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STRATEGIES EMPLOYED BY PLANTS TO CONSERVE WATER: CAN WE IMPROVE ON THEM?

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INTRODUCTION

Our title covers a very broad area, and it is necessary at the outset to define narrower limits within which our discussion will range. The strategies we intend to discuss are the physiological mechanisms that operate in the shoots of mesophytes (a) to reduce excessive water loss, and (b) to minimise and alleviate stress when the first lines of defence are inadequate.

Once a leaf has completed its development the only way it can control water vapour loss is via the movements of stomata. Structural modifications can occur in the leaves of mesophytes as they develop, but a fully grown leaf cannot change its construction in order to conserve water. It must, therefore, resort to physiological mechanisms and we shall consider these with regard to the possibility of inducing and/or mimicking them in order to regulate water stress in crops.

The use of film antitranspirants will not be discussed. These essentially represent an alteration in the structure of the leaf, giving the mesophyte the equivalent of some of the physical characteristics of xerophytes - for example, thickened cuticles and occluded stomatal pores. The number of practical situations in which this method can be used successfully is strictly limited.

PHYSIOLOGICAL MECHANISMS OF AVOIDING WATER STRESS

1. First lines of defence

The rapid responses of stomata to factors of the aerial environment constitute the primary mechanisms of stress avoidance. It is not a misrepresentation to regard stomata as sense organs capable of detecting changes in a remarkably wide range of environmental variables. Perhaps the most important of these in the present context are atmospheric water vapour content, and CO_2. Stomata are known to respond directly to changes in water vapour pressure deficit (VPD) and to variations in CO_2 concentration. Responses to VPD have been found in a wide variety of species (1, 2, 3) and they are both rapid and reversible (4). Closure occurs under conditions of high VPD, probably because the guard cells themselves are important sites of evaporation (5, 6). The stomata thus act as humidity sensors capable of protecting the leaf from excessive water loss in dry conditions. The same can be said for their response to CO_2. It has been known for many years that stomata close in response to small increases in CO_2 concentration in the sub-stomatal cavity. Such increases will occur, for example, as a result of wind causing a reduction in the diffusion resistance of the boundary layer. The response to CO_2 could constitue a way of reacting to wind, which is obviously a major stimulant to transpiration.

These first lines of defence are not always sufficient. When the supply of water to roots becomes limiting, any opening of stomata may be hazardous under nearly all atmospheric conditions. It is at this stage that further protective mechanisms are called upon.

2. Second lines of defence

The responses of stomata to VPD are thought to involve local changes in water potential in the epidermis, and they do not depend upon bulk changes in the tissue as a whole. The latter can trigger off events which exert both short and long term effects on stomata. Major participants in these are stress hormones, of which abscisic acid (ABA) may be the most important.

The discovery that ABA is formed in wheat leaves under water stress was made by Wright (7) and Wright and Hiron (8), and similar observations have since been made in studies by other workers on various species. More recently Wright (9) has shown that in wheat there is a smooth sigmoid relationship between ABA production and decreasing water potential, with a very steep rise as water potential falls from -9.5 to -11.0 bars.

When ABA is applied externally to plants it induces stomatal closure (10), and it probably influences guard cells directly since it affects stomata on isolated epidermis (11). The response of stomata to ABA provides the basis of one of the most sensitive bioassays for this hormone, which can detect concentrations down to 10^{-10}M (12).

Although the effects of ABA on stomata are thus well established, the precise part it plays in the sequence of responses to a fall in bulk water potential has not been determined. We need a better understanding of this role before we can evaluate fully the possibilities of exploiting these effects to the benefit of the water relations of crop plants. The following suggestions of the sequence of events which we are collectively calling the "second line of defence" are based mainly on factual information, but gaps in our knowledge result in some speculation.

(a) Stomatal closure which precedes a rise in ABA level

This is the main area in which we must speculate. Stomatal closure in response to a fall in water potential can occur before the level of ABA in the tissues has risen appreciably (13, 14). A number of possible reasons why this is the case could be suggested. For example, other compounds apart from ABA might be involved, because some which are capable of closing stomata are formed under water stress (15, 16, 17), and there may be others which are as yet unknown. Alternatively, the change in turgor in the epidermis may be larger than the change in turgor in the underlying mesophyll. Thus the stomata of some plants may respond to water stress before this stress is apparent in the mesophyll and is measured as a decrease in bulk leaf water potential. The most likely explanation is, however, as follows.

There is a store of "bound" ABA in the mesophyll chloroplasts of well-watered plants (A.R. Wellburn, personal communication). As soon as the bulk water potential falls sufficiently for the chloroplasts to become stressed, this ABA is converted to a "free" form, which can then move out into the cytoplasm, from where it is transported to the guard cells. Loveys (18) has shown that epidermal tissue (including guard cells) is not able to synthesize ABA, but that the amount in the epidermis of intact leaves increases greatly when they undergo water stress.

The only one of the above suggestions which is speculative is the proposed conversion of ABA from a "bound" to a "free" form when the tissue is under water stress. The hypothesis we offer is attractive because the organelles which are most sensitive to water stress are the mesophyll chloroplasts, and therefore the rapid transmission of a hormonal signal from them to the guard cells would be a logical protective mechanism.

(b) The long term effect of ABA on stomatal opening

The 'after effect' of water stress on stomata has been known for a long time: many mesophytes, even when they have regained full turgor, have reduced stomatal apertures for at least several days, and sometimes for weeks (19, 20, 21). There now seems little doubt that this prolonged effect is due to the accumulation of ABA in the leaves (14). Exogenous application of ABA can simulate the accumulation of endogenous ABA, and cause a reduction of stomatal openings for a week or more (22, 23). In plants where the decline in the level of endogenous ABA is not related to recovery of stomatal opening capacity (14) it is likely that bulk ABA levels in the leaf mask changes in the ABA levels in the guard cells, which are critical in this regard.

As part of the "second line of defence" for plants growing in natural communities, the advantages of this mechanism are clear enough: the plant, having once experienced water stress, is adopting a strategy which ensures a greater efficiency of water use (see the following section). This strategy does not continue indefinitely, but it is operative for a period during which the plant essentially "ascertains" that the renewed supply of water is sufficient for a return to full stomatal opening.

WHY ATTEMPT TO CONTROL STOMATA?

Since we have shown that plants already possess endogenous control mechanisms providing defence against water stress, this question must be considered, and the case for attempting to impose artificial controls needs careful examination. It is necessary first of all to point out the way in which stomatal closure can increase the efficiency of water use, and we shall also outline the damaging consequences of water stress.

The concept of water use efficiency

At the simplest level, transpiration can be described by the following equation

$$T = \frac{[H_2O]_{int} - [H_2O]_{ext}}{r_s + r_a}$$

where T is the transpiration flux from the leaf, H_2O_{int} and H_2O_{ext} are respectively, the densities of water vapour in the sub-stomatal cavities and at a point outside the leaf, r_s is the stomatal resistance to water loss, and r_a is the boundary layer resistance to water vapour transfer in the immediate vicinity of the leaf.

An analogous equation describes the diffusion of CO_2 from the air around the leaf to the sites of fixation:

$$P = \frac{[CO_2]_{ext} - [CO_2]_{int}}{r_a' + r_s' + r_m'}$$

where P is the rate of CO_2 uptake, $[CO_2]_{ext}$ and $[CO_2]_{int}$ are, respectively, the CO_2

TABLE 1. EFFECTS OF CHEMICALLY INDUCED CLOSURE OF STOMATA ON TRANSPIRATION AND GROWTH

(The data for phenylmercuric acetate (PMA) are taken from studies in which no foliar injury was reported).

author(s)/plant species/ treatments	observations
Zelitch & Waggoner (1962)(30) *Nicotiana tabacum* PMA: 3.3×10^{-4}M, 1×10^{-4}M and 3.3×10^{-5}M	$(P_t/P_o)/(T_t/T_o)$ ranged from 1.40 to 0.96 and was significantly above unity; $(P < 0.05)$; P_t, P_o = photosynthesis in treated and control plants; T_t, T_o = ditto, for transpiration
Shimshi (1963b)(31) *Nicotiana tabacum* PMA: 9×10^{-5}M	ratio of growth over transpiration increased from 0.78 to 1.00 in well-watered plants, and from 0.83 to 0.93 in water-stressed plants; growth was indicated by leaf area
Slatyer & Bierhuizen (1964)(42) *Gossypium* sp. PMA: 10^{-4}M	g transpiration/g dry tissue produced was 245 for controls and 180 for treated plants
Davenport (1967) (43) *Festuca rubra* PMA: $10^{-3.5}$M	g transpiration/g dry tissue produced was 2052 for controls and 1734 for treated plants
Jones & Mansfield (1972)(23) *Hordeum vulgare* ABA and its methyl and phenyl esters, all at 10^{-4}M	g transpiration/g dry tissue produced was 560 in controls, 410, 390 and 390 respectively, after treatment with ABA, methyl-ABA and phenyl-ABA
Mizrahi et al. (1974) (34) *Hordeum vulgare* ABA: 3.8×10^{-4}M	cumulative transpiration over 20 days was approximately 13 cm^3 in controls and 5 cm^3 in treated seedlings; total dry mass was 24% greater in treated plants (over unspecified period); these figures applied to non-watered plants
Raschke (1974) (33) *Xanthium strumarium* ABA: 10^{-5}M	g transpiration/g dry tissue was reduced by one half, while net photosynthesis was reduced only by one seventh

concentrations in the air and at the sites of carboxylation, and r_a', r_s' and r_m' are, respectively, the boundary layer, stomatal and mesophyll resistance to CO_2 transfer.

In C3 plants where r_m' is usually large, the existence of this extra resistance in the pathway for CO_2 transfer, compared with that for water vapour, means that a given degree of stomatal closure should exert a larger proportional effect on transpiration than on photosynthesis, in other words, there should be an increase in water use efficiency. This theoretical prediction has now been confirmed experimentally many times (24, Table 1).

Applications of compounds which cause stomatal closure may lead to a reduction of r_m' as a result of improved plant water balance. Such an effect will further increase the plant's water use efficiency. On the other hand some chemical antitranspirants apparently cause stomatal closure by first increasing the internal CO_2 concentration (25, 26). Such an effect must decrease the gradient $[CO_2]_{ext} - [CO_2]_{int}$ with the result that a decrease in water use efficiency is likely.

In the case of C4 plants r_m' is very low (because the CO_2-capturing enzyme, phosphoenolpyruvate carboxylase, is in the cytoplasm), and there is already a high water use efficiency which cannot be appreciably improved upon by inducing stomatal closure. There will, however, be a reduction in the absolute rate of water consumption, which in some circumstances may be beneficial. The disadvantages of a reduction in CO_2 fixation must be balanced against the considerable advantages of an increase in plant turgor, and a delay in the damaging effects of water stress.

The damaging effects of a reduction in plant turgor

It is well known that many plant processes are sensitive to a very small reduction in cell turgor (27). For example, enzyme levels, chlorophyll synthesis and cell growth may be reduced at bulk tissue water potentials higher than those which will cause stomatal closure. While some plants can maintain cell turgor at low water potentials (28), others require partial stomatal closure to re-establish turgor and essential processes (Fig. 1). The situation may be extremely critical in many crop varieties which are the result of extensive breeding programmes. Maximisation of yield is a primary consideration for plant breeders and unless selection programmes are carried out in environments dominated by water deficits it is possible that some physiological responses to stress will be bred out of modern cultivars. An example of such a phenomenon may be the lack of a CO_2 response by stomata of several crop plants (44). A reduction in the capacity to close stomata may be of some advantage in a crop plant constantly supplied with water. For example, reduced stomatal sensitivity to darkness and a removal of the stomatal rhythm will lead to maximum photosynthetic utilization of available light early in the morning, late in the afternoon and during a cloudy period. In addition, it is difficult to see how delayed stomatal opening following rewatering (29) can be of the same advantage to a crop plant as it may be to one in a natural ecosystem. Few growers can however guarantee maximum availability of soil water and an inadvertant reduction in stomatal sensitivity to stress as a result of breeding is likely to lead to a possibly damaging reduction in plant turgor. In this situation the artificial control of stomatal behaviour may be deemed to be highly desirable.

ARTIFICIAL CONTROL OF STOMATAL BEHAVIOUR

For an antitranspirant to be of practical interest it should be capable of inducing partial stomatal closure without interfering with the capacity of the

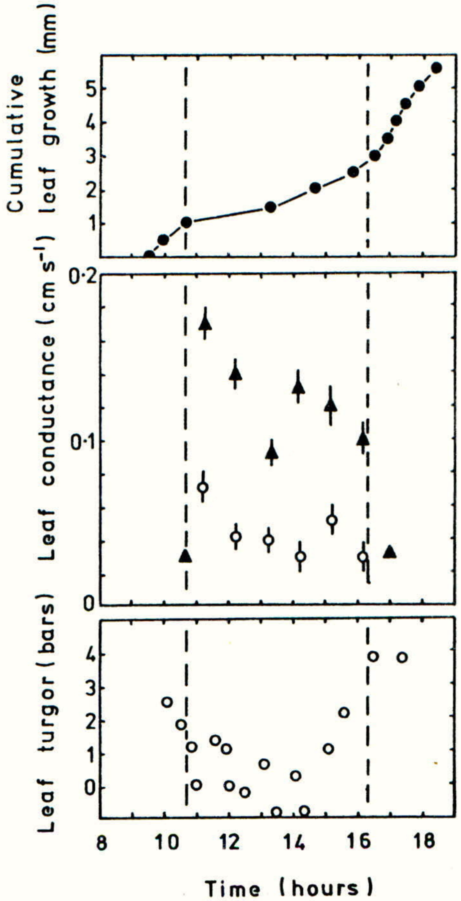

Fig. 1. The influence of variation in leaf conductance on leaf turgor and leaf extension of Zea mays (Lights on at 10.45 a.m. and off at 16.15 p.m.) (Abaxial stomata ▲, adaxial stomata ○).

underlying mesophyll to fix CO_2. Various chemicals have been tested in this regard but for various reasons have been deemed undesirable. Perhaps the most promising is phenylmercuric acetate (PMA), which when applied to tobacco leaves causes an increase in water use efficiency (30, 31, Table 1). In other circumstances, however, PMA has proved to be quite phytotoxic (32) and presumably a mercuric compound would be an undesirable environmental pollutant.

The discovery that compounds which cause stomatal closure are produced endogenously in response to water stress raises the question of whether there is any need to apply antitranspirants exogenously. If there proves to be such a need, presumably compounds the same as, or related to, those produced by the plant might prove to be suitable, non-toxic antitranspirants.

The application of aqueous solutions of ABA to leaves results in a very desirable modification of the water use efficiency of a number of plants (23, 33, Table 1). A single surface application of a 10^{-4}M solution of ABA to leaves of Xanthium resulted in a supression of stomatal opening for up to 9 days (22). A similar response was observed with barley. Exogenous application of all-trans farnesol to Sorghum plants resulted in stomatal closure with a proportionally similar reduction in transpiration and photosynthesis (17). The effect of the compound was to reduce transpiration by 75% but the effect wore off quite rapidly and was negligible after three days.

Mansfield (24) has noted that ABA has most of the properties of an ideal antitranspirant although applications to leaves may reduce the rate of shoot and root growth (34). Studies with ABA analogues (35) have not, as yet, produced a compound with more desirable properties than ABA itself. All-trans farnesol is insoluble in water and is applied to leaves as an emulsion. A water-soluble, active analogue of this compound must be found if it is to prove to be a practical antitranspirant. Considering the very large number of possible structural variations of the ABA and farnesol molecules, very few have so far been assayed. It is likely that there will be considerable rewards in this area of research.

Assuming that a non-toxic, active antitranspirant can be developed this compound could then be applied during the development of water stress in the plant. Work by Mizrahi et al (34) has shown that when water was withheld from barley seedlings exogenously applied ABA reduced the rate of water consumption and caused an increase in plant growth. This work suggests that in terms of water use efficiency there is an advantage in exogenously applying a stomatal closing compound before the stomata close in response to water stress. The effect of exogenous application of ABA to leaves of coffee plants subjected to a drying cycle is shown in Fig. 2, (Moreira, unpublished). The ABA spray significantly delayed the development of water stress in these plants.

We have some data which suggest that the application of (±) ABA to broad bean seedlings which show partial stomatal closure in response to water stress may reduce subsequent stomatal closure as water stress develops, compared to the situation in untreated seedlings (Fig. 3). Such a response to ABA could have an adverse effect on plant turgor. It is conceivable that exogenously applied ABA could control natural ABA levels either by facilitating conversion of endogenous ABA to a metabolite (36) or by inhibition of synthesis by end product control (37). The (-) enantiomer of ABA is much less active in inducing stomatal closure than the (+) enantiomer, but both (+) and (-) enantiomers may inhibit natural (+) ABA biosynthesis by means of an end product feedback control system. In Phaseolus, ABA is is rapidly metabolised to phaseic acid (39) but the (+) enantiomer is preferentially

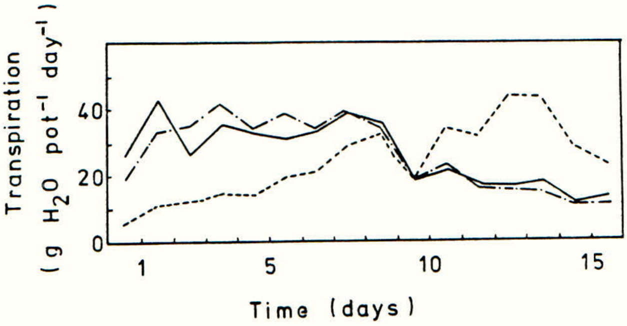

Fig. 2. The influence of two antitranspirants (ABA --- and Wilt Pruf ——) on the water loss of potted coffee plants (—·—·— Control). Plants not watered after day 1. (Wilt Pruf is a film-forming antitranspirant)

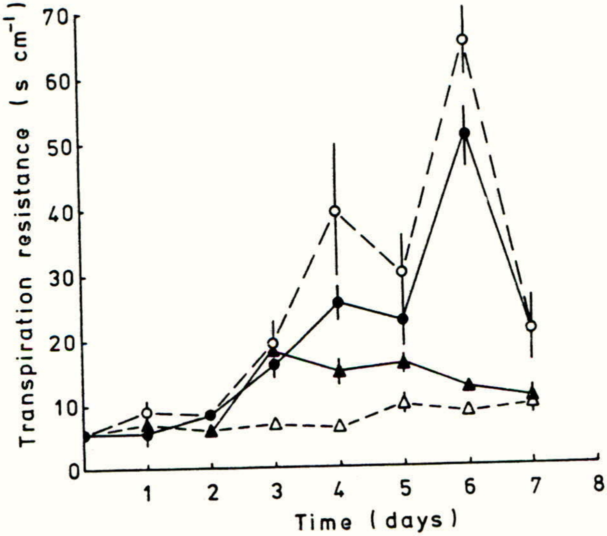

Fig. 3. Diffusive resistance of Vicia faba leaves subjected to water stress and/or treated with ABA on day 3. Control plants (△) watered every day, other treatments last watered on day 0. (○ - water-stressed plants, ● - water-stressed plus ABA, ▲ - well watered plus ABA). All plants rewatered on day 6.

converted (40). It may be supposed, therefore, that when synthetic racemic ABA is applied to leaves the (+) enantiomer will be metabolised in this way leaving a preponderance of the less active (-) enantiomer. Application of racemic ABA to leaves subjected to stress may inhibit the rate of increased (+) ABA biosynthesis since total ABA content would appear to be sufficient to cause stomatal closure. Whilst total ABA content of treated, stressed leaves may not be significantly different from that in untreated, stressed leaves, the concentration of the active (+) component could be lower with a consequent smaller reaction of the stomata. In addition to this, exogenous application of ABA to a plant producing ABA in response to stress may result in a supra-optimal concentration of the hormone in the plant and an unacceptable check in growth.

When ABA is applied to turgid broad beans which have a history of water stress, stomatal sensitivity to the hormone is apparently increased (41). This is not the case, however, in sugar beet where stomata of previously water stressed plants show decreased sensitivity to exogenous application of ABA (38). It would seem that stomatal responses of plants treated with naturally occurring water stress hormones are highly dependent upon the past and present water balance of the plant. The timing of the application in response to development of water stress may, therefore, be extremely critical.

CONCLUSIONS

The physiological defence mechanisms which have evolved to give protection against water stress to plants in natural communities are not always ideally suited for plants under cultivation. There is evidence, however, that some crop varieties which have been produced with high yield as the major objective have lost some of the more important physiological lines of defence. Exogenous applications of antitranspirants to these plants may result in a delay in the onset of the damaging effects of water stress. This can only be considered to be a temporary state of affairs and an increased supply of water must eventually be made available to the plants. Ultimately they must be considered unsuitable for growth in an environment dominated by stress. Many plants exhibit physiological mechanisms which enable them to survive periods of water stress. Identification and investigation of the function of water stress hormones may point the way to new mechanisms of survival and provide clues for plant breeders concerned with selecting for drought tolerance.

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CAN ABSCISIC ACID BE USED AS A METABOLIC INDICATOR OF DROUGHT RESISTANCE IN CEREALS?

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Summary

Eight cultivars of spring wheat were subjected to an increasing soil moisture deficit and the abscisic acid (ABA) content was determined for leaves as their water potential declined. Highly significant correlations between ABA content and leaf water potential were obtained for all cultivars but only one cultivar gave a regression slope different from the others. The eight cultivars gave a significant correlation between the ABA content of leaves from plants without water for six days and the ABA content of leaves from unstressed plants excised and desiccated to 90% fresh weight and incubated for four hours. Using a total of 25 cultivars a five fold range in the ABA accumulation in excised leaves was obtained. Excised leaves of relatively drought tolerant cultivars had low ABA contents. A tentative relationship between ABA accumulation and drought resistance in wheat is proposed.

INTRODUCTION

Stomatal conductance has for some while been regarded as an important physiological character in the study of drought resistance in cereals and there have been several attempts to define the type of stomatal response following the onset of drought that confers on a genotype a measure of drought resistance e.g.(1). Stomatal conductance is controlled by both physical and metabolic processes and the plant hormone abscisic acid (ABA) is known to have considerable effect on metabolism in the stomatal compled (2). Stomatal closure occurs in the presence of ABA and the degree of closure is proportional to the level of ABA in the tissues (3,4). The presence of ABA in plant tissues is determined particularly by plant water status and accumulation of ABA takes place as internal water stress increases. Hence a study of the relationship between the development of internal water stress, changes in stomatal conductance and the accumulation of ABA may reveal a pattern of response in ABA accumulation that would be beneficial in alleviating some of the effects of water stress.

In addition to the association between ABA and stomatal conductance there are many other physiological processes which are affected by the level of ABA present. Some of these effects would be beneficial under conditions of drought whilst others would not. Table 1 lists several of the major effects of enhanced ABA production and their consequences for yield in a cereal plant.

Table 1. Physiological responses to high ABA concentrations

Response	Value	Consequence
Decreased stomatal conductance	Beneficial	Prevents extravagant use of water and hence delays severe desiccation. Water-used efficiency is increased.
	Detrimental	Reduces photosynthesis and hence potential yield.
Decreased growth rate and reduced leaf area	Beneficial	Reduces the transpiring area and helps conserve water.
	Detrimental	Smaller plants give a reduced yield.
Decreased spikelet numbers per ear	Detrimental	Reduces grain number per ear and hence yield.

The importance attached to a particular response would depend upon the severity of the response and the type of drought expected. Thus, not only the production of ABA *per se* but also the magnitude of the resulting physiological responses to that level of ABA should be taken into account in any model designed to relate ABA accumulation to drought resistance.

Work in these laboratories has therefore been aimed at developing procedures to identify genotypic variation in the production of ABA in response to water deficits in wheat and for those genotypes which possess contrasting ABA accumulation characteristics to correlate the changes in ABA levels with other physiological processes that would eventually determine the yield. To complement this work, the response of genotypes to uniform levels of applied ABA is also under investigation.

1. Production of ABA in spring wheat plants in response to a soil water deficit

Preliminary studies with 50 cultivars of spring wheat have shown that after withholding water from plants grown in nutrient culture, a wide range in leaf water potential between the cultivars tested was found and that the level of ABA present in the tissues was generally well correlated with the leaf water potential. Eight cultivars were selected for more detailed investigation of the relationship between the accumulation of ABA and leaf water potential.

Cultivars, experimental method and results

The cultivars of spring wheat used were Maris Dove, Inia 66, Pitic 62, Sappo, Wagga-Wagga 15, TW 269/9/3/4, and two derivatives of Pitic 62: TGS 37/20/6/3 and TGS 37/50/10/3.

Germinated seeds of each cultivar were sown in pots containing 240 g of Lytag (an inert, porous, granular material). The seedlings were irrigated with nutrient solution (7), and were grown in controlled environment cabinets operating with a 16 hour photoperiod, a dry bulb temperature of 16^o and a dew point of 14^o. Water stress was applied to the plants as main stem leaf (MSL) 5 was emerging by making up all pots to the same weight with water (about 105 g water/pot) and then giving no more water.

Four plants per cultivar were harvested after four, five and six days without water, and measurements of leaf water potential and ABA content were determined for MSL 4. Leaf water potential was measured with a pressure chamber (8) and ABA was assayed according to the procedure of Quarrie (9).

The change in water potential with time is shown in Fig. 1. Although there was considerable genotypic variation in the rate at which water potential changed the accumulation of ABA was related to the change in leaf water potential (Fig. 2). Pitic 62 and TGS 37/20/6/3 accumulated ABA in a similar way to TGS 37/50/10/3 and for clarity are omitted from Fig. 2. ABA accumulation from control values of about 1 x 10^{-8} M (equivalent to 10 ng/g DW) occurred immediately the water potential began to fall, in contrast to the findings of Beardsell and Cohen (4) and Zabadal (10). Correlation coefficients for the relationship between ABA concentration and leaf water potential were highly significant for all cultivars ($r = 0.775$-0.963, 12 d.f.). Of the eight cultivars only Inia 66 showed a pattern of ABA accumulation in response to water stress different from the others.

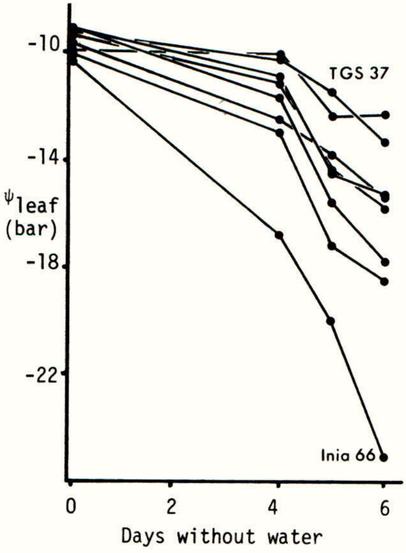

Fig.1. Change of leaf water potential (ψ_{leaf}) with time for 8 wheat cultivars.

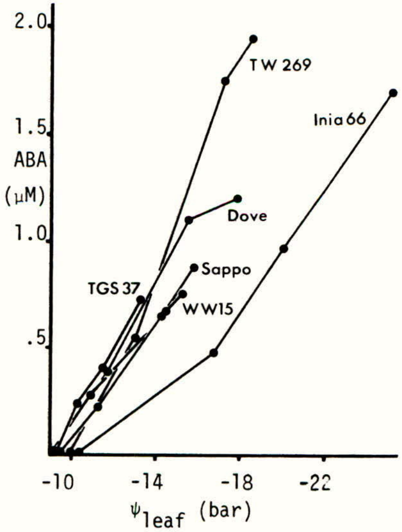

Fig.2. Relationship between leaf ABA concentration and leaf water potential for 6 wheat cultivars.

2. Production of ABA in partially desiccated spring wheat leaves

Recent studies by Larqué-Saavedra and Wain (11, 12) have shown that ABA accumulation in response to partial desiccation of excised leaves was greater for some drought resistant lines of maize and sorghum than it was for susceptible lines. We have now used a similar procedure to establish whether variation exists in ABA production in a selection of spring wheat cultivars with contrasting patterns of growth.

Cultivars, experimental method and results

Twenty five cultivars of spring wheat from Europe, North and Central America, and Australia, and including the eight used in experiment 1 were grown in compost in a glasshouse until MSL 5 had emerged. Each cultivar was maintained in a controlled environment cabinet with operating conditions as in experiment 1 for two to four days immediately prior to being sampled. Ten MSL 4 per cultivar were cut off and incubated for one hour in the cabinet with the cut end in distilled water (to allow each leaf to reach the same turgidity). The leaves were weighed and eight were then subjected to warm air treatment (35^0 C at the leaf surface) until 10% of the initial fresh weight had been lost (five to 30 min.). Each leaf was then placed in a boiling tube containing a strip of Whatman chromatography paper moistened with 1 ml water. The two remaining leaves were placed in boiling tubes containing an excess of water at the base into which the cut leaf ends were placed to give control values for ABA production. The tubes were sealed in polythene bags and incubated at $21\text{-}24^0$ C in dim light for four hours. At the end of the treatment each leaf was assayed for ABA.

No measurable accumulation of ABA took place in any of the control leaves after four hours (cultivar ABA concentrations varied from 2 to 10 $ng.g^{-1}$ FW and gave an overall mean of 5.3 $ng.g^{-1}$ FW), but there was a five fold difference, when stressed, in the mean ABA content between the cultivars with the highest and lowest values (Maris Butler and TGS 37/50/10/3 respectively). Table 2 gives ABA data for the eight cultivars used in experiment 1 together with the data for other selected cultivars.

Table 2. ABA concentration in leaves of spring wheat cultivars four hours after excision and desiccation to 90% FW ($ng.g^{-1}$ FW + SE).

Cultivar	ABA content
Maris Butler	82.0 ± 3.6
Inia 66	67.5 ± 11.5
TW 269/9/3/4	61.0 ± 6.1
TW 161	58.5 ± 4.0
WW 15	54.5 ± 3.4
Maris Dove	49.5 ± 3.5
Pitic 62	34.9 ± 4.1
Sappo	31.5 ± 3.3
Highbury	28.4 ± 2.6
Siete Cerros	20.0 ± 1.9
TGS 37/20/6/3	17.3 ± 4.4
TGS 37/50/10/3	15.5 ± 1.7
Mean of 25 cultivars	41.0

DISCUSSION

The range in ABA production by these spring wheat cultivars exceeded that observed by Larqué-Saavedra and Wain for their cultivars of maize and sorghum incubated for four hours. However, the maximum concentration of ABA in excised leaves for each cultivar, which would be reached after 10-20 hours (13, 14), may give a different order of ranking with a smaller overall range. Further work is at present underway to determine the kinetics of ABA production in excised leaves of some of these cultivars.

For the eight cultivars used in experiment 1 there was a significant correlation between ABA accumulation in MSL 4 after excision and desiccation to 90% FW and ABA concentration in leaves of intact plants after either four, five or six days without water. The ABA concentration in plants without water for six days in experiment 1 (Fig. 3) gave the highest correlation with the data from this experiment: r = 0.82, 6 d.f. The significance of the correlation suggests that the production of ABA in excised leaves may be a useful and simple technique to use for comparing genotypes. Although we have shown that genotypic variation exists in spring wheat in the accumulation of ABA, both after a given time and at a given level of internal stress, the existence of such variation in field grown material during a drought remains to be determined.

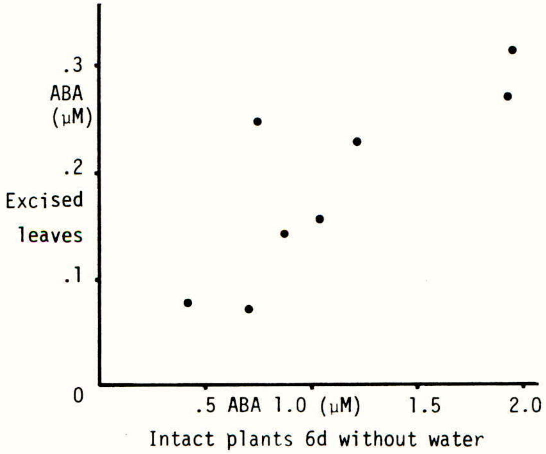

Fig. 3. The relationship between ABA accumulation in leaves excised and partially desiccated for 4 h and leaves from intact plants 6d without water.

The Relationship between ABA accumulation and Drought Resistance

Information on the response to drought in the field is, at present, available for only a few of the cultivars used in these experiments. Highbury has shown considerable drought resistance in the dry summers of recent years (G.C.M. Sage, personal communication) and the varieties Pitic 62 and S. Cerros are also reported to be drought tolerant in certain environments (15, 16). Excised leaves of these three varieties produced relatively small quantities of ABA. In contrast TW 161 is drought susceptible (17), and excised leaves of this cultivar accumulated a high concentration of ABA in response to partial desiccation. It is therefore possible that drought resistant lines of spring wheat are more likely to accumulate low levels of ABA in response to stress.

This conclusion with spring wheat appears to be the reverse of the findings of Larqué-Saavedra and Wain (12) who found that more ABA accumulated in drought resistant cultivars of maize and sorghum. This may be explained by the differing responses of the species to a drought. In many drought resistant cultivars of maize and sorghum (including the maize cultivar Latente used by Larqué-Saavedra and Wain) development during a drought effectively ceases until the stress is relieved. A high endogenous concentration of ABA would slow down the metabolic processes associated with growth and hence development. In wheat, however, resistance to a prolonged drought is frequently associated with earliness of maturity (e.g. 18) and a low accumulation of ABA would prevent a serious reduction in assimilation during the drought, allowing dry matter to accumulate and development to continue. However, a low accumulation of ABA in response to drought may not be characteristic of all types of drought resistance and is probably independent of the effect of other plant characters (such as rooting depth, e.g. 19) which have been shown to be associated with drought resistance, and which, in wheat, may override any detrimental effects of high ABA concentrations.

As stated earlier the developmental responses to high concentrations of ABA are also important in determining a cultivar's drought resistance. Homeostasis of spikelet production in the presence of ABA would therefore be a desirable character and we have found (Quarrie, unpublished results) genotypic variation in the reduction in spikelet numbers in response to ABA injection following the procedure of Quarrie and Jones, 1977. Of the 25 cultivars used in experiment 2, Inia 66 showed the least reduction in spikelet numbers following ABA treatment with 86.5% of the control spikelet numbers. We have also found (Quarrie, unpublished results) genotypic variation in the time to anthesis in response to applied ABA, and cultivars Maris Butler and Condor came to anthesis up to a week earlier than normal following treatment with ABA. This acceleration in development is a frequent occurrence in response to drought (e.g. 20) and would be of value in a situation where drought is prolonged (see above).

It may therefore be possible to improve drought resistance in wheat by selecting for a low production of ABA in response to drought and, in addition, resistance to some of the detrimental developmental responses of high ABA concentrations. However, further work with genotypes known to differ in their drought response characteristics is required before any relationship between ABA accumulation and drought resistance in spring wheat can be demonstrated.

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NOTES

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THE EFFECT OF ABSCISIC ACID ON THE WATER BALANCE AND GROWTH OF VEGETABLE TRANSPLANTS DURING RE-ESTABLISHMENT

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Summary Abscisic acid over a range of concentrations (3.8×10^{-4}M, 3.8×10^{-5}M and 3.8×10^{-6}M) applied to the shoots of well-watered bare-root tomato transplants one day before transplanting, failed to significantly improve the water balance and shoot growth of the plants during re-establishment.

Bare-root Brussels sprout plants treated with 3.8×10^{-4}M ABA had a significantly lower rate of stomatal water loss at transplanting. The treatment had no effect on plant water balance and shoot growth during re-establishment but did increase the length of new root by 33%.

It is suggested that any beneficial effects in transplant establishment derived from applied ABA are more likely to be the result of its role in stimulating root growth rather than its antitranspirant properties.

INTRODUCTION

The main factor in the 'check' undergone by a transplant is the degree of water stress caused by its inability to balance transpirational loss and water absorption from the soil. With bare-root transplants the imbalance is a direct result of the damage and disturbance caused to the root system at lifting. The rate of root replacement is probably the most important factor affecting the recovery from transplanting(1).

Antitranspirants were developed to increase survival and to speed the re-establishment of transplanted trees and shrubs by reducing the rate of water loss from their leaves so encouraging the early resumption of root growth (2). For example, Davenport (3) significantly reduced the degree of water stress in transplanted bare-root citrus trees by spraying them with a film-forming antitranspirant one day before transplanting. Few reports have been published on the use of antitranspirants as aids to the establishment of vegetable transplants. However, successful results have been reported for tomato (4, 5), celery (6) and Brassica (7) seedlings.

Recently, published work has suggested that abscisic acid (ABA), a non-toxic plant hormone which induces stomatal closure, may be the 'ideal' antitranspirant (8, 9). Endogenous ABA is known to play a role in the plant's natural response to water stress (10, 11).

As part of a larger programme of experiments on transplant physiology, experiments were conducted to examine the effect of applied ABA on the re-establishment of bare-root transplants of tomato and Brussels sprout.

GENERAL METHODS AND MATERIALS

Tomato (Lycopersicon esculentum Mill.) cv Moneymaker (Harrisons' Re-selected) and Brussels sprout (Brassica oleracea L. var gemmifera Zenber) F_1 Hybrid Quickline plants were raised in a heated glasshouse using supplementary illumination when required to give a 13 h day with mean temperature of 18°C. Seeds were sown to establish single plants in 3 inch plastic pots containing John Innes Potting Compost No.2. The plants were selected for uniformity before treatment and transplanted as bare-root transplants (plants with their root systems washed free of compost) into 6 inch plastic pots. The compost used for the experiments in which root length measurements were made was modified by excluding the peat fraction and replacing the sand with grit. These latter experiments were carried out in a growth room having a 16 hour day, and day/night temperatures of 18/15°C.

Solutions of 3.8×10^{-4}M, 3.8×10^{-5}M and 3.8×10^{-6}M ABA were prepared by dissolving ABA in a minimal volume of 100 mM potassium hydroxide, neutralizing with 0.1 N hydrochloric acid and diluting to the required concentrations using distilled water (12). The final solutions contained 0.01 % Nonidet P42, a non-ionic surfactant. Control plants were treated with 0.01 % Nonidet P42. The treatments were applied by spraying to run-off one day before transplanting.

Transpiration rates were measured gravimetrically with the surfaces of the pots sealed with a proprietary film (Clingfilm). Plant water potentials were measured using a pressure chamber (13), leaf areas using a photoelectric digital leaf scanner (14), root lengths by the line intercept method (15) and stomatal resistances using a diffusion porometer (16). Stomatal and cuticular rates of water loss were estimated from the drying curves of excised shoots (17). The growth of plants was followed by measuring changes in shoot fresh and dry weights and root length.

RESULTS

Effect of ABA on the re-establishment of bare-root transplants of tomato

Forty-day-old tomato plants were treated with 3.8×10^{-4}M, 3.8×10^{-5}M, 3.8×10^{-6}M ABA or 0.01 % Nonidet P42. The plants were transplanted into compost at a moisture content of 29 % w/w (-0.1 bar soil moisture potential). The compost was returned to its original moisture content 4, 7 and 12 days after transplanting. The stomatal resistances of the abaxial surfaces of the leaves increased rapidly over the first two days after transplanting, but decreased on the third day (Fig.1). There were no significant differences arising from the treatments. Stomatal resistances returned to pre-transplant levels by the ninth day after transplanting. Measurements taken 16 days after transplanting indicate that the plants had recovered to their pre-transplant water potentials and that the concentrations of ABA used had no significant effect on plant growth during the re-establishment period (Table 1).

Twenty three-day-old tomato plants pre-treated with 3.8×10^{-4}M ABA were transplanted into modified compost at an average moisture content of 20 % w/w (-0.2 bar soil moisture potential). The ABA treatment again had no significant effect on plant growth during the post-transplant period (Table 2). The lower soil moisture potential resulted in the failure of the plants to recover to their pre-transplant water potentials within 14 days of transplanting.

Figure 1

Effect of different concentrations of abscisic acid on the stomatal resistances (abaxial surfaces) of bare-root tomato transplants

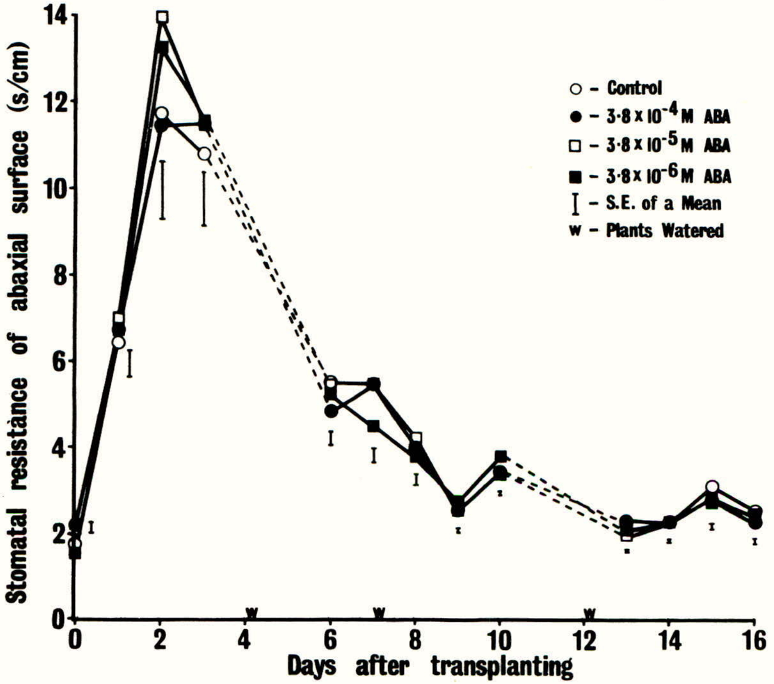

Table 1

Effect of different concentrations of abscisic acid on the water balance and growth of bare-root transplants of tomato measured 16 days after transplanting into compost at -0.1 bar soil moisture potential

	Control	$3.8x10^{-4}$M ABA	$3.8x10^{-5}$M ABA	$3.8x10^{-6}$M ABA
Plant Water Potential (bar)	-4.6	-4.6	-4.5	-4.4
S.E. Mean	0.13	0.13	0.13	0.18
Shoot Fresh Weight (g)	49.9	50.9	46.5	52.8
S.E. Mean	1.54	1.54	1.54	2.17
Shoot Dry Weight (g)	3.8	3.9	3.5	4.1
S.E. Mean	0.13	0.13	0.13	0.19

Table 2

Effect of 3.8x10^{-4}M abscisic acid on the water balance and growth of bare-root tomato plants transplanted into compost at -0.2 bar soil moisture potential

	Day 0	Day 3		Day 7		Day 14	
		-ABA	+ABA	-ABA	+ABA	-ABA	+ABA
Plant Water Potential (bar)	-7.5	-12.5	-12.1	-10.5	-10.8	-9.9	-11.1
S.E. Mean	0.6	0.3	0.4	0.5	0.3	0.2	0.1
Shoot Fresh Weight (g)	6.13	6.34	5.86	6.17	6.33	12.92	12.65
S.E. Mean	0.99	1.01	0.63	0.70	0.74	1.22	1.68
Shoot Dry Weight (g)	0.68	0.80	0.72	0.77	0.80	1.44	1.41
S.E. Mean	0.12	0.12	0.08	0.09	0.09	0.15	0.20
Root Length (m)	17.6	18.0	17.8	22.2	22.0	49.4	49.2
S.E. Mean	3.0	3.0	3.0	3.0	3.0	4.4	4.5
Transpiration Rate (g/dm^2/day)	-	2.9	3.2	4.8	4.2	10.0	9.7
S.E. Mean	-	0.4	0.4	0.7	0.4	1.0	1.3

Effect of 3.8 x 10^{-4}M ABA on the re-establishment of bare-root transplants of Brussels sprout

The shoots of 34-day-old Brussels sprout plants were sprayed with 3.8 x 10^{-4}M ABA one day before being transplanted as bare-root transplants. Similarly treated plants were used to estimate stomatal and cuticular rates of water loss at transplanting. The plants were transplanted into modified compost at a moisture content of 20 % w/w (-0.2 bar soil moisture potential) and were not subsequently watered.

The ABA significantly decreased the rate of stomatal water loss, but significantly increased the rate of cuticular water loss at transplanting (Table 3). Measurements made 8 days after transplanting show that ABA, although only slightly increasing plant water potential and shoot weight, significantly (P = 0.05) increased the length of new root by 33%.

Table 3

Effect of 3.8x10^{-4}M abscisic acid on (a) the rates of water loss at transplanting, and (b) the water balance and growth measured 8 days after transplanting, of bare-root Brussels sprout transplants

	3.8x10^{-4}M ABA	Control
(a) Rate of Stomatal Water loss(%IFW/min)	0.184**	0.328
Rate of Cuticular Water Loss(%IFW/min)	0.031**	0.025
(b) Plant Water Potential (bar)	-14.5	-15.9
S.E. Mean	0.4	0.7
Shoot Fresh Weight (g)	5.25	4.60
S.E. Mean	0.34	0.23
Shoot Dry Weight (g)	0.78	0.71
S.E. Mean	0.06	0.03
Length of New Root (cm)	780*	587
S.E. Mean	46	81

IFW- Initial fresh weight of excised shoot; * - significant at 5% level; ** - significant at 1% level.

DISCUSSION

Studies on the use of applied ABA as an aid to transplant establishment are complicated by the fact that it occurs naturally within the plant and is directly involved in responses to stress. The results are therefore discussed in terms of the effect of exogenous ABA on stomatal control before transplanting, plant water balance immediately after transplanting and plant growth and water balance during re-establishment.

The results indicate that there were differences in the responses of tomato and Brussels sprout plants to applied ABA. Concentrations ranging from 3.8×10^{-6}M to 3.8×10^{-4}M failed to induce significant increases in the stomatal resistances of the abaxial surfaces of tomato leaves, while 3.8×10^{-4}M ABA significantly reduced the rate of water loss through the stomata of excised shoots of Brussels sprout plants. The lack of effect of applied ABA on stomatal regulation of the tomato plants is inconsistent with previous reports. Abscisic acid has been found to be effective in inducing stomatal closure for a wide range of species over concentrations ranging from 10^{-6}M to 3.8×10^{-4}M (10), and to be directly involved in the stomatal regulation of tomato plants (18). However, there is some evidence to suggest that well-watered plants of certain species may not respond to exogenous applications of ABA (19, McKee unpublished data) and this may have been the situation in the present experiments.

The transplanting of bare-root transplants unavoidably results in loss of functional roots, the breakdown of the normal soil/root contact and the inability of the roots to absorb water from the soil. The production of functional roots is therefore essential to the re-establishment of bare-root transplants.

Pre-transplant treatment with ABA had no effect on the water balance and shoot growth of tomato transplants during re-establishment. Similar results were obtained with the Brussels sprout transplants treated with 3.8×10^{-4}M ABA. However, whereas 3.8×10^{-4}M ABA had no effect on the root growth of the tomato transplants, the treatment greatly increased the length of new root produced by the Brussels sprout transplants.

Following transplanting, untreated plants rapidly lose water resulting in a loss of turgor and stomatal closure. Under these circumstances the delay in the loss of turgor, even with a significantly lower rate of stomatal water loss, is limited by the amount of water held within the plant. With an estimated turgor loss point at 85% of initial shoot fresh weight, the Brussels sprout transplants of both treatments had wilted within 1½ hours of transplanting. The tomato transplants were also observed to respond in a similar way. Thus it is likely that the stimulation of root regeneration by the Brussels sprout plants resulted from the growth regulatory effect of ABA rather than its anti-transpirant properties.

The ABA was applied to the plants the day before transplanting. Therefore, assuming that foliar uptake occurred, the level within the plants was high for 24 hours whilst they were still at full turgor. Hiron's (20) results suggest that following the post-transplant stress, the levels in both the treated and untreated plants would be similar assuming no effect on the rate of recovery. Thus the stimulus to produce new roots probably occurred during the 24 hour period prior to transplanting.

In general, shoot growth appears to be stimulated by concentrations between 10^{-7} and 5×10^{-6}M ABA and inhibited by higher concentrations (21, 22), while root growth is inhibited by concentrations greater than 5×10^{-6}M even if applied to the shoot (21, 23). Although the results are again inconsistent with those reported in the literature, the previously published work did not involve transplanting where plant disturbance and water stress follow shortly after treatment.

In order to evaluate more fully the use of exogenous applications of ABA as an aid to transplant establishment, more information is required both on the effect of different water regimes before transplanting and the plant's inherent ability to withstand disturbance on its responses to the chemical.

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