaves. I. Effects of incubation time and severity of stress. Z. Pflanzenphysiol., 101:431-8, 1981.

HIRON, R.W.P. & WRIGHT, S.T.C. The role of endogenous abscisic acid in the response of plants to stress. J. Exp. Bot., 24:769-81, 1973.

HOAD, G.V. Effect of water stress on abscisic acid levels in white lupin (*Lupinus albus* L.) fruit, leaves and phloem exudate. Planta, 142:25-9, 1978.

HORTON, R.F. & MORAN, L. Abscisic acid inhibition of potassium influx into stomatal guard cells. Z. Pflanzenphysiol., 66:193-6, 1972.

HSIAO, T.C. Plant responses to water stress. Annu. Rev. Plant Physiol., 24:519-70, 1973.

HUMBLE, G.D. & RASCHKE, K. Stomatal opening quantitatively related to potassium transport. Plant Physiol., 48:447-58, 1971.

IMBER, D. & TAL, M. Phenotypic reversion of Flacca, a wilty mutant of tomato, by abscisic acid. Science, 169:592-3, 1970.

ITAI, C. & MEIDNER, H. Abscisic acid and guard cells of *Commelina communis* L. Nature, 271:652-3, 1978a.

ITAI, C. & MEIDNER, H. Functional epidermal cells are necessary for abscisic acid effects on guard cells. J. Exp. Bot., 29:765-70, 1978b.

JACKSON, C.A.D. & BLUNDELL, J.B. Effect of dormin on fruit-set in Rosa. Nature, 212:1470-1, 1966.

JEWER, P.C. & INCOLL, L.D. Promotion of stomatal opening in the grass *Anthephora pubescens* Nees by a range of natural and synthetic cytokinins. Planta, 150:218-21, 1980.

JONES, R.J. & MANSFIELD, T.A. Suppression of stomatal opening in leaves treated with abscisic acid. J. Exp. Bot., 21:714-9, 1970.

KARSSEN, C.M. Indirect effect of abscisic acid on the induction of secondary dormancy in lettuce seeds. Physiol. Plant., 54:258-65, 1982.

KIRKHAM, M.B. Effect of ABA on the water relations of winter-wheat cultivars varying in drought resistance. Physiol. Plant., 59:153-7, 1983.

KONDO, K. & YAGUCHI, Y. Stomatal responses to prey capture and trap closure in Venus's flytrap (*Dionaea muscipula* Ellis). II. Effects of various chemical substances on stomatal response and trap closure. Phyton, 43:1-8, 1983.

KRIEDEMANN, P.E.; LOVEYS, B.R. & DOWNTON, W.J.S. Internal control of stomatal physiology and photosynthesis. II. Photosynthetic responses to phaseic acid. Aust. J. Plant Physiol., 2:553-67, 1975.

KRIEDEMANN, P.E.; LOVEYS, B.R.; FULLER, G.L. & LEOPOLD, A.C. Abscisic acid and stomatal regulation. Plant Physiol., 49:842-7, 1972.

KUNDU, K.K. & AUDUS, L.J. Root growth inhibitors from root tips of *Zea mays* L. Planta, 117:183-6, 1974.

LARQUÉ-SAAVEDRA, A. & WAIN, R.L. Studies on plant growth-regulating substances. XLII. Abscisic acid as a genetic character related to drought tolerance. Ann. Appl. Biol., 83:291-7, 1976.

LE PAGE-DEGIVRY, M.T. & BULLARD, C. A radioimmunoassay for abscisic acid; properties of cross-reacting polar metabolites. Physiol. Veg., 22:215-22, 1984.

LITTLE, C.H.A. & EIDT, D.C. Effects of abscisic acid on budbreak and transpiration in wood species. Nature, 220:498-9, 1968.

LOVEYS, B.R. The intracellular location of abscisic acid in stressed and nonstressed leaf tissue. Physiol. Plant., 40:6-10, 1977.

LOVEYS, B.R. & KRIEDEMANN, P.E. Rapid changes in abscisic acid-like inhibitors following alterations in vine leaf water potential. Physiol. Plant., 28:476-9, 1973.

LUDLOW, M.M.; NG, T.T. & FORD, C.W. Recovery after water stress of leaf gas exchange in *Panicum maximum* var. trichoglume. Aust. J. Plant Physiol., 7: 299-313, 1980.

MCWHA, J.A. & JACKSON, D.L. Some growth promotive effects of ABA. J. Exp. Bot., 27:1004-8, 1976.

MANSFIELD, T.A. Delay in the response of stomata to abscisic acid in CO_2-free air. J. Exp. Bot., 27:559-64, 1976.

MANSFIELD, T.A. & DAVIES, W.J. Stomata and stomatal mechanisms. In: PALEG, L.G. & ASPINALL, D., eds. The physiology and biochemistry of drought in plants. San Francisco, Academic Press, 1981. p.314-46.

MANSFIELD, T.A. & JONES, R.J. Effects of abscisic acid on potassium uptake and starch content of stomatal guard cells. Planta, 101:147-58, 1971.

MAPELLI, S. & ROCCHI, P. Separation and quantification of abscisic acid and its metabolites by high-performance liquid chromatography. Ann. Bot., 52:407-9, 1983.

MARKHART, A.H. Amelioration of chilling-induced water stress by abscisic acid-induced changes in root hydraulic conductance. Plant Physiol., 74:81-3, 1984.

MARKHART, A.H.; FISCUS, E.L.; NAYLOR, A.W. & KRAMER, P.J. Effect of abscisic acid on root hydraulic conductance. Plant Physiol., 64:611-4, 1979.

MEIDNER, H. & MANSFIELD, T.A. Physiology of stomata. New York, McGraw-Hill, 1968. 178p.

MILBORROW, B.V. Abscisic acid and other hormones. In: PALEG, L.G. & ASPINALL, D., eds. The physiology and biochemistry of drought in plants. San Francisco, Academic Press. 1981. p.347-88.

MILBORROW, B.V. Antitranspirants and the regulation of abscisic acid content. Aust. J. Plant Physiol., 6:249-54, 1979.

MILBORROW, B.V. The chemistry and physiology of abscisic acid. Annu. Rev. Plant Physiol., 25:259-307, 1974.

MILBORROW, B.V. Inhibitors. In: WILKINS, M.B., ed. Advanced plant physiology. London, Pitman, 1984. p.77-110.

MITTELHEUSER, C.J. & STEVENICK, R.F.M. van. Stomatal closure and inhibition of transpiration induced by (RS) ± abscisic acid. Nature, 221:281-2, 1969.

MURPHY, G.J.P. Metabolism of R,S-(2-^{14}C) abscisic acid by nonstressed and water-stressed detached leaves of wheat (*Triticum aestivum* L.). Planta, 160:250-5, 1984.

NOGGLE, G.R. Plant growth substances; structure and physiological effects. In: NOGGLE, G.R. & FRITZ, G.J. Introductory plant physiology. Englewood Cliffs, Prentice-Hall, 1983. p.417-82.

OHKUMA, K.; ADDICOTT, F.T.; SMITH, O.E. & THIESSEN, W.E. The structure of abscisin II. Tetrahedron Lett., 29:2529-35, 1965.

ORTON, P.J. & MANSFIELD, T.A. The activity of abscisic acid analogues as inhibitors of stomatal opening. Planta, 121:263-72, 1974.

PIERCE, M. & RASCHKE, K. Correlation between loss of turgor and accumulation of abscisic acid in detached leaves. Planta, 148:174-82, 1980.

PIERCE, M. & RASCHKE, K. The relationship between abscisic acid and leaf turgor. Plant Physiol., 61:25, 1978. Supplement.

PIERCE, M. & RASCHKE, K. Synthesis and metabolism of abscisic acid in detached leaves of *Phaseolus vulgaris* L. after loss and recovery of turgor. Planta, 153:156-65, 1981.

PILET, P.E. The effect of abscisic acid on asseptically cultured maize roots. Physiol. Veg., 21:495-500, 1983.

QUARRIE, S.A. Genetic variability and heritability of drought-induced abscisic acid accumulation in spring wheat. Plant Cell Environ., 4:147-51, 1981.

QUARRIE, S.A. & HENSON, I.E. Abscisic acid accumulation in detached cereal leaves in response to water stress. II. Effects of leaf age and leaf position. Z. Pflanzenphysiol., 101:439-46, 1981.

QUARRIE, S.A. & JONES, H.G. Genotypic variation in leaf water potential, stomatal conductance and abscisic acid concentration in spring wheat subjected to artificial drought stress. Ann. Bot., 44:323-32, 1979.

RAJAGOPAL, V. & ANDERSEN, A.S. Water stress and root formation in pea cuttings. III. Changes in the endogenous level of abscisic acid and ethylene production in the stock plants under two levels of irradiance. Physiol. Plant., 48:155-60, 1980.

RASCHKE, K. Involvement of abscisic acid in the regulation of gas exchange; evidence and inconsistencies. In: WAREING, P.V., ed. Plant growth substances 1982. San Francisco, Academic Press, 1982. p.581-90.

RASCHKE, K. Simultaneous requirement of CO_2 and ABA for stomatal closing in *Xanthium strumarium*. Planta, 125:243-59, 1975a.

RASCHKE, K. Stomatal action. Annu. Rev. Plant Physiol., 26:309-40, 1975b.

RASCHKE, K. & FELLOWS, M.P. Stomatal movement in *Zea mays;* shuttle of potassium and chloride between guard cells and subsidiary cells. Planta, 101:296-316, 1971.

RASCHKE, K. & ZEEVAART, J.A.D. Abscisic acid content, transpiration, and stomatal conductance as related to leaf age in plants of *Xanthium strumarium* L. Plant Physiol., 58:169-74, 1976.

REHM, M.M. & CLINE, M.G. Rapid growth inhibition of Avena coleoptile by abscisic acid. Plant Physiol., 51:93-6, 1973.

RIKIN, A.; ATSMAN, D. & GITLER, C. Chilling injury in cotton (*Gossypium hirsutum* L.); prevention by abscisic acid. Plant Cell Physiol., 20:1537-46, 1979.

RIKIN, A.; BLUMENSFIELD, A. & RICHMOND, A.E. Chilling resistance as affected by stressing environments and ABA. Bot. Gaz., 137:307-12, 1976.

SINGH, B.N.; GALSON, E.; DASHEK, W. & WALTON, D.C. Abscisic acid levels and metabolism in the leaf epidermal tissue of *Tulipa gesneriana* L. and *Commelina communis* L. Planta, 146:135-8, 1979.

SNAITH, P.J. & MANSFIELD, T.A. Control of CO_2 responses of stomata by IAA and ABA. J. Exp. Bot., 33:360-5, 1982.

SQUIRE, G.R. & MANSFIELD, T.A. Studies on the mechanism of action of fusicoccin, the fungal toxin that induces wilting, and its interaction with abscisic acid. Planta, 105:71-8, 1972.

STEWART, C.R. The mechanism of abscisic acid-induced proline accumulation in barley leaves. Plant Physiol., 66:230-3, 1980.

TAL, M. & IMBER, D. Abnormal stomatal behavior and hormonal imbalance in Flacca, a wilty mutant of tomato. Plant Physiol., 46:373-6, 1970.

TAYLOR, H.F. & BURDEN, R.S. Preparation and metabolism of 2-(^{14}C)-cis, transxanthoxin. J. Exp. Bot., 24:873-80, 1973.

TAYLOR, H.F. & BURDEN, R.S. Xanthoxin, a recently discovered plant growth inhibitor. Proc. R. Soc. London Ser. B., 180:317-46, 1972.

TINELLI, E.T.; SONDHEIMER, E.; WALTON, D.C.; GASKIN, P. & MACMILLAN, J. Metabolites of 2-(^{14}C)-abscisic acid. Tetrahedron Lett., (2):139-40, 1973.

UEHARA, Y.; OGAWA, T. & SHIBATA, K. Effects of ABA and its derivates on stomatal closing. Plant Cell Physiol., 16:543-7, 1975.

WALTON, D.C. Biochemistry and physiology of abscisic acid. Annu. Rev. Plant Physiol., 31:453-89, 1980.

WALTON, D.C.; DASHEK, W. & GALSON, E. A radioimmunoassay for abscisic acid. Planta, 146:139-45, 1979.

WALTON, D.C.; GALSON, E. & HARRISON, M.A. The relationship between stomatal resistance and abscisic acid level in leaves of water-stressed bean plants. Planta, 133:145-8, 1977.

WALTON, D.C. & SONDHEIMER, E. Metabolism of 2-^{14}C-(±)-abscisic acid in excised bean axes. Plant Physiol., 49:285-9, 1972.

WAREING, P.F. & SOUNDERS, P.F. Hormones and dormancy. Annu. Rev. Plant Physiol., 22:261-88, 1971.

WEILER, E.W. Immunoassay of plant growth regulators. Annu. Rev. Plant Physiol., 35:85-95, 1984.

WEILER, E.W. Radioimmunoassay for the determination of free and conjugated abscisic acid. **Planta, 144**: 255-63, 1979.

WEILER, E.W. Radioimmunoassay for the differential and direct analysis of free and conjugated abscisic acid in plant extracts. **Planta, 148**:262-72, 1980.

WEILER, E.W.; SCHNABL, H. & HORNBERG, C. Stress-related levels of abscisic acid in guard cell protoplasts of *Vicia faba* L. **Planta, 154**:24-8, 1982.

WILLMER, C.M. **Stomata.** London, Longman, 1983. 166p.

WILLMER, C.M. & RUTTER, J.C. Guard cell malic acid metabolism during stomatal movements. **Nature, 269**:327-8, 1977.

WRIGHT, S.T.C. An increase in the "inhibitor-β" content of detached wheat leaves following a period of wilting. **Planta, 86**:10-20, 1969.

WRIGHT, S.T.C. The relationship between leaf water potential (ψ_{leaf}) and the levels of abscisic acid and ethylene in excised wheat leaves. **Planta, 134**:183-9, 1977.

WRIGHT, S.T.C. & HIRON, R.W.P. (+)-abscisic acid, the growth inhibitor induced in detached leaves by a period of wilting. **Nature, 224**:719-20, 1969.

ZABADAL, T.J. A water potential threshold for the increase of abscisic acid in leaves. **Plant Physiol., 53**:125-7, 1974.

ZEEVAART, J.A.D. Changes in the levels of abscisic acid and its metabolites in excised leaf blades of *Xanthium strumarium* during and after water stress. **Plant Physiol., 66**:672-8, 1980.

ZEEVAART, J.A.D. & BOYER, G.L. Accumulation and transport of abscisic acid and its metabolites in *Ricinus* and *Xanthium*. **Plant Physiol., 74**:934-9, 1984.

ZEIGER, E. The biology of stomatal guard cells. **Annu. Rev. Plant Physiol., 34**:441-75, 1983.