Plant Physiol. (1979) *64*, 611–614
0032-0889/79/64/0611/04/$00.50/0

Effect of Abscisic Acid on Root Hydraulic Conductivity[1]

Received for publication December 15, 1978 and in revised form April 17, 1979

ALBERT H. MARKHART III[2,3], EDWIN L. FISCUS[4], AUBREY W. NAYLOR[2], AND PAUL J. KRAMER[2]
Department of Botany, Duke University, Durham, North Carolina 27706 and United States Department of Agriculture, Science and Education Administration, Agricultural Research, Crops Research Laboratory, Colorado State University, Fort Collins, Colorado 80523

ABSTRACT

Reports of the effects of abscisic acid (ABA) on ion and water fluxes have been contradictory. Some of the confusion seems due to the interaction of ion and water transport across membranes. In these experiments root systems were subjected to hydrostatic pressures up to 5.0 bars to enable measurement of root conductance that was independent of measurement of osmotic potentials or ion fluxes.

ABA between 5×10^{-5} molar and 2×10^{-4} molar resulted in a decrease in the conductance of the soybean root systems as compared with the controls. ABA treatment also eliminated the discontinuity in the Arrhenius plot of total flow *versus* reciprocal temperature at constant pressure. The results suggest that ABA acts at the membrane that is rate-limiting to water flow directly, or by altering metabolism that in turn affects the membrane.

The dramatic increase in ABA content in stressed plants (20, 23) has stimulated research into possible mechanisms or functions of ABA. One area of considerable interest has been the effects on ion transport and hydraulic conductivities of plant cells. The results have often been contradictory.

ABA decreased ion transport to the shoots in corn and barley roots (3, 21). In aged beet root discs ABA temporarily inhibited K^+ uptake, stimulated Na^+ and Cl^- uptake, and altered the K^+/Na^+ selectivity (22). Others have reported increased ion transport, measured either as transport to shoots of whole plants, or as exudation from excised roots (2, 11, 16). Most recently, Karmoker and van Steveninck (12) found that ABA stimulated ion uptake in *Phaseolus* roots, whereas Erlandsson and co-workers (4) observed a decrease in ^{86}Rb uptake in sunflower roots.

Cram and Pitman (3) reported that ABA did not change the hydraulic conductivity of maize or barley roots, but Glinka and Reinhold (9, 10) reported that ABA increased both the diffusional permeability and hydraulic conductivity of roots to water flow under a pressure of 0.8 bar. Most recently, ABA was reported to have no effect on osmotically driven water flow in excised sunflower roots (4). Pitman and Wellfare (18) found dramatic decreases in barley root exudation rates treated with ABA, but concluded that this was due to an inhibition of active salt transport and not hydraulic conductivity.

These contradictions could be due to the difficulty in evaluating a complex transport system as a root. The standing osmotic gradient (1) and the removal of ions from the ascending xylem water (13) make determination of the osmotic driving force for water flow difficult. At low hydrostatic pressures the interaction between osmotic and hydrostatic driving forces makes it impossible to assess whether a change in flow rate is due to a change in hydraulic conductivity or ion movement (6). The analytical technique of Fiscus (6) provides a method of measuring the hydraulic conductance (L)[5] of a root system independent of ion transport. The experiments reported here use applied hydrostatic pressures to determine the effect of ABA on water flux through root systems, to determine if changes in flux are due to changes in L and to determine the nature of the changes ABA induces in the rate-limiting barrier to water flux.

MATERIALS AND METHODS

Culture conditions and basic experimental procedures have been previously described (15). Soybean plants were grown in a half-strength Hoagland nutrient solution under a 14-h photoperiod and a thermoperiod of 28/23 C. Root systems of decapitated plants were sealed in a 10.3-liter pressure chamber filled with fresh nutrient solution. Hydrostatic pressures were applied using compressed air bubbled through the solution to ensure mixing and aeration. In all experiments we measured steady-state flow rates (Q) in $cm^3\ s^{-1}$, and osmotic concentrations of the exudates (π^i) in milliosmoles and converted to bars according to the Van't Hoff equation. In the pressure series flow rates were normalized by expressing the rates as a fraction of the rate at 5 bars and 25 C in the absence of ABA. In the time course the flow rate at the time of ABA addition was set equal to 1 and used to normalize the rest of the flow rates. In the temperature experiments, Q at 5 bars and 25 C in the presence of ABA for the treated root systems, and in plain nutrient solution for the control system, was used to normalize the data. ABA, mixed isomers, or pure c-t isomer, was purchased from Sigma Chemicals. HPLC analysis showed a 60/40 t-t to c-t ratio in the mixed isomers. The time course experiments were repeated with both the c-t and the mixed isomers with similar results. All other experiments used the mixed isomer ABA.

Time Course. Root systems were pressurized to a constant pressure of 2.0 bars until Q and a sap concentration were reached. ABA dissolved in sufficient 95% ethanol and diluted with distilled H_2O was injected into the chamber to a final concentration of 5×10^{-5} M. Flow rates and exudate potentials were measured at 15-min intervals for the next 10 h. A control was run in a similar fashion, injecting ethanol in place of the ABA solution.

Pressure Series. Root systems were pressurized in steps between

---

[1] Research supported by National Science Foundation Grants PCM76-11142-A01-2 to Dr. P. J. Kramer and DEB77-15845 to Dr. Henry Hellmers for the Duke University Phytotron.

[2] Department of Botany, Duke University.

[3] Current address: Botanisches Institut der Tievärztlichen Hochschule Hannover, Hannover, Federal Republic of Germany.

[4] USDA SEA-AR, Crops Research Laboratory, Colorado State University.

[5] Abbreviations: L: hydraulic conductance; Q: steady-state flow rate; π^i: osmotic concentrations of exudates; HPLC: high performance liquid chromatography; σ: reflection coefficient; π^0: effective outside osmotic potential; Ea: activation energy; C-t: *cis-trans*; t-t: *trans-trans*.

0.2 and 5.0 bars at 25 C, with Q and π^i measured at each pressure. The pressure was then dropped to 0.2 bar and ABA, dissolved in minimal 95% ethanol, and diluted with distilled H_2O, was injected to yield net solution concentrations of 5×10^{-5} M, 1×10^{-4} M, or 2×10^{-4} M. After a 6- to 8-h incubation the pressure was increased in steps to 5.0 bars, and Q and π^i were measured at each pressure. The conductance (L) in cm^3s^{-1} bar^{-1}, the reflection coefficient (σ), and the effective outside osmotic potential (π^0) in bars were calculated according to Fiscus (6).

Temperature Series. Root systems were pressurized to 0.2 bar at 25 C in 2×10^{-4} M ABA in half-strength Hoagland solution. After a 6- to 8-h incubation period, the pressure was increased to 5.0 bars and the temperature dropped to 7 C in three steps. Q and π^i were measured at each temperature.

In all experiments except the temperature series, root systems treated with only 95% ethanol were run in similar manner as controls. In the temperature series, one root system was treated with 95% ethanol and four others run with no treatment served as controls. The time course and temperature series were run in triplicate. Each concentration of ABA in the pressure series was run in duplicate.

RESULTS

Time Course. When root systems were held at a constant pressure of 2 bars the addition of ABA (5×10^{-5} M) decreased Q by 40% over the ethanol control after 3 h (Fig. 1). In all experiments Q began to decrease after 1 to 3 h, with the rate reduced 20 to 40% after 6 h. In one of the three replicates there was a 10% increase in Q after 1 h, but this lasted only 45 min, after which Q declined.

Since the amount of ethanol varied with the amount of ABA, an excess amount of ethanol (5 ml) was used as a control so that future comparisons could be made and the effect attributed to ABA. Figure 2 shows that with the addition of the ethanol, Q dropped almost immediately by 4%, and then remained constant for the next 8 to 10 h.

π^i decreased in both the ethanol and the ABA-treated roots with the decrease more dramatic in the ethanol treatment. The decrease in the ethanol-treated roots began almost immediately, suggesting that it was due to the appearance of the added ethanol in the exudate. In the ABA-treated roots π^i decreased only 0.05 bar after the addition of the ABA solution, but then decreased another 0.16 bar in parallel with the decreasing Q (Fig. 1).

Pressure Series. Figure 3 shows that root systems treated with 5×10^{-5} M, 1×10^{-4} M, and 2×10^{-4} M ABA had significantly decreased L. The effect was concentration-dependent with L decreasing 17, 27, and 60% for the 5×10^{-5} M, 1×10^{-4} M, and 2×10^{-4} M ABA, respectively. In no case did the ambient π^i increase more than 0.12 bar following addition of ABA. Each ABA curve is the average of data from two root systems. The addition of 2.5 ml of 95% ethanol had no effect on the L of the root system. The control curve in Figure 3 is therefore the flow curve for the roots before the ABA addition, and the flow curves before and after the addition of the ethanol.

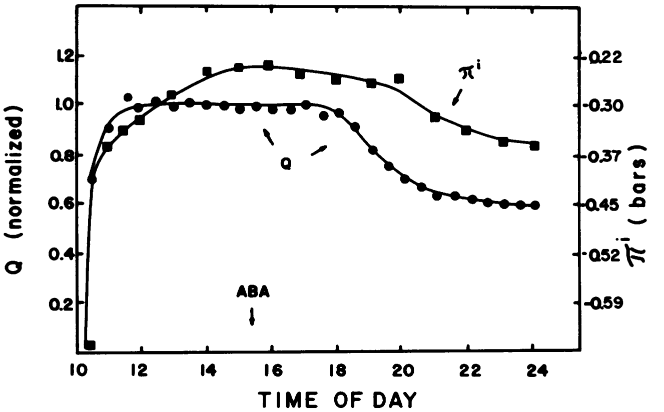

FIG. 1. Effect of ABA on Q (●) and π^i (■) from an excised soybean root system. Roots were held at a constant 2.0 bars hydrostatic pressure at 25 C. Roots were pressurized at 10:00 with 5×10^{-5} M ABA dissolved in 2.1 ml of 95% ethanol injected at 15:45. Flow rates were normalized to the rate at the time of ABA injection.

π^i of the exudate at high pressures decreased in all ABA treatments (Fig. 4). Since π^i is dependent on Q, filter effectiveness (σ), and active uptake of ions, π^i was plotted *versus* reciprocal flow rate. This was useful for several reasons. The *y* intercept, π^i at infinite flow rate, provided a basis for comparing solute flux independent of flow. This value was used in conjunction with the

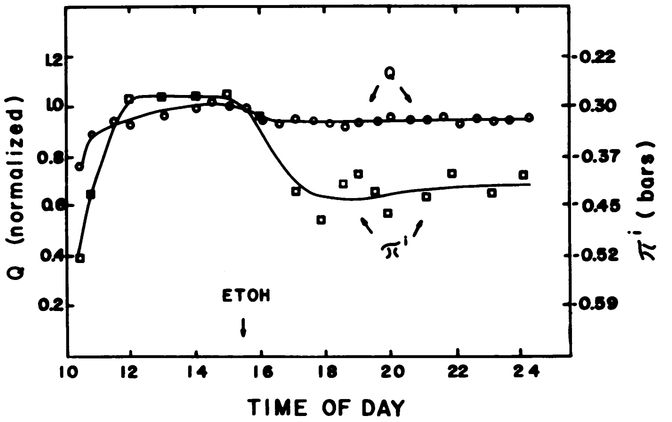

FIG. 2. Effect of 5 ml of ethanol on Q (○) and π^i (□) from an excised soybean root system. Roots were held at a constant 2 bars hydrostatic pressure at 25 C. Roots were pressurized at 10:00 and 5 ml of ethanol added at 15:45. Flow rates were normalized to the rate at the time of ethanol injection.

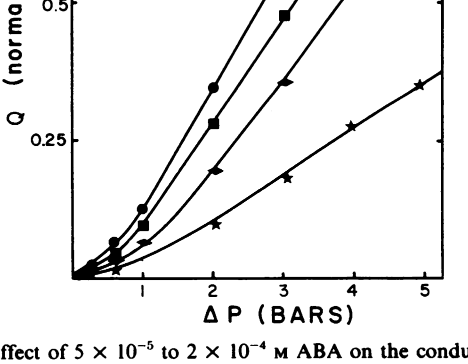

FIG. 3. Effect of 5×10^{-5} to 2×10^{-4} M ABA on the conductance of excised soybean roots. Control and experimental pressure series were run on the same root system. Q was normalized to the rate of the control at 5.0 bars. Each experimental curve is the average of two root systems. The conductance, L, is represented by the slope of each straight line formed by the data.

x intercept of the straight line portion of the Jv:ΔP curve to calculate values for σ and π^0 according to equations developed by Fiscus (5, 6). Because of large variations between roots, the data from one representative ABA and ethanol experiment were plotted in Figure 4, while data from all experiments are summarized in Table I. In all cases ABA decreased π^i relative to the first pressure series. In contrast to this, in the ethanol control, or the second curve of untreated roots, π^i increased relative to the first pressure series. In all ABA treatments π^i at infinite flow rate decreased 0.05 bar to −0.15 bar, whereas there was no change in the ethanol control (Fig. 4).

Temperature Effects. We have shown previously that a decrease in temperature decreased the L of a root system (15). These results are similar to the effect of treatment with ABA. We therefore decided to investigate the interaction of ABA and temperature. Root systems pressurized to 0.2 bar were treated with 2×10^{-4} M ABA for 6 to 8 h at 25 C. The pressure was then increased to 5.0 bars and the temperature decreased in steps to 7.0 C. Figure 5 compares the Arrhenius plot of the mean of three ABA-treated root systems with the plot of the mean of five control root systems. Flow rates were again normalized to the rate at 5 bars and 25 C of each root system.

Pretreatment with ABA eliminated the discontinuity in the Arrhenius plot. The activation (Ea) of the ABA-treated roots averaged 25 kcal/mol. This Ea was intermediate between that above the break point, 4 kcal/mol, and that below the break point, 38 kcal/mol, of the untreated roots. π^i remained unchanged during the temperature drop, averaging −0.1 to −0.19 bars.

DISCUSSION

In mature soybean root systems, concentrations of ABA between 5×10^{-5} M and 2×10^{-4} M markedly reduced the L of the root system. These results differ from those of Glinka (8) and Glinka and Reinhold (9, 10) who found, in carrot storage tissue and excised barley roots, that ABA increased the diffusive permeability and the hydraulic conductivity of the tissue. Glinka (8) used suction to induce hydrostatic pressure gradients, and interpreted increases in flow as an increase in the L of the root. The differences between Glinka's work and the results reported here could be due to the use of pressures below 1 bar where there is considerable interaction between the osmotic driving forces and the hydrostatic forces (6), or waiting only 1 h after adding ABA to measure the flow rate. At pressures below 1 bar our experiments showed that steady-state rates were not reached until 3 or 4 h after the initial pressure was applied (Fig. 1). A basic assumption in the derivation of the basic flow equation is that fluxes are in a steady-state, and unless Glinka's plants reached a steady-state much faster than ours, his rates do not appear to meet this criterion.

The situation is confused further because different species, different cultural practices, and different concentrations of ABA were used. Glinka's concentrations were about 10-fold less than

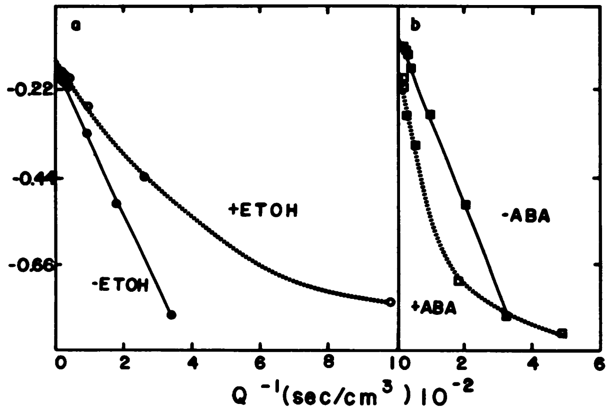

FIG. 4. Effect of 2×10^{-4} M ABA on π^i expressed as a function of the reciprocal of flow rate (Q^{-1}). Curves are for representative root systems.

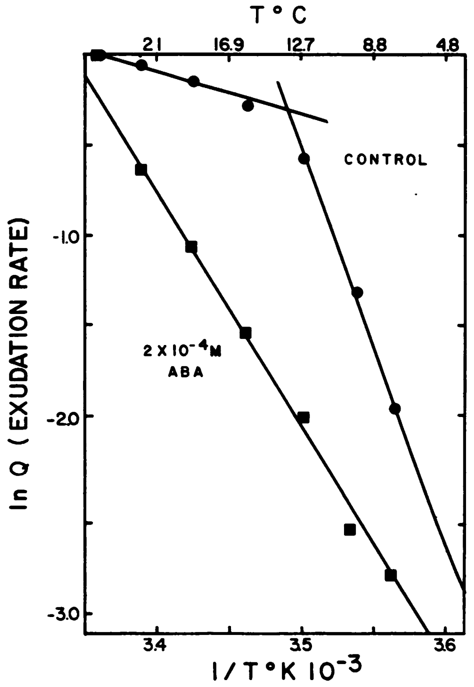

FIG. 5. Effect of ABA on Arrhenius plot of Q *versus* reciprocal temperature at 5 bars hydrostatic pressure. Curves are the averages of four experiments and three experiments for control (●) and ABA treatment (■), respectively. Q was normalized to the rate at 25 C.

Table I. *Summary of Data from ABA Pressure Series*

Values from the pre-ABA control curve appear under the heading C. Values obtained from the ABA-treated experimental curve appear under column E.

Treatment	Replicate	ΔP (Bars)		π^i (Bars)		σ		π^o (Bars) Calculated		Lp (%)
		C	E	C	E	C	E	C	E	
5×10^{-5} M	1	−0.50	−0.60	−0.15	−0.18	0.80	0.81	−0.78	−0.93	−10
ABA	2	−0.50	−0.51	−0.10	−0.15	0.85	0.81	−0.70	−0.78	−24
1×10^{-4} M	1	−0.36	−0.68	−0.07	−0.10	0.85	0.89	−0.50	−0.88	−21
ABA	2	−0.32	−1.15	−0.07	−0.14	0.84	0.90	−0.46	−1.42	−32
2×10^{-4} M	1	−0.32	−0.96	−0.10	−0.16	0.81	0.87	−0.47	−1.27	−66
ABA	2	−0.52	−1.20	−0.07	−0.14	0.89	0.90	−0.66	−1.47	−56
2.5 ml	1	−0.45	−0.45	−0.11	−0.11	0.83	0.83	−0.66	−0.66	0
Ethanol	2	−0.30	−0.31	−0.08	−0.08	0.82	0.83	−0.45	−0.46	−1.5

those used here. Pitman *et al.* (17) showed that the growth environment of the plants could influence the response of the plant to exogenous ABA.

The salt flux data were not as clear-cut as the volume flow results. Although σ increased in three of the six ABA-treated root systems, the increase did not exceed 0.06. In two of the root systems the values remained unchanged and in one there was a slight decrease (Table I). What is most perplexing is the 2- to 3-fold decrease in calculated π^0 due to ABA treatment. This decrease was not seen in the ethanol control, and seems to be due, at least mathematically, to the large increase in the *x* intercept of the Jv: ΔP curve. The π^i at infinite flow rate consistently decreased with ABA treatment. There was no change in the ethanol control, an observation that at first is difficult to explain. Since ethanol is freely permeable to root systems, we expected that π^i should decrease by an amount equivalent to the amount of ethanol added as was seen in the initial time course experiments (Fig. 2). The failure to see this increase in the longer term experiments appears due to the removal of the ethanol from the solution either by evaporation or metabolic activity of the roots or the bacteria growing in the pot. Measurements of the ambient solution osmotic potential at the end of the experiment showed no significant difference from the potential at the beginning of the experiment. If the endodermis is in fact the rate-limiting step and the major ion barrier, then the large ABA-induced increase in the effective π^0 could be caused by a loss of ions from the surrounding cortex to the apoplast. However, one must remember that the term reflection coefficient, when applied to a large root system, cannot be interpreted in the same way as in a simple membrane system. In the root system the apparent σ is the sum of many active and passive forces and compartments which contribute to net ion flux (6). Consequently, we were unable to interpret fully the effect of ABA on salt flux from a mechanistic standpoint.

Markhart *et al.* (15) presented data supporting the hypothesis that the hydraulic conductivity of mature soybean roots was determined by the character of its lipid membranes. The significant effect ABA has on the L of the roots suggests that ABA interacts with, or in some way alters, the rate-limiting membrane. The effect of ABA on the Arrhenius plot provides important insight into the physical changes that occur in the membrane, leading to a decrease in L. The slope of the straight line portions of an Arrhenius plot is in part determined by the mobility of the hydrocarbon chains in the membrane (7). The less viscous the lipid, the lower the activation energy; the more viscous the lipid, the higher the activation energy. We, therefore, propose that the flattening out of the break in the Arrhenius plot caused by ABA (Fig. 5) is due to a change in the mobility of the hydrocarbon chains. Whether the alteration is a primary effect of ABA's binding to the membrane, or whether it is a secondary effect of ABA's altering the metabolism of the cell, which in turn affects the membrane, is not clear. However, the secondary effect seems more likely considering the time course of development of the effect.

Several investigators have suggested that cold hardening may in fact be related to an increase in membrane flexibility, at low temperatures (14, 19). Membranes are relatively fragile components of cells, and may be injured by a variety of stress conditions (14). We propose that a major role of ABA is to protect the membranes from damage. We also suggest that the large increases in ABA under stress conditions are part of this adaptive response. The data of Rikin and co-workers (20) support this hypothesis. They found that nutrient, salt, and water stress all increased the levels of ABA in the stressed tissue. In addition, all three treatments, as well as the application of exogenous ABA, increased the plant's resistance to cold. Rikin has elegantly demonstrated that the generalized nature of hardening is linked to ABA increases. The results present here a general mechanism for ABA's role in hardening. ABA may directly or indirectly stabilize cell membranes, thereby increasing their resistance to damage from future stress.

LITERATURE CITED

1. Anderson WP, DP Aikman, A Meiri 1970 Excised root exudation a standing gradient osmotic flow. Proc R Soc Lond B 174: 445–458
2. Collins JC, AP Kerrigan 1973 Hormonal control of ion movements in the plant root? *In* WP Anderson, ed, Liverpool Workshop on Transport in Plants. Academic Press, London
3. Cram WJ, MG Pitman 1972 The action of abscisic acid on ion uptake and water flow in plant roots. Aust J Biol Sci 25: 1125–1132
4. Erlandsson GS, S Pettersson, S Svensson 1978 Rapid effects on ion uptake in sunflower roots. Physiol Plant 43: 380–384
5. Fiscus EL 1975 The interaction between osmotic and pressure induced water flow in plant roots. Plant Physiol 55: 917–922
6. Fiscus EL 1977 Determination of hydraulic and osmotic properties of soybean root systems. Plant Physiol 59: 1013–1020
7. Fox CF 1975 Phase transitions in model systems and membranes. *In* J Frei, M Jemelin, eds, Biochemistry of Cell Walls and Membranes. University Park Press, Baltimore
8. Glinka, Z 1977 Effects of abscisic acid and hydrostatic pressure gradients on water movement through excised sunflower roots. Plant Physiol 59: 933–935
9. Glinka Z, L Reinhold 1971 Abscisic acid raises the permeability of plant cells to water. Plant Physiol 48: 103–105
10. Glinka Z, L Reinhold 1972 Induced changes in permeability of plant cell membranes to water. Plant Physiol 49: 602–606
11. Imber D, M Tal 1970 Phenotypic reversion of flacca, a wilty mutant of tomato, by abscisic acid. Science 169: 592–593
12. Karmoker JL, RFM van Steveninck 1978 Stimulation of volume flow and ion flux by abscisic acid in excised root systems of *Phaseolus vulgaris* cv. Redland Pioneer. Planta 141: 37–43
13. Klepper B 1967 Effects of osmotic pressure on exudation from corn roots. Aust J Biol Sci 20: 723–735
14. Lyons JM 1973 Chilling injury in plants. Annu Rev Plant Physiol 24: 445–466
15. Markhart AH, III, EL Fiscus, AW Naylor, PJ Kramer 1979 Effect of temperature on water and ion transport in soybean and broccoli root systems. Plant Physiol 64: 83–87
16. Mizrahi Y, AE Richmond 1972 Hormonal modification of plant response to water stress. Aust J Biol Sci 25: 437–443
17. Pitman MG, U Lüttge, A Lauchli, U Ball 1973 Action of abscisic acid on ion transport affected by root temperature and nutrient status. J Exp Bot 25: 147–155
18. Pitman MG, O Wellfare 1978 Inhibition of ion transport in excised barley roots by abscisic acid; relation to water permeability of the roots. J Exp Bot 29: 1125–1138
19. Pomeroy MK 1976 Swelling and contraction of mitochondria from cold-hardened and nonhardened wheat and rye seedlings. Plant Physiol 57: 469–473
20. Rikin A, A Blumenfeld, AE Richmond 1976 Chilling resistance as affected by stressing environment and ABA. Bot Gaz 137: 307–312
21. Shaner DL, M Mertz, CJ Arntzen 1975 Inhibition of ion accumulation in maize roots by abscisic acid. Plant 122: 79–90
22. Van Steveninck RFM 1972 Abscisic acid stimulation of ion transport and alteration in K^+ - Na^+ selectivity. Z Pflanzenphysiol 67: 282–286
23. Wright STC, RWP Hiron 1969 (+) Abscisic acid the growth inhibitor induced in detached wheat leaves by a period of wilting. Nature 224: 719–720