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The effect of abscisic acid on cell turgor pressures, solute content and growth of wheat roots

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Abstract. Abscisic acid (ABA) was shown to influence turgor pressure and growth in wheat (*Triticum aestivum* L.) roots. At a concentrations of 25 mmol·m^{-3}, ABA increased the turgor pressure of cells located within 1 cm of the tip by up to 450 kPa. At 4 to 5 cm from the root tip this concentration of ABA reduced the turgor pressure of peripheral cells (epidermis and the first few cortical cell layers) to zero or close to zero while that of the inner cells was increased. Increases in sap osmolality were dependent on the concentration of ABA and the effect saturated at 5 mmol·m^{-3} ABA. The increase in osmolality took about 4 h and was partly the result of reducing-sugar accumulation. Levels of inorganic cations were not affected by ABA. Root growth was inhibited at ABA concentrations that caused a turgor-pressure increase. The results show that while ABA can affect root cell turgor pressures, this effect does not result in increased root growth.

Key words: Abscisic acid and turgor – Root growth – Solute relations – *Triticum* (roots) – Turgor pressure – Water relations.

Introduction

Abscisic acid (ABA) is known to accumulate in higher plants in response to drought stress (Wright and Hiron 1969; Wright 1977) and can influence a number of processes such as stomatal movement (for a review, see Raschke 1979), phloem transport and assimilate partitioning (Karmoker and van Steveninck 1979; Vreugdenhil 1983; Saftner and Wyse 1984), and ion and water transport in roots (Behl and Jeschke 1979; Behl and Raschke 1986; van Steveninck 1984). The effect of ABA on some of these processes is consistent with a proposed role in the adaptation of plants to drought stress (for reviews, see Davies et al. 1980; Bradford and Hsiao 1982). Abscisic acid is therefore thought to act as a signal for the initiation of regulatory processes involved in adaptation.

This hypothesis has been strengthened by the observation that application of ABA to fully hydrated plants can increase the root:shoot ratio (Davies et al. 1980; Watts et al. 1981; Bradford and Hsiao 1982), a response also observed in drought-stressed plants (Sharp and Davies 1979; Bradford and Hsiao 1982 and references therein). Root growth might be less affected than shoot growth because increased accumulation of solutes helps to maintain root cell turgor pressure during drought stress (Sharp and Davies 1979). Abscisic acid has been shown to affect ion and sugar accumulation in roots (see van Steveninck 1984) which indicates that maintenance of root cell turgor pressure in drought-stressed plants could be mediated by ABA. If this is the case, ABA should influence the turgor pressure of root cells. In this study we have used a pressure probe (Hüsken et al. 1978) to determine the effects of ABA on the turgor pressure of individual cells of wheat roots. The effects of ABA on root growth were also examined.

Material and methods

Plant material. Seeds of wheat (*Triticum aestivum* L., cv. Flanders; Rank, Hovis, McDougall, High Wycombe, Bucks, UK) were surface sterilised for 15 min in 1% (v/v) sodium-hypochlorite solution, washed in distilled water and then germinated in the dark at 23° C on filter paper moistened with half-strength Hoagland No. 1 solution, pH 6.3 (Hoagland and Ar-

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Abbreviation: ABA = abscisic acid

Table 1. Effect of treatment with 25 mmol·m^{-3} ABA for 48 h on the turgor pressure of cells located 0–10 mm and 40–50 mm from the tips of intact wheat roots. Values are given as mean±SD; number of cells in brackets

Cell type	Turgor pressure (kPa)			
	0–10 mm from tip		40–50 mm from tip	
	Control	ABA	Control	ABA
Epidermis	560±30 (3)	970 (1)	480, 540 (2)	0[a]–30 (3)
Cortical 1	610±10 (8)	970 (1)	610±10 (3)	0[a]–30 (2)
Cortical 2	610±10 (6)	960±100 (3)	490, 630 (2)	60, 90 (2)
Cortical 3	620±10 (3)	1,070±150 (4)	600, 600 (2)	1,030±130 (4)
Cortical 4	590, 630 (2)	1,030 (1)	640 (1)	1,060± 80 (4)
Endodermis/Stele	620±20 (6)	1,020±350 (2)	640±40 (3)	1,300±240 (7)

[a] It is not possible to measure zero turgor pressure with the pressure probe. This 0 value indicates a number of cells that appeared flaccid when prodded by the probe. The number in bracket refers to cells in which a turgor pressure could be measured

non 1950). After 48 h the germinated seedlings were transferred to 4-mm-aperture nylon mesh suspended over aerated half-strength Hoagland solution, and further grown in a Fisons 600 H growth cabinet (Fisons Scientific Apparatus, Loughborough, Leics., UK) with a fluence rate of 280 μmol photons·m^{-2}·s^{-1}, a daylength of 16 h, day/night temperatures of 25° C/20° C and a relative humidity of 50%. In some experiments a Saxcil growth cabinet was used instead and the corresponding conditions were 425 μmol photons·m^{-2}·s^{-1}, 14 h light period, 20° C/16° C temperature cycle and 80% relative humidity. The growth conditions did not appear to influence the results. Plants were used for experiments when 7–10 d old unless otherwise stated. Solutions were changed every 2 d.

Abscisic-acid solutions. The naturally occurring isomer 2,4-*cis, trans* ABA (Sigma Chemical Co., Poole, Dorset, UK) was first dissolved in 0.5 cm^3 of ethanol and then made up to a concentrated stock solution by adding half-strength Hoagland solution. This stock was diluted with nutrient solution for use. The final concentration of ethanol in the solutions was very low, nevertheless, appropriate amounts of ethanol were also added to the controls. This was done because similar concentrations of dimethylsulphoxide added for the same purpose have been shown to influence the water relations of cells (Eamus and Tomos 1983). Roots of intact plants were subjected to ABA treatment by immersing the entire root systems into ABA-nutrient solution.

Pressure-probe measurements. Turgor pressure (P) was measured on roots of intact seedlings using a pressure probe as previously described (Hüsken et al. 1978; Jones et al. 1983). Roots were held in position by carefully placing the first few centimeters into a 1-mm-diameter channel in the base of a plexiglass dish. The roots were clamped by two small plexiglass covering plates which screwed into the base of the dish. At the centre of the channel there was a 1-cm-long side opening which gave the microcapillary of the pressure probe access to the root. The depth of insertion of the microcapillary was always recorded in order to determine in which cell layer the tip was located. Cell dimensions were assumed to be similar to those reported by Jones et al. (1983). Roots were irrigated with ABA-nutrient solutions throughout the measurement period.

Sap extraction and analyses. Sap was extracted from the apical 20 mm of roots by the method of Gorham et al. (1984). Osmotic pressure of undiluted sap was measured using a Wescor 5100B or 5100C vapour pressure osmometer (Wescor Inc., Logan, U., USA). Cations, phosphorus, and sulphur were measured using an inductively coupled plasma optical emission spectrometer after dilution of sap in 600 mol·m^{-3} HCl. Reducing sugars were estimated using the Somogyi-Nelson method (Somogyi 1952). Sucrose was determined as previously described (Leigh et al. 1979). Solute concentrations were converted to osmotic pressure using the osmotic coefficients given by Wyn Jones and Gorham (1983).

Growth measurement. At the end of the treatment period, roots were harvested and their total length measured.

Results

Treatment of wheat roots with ABA for 48 h increased the turgor pressure in cells but the effect was dependent on the cell type, the distance from the root tip and the concentration of ABA. In roots treated with 25 mmol·m^{-3} ABA, increases in turgor pressure of between 350 and 450 kPa were observed in cells located within 1 cm of the root tip (Table 1). However, in cells located 4 to 5 cm from the tip, turgor pressure was reduced to nearly zero in peripheral cells (epidermal and outer cortical cells) but was almost doubled in inner cells (Table 1). A concentration of 1 mmol·m^{-3} ABA had no significant effect on the turgor pressure of any of the cells.

As expected from the turgor-pressure measurements, high ABA concentrations increased the osmolality of sap extracted from the apical 2 cm of roots. The osmolality measured after a 12-h treatment, increased with increasing ABA concentration and the response saturated at 5 mmol·m^{-3}

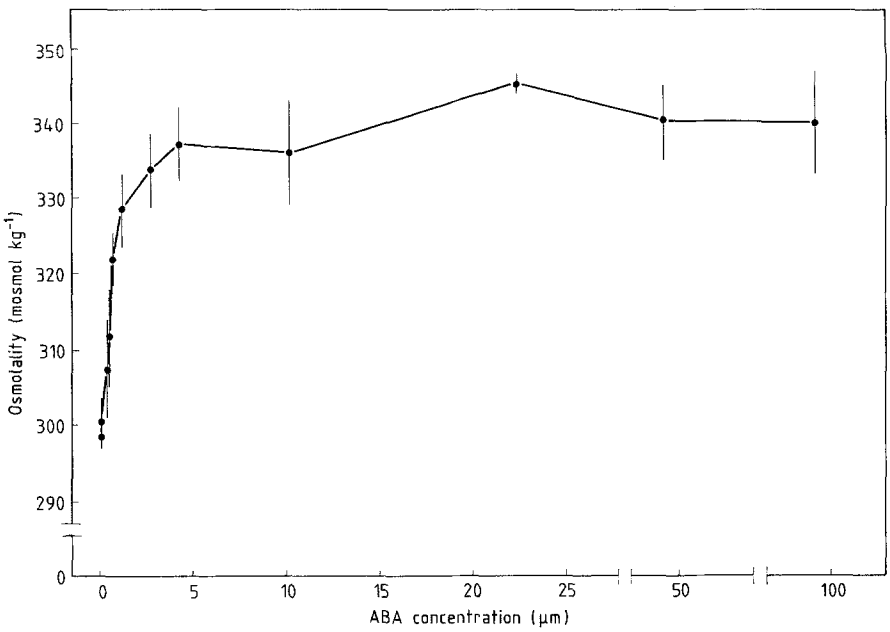

Fig. 1. Osmolality of cell sap extracted from the apical 2 cm of wheat roots treated for 12 h with various concentrations of ABA. Bars indicate SD ($n=4$). Note breaks in axes

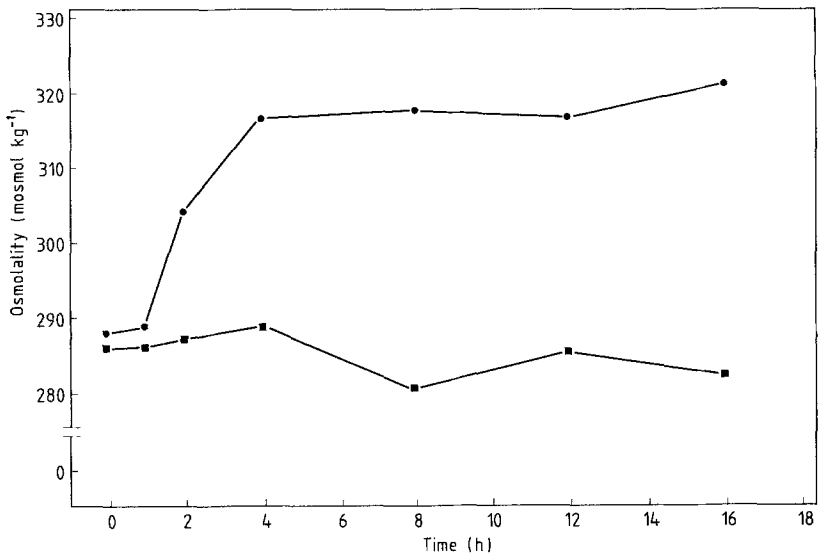

Fig. 2. Time course of the effect of 0 (■—■) or 5 (●—●) mmol·m^{-3} ABA on the osmolality of sap from the apical 2 cm of wheat roots. Note break in ordinate

ABA (Fig. 1). With the latter concentration of ABA, the osmolality reached its new value after 4 h of treatment and then remained constant (Fig. 2). Roots not treated with ABA showed no change in osmolality over the same period. The increases in osmolality induced by ABA were not, however, in quantitative agreement with the turgor-pressure measurements. Whereas turgor in the apical 1 cm increased by about 400 kPa (Table 1) the increases in osmolality were equivalent to a change in turgor of only about 100 kPa (Figs. 1, 2). This was probably the result of contamination of sap with the dilute solutions from the extracellular space, the xylem and the flaccid cells located in the basal regions of the root segments (Table 1).

Measurement of the solute contents of roots indicated that the increase in osmolality was caused, in part, by an increase in reducing-sugar concentration (Table 2). The measured change in reducing sugars accounted for approx. 50% of the measured increase in osmolality. Although K^+ salts accounted for 80% of the osmotic pressure in untreated roots, ABA did not affect K^+ concentrations (Table 2). Concentrations of Na^+, Ca^{++}, Mg^{++}, P and S were also unaffected by ABA (not shown). These data indicate that changes in

Table 2. Effect of treatment with 5 mmol·m^{-3} ABA for 24 h on the solute concentrations and osmolality of sap from wheat root tips. Values are given as mean ± SD (n = 3)

Treatment	Solute concentration (mol·m^{-3})			Osmolality (mosmol·kg^{-1})
	K	Reducing sugars	Sucrose	
Control $t=0$ h	101.0±0.8	17.75±3.04	2.76±0.23	258.75±10.2
Control $t=24$ h	104.9±4.5	19.90±2.64	3.06±1.21	264.08± 2.08
ABA $t=24$ h	99.1±4.4	53.41±6.03	7.82±3.13	324.92± 2.22

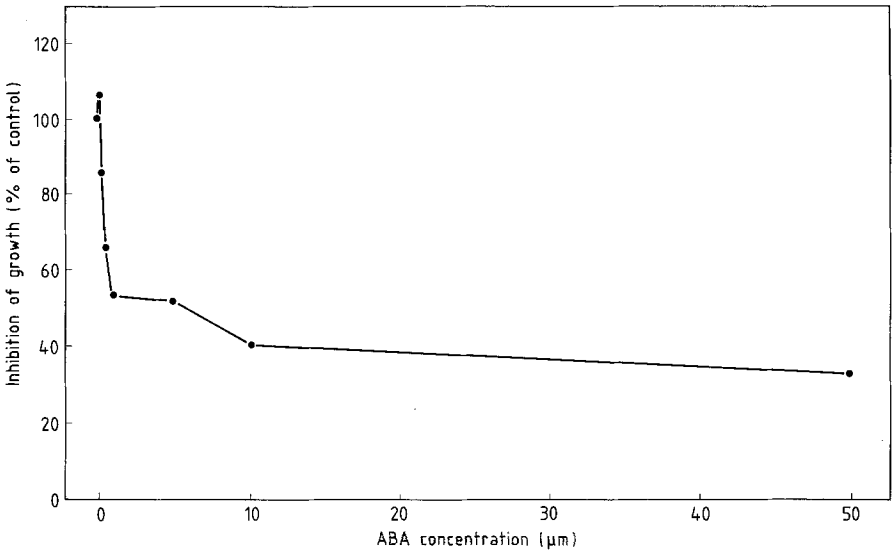

Fig. 3. The effect of ABA concentration on the growth of roots. 100% represents growth in the absence of ABA – 25.30 ± 2.03 cm in 4 d (mean ± SD, $n=8$)

ion transport did not contribute to the increase in turgor. The identity of the solutes that contributed the remaining 50% of the increase in osmolality was not investigated.

Based on the responses of turgor and osmolality to ABA, it might be expected that growth of wheat roots would increase with ABA concentration up to 5 mmol·m^{-3}. When the effect of a range of ABA concentrations was investigated root growth was unaffected by 0.1 mmol·m^{-3} ABA but was inhibited at all higher concentrations tested (Fig. 3). In all cases, however, the inhibition was less than that observed for shoot growth so that ABA caused an increase in root: shoot ratio (Jones 1985).

Discussion

It is evident that ABA can influence the processes controlling turgor pressure in wheat root cells but the responses depend on the cell type and location. This indicates the advantage of using the pressure probe to investigate these effects because such differential changes would be undetectable using averaging techniques such as osmometry. It emphasises the danger of trying to understand such responses in terms of the 'average' root cell and indicates that more attention will have to be paid to responses of different cell types.

Although the outer, older cells lost turgor pressure when treated with ABA (Table 1), this may not have been a different response from that observed in the younger cells. Abscisic acid may also have induced high turgor pressures in these older cells but their walls may have been too weak to sustain the high internal hydrostatic pressure and so they burst. This could be tested by examining the time course of the effects of ABA on these older cells.

Surprisingly, the ABA-induced change in turgor did not involve increases in cation concentrations but were partly the result of increased reducing-sugar accumulation. Similar results were obtained by Karmoker and van Steveninck (1979) using roots of intact bean seedling. These observations are in contrast to those obtained on excised

roots (e.g. Cram and Pitman 1972; Behl and Jeschke 1979; Behl and Raschke 1986) which show that ABA causes increased accumulation of ions, particularly Na^+. It seems that some of the effects of ABA on ion transport are an artefact of excision which removes the normal supply of carbohydrate from the shoot. It is unclear why potentially useful carbohydrate is placed into a relatively unproductive osmotic role when adequate supplies of inorganic osmotica were available in the external medium. The increase in reducing sugars may represent either an increased supply of carbohydrate from the shoot in response to ABA or an accumulation of material that would normally be used in synthesis if growth were not inhibited by the hormone. In potato roots, ABA has been shown to reduce nitrate-reductase activity (Palmer 1985); thus an alternative explanation of the increase in carbohydrate may be that it is the consequence of the cessation of this energy-demanding activity.

The results clearly indicate that increased turgor pressure does not result in the increased growth (compare Figs. 1 and 3). In principle turgor-driven extension growth can be regulated by solute transport, water transport or wall mechanical properties (Tomos 1985 and references therein). The lack of correlation with increased solute content indicates that either water transport or wall properties are more important in the system studied here. However, preliminary observations (Jones 1985) indicate that neither membrane hydraulic conductivity nor cell instantaneous volumetric elastic modulus were influenced by ABA. This indicates that some other water-relation parameter, which is not measurable with the pressure probe, is being altered by ABA. Recent experiments in this laboratory (Pritchard et al. 1987) indicate that growth of wheat roots may be related to the plastic extensibility of cell walls in the growing zone (measured using an Instron-type tensiometer). It is possible that ABA influences this parameter and this is currently being tested.

Finally, it should be stressed that while ABA increases turgor pressure in the roots grown in nutrient solution as used here, this may not be the case in drought-stressed roots. In the latter, the changes in external water potential may offset any increase in internal osmotic pressure and hence minimise turgor changes.

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