Effects of Abscisic Acid and Cytoplasmic pH on Potassium and Chloride Eﬄux in Arabidopsis thaliana Seedlings

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The effects of ABA, isobutyric acid (IBA) and nicotine on K⁺ and Cl⁻ eﬄux were studied in Arabidopsis thaliana seedlings, and the role of pH_{cyt} and E_m in the regulation of the eﬄux of these ions was discussed. The data show that treatments with IBA and nicotine inﬂuenced in opposite directions the eﬄux of either K⁺ or Cl⁻: K⁺ eﬄux was increased by nicotine and reduced in the presence of IBA, whereas Cl⁻ eﬄux was stimulated by IBA and decreased by nicotine treatment. Under all the conditions tested ABA induced cytoplasmic acidification and inhibition of K⁺ and Cl⁻ net eﬄux. Experiments aimed to estimate the individual contribution of pH_{cyt} and E_m in modulating K⁺ eﬄux indicated that, within the range of acidic pH_{cyt} values, a regulation of K⁺ eﬄux was imposed by pH_{cyt} on the control exerted by E_m, the eﬄux being inhibited by lower pH_{cyt} values. Conversely, in the alkaline side of pH_{cyt}, K⁺ eﬄux seemed linked only to the E_m values. These results are consistent with the hypothesis that the decrease in K⁺ eﬄux observed in non-stomatal tissues in the presence of ABA may be mediated by the cytoplasmic acidification induced by the hormone.

Key words: ABA — Arabidopsis thaliana — Cl⁻ and K⁺ eﬄux regulation — Cytoplasmic pH.

A number of studies carried out with stomata guard cells indicate that ABA, known to be synthesized and accumulated in the leaf tissues of higher plants under water deﬁcit conditions, acts as physiological signal and mediates stress-induced closure of stomata. This eﬀect is achieved by the induction of a net loss of potassium salt from the guard cells, with consequent reduction in turgor, cell shrinkage and closure of the stomatal pore. The mechanisms by which ABA brings about these eﬀects are not yet completely known; several ABA-induced changes have been observed in stomatal guard cells, but the cause-eﬀect relationships between these events are not well deﬁned. Anyway, beyond a number of evidence indicating that an E_m change may act as a signal evoking channel gating (Tester 1990, MacRobbie 1990, Irving et al. 1992, Schroeder and Keller 1992), at least two distinct signal cascades have been outlined in the recent years: one passing through the cytoplasmic [Ca^{2+}], and the second through the intracellular pH. An ABA-induced increase in [Ca^{2+}], due to the release of Ca^{2+} from internal stores (Gilroy et al. 1991, Lemtiri-Chlieh and MacRobbie 1994) has been reported to be linked either to the inactivation of Ca^{2+}-sensitive and inward rectifying K⁺ channels or to the activation of voltage-dependent depolarizing anion channels (Schroeder and Hagiwara 1989, Schroeder and Keller 1992), and also to the K⁺ release from the vacuole through a Ca^{2+}-activated K⁺ channel located at tonoplast, as suggested by Ward and Schroeder (1994).

As far as a role for the intracellular pH as a second messenger is concerned, a class of outward-rectifying K⁺ channels, moderately voltage-sensitive, Ca^{2+}-insensitive and activated by alkaline-going pH_{cyt}, has been described by Blatt (1992; Blatt and Armstrong 1993) and further characterized by Lemtiri-Chlieh and MacRobbie (1994). These authors hypothesized that this class of K⁺ channels might be responsible for the Ca^{2+}-activated flux changes corresponding roughly in time with the pH_{cyt} increase occurring in the stomatal guard cells in the presence of ABA (Irving et al. 1992).

In non-specialized cells less attention has been paid up to now to the eﬀects of ABA on [Ca^{2+}] and intracellular pH, and to the possible correlations between the changes of these parameters and the behaviour of K⁺ and Cl⁻ ﬂuxes, and only a few literature data on this topic are available. An ABA-evoked rapid rise in cytosolic pH and a concomitant increase in [Ca^{2+}] have been detected in corn coleoptiles and parsley roots and hypocotyls by Gehring et al. (1990), and a cytosolic alkalization was measured within 45 min after ABA treatment in barley aleurone protoplasts by van der Veen et al. (1992). Conversely, previous work carried out in this laboratory indicated that in leaves of barley and of Elodea densa (a water plant without stomata) ABA treatment induced a significant decrease in pH_{cyt}, accompanied by a strong inhibition of Cl⁻ eﬄux (Beffagna 1992, Beffagna et al. 1995). In these last cases, pH_{cyt} and Cl⁻ eﬄux appeared to be influenced by ABA in a way opposite to that described for stomatal guard cells. Anyway, from a physiological point of view, is seems reasonable to

Abbreviations: BTP, bis-tris propane (1,3-bis[tris(hydroxy-methyl)methylamino]propane); DMO, 5,5-dimethylxazolidine-2,4 dione; E_m, transmembrane electrical potential difference; FC, fusicoccin; H₂O₅, intracellular water; IBA, isobutyric acid; pH_{cyt}, cytoplasmic pH.
think that the requirements to efficiently contrast water stress situations should be opposite for stomatal guard cells and for unspecialized adult tissues: the former need to lose water to achieve the closure of the stomatal pore and reduce transpiration, and the latter need to retain water to maintain the turgor pressure. Organic compounds are used for osmotic adjustment in shoots of higher plants (Morgan 1984), but in case of short-term physiological responses the use of inorganic ions and, in particular, the retention of salts inside the cell membrane might be the more rapid way to restrict the loss of water from the tissues and attain osmotic adjustment under stress conditions. In agreement with this hypothesis are the data reported by Pennarun and Maillot (1988) showing that in Acer pseudoplatanus cells an inhibition of Cl\textsuperscript{−} efflux is the first response to a turgor drop.

The results previously obtained in our laboratory with barley and Elyddea densa leaves, together with the considerations above reported, suggested the possibility that, as well as for Cl\textsuperscript{−} efflux, also K\textsuperscript{+} efflux were inhibited by ABA in non-stomatal tissues. On the other hand, on the basis of the results obtained by Blatt (1992), who detected an inhibition of the K\textsuperscript{+} outward rectifying channels by acidification of the cytoplasm after addition of butyric acid to the incubation medium, the ABA-induced pH_{cyt} decrease observed in barley and Elyddea densa leaves would be compatible with a decreased K\textsuperscript{+} efflux.

Aim of our work was: (a) to verify whether the cytoplasmic acidification and the inhibition of Cl\textsuperscript{−} efflux previously detected in barley and Elyddea densa leaves in the presence of ABA were events common to other non-stomatal tissues, and to characterize the effect of ABA on K\textsuperscript{+} efflux in this kind of materials, and (b) to study the features of K\textsuperscript{+} efflux, compared with those of Cl\textsuperscript{−} efflux, as a function of intracellular pH. To investigate these points 72 h old etiolated seedlings of Arabidopsis thaliana were used, a material particularly suitable for transport studies and measurements of cytoplasmic pH (Beffagna and Romani 1994).

In the present paper the results are reported of some experiments in which ABA effects on pH_{cyt} and on K\textsuperscript{+} and Cl\textsuperscript{−} fluxes were studied both in the absence of permeating cations in the external medium, and in the presence of IBA or nicotine, used as acidifying and alkalinizing agents. Results are also presented of some experiments in which the behaviour of K\textsuperscript{+} efflux in response to a number of effectors known to differently modify both pH_{cyt} and E_{m} was examined, and the individual contribution of these two parameters in the regulation of K\textsuperscript{+} efflux is discussed.

Materials and Methods

Plant material and general conditions—Seed stocks of Arabidopsis thaliana (L.) derived from the pure line erecta of the ecotype Landsberg were purchased from the Nottingham Arabidopsis Stock Centre. The experiments were carried out in whole etiolated seedlings, grown for 72 h at 24°C in liquid mineral medium supplemented with 0.5% (w/v) sucrose. Seeds were first surface sterilized for 5 min in a solution containing 80% absolute ethanol and 0.001% Tween 20 (60 mg seeds in 10 ml) and then washed for 5 min with a 1% solution of NaOCl containing 0.001% Tween 20. After repeated washings with sterile water, the seeds were drained and kept in glass tubes for 4 d at 4°C in the dark to obtain synchronized germination. At the end of the vernalization each lot of seeds was further sterilized for 5 min in a 0.5% solution of NaOCl. After rinsing, the seeds were transferred into 1,000 ml Erlenmeyer flasks with 200 ml of sterilized mineral medium (pH 6), containing NH4NO3 as nitrogen source and supplemented with 0.5% (w/v) sucrose (for a more detailed description of the standard mineral medium composition, see Beffagna and Romani 1994), and maintained for 72 h in the dark at 24°C under agitation (200 rpm). At the end of the growth period, the seedlings mostly consisted of a thin hypocotyl (about 5–6 mm long, with a diameter of 0.5 mm). The root was about 3–4 mm long (with a diameter of 0.1 mm), the size of the two cotyledons was about 1 mm\textsuperscript{2} or less, and leaves were not yet present. On the basis of observations performed by optical microscope, almost all the cells appeared to be fully vacuolate, the contribute by meristematic tissues being essentially negligible. Sample preparation was as described in a previous paper (Beffagna and Romani 1994).

Cell sap preparation and determination of K\textsuperscript{+} and Cl\textsuperscript{−} content—Samples of 200 mg FW were pretreated for 120 min at 24°C under agitation in 10 ml of a solution containing 0.5 mM CaSO\textsubscript{4}, 2 mM KCl, 5 mM DCMU and 10 mM MES, adjusted to pH 6 with BTP (basal solution), unless otherwise specified. After this pretreatment (required to carry out the experiments in the same conditions adopted for the K\textsuperscript{+} and Cl\textsuperscript{−} uptake experiments), the seedlings were washed for 3 min at 0°C in 0.5 mM CaSO\textsubscript{4}, then transferred in basal medium free from KCl and containing the different effectors, and incubated for a further 30 min. The samples collected at the desired times were then rinsed for 3 min under agitation with ice-cold 0.5 mM CaSO\textsubscript{4} to remove solutes originating from the external medium from the free space, drained, transferred into plastic syringes and frozen at −30°C for at least 3 hours. The cell sap was obtained by squeezing the seedlings after freezing and thawing. Aliquots of the cell sap were utilized to measure (after appropriate dilutions) the contents of K\textsuperscript{+} and Cl\textsuperscript{−}. Potassium content was determined by flame spectrophotometry (Varian Techtron SpectraAA-Plus), and chloride content by potentiometric measurements, using a solid state membrane selective electrode (Radiometer F1012Cl Selectrode). K\textsuperscript{+} and Cl\textsuperscript{−} contents have been expressed throughout the text as µmol ml\textsuperscript{−1} intracellular water (H\textsubscript{2}O\textsubscript{m}, see below).

Measurements of cytoplasmic pH—pH_{cyt} values were calculated by the distribution at equilibrium of the weak acid DMO, after correcting the uptake values for the contribution of metabolism (evaluated by thin layer chromatographic analyses, see Beffagna and Romani 1989). The calculations were carried out by the following equation derived from that of Henderson-Hasselbalch:

\[ pH_{cyt} = pK + \log \left( \frac{1}{V_c} [C_i - V_e] \times (AH + 10^{pH - pK + \log AH}) - AH \right) - \log AH \]

where pK = pK\textsubscript{s} of the weak acid, V\textsubscript{c} and V\textsubscript{e} are the volume of cytoplasm and of vacuole, respectively, C\textsubscript{i} is the total intracellular con-
centration of the weak acid, AH is the concentration of the uncharged form of the probe at equilibrium, and pH is the vacuolar pH. For a detailed description of the principles of the weak acid distribution method see Marrè et al. (1987). As previously described for the calculation of pH_{	ext{in}}, in this plant material, the values of vacuolar pH were substituted with those of the cell sap (directly measured in the sap by a flat tip combined electrode—Radiometer G732511), this replacement resulting in only a slight increase in the absolute values of pH_{	ext{in}} (Belfagna and Romani 1994).

To know the actual concentration of the weak acid accumulated inside the tissue, the intracellular water (H_{2}O_{i}) volume was calculated by subtracting from the fresh weight of the samples (100 mg) their dry weight (4-5 mg) and the extracellular water weight, evaluated by the use of labelled sorbitol, as previously described (Belfagna and Romani 1994). In the calculation 1 mg was taken as equal to 1 μl, and a ratio between vacuolar and cytoplasmic volume of 0.9/0.1 (usually adopted for mature cells) was assumed. On the other hand, it must be noted that vacuolar and different effector values, would not significantly influence the pH differences induced by treatments (Marrè et al. 1986).

Uptake experiments—For the uptake experiments batches of 100 mg FW of seedlings were used. The accumulation of the weak acid DMO (pK 6.4), used as pH_{	ext{in}} probe, was measured by the uptake in the tissue of [2-^{14}C]-DMO. The radioactivity supplied was 10 kBq per sample, the initial concentration of the probe being 5 μM. Samples were pretreated in a solution containing 0.5 mM CaSO_{4}, 5 μM DCMU, 10 mM MES/BTP (pH 6) and DMO for 90 min (required for the equilibration between the intra- and extra-cellular concentration of the probe) at 24°C under agitation. The samples were then incubated for a further 30 min after addition of the different effectors. At the end of the incubation with the labelled probe the seedlings were rinsed for 3 min under agitation with the corresponding ice-cold unlabelled solution.

Chloride influx was measured as 36Cl\textsuperscript{−} uptake. The radioactivity (14 kBq per sample) was provided as Na\textsuperscript{36}Cl. After incubation for different times (up to 120 min) in basal medium, the samples were washed for 3 min under agitation with the corresponding ice-cold unlabelled solution. In the experiments in which Cl\textsuperscript{−} release was evaluated, samples were preloaded for 120 min in 36Cl\textsuperscript{−} under the same conditions adopted for the uptake experiments. The preloaded samples were then rinsed for 3 min under agitation with basal ice-cold unlabelled solution and successively transferred into different KCl-free solutions always containing 0.5 mM CaSO_{4}, 5 μM DCMU, 10 mM MES/BTP (pH 6), and various effectors (ABA, IBA, nicotine) as specified in the individual experiments. Chloride efflux was evaluated as the difference between the radioactivity in the tissue at the end of the loading and that remaining after 30 min of treatment in the different experimental conditions.

Potassium influx was measured as 86Rb\textsuperscript{+} uptake. The radioactivity (33 kBq per sample) was provided as 86RbCl. To evaluate K\textsuperscript{+} influx and release, the same experimental procedure adopted to measure Cl\textsuperscript{−} efflux was followed, except for the rinsing of the samples that, in this case, consisted of two 8-min washings with the basal ice-cold unlabelled solution.

The radioactivity incorporated was measured by liquid scintillation counting, after digestion and bleaching of the tissue carried out as described in a previous paper (Romani and Belfagna 1991), and determined by a Packard TriCarb counter after addition of 10 ml scintillation liquid (Hionic Flour, Packard).

Transmembrane potential difference (E_{m}) measurements—E_{m} was evaluated by the conventional procedure, as described by Ulrich-Eberius et al. (1983). Seedlings (10-12) were mounted in a 5 ml plexiglas chamber under continuous flow (15 ml min\textsuperscript{−1}) of aerated medium at 24°C. The control medium to which the different effectors were added, as indicated in Table 2, contained 0.5 mM CaSO\textsubscript{4}, 5 μM DCMU, 10 mM MES/BTP (pH 6). Micropipettes with a resistance of 10-20 MΩ filled with 2 M KCl were used as salt microbridges to Ag/AgCl electrodes, and were inserted vertically in the hypocotyl of the seedlings by means of a Leitz micromanipulator. E_{m} was recorded with a high impedance electrometer amplifier (WPI K5-700) and a chart recorder.

Results

Effects of ABA, IBA and nicotine on cytoplasmic pH and on K\textsuperscript{+} and Cl\textsuperscript{−} fluxes—Preliminary experiments were carried out to test the sensitivity of Arabidopsis seedlings to ABA, and to IBA and nicotine, used as acidifying and alkalizing agents. The capability of ABA to inhibit the K\textsuperscript{+}-stimulated net H\textsuperscript{+} extrusion was taken as a parameter indicative of the hormonal action, whereas the optimal concentrations of IBA and nicotine were selected on the basis of their capability to significantly modify the pH_{	ext{in}}. On the basis of the results obtained (not shown) 10 μM ABA, 2 mM IBA and 1 mM nicotine were chosen and routinely used for the experiments reported herein after, unless otherwise specified.

The ABA effects on pH_{	ext{in}} in the different experimental conditions settled were first investigated. Figure 1 shows the changes of pH_{	ext{in}}, induced by IBA and nicotine after 30 min of incubation with or without ABA. The data reported indicate that the pH_{	ext{in}} of the ABA-treated samples was significantly lower than that of the controls, and that the occurrence of the decrease in pH was independent of the absolute pH values determined by the presence of the different
Table 1  Short time ABA effect on cytosolic pH

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<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>30 min</th>
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<tr>
<td>Control</td>
<td>7.46±0.008</td>
<td>7.47±0.019</td>
<td>7.48±0.023</td>
<td>7.48±0.024</td>
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<tr>
<td>10 μM ABA</td>
<td>7.41±0.018</td>
<td>7.39±0.017</td>
<td>7.40±0.023</td>
<td>7.41±0.018</td>
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Cytoplasmic pH was calculated from the accumulation of DMO at the indicated times. Treatments were supplied after equilibrating the labelled probe for 90 min in 0.5 mM CaSO₄, 5 μM DCMU and 10 mM MES, adjusted to pH 6 with BTP. Values are means (n=6)±SE.

The effects of ABA and of IBA or nicotine on K⁺ and Cl⁻ fluxes were then studied. In Figure 2 the results are reported of some experiments in which the influx and the efflux of K⁺ and Cl⁻ were followed as ⁸⁶Rb⁺ and ³⁶Cl⁻ uptake and release, respectively. The data show that: (a) an inhibition of K⁺ influx (20-25%) was induced by ABA, whereas no effect of the hormone was detectable on Cl⁻ influx (inserts of Fig.2A, B), in agreement with previous results obtained in barley and Elodea densa leaves (Beffagna et al. 1995); (b) in all the conditions tested ABA induced a significant decrease in the efflux of either K⁺ or Cl⁻; (c) treatments with IBA and nicotine, in the absence of the hormone, affected in opposite directions the efflux of the two ions. In fact, K⁺ efflux was consistently increased in the presence of nicotine, and markedly reduced by IBA (Fig.2A). Conversely, Cl⁻ efflux was strongly stimulated by IBA and slightly but significatively reduced by nicotine treatments (Fig.2B). However, the use of the labelled ions, which allowed to detect and emphasize even small differences in K⁺ and Cl⁻ fluxes, could not give any quantitative information about the efflux of these ions, the specific activity of which during the release was unknown. Thus, the effects of the different treatments on K⁺ and Cl⁻ fluxes were also evaluated as changes of K⁺ and Cl⁻ intracellular contents.

Figure 3 shows the net effluxes of K⁺ and Cl⁻ calculated by subtracting from the values of K⁺ and Cl⁻ contents at t₀ (120 min of preincubation in basal medium) those found after 30 min of treatment with the different effectors. The data reported indicate that the pattern of K⁺ and Cl⁻ efflux obtained with this kind of measurement closely matched with that obtained with the labelled ions. This finding made us confident in using the determination of K⁺ and Cl⁻ contents as an alternative method to satisfactorily evaluate either the direction or the entity of the changes in K⁺ and Cl⁻ fluxes, in spite of the fact that, in particular for K⁺, the entity of the changes induced by the different treatments were very small compared to the overall ionic content.

Changes of K⁺ and Cl⁻ efflux as a function of cytoplasmic pH—The finding that treatments with IBA and nicotine, leading to opposite changes of pHcyt, influenced
ABA and pH_{so} on K^+ and Cl^- efflux

![Graphs showing net fluxes of K^+ and Cl^-](Fig. 3)

**Fig. 3** ABA and pH_{so} effects on K^+ and Cl^- net fluxes. The net efflux of K^+ (△K^+) and Cl^- (△Cl^-) was calculated by subtracting from the values of K^+ and Cl^- contents at the end of the 120 min preincubation in basal medium (t0) those found after 30 min of treatment in the same KCl-free medium with or without the different effectors fed at the concentrations indicated in Figure 1. K^+ and Cl^- contents at t0 were 119 μmol ml^-1 H_2O_{so} and 63 μmol ml^-1 H_2O_{so}, respectively. Values are means from at least four experiments run in triplicate. SE for K^+ and Cl^- net efflux did not exceed ±5%.

in opposite directions the release of either K^+ or Cl^- was consistent with a possible role for the pH_{so} in regulating the efflux of these ions, as previously proposed by Blatt (1992) for K^+ and by Beffa et al. (1995) for Cl^- A further indication in this direction comes from the data of Figure 4, illustrating the results of a series of experiments in which different concentrations of IBA and nicotine were used to differently modify the values of pH_{so}, and K^+ and Cl^- contents were evaluated. Since the nicotine concentrations here adopted could give only small increases in pH_{so}, and, on the other hand, higher nicotine concentrations resulted to cause an increase in the aspecific permeability of the cell membranes (not shown), the pH_{so} was also indirectly modified by supplying Rb^+ (fed as sulphate salt) which determined a larger cytoplasmic alkalinization due to the activation of net H^+ export via the plasmalemma H^+ pump. The data reported show that the progressive decrease in pH_{so} induced by increasing concentrations of IBA (0.5, 1 and 2 mM) was accompanied by a corresponding increase in K^+ retention (Fig. 4B) and decrease in Cl^- content (Fig. 4C). Conversely, shifting pH_{so} alkaline-going determined a marked decrease in K^+ content and only a small decrease in Cl^- content, significantly lower than that observed in the control. These effects were somewhat more evident in the presence of Rb^+. No significative difference appeared between the changes in K^+ and Cl^- contents oc-
currying in the presence of the two nicotine concentrations tested (1 and 1.5 mM), the weak base-induced pH_{cyt} increase being very similar for the two conditions.

Changes of $K^+$ efflux as a function of different pH_{cyt} and $E_m$ values—An attempt was made to investigate the relative importance of pH_{cyt} and $E_m$ in the regulation of $K^+$ efflux, and possibly to individuate the specific contribution of these two systems in this process. With this purpose, some experiments were carried out in which a number of different working conditions known to modify the activity of the plasmalemma H^+ pump and, consequently, the values of both pH_{cyt} and $E_m$ were chosen, and the behaviour of $K^+$ efflux was followed. For these experiments fusicoccin (FC) and IBA were used to directly or indirectly activate the H^+ pump (Rasi-Caldogno et al. 1986, Beffagna and Romani 1991) and get similar and highly hyperpolarized $E_m$ values (in the absence of permeating cations) and, at the same time, very different pH_{cyt} values; NH_4^+ and Rb^+ were used to obtain a partial stimulation of the H^+ pump, similar $E_m$ depolarization (somewhat higher with Rb^+) and dissimilar pH_{cyt} values; nicotine and IBA plus Rb^+ were used to get similar weak $E_m$ depolarization and opposite pH_{cyt} changes (for an overview of the typical values of $E_m$ and pH_{cyt} measured in different plant materials in the experimental conditions reported above, see Marrè et al. 1983, 1989, 1995, Beffagna et al. 1995). Table 2 illustrates the $E_m$ values measured in Arabidopsis seedlings in the same experimental conditions adopted in the present study and presented in Figure 5.

Figure 5 shows the net efflux of $K^+$ calculated by subtracting from the values of $K^+$ content at $t_0$ those found after 30 min of treatment with the different effectors. The comparison between the changes in $K^+$ content induced by the conditions which more apparently determined similar pH_{cyt} values and different $E_m$ values or, alternatively, similar $E_m$ values and different pH_{cyt} values shows that: (a) with the higher (2 mM) IBA concentration and with FC (two situations of high $E_m$ hyperpolarization) both $K^+$ efflux and pH_{cyt} were quite different, the efflux of $K^+$ being approximately twofold larger in the more alkaline FC-treated samples; (b) with nicotine and IBA + Rb^+ (two conditions of weak $E_m$ depolarization) $K^+$ efflux was as well markedly higher in the alkaline nicotine-treated samples; (c) 1 mM IBA and 2 mM IBA + Rb^+ (characterized by high $E_m$ hyperpolarization and weak $E_m$ depolarization, respectively) exhibited equal acidic pH_{cyt} values and the same $K^+$ efflux; (d) in the presence of NH_4^+, a condition in which $E_m$ is depolarized and pH_{cyt} is unvaried respect to that of the control, $K^+$ efflux was approximately 50% larger than that observed in the control; (e) with nicotine and FC (showing the same pH_{cyt}, and $E_m$ weakly depolarized and highly hyperpolarized, respectively) $K^+$ efflux resulted more than twofold larger in the weak base-treated samples.

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<tr>
<th>Table 2</th>
<th>$E_m$ values after 30 min of incubation in different experimental conditions</th>
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<tr>
<td>FC</td>
<td>2 mM IBA</td>
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<tr>
<td>mV</td>
<td>-240 ± 10</td>
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<tr>
<td>n</td>
<td>5</td>
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$E_m$ measurements were carried out for up to 30 min after the addition of the different effectors to the control medium (0.5 mM CaSO_4, 5 μM DCMU and 10 mM MES, adjusted to pH 6 with BTP). FC concentration was 2 μM, NH_4^+ and Rb^+ (fed as sulphate salt) were 2 mM, and nicotine was 1 mM. Data are means from n experiments for each condition (as indicated in the second line of the table) ± SE.
ABA and pH_{opt} on K^{+} and Cl^{-} efflux

Discussion

The results presented in this paper show that in Arabidopsis thaliana seedlings treatment with 10 µM ABA induced a cytoplasmic acidification and an inhibition of the net efflux of K^{+} and Cl^{-} towards the K^{+} and Cl^{-}-free external medium. These effects occurred in all the conditions tested, independently of the absolute values of either pH_{opt} or E_{m} determined by the different effectors utilized. The data also show that, in the absence of ABA, treatments with IBA and with nicotine or Rb^{+}, inducing opposite pH_{opt} changes, influenced in opposite directions the efflux of either Cl^{-} or K^{+}, in agreement with a possible role for the pH_{opt} in regulating the efflux of these ions.

However, it is to consider that the changes of K^{+} and Cl^{-} fluxes observed in the presence of the different acidifying or alkalinizing agents, and interpreted as a consequence of the imposed pH_{opt} changes, might be as well explained as the result of the E_{m} changes occurring at the same time. In fact, the experimental conditions adopted to decrease the pH_{opt} values determined an E_{m} hyperpolarization too, which might be responsible for both the decrease in K^{+} efflux and the increase in Cl^{-} efflux. Again, the cytoplasmic alkalinization was obtained by treatments inducing a (weak or marked) E_{m} depolarization, which might account for either the K^{+} release or the Cl^{-} retention. Several examples of ion channel regulation by E_{m} have been reported in literature also for non-specialized tissues of higher plants (Tester 1990). For K^{+} fluxes, in particular, patch-clamp studies have demonstrated also in Arabidopsis thaliana protoplasts the existence in the plasmalemma of two classes of channels playing different physiological roles: the hyperpolarization-activated inward rectifying channels, and the depolarization-activated outward rectifying channels (Cerana and Colombo 1993).

As far as Cl^{-} is concerned, a possible causal relationship between E_{m} and Cl^{-} efflux changes seemed to be excluded by the finding that in Elodea densa leaves, in experimental conditions closely comparable with those adopted here, ^{36}Cl^{-} releases quite similar were found in conditions determining markedly different E_{m} values and, vice versa, very different ^{36}Cl^{-} releases were found in case of treatments leading to E_{m} values very close to each other (Beffagna et al. 1995).

As regards K^{+}, the results reported in Figure 5 and Table 2 indicate that within the whole range of conditions tested it is possible to identify a regulation of this efflux by E_{m} since, for the most part of the cases, for E_{m} values progressively decreasing (FC≥2 mM IBA>1 mM IBA>control>2 mM IBA+Rb^{+}>nicotine>NH_{4}^{+}>Rb^{+}) a corresponding increase in net K^{+} efflux resulted. However, an exception to this rule was evident for two conditions (FC and IBA+Rb^{+}) in which K^{+} efflux was larger or smaller, respectively, than expected on the basis of the E_{m} value. In fact, a marked reduction of K^{+} efflux, at least as large as that induced by 2 mM IBA, should be detected in the presence of FC, given the similar and highly hyperpolarized E_{m} values measured in these two conditions. On the contrary, K^{+} efflux was approximately twofold larger in the FC-treated samples. Again, with IBA+Rb^{+} the behaviour of K^{+} efflux would be expected to closely resemble that found for the nicotine-treated samples, given the similar E_{m} values (weakly depolarized) induced by these two effectors. Conversely, the amplitude of K^{+} efflux with nicotine was nearly twofold greater than with IBA+Rb^{+}. The comparison between the pH_{opt} values of these couples of samples suggested that indeed the absolute pH_{opt} values might give a key for these "anomalous" results. In fact, in both cases, for equal E_{m} and independently of the E_{m} absolute values, the lower K^{+} efflux was measured in the samples showing an apparently acidic pH_{opt}. However, a correlation between pH_{opt} and K^{+} release was evident only for conditions leading to a low pH_{opt}, and no more perceivable for conditions determining alkaline-going pH_{opt} changes. In fact, an equal K^{+} efflux was apparent for samples with acidic pH_{opt} values substantially alike and E_{m} values markedly different, such as 1 mM IBA and 2 mM IBA+Rb^{+}, for which a very different K^{+} release would be expected on the basis of a mere control by E_{m}. Conversely, samples with the same alkaline pH_{opt} and very different E_{m} values, such as those treated with FC and nicotine or with NH_{4}^{+} (which does not alter the pH_{opt} respect to that of the control), showed K^{+} releases quite different, the larger efflux occurring for the conditions determining the more depolarized E_{m} values.

Taken as a whole, these results indicate that, at least within the range of acidic pH_{opt} values, a regulation of K^{+} efflux driven by pH_{opt} overlays the control exerted by E_{m}, whereas in the alkaline side of pH_{opt} this efflux seems to be linked only to the E_{m} values. Similar results have also been recently obtained in Elodea densa leaves (Meraviglia et al. 1996).

As far as the possible connection between the ABA-induced decrease in pH_{opt} and the inhibition of K^{+} and Cl^{-} efflux is concerned, the following considerations can be made: (a) the changes in Cl^{-} efflux observed in Arabidopsis seedlings in the experimental conditions reported above confirm the results previously obtained in Elodea densa leaves (Beffagna et al. 1995) indicating that the ABA-induced cytoplasmic acidification cannot be the cause of the declined Cl^{-} efflux, since this efflux, by itself, is enhanced by acidic pH_{opt} values (Fig. 4C); (b) the behaviour of K^{+} efflux, larger at alkaline pH_{opt} than at low pH_{opt} values (Fig. 4B) and, above all, apparently regulated also by the pH_{opt} in the range of the acidic pH values (Fig. 5), is consistent with the hypothesis that also in this material, as already proposed for stomatal guard cells (Blatt and Amstrong 1993), the changes of K^{+} efflux observed in the
The presence of ABA may be mediated by the pH\textsubscript{cyt} changes induced by the hormone. If this is true, the suppressive effect of ABA on K\textsuperscript{+} efflux should be detected also after very short times of treatment, given the rapidity of the ABA effect on pH\textsubscript{cyt}. However, for an accurate analysis of the short time effects of ABA, devices more specific than those adopted in the present study are required (e.g., selective microelectrodes), allowing to obtain early and simultaneous measurements of the changes of pH\textsubscript{cyt} and K\textsuperscript{+} fluxes. Work in this direction is presently in progress in this laboratory.

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References


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