Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Raffaella Cerana, Franca Rasi-Caldogno

Cell enlargement and H^+/K^+ exchange in maize coleoptile segments treated with auxin and fusicoccin

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **60** (1976), n.5, p. 680–685.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1976_8_60_5_680_0>

Fisiologia vegetale. — *Cell enlargement and* H^+/K^+ *exchange in maize coleoptile segments treated with auxin and fusicoccin.* Nota di RAFFAELLA CERANA e FRANCA RASI-CALDOGNO, presentata (*) dal Corrisp. E. MARRÈ.

RIASSUNTO. — I dati riportati in questo lavoro estendono ai coleottili di mais i risultati ottenuti con stimolatori della crescita per distensione su altri materiali: 1) IAA e FC stimolano la crescita per distensione e parallelamente l'estrusione di protoni e l'assunzione di K^+; 2) la presenza di K^+ nel mezzo di incubazione stimola la secrezione di H^+ e tale stimolo è sinergico con quello indotto da IAA e FC; 3) il Na^+ quale catione monovalente sostituisce solo parzialmente il K^+ nel sistema di scambio H^+/K^+ attivato da IAA e FC.

INTRODUCTION

Recent data show that the promoting effect of auxin (IAA) and of fusicoccin (FC) in pea internode segments [1, 2, 3] and of FC in root segments [4, 5] on cell enlargement is accompanied by: 1) active H^+ secretion into the incubation medium [1-4], 2) stimulation of the uptake of K^+ and (to a lesser extent) of other monovalent cations [4, 6, 7, 8, 9, 10], 3) correlation between rate of monovalent cation uptake and H^+ extrusion [8, 9], 4) hyperpolarisation of the transmembrane potential difference [7, 8, 10, 11].

The parallelism and the high correlation between these phenomena agree with the view that IAA and FC activate an electrogenic mechanism of H^+/monovalent cation exchange [7, 9, 12].

In pea stems and in roots this mechanism has a high affinity with K^+ as the monovalent cation: in fact at low concentrations K^+ is much more effective than other monovalent cations tested in promoting H^+ secretion. The promoting effect of K^+ on H^+ secretion is synergistic with that of IAA or FC [6, 9].

The experiments on maize coleoptiles reported in this work are aimed at generalizing the data obtained with stem and root segments.

MATERIALS AND METHODS

Maize (Zea mays L. cv. Dekalb XL 342) seeds were germinated for ca. 80–90 hours on poplar sawdust in the dark at 28 °C.

Coleoptile segments, 3 mm long, were cut from the region between 5 and 15 mm from the tip.

(*) Nella seduta dell'8 maggio 1976.

After harvesting, they were incubated for ca. 30′ in 5×10^{-4} M $CaCl_2$ and 2.5×10^{-4} M $MgCl_2$, then rapidly washed and transferred to the various media as specified in the single experiments.

5×10^{-4} M $CaCl_2$ and 2.5×10^{-4} M $MgCl_2$ were always present in every treatment. The experiments were run in the dark in a thermoregulated water bath with shaking (50 spm) at 28 °C.

Growth was measured as increase in length. Measurements of pH, titrations of H^+ released in the media at the end of incubation and assay of K^+ concentration by atomic absorption were performed as already described [6].

In the experiments of tracer uptake $^{86}Rb^+$ was used as the tracer for K^+. After incubation in labelled solution the samples were rapidly rinsed with ice-cold water (20 ml) then incubated for 15′ in 15 ml ice-cold unlabelled solution of 5×10^{-4} M KCl, then rinsed again with ice-cold water (15 ml). The tissue, homogenized in 4 ml of water, was added with 10 ml Instagel and the radioactivity of the samples was determined by a Packard Tricarb Scintillator.

RESULTS AND DISCUSSION

I. *Effects of* IAA *and of* FC *on cell enlargement and* H^+ *secretion.*

The data in fig. 1 show, also in this material, the correlation between IAA- and FC- induced cell enlargement and acidification of the incubation medium. FC is clearly more active than IAA in promoting maize coleoptile

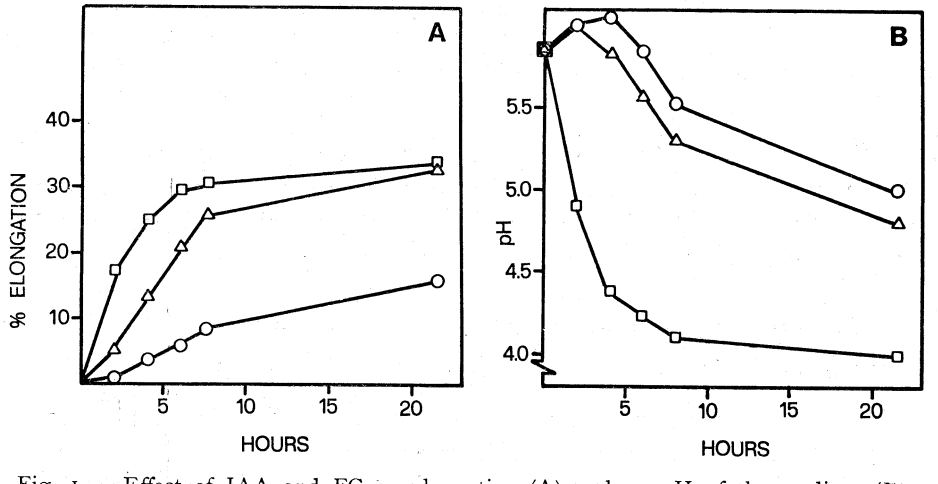

Fig. 1. – Effect of IAA and FC on elongation (A) and on pH of the medium (B). The sections (350 mg/10 ml) were incubated ± 2×10^{-5} M IAA or FC. Control (○), IAA (△), FC (□).

elongation and H^+ extrusion. FC-induced stimulation of cell enlargement is maximum within a short time, then rapidly decreases. IAA-induced stimulation is constant for 7–8 h and then decreases. The decrease of the growth rate might

depend *a*) on the existence of an upper limit of extensibility reached with FC in the early phase of treatment; *b*) on the possibility that in the FC-treated segments the pH in the wall space drops to a value lower than the optimum one for growth (see fig. 2).

II. pH-*dependence of elongation of maize coleoptile sections.*

The data in fig. 2 show that also in the corn coleoptile (as in several other materials [4, 13, 14, 15, 16, 17]) a low pH of the medium can induce a marked enhancement of elongation. The same data also show that in the earlier 3 h period of treatment maximal elongation is observed at pH 4, while (fig. 2 A)

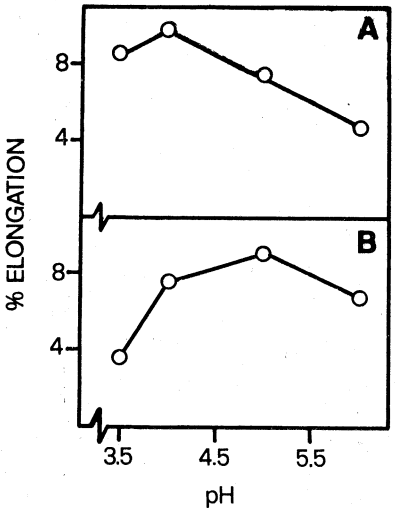

Fig. 2. – Effect of pH of the medium on maize coleoptile elongation. The sections (350 mg/10 ml) were incubated in 3×10^{-3} M citrate-Na-phosphate buffer at different pHs. A) % increase in length after 3 h. B) % increase in length between 3rd and 5.5th h.

in the following 2.5 hours the maximal growth rate occurs at pH 5 (fig. 2 B). This shift from pH 4 to pH 5 might depend on the time needed to reach a steady pH situation in the cell wall. If this is true, maximal elongation would correspond to a pH value in the free space close to 5.

III. *Release and re-absorption of "easily removable"* K^+ *and effects of* K^+ *on proton extrusion in* IAA- *and* FC-*treated maize coleoptile sections.*

The data in fig. 3 show that a consistent amount of K^+ is rapidly released by freshly prepared maize coleoptile segments when transported into a K^+-free medium. In the following period the K^+ thus released is reabsorbed by the tissue, the rate of re-absorption being maximal for the FC-treated, intermediate for the IAA-treated and minimal for the control sections. These different rates of re-absorption correspond to the capacity of both IAA and FC to stimulate K^+ uptake [6, 9, 18], also in this material. Table I shows the values of K^+ uptake from a 5×10^{-4} M KCl solution (^{86}Rb was used as the tracer) in the presence and absence of FC. Already at concentrations (2×10^{-7} M

and 2×10^{-6} M) which are suboptimal for growth, FC induces a marked increase in K^+ uptake; this promoting action is even greater at the optimal concentration for growth (2×10^{-5} M). Moreover, other data obtained in this

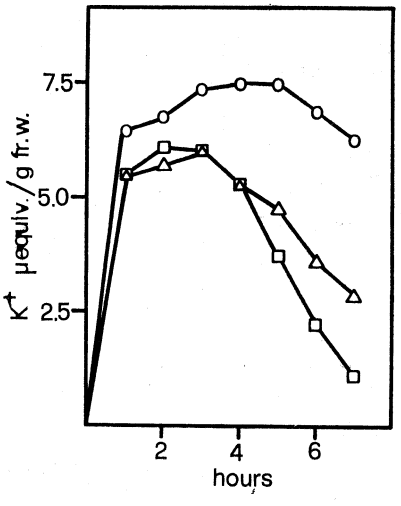

Fig. 3. – Effect of IAA and FC on net efflux of K^+. The sections (450 mg/5 ml) were incubated $\pm 2 \times 10^{-5}$ M IAA or FC. Control (○), IAA (△), FC (□).

laboratory (M. C. Cocucci *et al.*, unpublished data) under slightly different experimental conditions, show that IAA increases the uptake rate of 10^{-2} M K^+ in this material by ca. 80 % and FC by ca. 200 %.

TABLE I.

Effect of FC *on* K^+ *uptake rate.*

	Control	FC		
		2×10^{-7} M	2×10^{-6} M	2×10^{-5} M
µequiv $K^+/g \times h$ . . .	0.8	1.04	1.41	1.66
stimulation by FC . .	—	+30%	+76%	+107%

Sections (130 mg/8 ml) were preincubated for 3 h in 5×10^{-4} M $CaCl_2$ and 2.5×10^{-4} M $MgCl_2$, rapidly rinsed, then incubated for 30′ in labelled solution of 5×10^{-4} M KCl ± different concentrations of FC.

The data in fig. 4 show that in the maize coleoptile sections, just as in the pea internode segments [6], pretreatment with acidic buffer accelerates the " net leakage " of " easily removable " K^+. When sections pretreated at pH 3.5 are transported to a K^+-free medium, very little further release of K^+ is observed, and the effects of salt additions on proton extrusion can be studied under conditions of reduced interference of endogenous K^+.

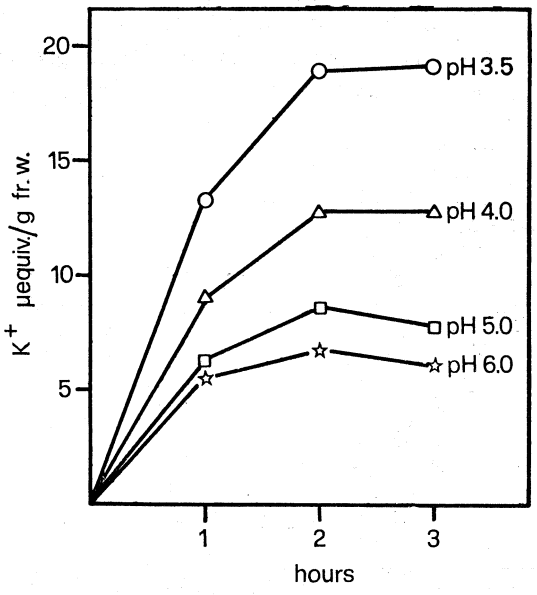

Fig. 4. – Effect of pH of the medium on net K^+ efflux. The sections (450 mg/5 ml) were incubated in 3×10^{-3} M citrate-Na-phosphate buffer at different pHs. pH 6.0 (☆), pH 5.0 (□), pH 4.0 (△), pH 3.5 (○).

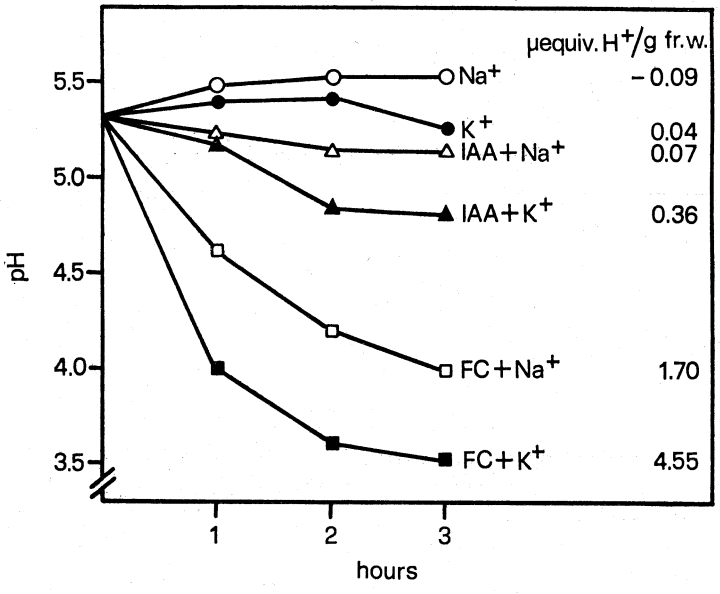

Fig. 5. – Effect of Na^+ or K^+ on FC- or IAA-promoted proton extrusion in maize coleoptiles pretreated with acid buffer. Sections (450 mg/5 ml) were preincubated in 3×10^{-3} M citrate-Na-phosphate buffer pH 3.5 for 2 h, then rapidly rinsed, washed two times for 4′ and rapidly rinsed again in the solutions used in the following treatments. Then the sections were incubated with 2×10^{-2} M NaCl (open symbols), 2×10^{-2} M KCl (closed symbols). Control (◑), 2×10^{-5} M IAA (◭), 2×10^{-5} M FC (◧). The values of H^+ extruded in the different conditions, titrated at the end of treatment (3 h), are shown in the figure.

The data in fig. 5 show the results of an experiment of this type. The data clearly show that also in maize coleoptile sections, as in all of the other materials as yet investigated [4, 6, 8, 9], proton extrusion expressed either as pH decrease or as increase of titratable acidity in the medium is higher in the presence of K^+ than in the presence of Na^+ both in the controls and in IAA- or FC-treated segments. It is also shown that the effect of K^+ is synergistic with those of IAA and FC.

The data reported above indicate that also in maize coleoptile tissue, as in other materials, the promoting effect of IAA and of FC on cell enlargement is associated with the simultaneous stimulation of proton extrusion and K^+ uptake, and that the IAA- and FC-enhanced H^+/K^+ exchange system has a high affinity with K^+ versus Na^+.

These data make it possible to generalize the correlation between the effects of growth promoters on proton/monovalent cation exchange and those on cell enlargement.

REFERENCES

[1] E. MARRÈ, P. LADO, F. RASI CALDOGNO and R. COLOMBO (1973) – « Plant Sci. Lett. », *1*, 179.
[2] E. MARRÈ, P. LADO, F. RASI CALDOGNO and R. COLOMBO (1973) – « Plant Sci. Lett. », *1*, 185.
[3] Y. YAMAGATA and Y. MASUDA (1975) – « Plant Cell Physiol. », *16*, 41.
[4] P. LADO, M. I. DE MICHELIS, R. CERANA and E. MARRÈ (1976) – « Plant Sci. Lett. », *6*, 5.
[5] P. E. PILET (1975) – « Plant Sci. Lett. », 5, 137.
[6] E. MARRÈ, P. LADO, F. RASI CALDOGNO, R. COLOMBO and M. I. DE MICHELIS (1974) – « Plant Sci. Lett. », *3*, 365.
[7] E. MARRÈ, P. LADO, F. RASI CALDOGNO, R. COLOMBO, M. COCUCCI and M. I. DE MICHELIS (1975) – « Physiol. Vég. », *13*, 797.
[8] M. G. PITMAN, N. SCHAEFER and R. A. WILDES (1975) – « Planta » (Berl.), *126*, 61.
[9] E. MARRÈ, P. LADO, F. RASI CALDOGNO, R. COLOMBO and M. I. DE MICHELIS (1976) – « Plant Sci. Lett. », 7, 199.
[10] M. COCUCCI, E. MARRÈ, A. BALLARIN DENTI and A. SCACCHI (1976) – « Plant Sci. Lett. », 6, 143.
[11] E. MARRÈ, P. LADO, A. FERRONI and A. BALLARIN DENTI (1974) – « Plant Sci. Lett. », *2*, 257.
[12] E. MARRÈ, P. LADO, F. RASI CALDOGNO and R. COLOMBO (1974) – « Rend. Accad. Naz. Lincei », *57*, 690.
[13] J. BONNER (1934) – « Protoplasma » (Wien), *21*, 406.
[14] D. L. RAYLE and R. CLELAND (1970) – « Plant Physiol. », *46*, 250.
[15] M. L. EVANS, P. M. RAY and L. REINHOLD (1971) – « Plant Physiol. », *47*, 335.
[16] A. HAGER, H. MENZEL and A. KRAUSS (1971) – « Planta » (Berl.), *100*, 47.
[17] K. L. EDWARDS and T. K. SCOTT (1974) – « Planta » (Berl.), *119*, 27.
[18] R. CLELAND (1976) – « Biochem. Biophys. Res. Commun. », *69*, 333.