

Effects of Abscisic Acid Application to Roots on Water and Ion Uptake and Leaf Conductance of Soybean

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ABSTRACT. Reported effects of applied abscisic acid (ABA) on water and ion uptake rates of excised leaves and roots are many and contradictory. Seedlings (8 d) of soybean (*Glycine max* (L.) Meer. cv Bragg) were trimmed to three uniform root branches and grown in nutrient solution. Seedlings (18 d) were transferred to a continuously aerated circulating nutrient solutions in 3 glass tubes mounted together. An automatic system was used to monitor water and ion uptake at 10 min intervals. Concentrations of ABA from 10^{-5} to 5×10^{-4} M were applied to one root division. The other two divisions were used as controls. Water uptake was enhanced by 10^{-4} to 5×10^{-4} M ABA in the treated root portion for a short time and then reduced throughout the treatment. When ABA was replaced with nutrient medium, treated roots began to recover slowly. Complete recovery required more than 24 h. Water uptake of untreated roots was reduced less than treated roots and returned more rapidly to normal water uptake. Reductions of water uptake rates were less during applications of 3.16×10^{-5} and 10^{-5} M ABA. Ion uptake rates were reduced in treated root portions during application of all ABA concentrations, while untreated root portions were not affected. Patterns of nutrient uptake reduction were unrelated to ABA concentration.

ABA applied to roots reduced stomatal aperture as evidenced by consistent reductions in leaf conductance and in total water uptake.

The effects of exogenous ABA on the hydraulic conductivity of roots and the flow of water and ions through root systems are many and contradictory. Root permeability, ion uptake, and root exudation were increased When 10^{-8} to 4×10^{-6} M ABA was applied to excised roots (Collins and Kerrigan 1974, Glinka 1973, and

1977). However, ion uptake was inhibited, while water flow was not affected in excised barley roots during application of 10^{-5} M ABA (Pitman and Wellfare 1974). Ion uptake was transiently stimulated by 10^{-5} M ABA, then rapidly inhibited in excised roots (Pitman *et al.* 1974A). Applying 4 to 9×10^{-3} M ABA to excised roots of barley inhibited ion transport (Cram and Pitman 1972), which may have resulted from a reduction in release of ions to the xylem (Pitman *et al.* 1974B). ABA at 5×10^{-6} M severely inhibited K^+ transport and favored the uptake of Na^+ in excised barley roots (Behl and Jeschke 1979), although the flux of K^+ did not depend on ABA application in excised lupin roots (Van Steveninck *et al.* 1988). Differences in plant species, age, and ABA concentrations may have contributed for some of these reported contradictions (Barlow 1987, Firn 1986, Lüttge 1974, and Trewavas 1987). Applied ABA to leaves was reported to have a direct effect on guard cells and is involved in stomatal closure (Hartung 1983, Loveys 1984, Raschke 1987). Application of ABA to leaves promoted increases in the cytosolic Ca^{2+} of guard cells prior to stomatal closure (McAinsh *et al.* 1990). However, because of possible changes in the physiology and biochemistry of excised tissues, this study indicates patterns of water and ion uptake rates of intact plants at different concentrations of ABA applied to roots.

Materials and Methods

Growth Conditions. Seeds of soybean (*Glycine max* (L.) Merr. cv Bragg) were germinated in sand. After eight days, seedlings were washed. The primary and lateral roots of every plant were removed except for three adventitious roots of uniform size. The plants were transferred to flasks with 750 mL of continuously aerated nutrient solution. Because of limitations in instrumental and growth chamber capacities, only two experimental plants could be monitored at a time. Experiments, each with two parts, were repeated three times. The nutrient solution was quarter strength for five days and half strength until harvest time. The full strength nutrient solution was modified from Claassen and Barber (1974) and contained 10 mM KNO_3 , 3 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 1 mM $Mg(NO_3)_2$, 1 mM K_2HPO_4 , 1 mM KH_2PO_4 , 46 μ M B, 9 μ M Mn, 0.8 μ M Zn, 0.3 μ M Cu, 0.8 μ M Mo, and 75 μ M Fe as FeEDTA. Nine days later, the three root divisions were transferred to three separated glass cylinders mounted together (2.2 cm id x 43 cm long surrounded by a glass jacket to contain circulating water at $27 \pm 1^\circ C$). The nutrient solutions were continuously aerated and circulated in these tubes. The growth chamber was maintained with steady horizontal air flow, $27 \pm 1^\circ C$ day and night, and 23 to 39% RH. Light flux from fluorescent and incandescent lamps was $0.48 \text{ mmol m}^{-2} \text{ s}^{-1}$ from 800 to 2300 h except from 1200 to 1600 h, when it was increased to $1.5 \text{ mmol m}^{-2} \text{ s}^{-1}$ by Lucalox lamps. A horizontal plywood partition was used to minimize light flux in the area of root growth.

Measurements. Water absorption rate, nutrient solution conductance, air and solution temperatures, light flux, and relative humidity (RH%) were measured automatically at 10 min intervals with a Campbell CR-5 data logger (Campbell Sci., Logan, UT). To reduce noise, the data are reported as running means of three consecutive values. The number of calibrated increments of water added to maintain water level, sensed by a thermistor circuit, was used to calculate water absorption rate. Stainless steel electrodes precalibrated using standard nutrient solution described above for a series of dilutions ($r^2 = 0.9998$, $N = 7$, $n = 42$) were used to measure the nutrient solution conductances, which were converted to nutrient solution concentrations. Light flux was monitored with a LiCor quantum sensor (LiCor, Inc., Lincoln, NE). The temperatures of root portions and the growth chamber were monitored using thermocouples. Total leaf conductance was calculated from measured rates of water uptake during periods when plant water status was not changing and air vapour pressure deficit (VDP).

Applications of ABA. Trizma base and authentic ABA were from Sigma (Sigma Chemical Co., St. Louis, MO). A series of dilutions from Trizma base (2 to 25 mM), Na_2CO_3 (10 mM to 100 mM), NaOH (10 to 100 mM), with and without pH adjustment, and ethanol (0.05% to 0.1%) were tested for their effects on water and ion uptake rates with no ABA added. All the solutions affected water and ion uptake rates, except 0.05% ethanol. A final dilution to 0.04% ethanol represented the least amount of absolute ethanol required to dissolve the highest concentration of ABA applied and this was used to add ABA to half strength nutrient solution. ABA concentrations were added to single portions of the divided root systems and the untreated root portions were used as controls. ABA was added at 800 h and removed 24 h later. The concentrations of ABA and the days of treatment are reported with the results.

Results

Results from a soybean plant during application of 3.16×10^{-4} M ABA are presented in Figure 1. Root portions 1 and 2 (roots 1 and 2) had comparable water uptake rates before application of ABA to root 2. However, the third root portion was usually different in water and ion uptake rates than first and second root portions before ABA treatment, thus was not used for comparisons. The ABA enhanced water uptake of treated root 2 during the first two hours more than the same period of the day before treatment, then water uptake slowed. A reduction in water uptake rate continued, reaching a maximum during the dark period. When ABA was removed at 800 h the next day, water uptake in root 2 was restarted after a short lag time of less than 30 min. Water uptake rate in root 2 recovered slowly during 10 h. Complete recovery of water uptake required roughly two days (shown later).

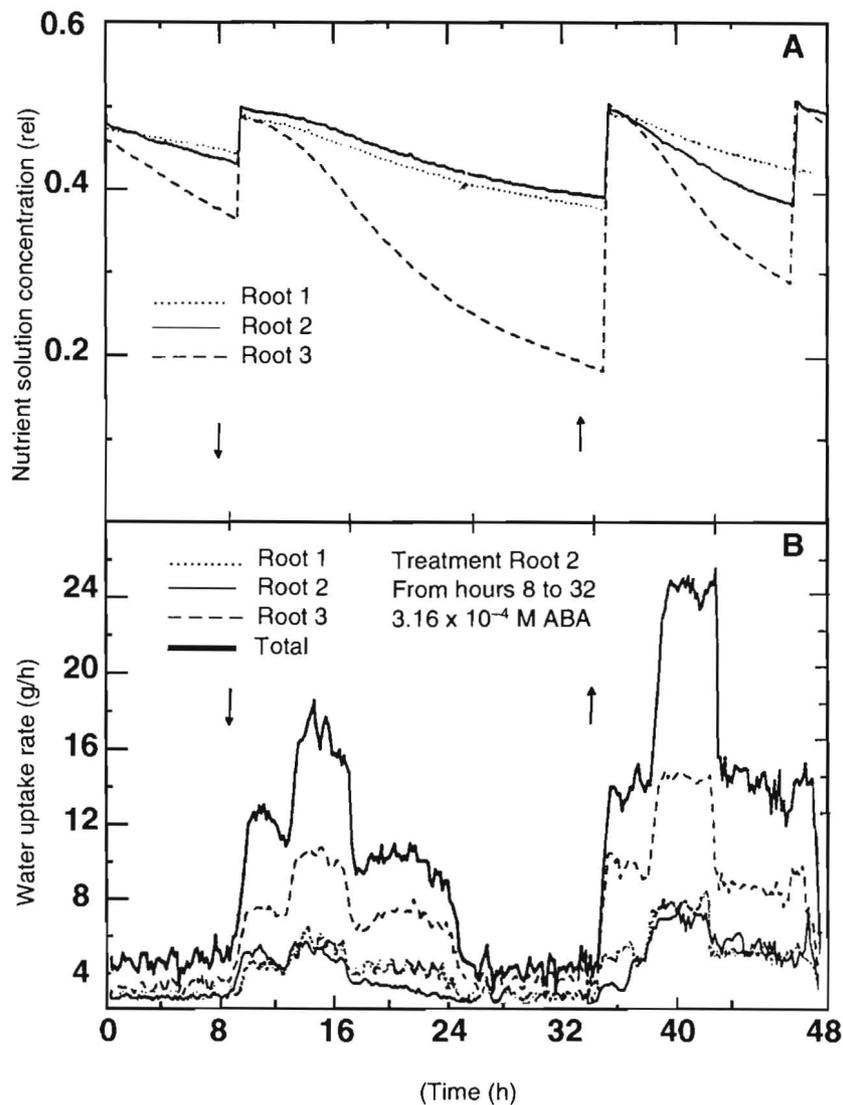


Fig. 1. A typical record showing effects on water and ion uptake of ABA applied to a portion of a soybean root system. \downarrow 3.16×10^{-4} M ABA applied to Root 2; \uparrow ABA removed; Time 0 = midnight (2400 h).

Water uptake rates in the untreated root portions and total water uptake rates were reduced during ABA treatment of root 2. Water uptake rates in the untreated root portions increased with no lag time to normal levels after replacing ABA with normal nutrient solution (Fig. 1).

Ion uptake rates were determined by comparing slopes of the nutrient solution concentrations vs time. Root 2 was slightly higher in nutrient uptake rate than root 1 during the dark prior to ABA application (Fig. 1). Addition of ABA to root 2 reduced ion uptake, which became similar to root 1. Replacing ABA with normal nutrient solution in root 2 resulted in a relatively faster recovery in ion uptake compared to recovery in water uptake. Figure 1 shows an increasing effect of ABA with time. Data from different periods following introduction of ABA were similar with consistency in the patterns of water and ion uptake. The differences were in the relative increases in uptake rates with higher light flux. Data from the middle part of high light were chosen to represent the water and ion uptake patterns (Figs. 2, 3, 4, 5). Water uptake rates were reduced markedly in root 2 during the treatment on experiment day 6 with 5×10^{-4} M ABA in both plants (Fig. 2). The increase in water uptake on day 7 was not high enough to meet an interpolated middle point between day 5 and day 8. This indicated incomplete water uptake recovery the day after treatment. Similar results occurred from 10^{-4} M (Figs. 2, 3) and 3.16×10^{-4} M ABA application (Figs. 4, 5). The change from medium to high light flux triggered more water uptake in both untreated and treated plants (Fig. 1). Averages of 8 h (data not shown) showed water uptake rate patterns similar to those of 2 h in Figures 2 and 3, thus confirm consistency in response of soybean plants during and following ABA application.

Treatment with 3.16×10^{-5} M and 10^{-5} M ABA slightly reduced the water uptake rates in treated plant 2 (Fig. 3). The exponential increase in water uptake rate slowed after ABA was replaced in both concentrations. Total water uptake rates were reduced and also a short delay occurred before complete recovery to normal water level in both treatments.

The circuit recording water uptake for root 3, plant 1, part 2, began to malfunction on experimental day 10; therefore further data from that root and totals were omitted from Figures 3 and 5.

All ABA concentrations used (from 5×10^{-5} M to 10^{-4} M ABA) reduced ion uptake rates in the treated root portions with no effect on the untreated root portions (Figs. 4, 5). The total nutrient depletion rate also slowed slightly (Figs. 4, 5). Because some lower ABA concentrations reduced ion uptake rates more than higher concentrations, the amount of ion uptake reduction did not appear to be related to

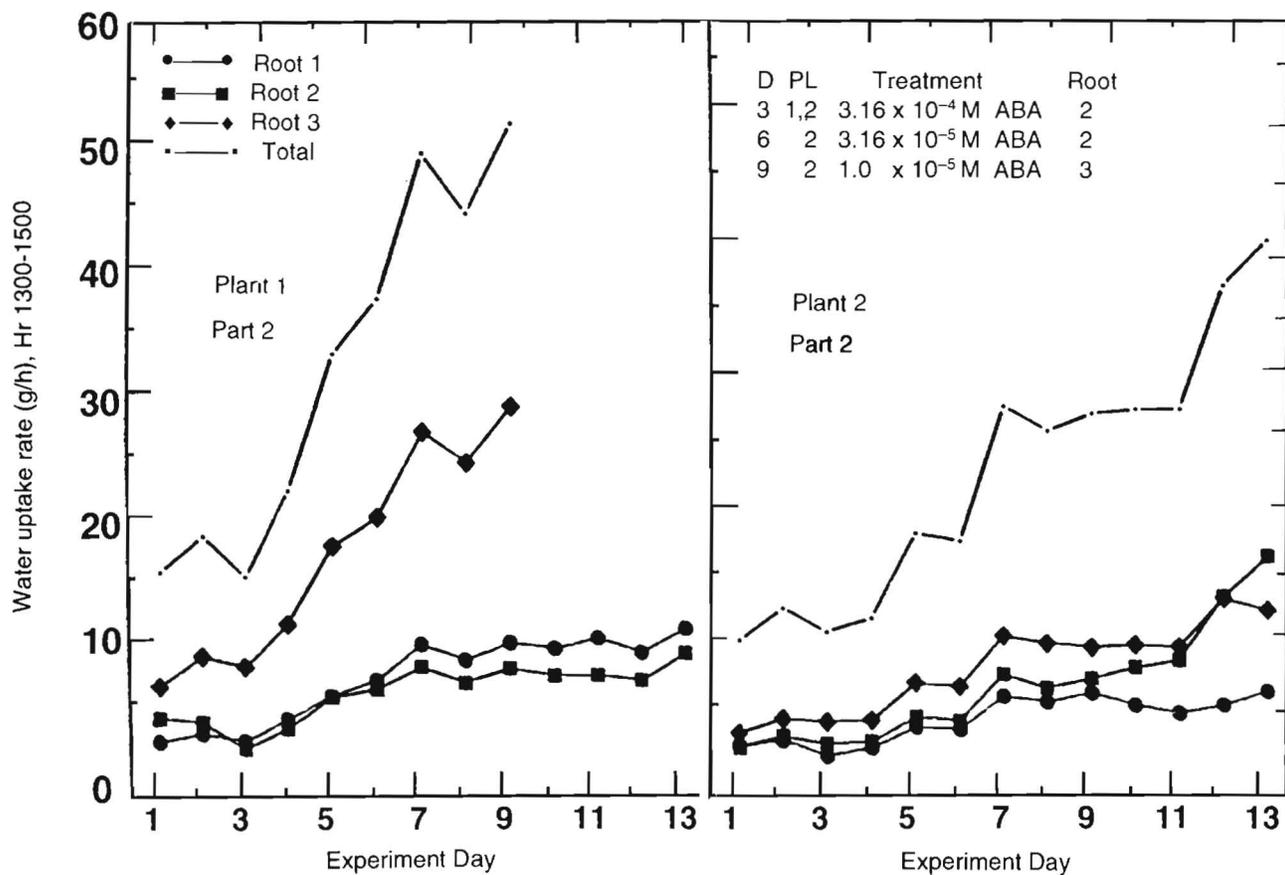


Fig. 2. Averages of water uptake rates in treated and untreated roots 5 to 7 h following additions to nutrient solution of ethanol alone (final concentration = 0.05%) and ABA in ethanol (final concentrations of ABA were 10^{-4} M and 5×10^{-4} M and of ethanol was 0.04%). Light = $1.5 \text{ mmol m}^{-2} \text{ s}^{-1}$). Experiment 3, Part 1.

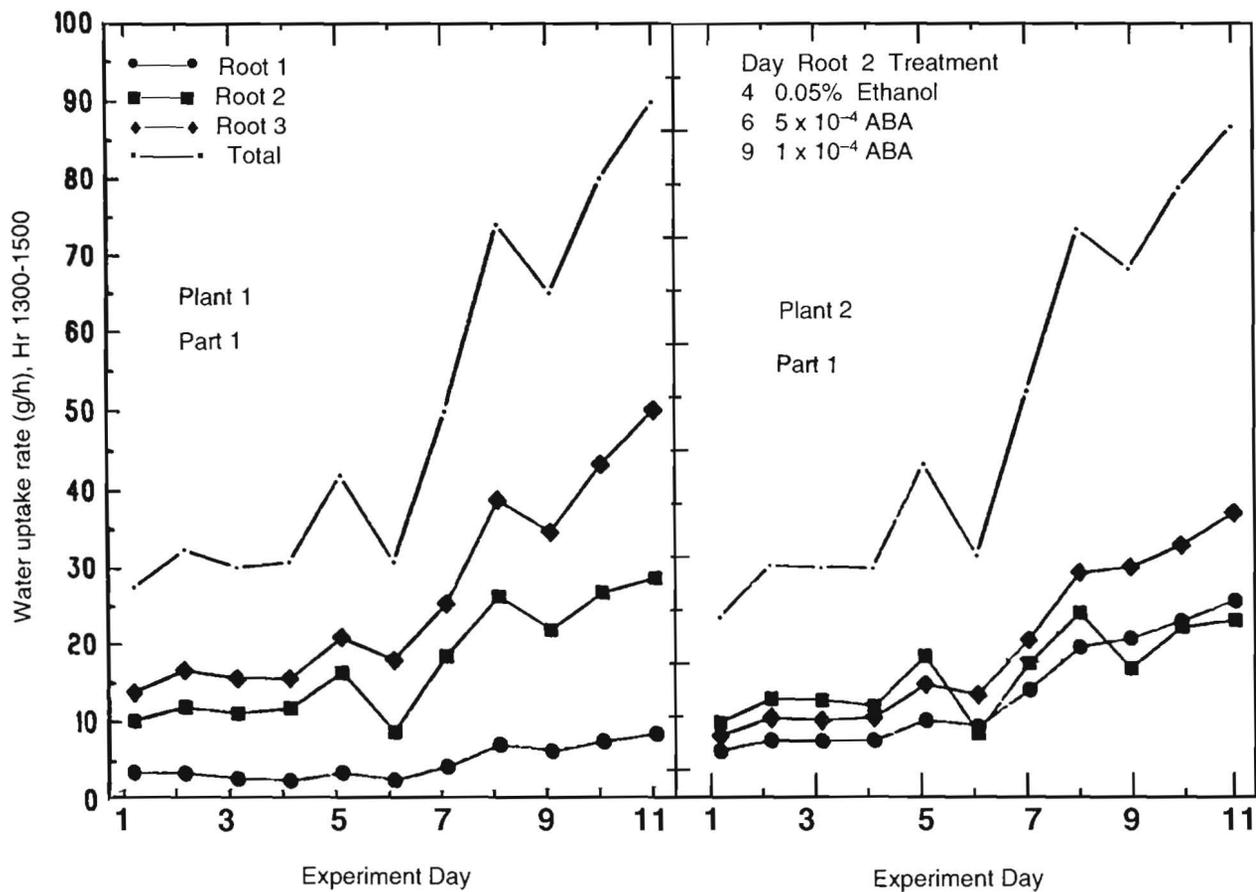


Fig. 3. Averages of water uptake rates in treated and untreated roots 5 to 7 h following additions of ABA in ethanol (final concentrations of ABA were 10^{-5} , 3.16×10^{-5} , and 3.16×10^{-4} M and of ethanol was 0.04%). Light flux was high ($1.5 \text{ mmol m}^{-2} \text{ s}^{-1}$). D = treatment day, PL = plant treated. Experiment 3, Part 2.

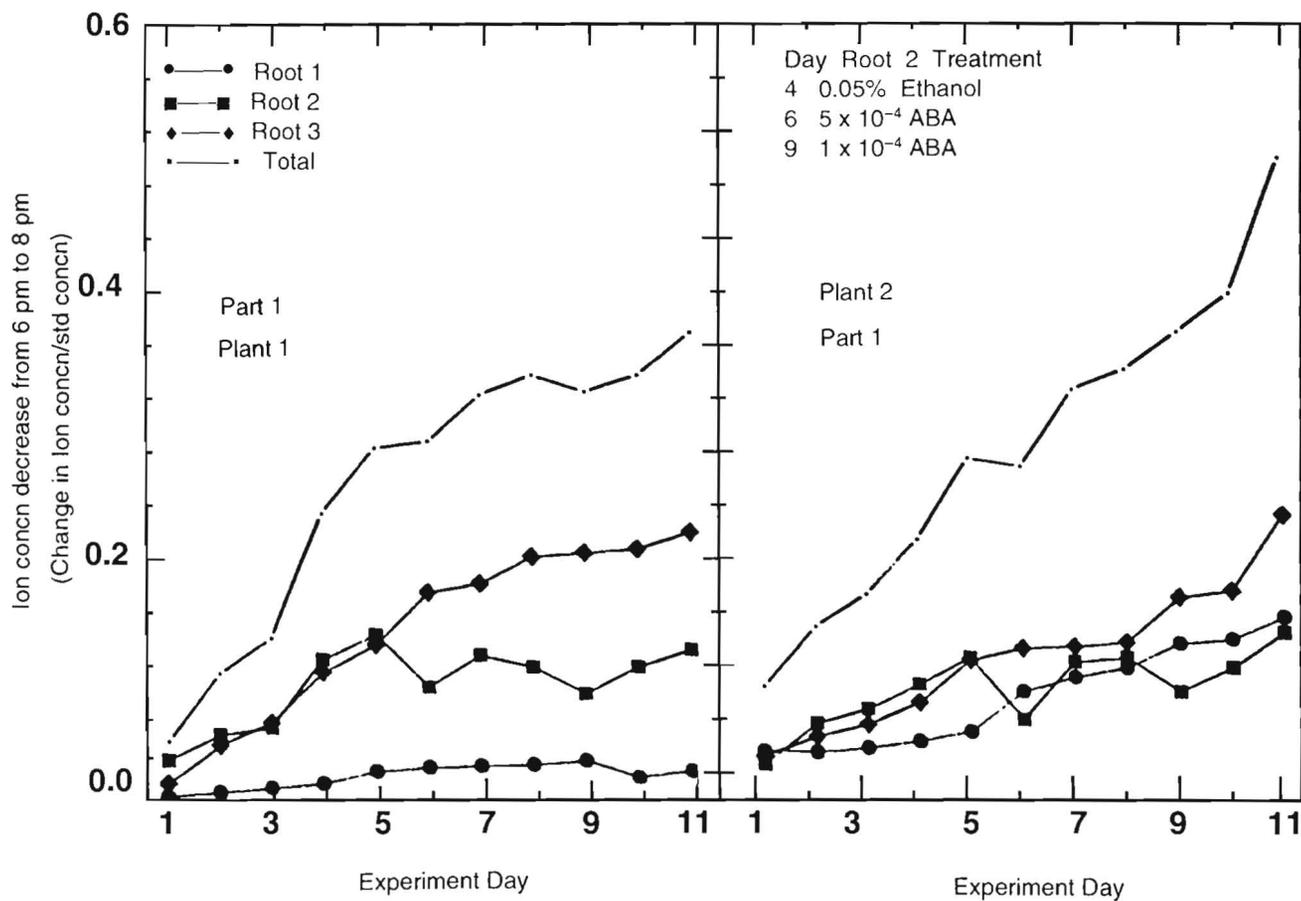


Fig. 4. Ion uptake by treated and untreated root portions of plants during 2 h periods, with additions of the following only to one root portion on the days indicated (final concentration given): 0.05% ethanol on day 4, 5×10^{-4} M ABA + 0.04% ethanol on day 6, and 1×10^{-4} M ABA + 0.04% ethanol on day 9. Light flux was high ($1.5 \text{ mmol m}^{-2} \text{ s}^{-1}$). Experiment 3, Part 1.

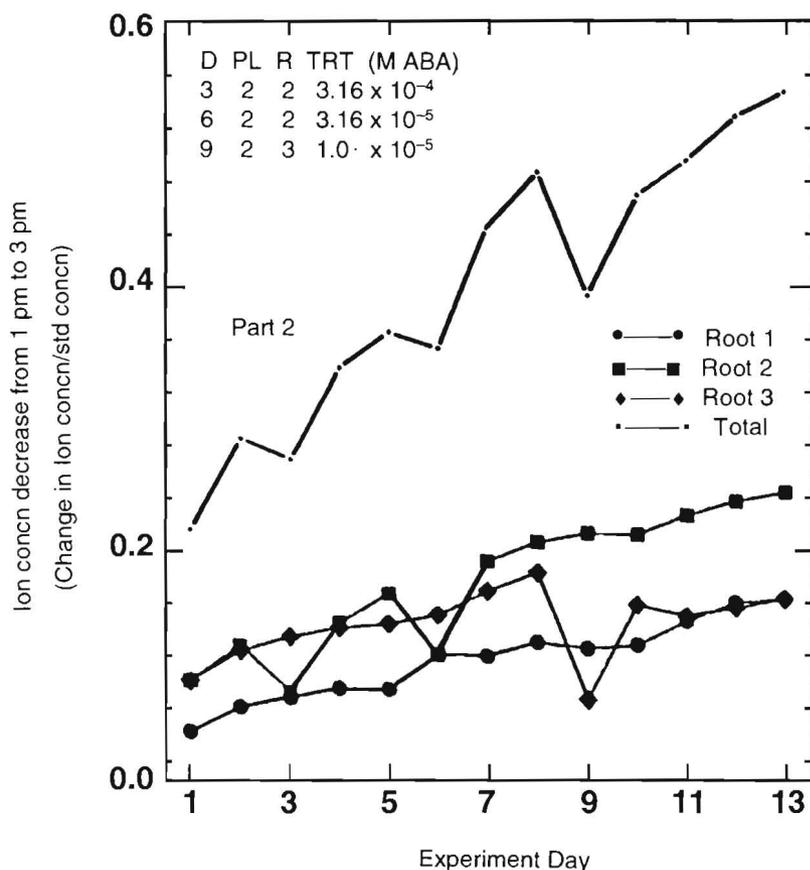


Fig. 5. Ion uptake by treated and untreated root portions of plants during 2 h periods, with additions of the following to one root portion at a time on the days indicated (final concentration given): 3.16×10^{-4} M ABA + 0.04% ethanol on day 3, 3.16×10^{-5} M ABA + 0.04% ethanol on day 6 for root 2; and 1×10^{-5} M ABA + 0.04% ethanol on day 9 for root 3. Light flux was high ($1.5 \text{ mmol m}^{-2} \text{ s}^{-1}$). D = Treatment day, PL = plant, R = treated root division, and TRT = ABA concentration. Experiment 3, Part 2.

concentration. The flow of particular ion species may be reduced, promoted or remain unchanged at different concentrations of applied ABA.

Reports in which different agents were used to dissolve ABA did not test these chemicals for any effect on water and ion flow rates in the absence of ABA. As indicated in the methods section, preliminary tests showed that all but low

concentrations of ethanol reduced water and ion uptake rates. The insignificant effects of 0.05% ethanol are shown in Figures 2 to 4.

Stomatal closure in response to ABA applied to a root portion is best described by leaf conductance measurements in Figure 6. Leaf conductances were not reduced during addition of 0.05% ethanol to the nutrient solution of root 2. All applied concentrations of ABA reduced leaf conductances.

Discussion

The enhancement of water uptake could have resulted from a temporary direct effect of ABA on the hydraulic conductivity of the treated root (Pitman *et al.* 1974A). The reduction in water uptake following the enhancement could be an indirect effect of ABA because of the disturbance of endogenous levels of plant hormones (Pitman *et al.* 1974B).

The delay in water uptake in root 2 after 800 h the next day could be a result of long term alteration in root permeability to water (Passioura 1988). Alteration in water uptake rates during and after ABA application during the 10 h might involve some active component(s) of water uptake (Kramer 1983, Turner 1986). The reduction in water uptake of the untreated roots was directly related to stomatal closure during ABA application where it causes stomatal closure and prevents reopening of the closed stomata (Raschke 1987, Zeevaart and Creelman 1988). The very short lag time of the untreated roots occurred only for the treated root portion, which may indicate that there was no movement of ABA from the treated to the untreated root portions. The mechanisms of communication and the exact biochemical changes that contribute to the changes in plant responses to a different water status induced by ABA are unclear (Harris *et al.* 1988). Replacing ABA with normal nutrient solution in root 2 resulted in a relatively faster recovery in ion uptake compared to the recovery in water uptake is supporting a relative independence of ion and water uptake (Van Steveninck *et al.* 1988). The increase in water uptake on day 7 was not high enough to meet an interpolated middle point between day 5 and day 8. This indicated incomplete water uptake recovery the day after treatment. Such long term internal alteration in the treated roots could be responsible for this prolonged effect.

Changes in ion uptake rates during ABA application have been reported to be conditioned by environmental parameters (Collins and Kerrigan 1974, Pitman *et al.* 1974A).

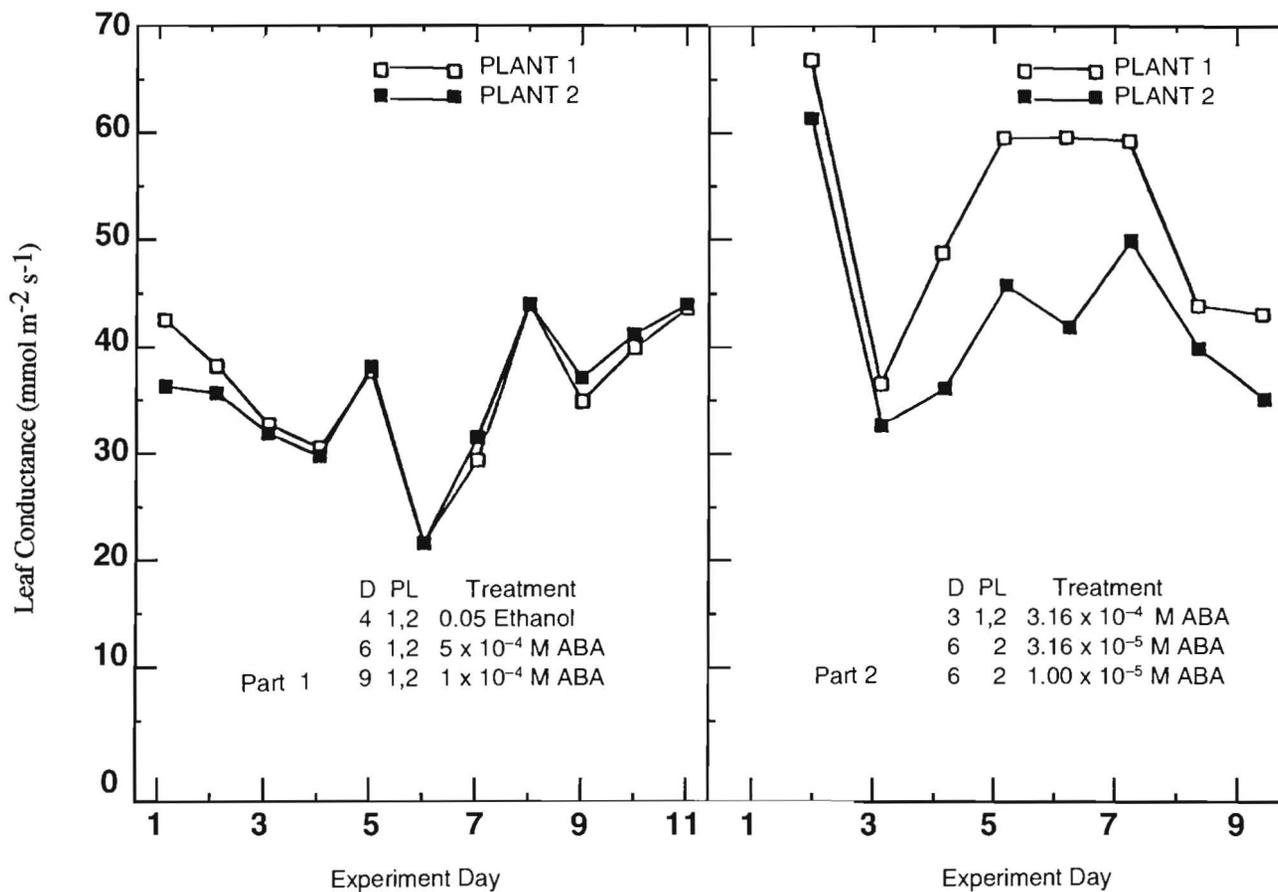


Fig. 6. Total leaf conductance throughout the experiment days. D = treatment day, PL = plant. Experiment 3, Parts 1 and 2.

From these results we conclude that (1) ABA application reduced water and ion uptake in treated root portions over the range of 5×10^{-4} to 10^{-5} M ABA; (2) water uptake processes are different from ion uptake processes; (3) water uptake rates were reduced in the untreated roots, while ion uptake rates were not affected in untreated roots during ABA application; (4) temporary enhancements of water uptake in the first 30 min of ABA application were followed by reduction in water uptake; (5) untreated root portions returned to normal water uptake levels immediately after replacement of ABA with nutrient solution; (6) the delay for treated root portions in returning to normal water uptake levels indicated an alteration in root hydraulic conductivity; (7) ABA probably did not move from the ABA treated portions to untreated roots; (8) stomatal closure was a direct effect of ABA application to the root portion(s), as indicated by reductions in total water uptake and in leaf conductances; and (9) reductions in ion uptake occurred with ABA applications but were not related to the concentrations of ABA applied.

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تأثير معاملات الجذور بهرمون حمض الأبسيسك على معدلات امتصاص الماء والأملاح وتوصيلية الأوراق في نبات فول الصويا

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ان النتائج المنشورة للدراسات حول تأثير معاملات هرمون حمض الأبسيسك على معدلات امتصاص الماء والأملاح الأيونية للأوراق والجذور عديدة ومتباينة . في هذه الدراسة تم تهذيب جذور نبات فول الصويا نوعية براج (٨ أيام) إلى ثلاثة أفرع ملتصقة بنفس النبات و متطابقة في محلول غذائي مشتق من هوجلاند . في اليوم الثامن عشر نقلت النباتات إلى تركيب زجاجي مكون من ثلاث أسطوانات ملتصقة بحيث يوجد في كل أسطوانة زجاجية فرع جذري يدور فيها محلول غذائي دائم التهوية بواسطة مولد غازي متصل بأنابيب مد هوائي وأيضا متصل بجهاز آلي لرصد معدل امتصاص الماء والأملاح كل عشر دقائق ويمكن نقله آليا إلى الحاسب الآلي . أضيفت تركيزات من هرمون حمض الأبسيسك تتراوح من 10^{-10} إلى 10^{-5} جزئ إلى أحد الجذور بينما استخدم الجزء ان الآخران من الجذور كأجزاء قياسية لمعدلات امتصاص الماء والعناصر الملحية . تم زيادة معدل امتصاص الماء في الجزء الجذري المعامل وسطه بتركيزات الهرمون بالمحلول الغذائي الخالي منه تم ببطء العودة للمعدلات القياسية حيث تطلب ذلك أكثر من ٢٤ ساعة . كان النقصان في امتصاص الماء لجزئي الجذور القياسية أقل من النقصان للجزء المعامل بالهرمون . عاد الجزءان القياسيان للمعدلات القياسية مرة أخرى أسرع كثيراً من الجزء المعامل

بالهرمون . بتخفيف تركيز الهرمون إلى ١٦ ، ١٠×٣^{-٥} و $١٠^{-٥}$ جزئاً قل نقص امتصاص الماء عن التركيزات الأخرى . قل امتصاص الأيونات في الجزء الجذري المعامل وسطه بالهرمون في كل تركيزاته بينما لم تتأثر الأجزاء الجذرية الغير معاملة بهرمون . لم يكن هناك علاقة إيجابية بين طرز امتصاص الماء و طرز امتصاص الأيونات . تسبب وجود الحمض الهرموني في أختزال توصيلية الأوراق والتي تدل على تدخله جزئياً في غلق الثغور .