

## INFLUENCE OF EXCISION AND GROWTH SOLUTIONS ON POTASSIUM INFLUX INTO ROOTS OF RICE AND WHEAT SEEDLINGS UNDER ACIDIC STRESS

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### Abstract

Responses of intact and excised roots of rice (*Oryza sativa* L. cv. Dungha Shali) and winter wheat (*Triticum aestivum* L. cv. GK Szeged) to low pH stress in  $K^+$  ( $^{86}Rb^+$ ) influx were studied and compared. Excised rice roots grown in 0.5 mM  $CaSO_4$  solution show an increased (anomalous)  $K^+$  influx responding to  $H^+$  stress (pH 3). Such an influx anomaly, however, was not detected in intact roots of rice seedlings grown in full nutrient solution. In the case of wheat seedlings, the  $K^+$  influx anomaly was not observed. Strictly speaking, the  $K^+$  influx anomaly could be detected only in case of „stress-sensitive” plants (e.g. rice) grown under insufficient ionic conditions (e.g. in 0.5 mM  $CaSO_4$ ), and if excised roots were used for  $K^+$  influx experiments. In conclusion we emphasize that results obtained with excised roots of stress-sensitive plants grown in 0.1–0.5 mM  $CaSO_4$  or  $CaCl_2$  solutions do not apply to intact plants, whereas stress-resistant plants (e.g. wheat) do not seem to have such  $K^+$  influx problems.

*Key words:*  $Ca^{2+}$ ,  $K^+$ , influx anomaly, pH, stress sensitivity

### Introduction

Various physiological changes influence the enhancement of ion uptake resulting from excision and washing or aging (FERRARI and RENOSTO, 1982; JACOBSON and YOUNG, 1975; LEONARD and HANSON, 1972; PARRANDO and SMITH, 1976). In spite of the fact that this issue is very interesting from many aspects, we do not know enough about the mechanism causing this effect. There are conflicting reports in the literature concerning the effects of excision on ion uptake (CSEH, 1974; FRICK et al. 1977; FRICK, 1980). Several authors state that the enhancement of ion uptake as a consequence of root excision is due to injury followed by recovery (GLASS, 1978; GRONEWALD et al. 1979; JACOBSON and YOUNG, 1975). The enhancement of ion uptake associated with aging in excised root tissues is thought not to be detectable in fresh excised roots (GLASS, 1978).

Growth conditions also influence the ion uptake processes. As for the ionic state of plants, it has been shown that winter wheat seedlings grown in 0.5 mM  $CaSO_4$  solution resembled to seedlings grown in tridistilled water rather than to the plants grown in  $K^+$ -less nutrient solution (BÉRCZI et al. 1984a,b). It is not yet known, however, how the components of the ionic state of the plant influence ion uptake.

The experiments described below were conducted as part of a continuous study of regulation of  $K^+$  uptake in response to low pH stress. We noted earlier that excised primary roots of low salt rice seedlings responded to  $H^+$  stress by increased

K<sup>+</sup> uptake and decreased K<sup>+</sup> content, suggesting an increased anomalous exchange between the cytoplasmic K<sup>+</sup> pool and the external medium (ZSOLDOS and ERDEI, 1981). Our earlier data also showed that under acidic stress conditions excised roots underwent changes in plasma membrane composition and structural organization (ERDEI et al. 1981; BÉRCZI et al. 1981), resulting in K<sup>+</sup> anomaly and leakage. To understand the anomaly more deeply, further K<sup>+</sup> uptake experiments were performed with excised roots as well as with intact plants of rice and winter wheat seedlings under acidic stress conditions.

### Materials and methods

Rice (*Oryza sativa* L. cv. Dunghan Shali) and winter wheat (*Triticum aestivum* L. cv. GK Szeged) seeds were washed in running tap-water for 4–6 h and then germinated in Petri dishes for 2 days (rice) or one day (wheat) at 24 °C. After germination the seedlings were cultured in 0.5 mM CaSO<sub>4</sub> or diluted full nutrient solution (0.5 mM CaSO<sub>4</sub>, 0.1 mM KH<sub>2</sub>PO<sub>4</sub>, 0.1 mM NH<sub>4</sub>NO<sub>3</sub>, 0.1 mM NaCl, 0.1 mM MgSO<sub>4</sub>, micro nutrients as in BÉRCZI et al. 1982) in a Conviron phytotron (Cabinet Model EF7), under about 80 Wm<sup>-2</sup> light intensity and 16 h photoperiods. The relative humidity was about 75% and 60% for rice and wheat, respectively. The day/night temperature was 24/20 °C.

The plants used in the K<sup>+</sup> uptake experiments were germinated for 7 days (rice) or 5 days (wheat), when their roots were about 7 cm long. In K<sup>+</sup> influx experiments <sup>86</sup>Rb<sup>+</sup> was used as label for K<sup>+</sup>. The influx experiments were performed at room temperature in 1 mM KCl solution in the absence or presence of 1 or 10 mM CaCl<sub>2</sub>. The specific activity of the uptake solution was 555 kBq.(mmol K)<sup>-1</sup>. The amount of isotope taken up by the roots was measured with a γ-spectrometer counter (Gamma NK-350, Hungary). The pH of the uptake solution was adjusted to the appropriate value with 0.1 M HCl and they were also tested after the influx experiments lasting 1 h. Roots were excised within 10 min before the beginning of experiments. At the end, roots were rinsed three times in 400 ml distilled water for 1 min. There was no any essential difference between the results when rinsing had been performed either in tridistilled water or in inactive uptake solution.

In every series of experiments triplicate samples were measured. The deviations between the results from individual determinations were less than 10% of the arithmetical mean value. Potassium and calcium contents of roots were determined as described earlier (BÉRCZI et al. 1982) both before and after the K<sup>+</sup> influx experiments.

### Results and discussion

Ion uptake of roots can be studied either with excised roots or with roots of intact seedlings (intact roots). They represent two different conditions in uptake experiments. Potassium influx (or uptake) of roots has been studied mostly with low K roots. To obtain such roots, plants had to be grown either in 0.1–0.5 mM CaSO<sub>4</sub> or CaCl<sub>2</sub> solution or in diluted complete nutrient solution. These treatments represent two different conditions for growth. In spite of the fact that these growth conditions result in nearly similar low K roots, the ionic state of the roots, which involves not only the K content but also the Na, Mg and Ca content, do differ (BÉRCZI et al. 1982, 1984a,b). The two-times-two different conditions allow us to study the pH-dependent anomalous K<sup>+</sup> influx of roots under four circumstances.

The pH-dependent anomalous K<sup>+</sup> influx means that K<sup>+</sup> influx at pH 3 is higher than that at pH 6 (ZSOLDOS and ERDEI, 1981). In Table 1 it can be seen that the anomalous K<sup>+</sup> influx can only be observed if 1/ rice seedlings were grown in 0.5



Table 1.  $K^+$  ( $^{86}Rb^+$ ) influx of roots of rice seedlings. Results are means  $\pm$  S.D. of experiments of four independent cultivations.

| State of roots | $Ca^{2+}$ |      | $K^+$ influx of roots ( $\mu\text{mol} \times (\text{g DW})^{-1} \times \text{h}^{-1}$ , grown in |                   |
|----------------|-----------|------|---|-------------------|
|                | pH        | (mM) | 0.5 mM $CaSO_4$   | nutrient solution |
| Excised roots  | 3         | —    | 86.8 $\pm$ 6.8  | 60.7 $\pm$ 6.0    |
|                | 3         | 1    | 99.3 $\pm$ 5.9  | 71.5 $\pm$ 4.6    |
|                | 3         | 10   | 112.0 $\pm$ 3.3   | 79.0 $\pm$ 9.3    |
|                | 6         | —    | 69.0 $\pm$ 6.2  | 70.4 $\pm$ 9.9    |
|                | 6         | 1    | 93.0 $\pm$ 8.1  | 87.1 $\pm$ 8.1    |
|                | 6         | 10   | 124.3 $\pm$ 5.3   | 106.7 $\pm$ 1.9   |
|                |           |      |   |                   |
| Intact roots   | 3         | —    | 86.8 $\pm$ 8.7  | 70.5 $\pm$ 6.4    |
|                | 3         | 1    | 97.8 $\pm$ 8.0  | 77.8 $\pm$ 5.5    |
|                | 3         | 10   | 112.3 $\pm$ 4.5   | 92.7 $\pm$ 5.4    |
|                | 6         | —    | 87.2 $\pm$ 5.7  | 86.2 $\pm$ 6.6    |
|                | 6         | 1    | 112.8 $\pm$ 9.5   | 104.8 $\pm$ 3.5   |
|                | 6         | 10   | 153.0 $\pm$ 4.2   | 134.7 $\pm$ 4.8   |
|                |           |      |   |                   |

mM  $CaSO_4$  solution, 2/ the uptake experiments were carried out with excised roots, 3/ there was no  $Ca^{2+}$  present in the uptake solution. For comparison, the  $K^+$  influx of wheat roots is presented in Table 2. It can be seen that neither excised nor intact roots showed the pH anomaly of  $K^+$  influx. This latter result is in a good agreement with earlier data obtained with barley (FAWZY *et al.* 1954) or with perennial ryegrass (MURPHY, 1959). The VIETS-effect (VIETS, 1944) can be observed in all cases. The disappearance of the pH-dependent anomalous  $K^+$  influx in rice with increasing  $Ca^{2+}$  concentration in the uptake solution verifies our earlier results (ZSOLDOS and ERDEI, 1981).

The K and Ca contents of the roots before the  $K^+$  uptake experiments are summarized in Table 3. The data clearly show that K is only partly replaced by Ca; i.e. the cation equivalent constancy is not fulfilled here (BEAR and PRINCE, 1945; LUCAS and SCARSETH, 1947; BÉRCZI *et al.* 1984c). In the case of rice at pH 3, the K content of roots decreased, while Ca content did not change if  $Ca^{2+}$  was absent in the uptake solution at all four cases defined above.

In case of wheat at pH 3, however, not only the K content but also the Ca content of roots decreased during the  $K^+$  influx experiments if  $Ca^{2+}$  was absent in the uptake solution (data not shown). This latter observation is consistent with earlier results; i.e. we know that 1/ the  $K^+$  uptake of plants is regulated by a  $H^+$ -pumping ATPase (SZE, 1984), 2/ H/Ca exchange occurs in the cell wall under sudden acidic stress (SENTENAC and GRIGNON, 1981), and 3/ structural changes in the plasma membrane result in an increased leakage and possibly in  $K^+$  loss (BÉRCZI *et al.* 1981). The first two statements are valid for rice too, but the third one is not

Table 2.  $K^+$  ( $^{86}Rb^+$ ) influx of roots of wheat seedlings. Results are means  $\pm$  S.D. of experiments of four independent cultivations.

| State of roots | $Ca^{2+}$ |      | $K^+$ influx of roots ( $\mu\text{mol x(g DW)}^{-1} \times \text{h}^{-1}$ ), grown in |                   |
|----------------|-----------|------|---|-------------------|
|                | pH        | (mM) | 0.5 mM $CaSO_4$   | nutrient solution |
| Excised roots  | 3         | —    | 55.3 $\pm$ 6.1  | 22.1 $\pm$ 2.2    |
|                | 3         | 1    | 68.2 $\pm$ 5.8  | 43.3 $\pm$ 4.1    |
|                | 3         | 10   | 75.2 $\pm$ 6.6  | 68.6 $\pm$ 5.2    |
|                | 6         | —    | 73.6 $\pm$ 5.8  | 79.7 $\pm$ 6.9    |
|                | 6         | 1    | 81.0 $\pm$ 6.2  | 89.4 $\pm$ 6.2    |
|                | 6         | 10   | 111.6 $\pm$ 5.9   | 123.3 $\pm$ 5.3   |
| Intact roots   | 3         | —    | 52.3 $\pm$ 5.5  | 19.8 $\pm$ 3.1    |
|                | 3         | 1    | 62.6 $\pm$ 6.1  | 37.7 $\pm$ 4.8    |
|                | 3         | 10   | 74.8 $\pm$ 5.8  | 65.6 $\pm$ 6.3    |
|                | 6         | —    | 84.5 $\pm$ 6.8  | 73.6 $\pm$ 4.8    |
|                | 6         | 1    | 97.4 $\pm$ 3.9  | 89.2 $\pm$ 5.3    |
|                | 6         | 10   | 128.5 $\pm$ 4.7   | 121.3 $\pm$ 8.3   |

Table 3. Potassium and calcium content of rice and wheat roots before the  $K^+$  uptake experiments. Data are means  $\pm$  S.D. of experiments of four independent cultivations.

| Plant | $K$ and $Ca$ content of roots ( $\mu\text{mol x(g DW)}^{-1}$ ) grown in |                |                   |                |
|-------|---|----------------|-------------------|----------------|
|       | 0.5 mM $CaSO_4$   |                | nutrient solution |                |
|       | (K)   | (Ca)           | (K)               | (Ca)           |
| Rice  | 383 $\pm$ 21  | 52.6 $\pm$ 1.6 | 648 $\pm$ 50      | 43.5 $\pm$ 1.8 |
| Wheat | 291 $\pm$ 24  | 41.3 $\pm$ 0.9 | 387 $\pm$ 7       | 38.7 $\pm$ 0.5 |

supported by ESR results (BÉRCZI et al. 1981). The lack of ions other than  $Ca^{2+}$  in the  $CaSO_4$  growth solution, however, cannot alone be responsible for the pH dependent anomalous  $K^+$  influx of excised rice roots, because the anomaly could not be observed with intact roots grown in the same solution. Our preliminary experiments with maize seedlings show similar results.

Thermophilic plants, which show not only the pH-dependent but also the temperature-dependent anomalous  $K^+$  influx phenomenon (ZSOLDOS, 1968; ZSOLDOS and KARVALY, 1979), seem to be sensitive to excision. Taking into account both the

pH- and the temperature-dependent  $K^+$  influx anomaly has been measured on excised roots, we think that the excision itself should be considered as a primary stress for the ion uptake of roots. Experiments and results obtained with „stress sensitive“ plants and their explanation need close attention. In such cases, as our present paper suggests, results obtained with excised roots must not be automatically extrapolated to roots of intact plants.

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