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Influence of Excision and Aging upon K^+ Influx into Barley Roots

RECOVERY OR ENHANCEMENT?[1]

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ABSTRACT

The influx of K^+ from ^{86}Rb-labeled solutions in the concentration range 0.008 to 0.2 mM into roots of intact plants and excised roots of barley plants (*Hordeum vulgare* [L.]) previously grown in 5 mM $CaSO_4$ (low K^+ roots) or 0.5 mM $CaSO_4$ plus 5 mM KCl (high K^+ roots) was measured. A consistent observation of these experiments was a substantial reduction of influx (usually by about 50%) following excision. The possible leakage of K^+ into the medium and subsequent dilution of specific activity of labeled solutions was eliminated as an explanation for influx reduction in excised low K^+ roots. Reduction of transpirational rates was also without effect upon influx into low K^+ roots. Excision followed by 2 hours aging in 0.5 mM $CaSO_4$ solution revealed that influx values recovered within the 2 hours to the values obtained in intact roots. It is concluded that much of the literature which describes the enhancement of ion uptake following excision actually describes excision damage followed by recovery.

The measurement of ion uptake by higher plant roots has been routinely obtained by the use of excised roots and sliced storage roots. Fewer studies have examined the kinetics of ion uptake into roots (particularly during short influx periods) using intact plants. In recent years several workers have reported the observation that various physiological changes, which may result in enhancement of ion uptake as well as hyperpolarization of transmembrane potential differences, result from the excision and "washing" or "aging" of root tissue (3, 5, 7, 9, 11). The mechanisms underlying these effects have, nevertheless, not yet been satisfactorily clarified. Leonard and Hanson (9) have reported that washing of excised corn root segments, which leads to enhanced uptake of ^{32}Pi, ^{86}Rb, and ^{36}Cl, was correlated with increased ATPase activity. Jacobson (7) has suggested that the blockage of wounded xylem vessels, which occurs following excision, prevents the loss of absorbed ions during subsequent uptake periods and hence is responsible for the observed "enhancement." While such an explanation may account for increased retention of absorbed ions during relatively long uptake periods it is difficult to account for increased retention of labeled ions during short influx periods, particularly in low K^+ roots which usually do not begin to deliver labeled ions to the stele for some time after initial exposure to radioactive solutions. Other workers (11) have suggested that the loss, during aging, of water-soluble inhibitory compounds originating in the root tip is responsible for the enhancement effect. Similar enhancement effects observed for membrane potentials have been attributed to compounds, perhaps hormones, originating at the root meristem (12). An important recent observation by Mertz and Higinbotham (10) is that the depolarization and subsequent repolarization of membrane potentials in excised roots are limited to cells adjacent to the cut root surface. Perhaps even more importantly, their observations may stress recovery rather than enhancement of membrane properties since although repolarized cells eventually became more negative than cells in intact roots by about 25 mv, the initial depolarization was by 90 mv.

Despite continuing reports of the enhancement of ion uptake associated with aging in excised root tissue, it cannot be overlooked that the literature also provides abundant testimony to the constancy of ion uptake (3, 6, 8, 15) for periods in excess of 2 hr. The reasons for this apparent discrepancy are not clear. Perhaps the enhancement of ion uptake does not occur in the presence of the ion whose uptake is being measured. Alternatively if the enhancement of ion uptake would be balanced by an equivalent reduction of influx associated with the increased internal concentration of the ion under examination (4) then no net enhancement would be observed. It may even be that we must consider that enhancement is species- or variety-specific. There are almost certainly differences in the pattern of time-dependent uptake according to the ion in question. The uptake of nitrate, *e.g.* displays an enhancement during the first 3 hr of exposure to nitrate (1). Manganese uptake by rice also displays rather unusual time-dependent increases during the first 2 hr of exposure to this ion (13).

The experiments described below were conducted as part of a continuing study of the regulation of K^+ influx in response to internal K^+ concentration (4). The experimental format was designed to permit estimates of K_m and V_{max} for K^+ influx into high and low K^+ roots to be made free from the complications of excision. In particular the author was concerned about the possible dilution of the specific activity of labeled uptake solutions by K^+ which might exude from cut surfaces. While these studies confirmed the general pattern of regulation of K^+ influx by internal K^+ previously observed with excised roots (4) a consistently observed effect of excision was a substantial reduction of measured influx (compared to intact roots) in both low and high K^+ roots. The results are discussed in terms of the aging phenomenon.

MATERIALS AND METHODS

Barley seeds (*Hordeum vulgare* [L.] cv. Conquest) were germinated as described previously (4). Ten-g batches of imbibed seeds were sown upon two layers of cheesecloth stretched across and glued to Plexiglas discs 12 cm in diameter from which a central disc, 8 cm in diameter, had been cut to permit the roots to grow down into the various growth media. These discs fitted over cylinders containing 1.8 liters of influx medium so that measurements of influx could be made with intact roots. When influx measurements were required using excised roots, roots were excised just below the cheesecloth and four 1-g samples were weighed into cheesecloth "teabags." As in the intact root experiments, roots were immersed in 1.8 liters of labeled uptake solution.

Plants were grown for 6 days at 26 C in the dark in 0.5 mM $CaSO_4$ solution or 0.5 mM $CaSO_4$ solution plus 5 mM KCl to produce low K^+ or high-K^+ roots, respectively. Prior to influx measurements intact roots were rinsed for 5 min in fresh $CaSO_4$

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solution. Except in the experiment designed to examine aging over a 6-hr period in $CaSO_4$ solution, excision of roots was performed within 3 min of the beginning of the influx period. All influx media contained 0.5 mM $CaCl_2$ plus 0.05 mM KCl except in the experiment (Fig. 1) where K^+ concentrations are shown. K^+ solutions were labeled with ^{86}Rb.

Uptake of labeled K^+ was determined for a 10-min period at 26 C followed by a 5-min rinse in ice-cold unlabeled uptake medium. Roots were then excised from intact plants, excess water removed by a 15-sec spin in a basket centrifuge, and 1-g samples weighed into scintillation vials. Root samples were then ashed at 500 C for 1 hr and the radioactivity of the ash determined by Cerenkov counting in a Searle Isocap/300 scintillation spectrometer. In the experiment which examined the influence of aging upon influx, roots were excised into 0.5 mM $CaSO_4$ and aerated for 6 hr at 30 C. Samples were removed at 2-hr intervals for influx measurements at 30 C. K^+ concentrations were determined by ashing root samples for 1 hr at 500 C followed by analysis of the aqueous extracts by flame photometry.

RESULTS AND DISCUSSION

Figure 1 records the isotherm for K^+ influx into excised (●) and intact low K^+ (○) roots of barley. K_m and V_{max} values determined by linear regression of the Hoffstee plots (v against v/s) for these data were 0.028 mM and 6.1 μmol K^+ g fresh wt^{-1} hr^{-1}, respectively, for excised roots and 0.024 mM and 9.9 μmol g fresh wt^{-1} hr^{-1} for intact roots. This observation is similar to that of Leonard and Hanson (6) who observed that after 8-hr washing the K_m for ^{32}Pi and ^{86}Rb had not significantly changed although M_{max} values increased by a factor of three. Similar reductions in influx were observed for high K^+ roots. K_m and V_{max} values for excised roots were 0.09 mM and 0.48 μmol g fresh wt^{-1} hr^{-1}, respectively, and for intact roots, 0.07 mM and 1.1 μmol g fresh wt^{-1} hr^{-1}. It seems unlikely that the exudation of K^+, from cut root surfaces, into the large volumes of uptake medium (1.8 liters), particularly in low K^+ roots which contain about 15 to 20 μmol K^+ g fresh wt^{-1}, could be responsible for the observed reduction of influx. Moreover, over the range of external K^+ concentration in Figure 1 from 0.008 mM to 0.025 mM the reduction of influx (46.8 ± 2%) was remarkably independent of initial external K^+ concentration. Any dilution of specific activity in the uptake

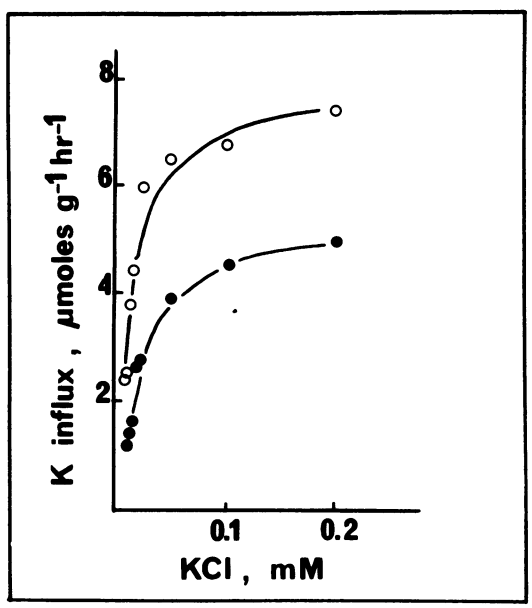

FIG. 1. Isotherm for K^+ influx into excised roots (●) and into roots of intact barley plants (○).

Table I

The influence of transpiration, shoot and root excision upon K^+ influx

Treatment	Influx (μmoles $g.f.w.^{-1}hr^{-1}$)
(a) Intact roots (normal transpiration)	6.83 ± 0.2
(b) Intact roots (low transpiration)	6.64 ± 0.3
(c) Intact roots (shoots excised)	7.84 ± 0.2
(d) Excised roots (immersed in uptake solution)	3.51 ± 0.4

solution by exudation from the roots would have been expected to be most pronounced at the lowest K^+ concentration. Nevertheless, the exudation question was examined by incubating 3 g of excised roots for 10 min at 30 C in 500 ml of 0.5 mM $CaSO_4$ under the same conditions as were used for influx determinations. The K^+ concentration of the resulting solution was too small to be measured by flame photometry.

Next I considered that perhaps the influence of the transpirational water flux through intact roots might be important. It might be argued that the high rates of plasmalemma influx in the low K^+ roots resulted in rapid depletion of K^+ in the apoplast. Replenishment of this ion in excised roots, by diffusion, might be anticipated to be much slower than by bulk solution movements in intact plants. The similarity of K_m values in intact and excised roots argues against such an interpretation. Nevertheless transpirational rates were modified by enclosing samples in 2-liter Plexiglas chambers 18 hr prior to influx measurements. The guttation of liquid from leaves of the latter plants was evidence of the high humidity and low transpiration rate. Shoots of other samples in the same growth cabinet were excised (15 min before the uptake experiment) as close to the seed as possible so that the roots remained intact in the gauze and no damaged tissue was subsequently immersed in uptake medium. Table I indicates that transpirational effects do not influence influx in these low K^+ roots (2). The apparently higher influx value for treatment (c) in which shoots were excised was not confirmed in subsequent experiments and must be attributed to variance between individual groups of plants. The major differences between treatment (c) and (d) (Table I) appear to be that only in (d) were excised tissues immersed in the uptake medium and only in (d) were roots injured. Because of the large volumes of uptake medium employed it is difficult to imagine that the exudation of inhibitory compounds is responsible for influx reduction. The known sensitivity of plant tissues to low concentrations of plant hormones makes it possible that the release of such compounds from damaged tissue might be responsible for the observed effects. However, in none of the "enhancement" studies on ion uptake, that I am aware of, were influx measurements simultaneously made upon intact roots in addition to excised roots.

Figure 2 records the results of an experiment in which excised roots were aged for 6 hr in 0.5 mM $CaSO_4$ solution. It can be seen that following excision there was the usual reduction of influx compared to intact roots. However after 2 hr, influx values had recovered to values comparable with those for intact plants. Note that after recovery there was no further significant increases of influx. Leonard and Hanson (9) observed that in "culture-grown" corn plants influx values, for ^{32}Pi, into freshly excised roots were similar to values for "washed" excised roots of "tray-grown" plants. However, they also observed an enhancement of ^{32}Pi uptake following 2-hr washing even in intact "culture-grown" roots. The present results indicate that a substantial component of what has been termed "enhancement" of uptake associated with the 'aging' of excised roots is actually a *recovery* from an initial wounding effect. Thus, although Leonard and Hanson (9) claim that "wounding is not involved; it is the washing which serves as the inducing act" it is clear from the above experiments that wounding does result in a reduction of influx and that recovery from this wounding effect is achieved within 2 hr. It is highly significant that in the recent publication by Parrondo and Smith

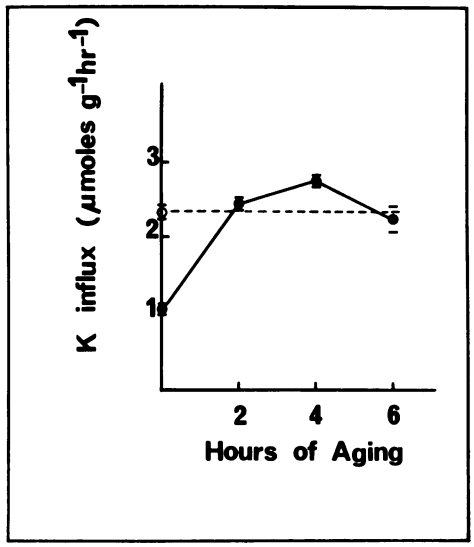

FIG. 2. Changes of influx values following excision and aging in 0.5 mM $CaSO_4$ solution for various times (●). The value for influx into roots of intact plants (○) is given for comparison.

(ref. 8, Figs. 2 and 3) the "enhancement" of Rb^+ absorption by excised corn roots was complete by 2 hr. I do not attempt to discredit real "enhancement" but warn that unless workers take care to measure influx in intact plants a substantial component of observed "enhancement" may actually be an observation of recovery from excision. In what appears to be a preoccupation with mechanisms of enhancement we are overlooking some basic excision damage.

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