Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Luciana Petruzzelli, Lucia Lioi, Silvia Morgutti, Sergio Colucci

Effect of fusicoccin and K^+ on the viability of aged wheat seeds

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **71** (1981), n.3-4, p. 37–43.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1981_8_71_3-4_37_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1981.

SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Fisiologia vegetale. — *Effect of fusicoccin and* K^+ *on the viability of aged wheat seeds.* Nota (*) di LUCIANA PETRUZZELLI (**), LUCIA LIOI (**), SILVIA MORGUTTI (***) e SERGIO COCUCCI (***), presentata dal Corrisp. E. MARRÈ.

RIASSUNTO. — Si sono studiati gli effetti della fusicoccina e del K^+ sulla germinazione e sulla crescita di due lotti di semi di grano di differente vitalità (non invecchiati ed invecchiati). I risultati ottenuti sia con i semi interi che con gli embrioni isolati mostrano che entrambi i trattamenti sono in grado di ripristinare la germinabilità del materiale invecchiato; contemporaneamente, è stato posto in evidenza che l'azione della fusicoccina e del K^+ si accompagna, negli embrioni isolati invecchiati, ad un aumento della capacità di acidificare il mezzo di incubazione. L'effetto della fusicoccina e del K^+ sul recupero della germinabilità viene discusso in relazione ad un probabile effetto dell'invecchiamento sull'attività del meccanismo di scambio H^+/K^+ a livello di membrana.

INTRODUCTION

The loss of seed viability during the time course of ageing is always correlated with degradative modifications of plasma lemma [3, 4, 8, 16]. Moreover, seed viability is negatively correlated with the leakage of electrolytes from the imbibing seed [1, 17], the leakage of electrolytes being certainly linked to the functional state of the transport mechanisms at the plasmalemma level. It has been recently pointed out that : *a*) proton extrusion is an important factor in directly or indirectly driving the transport of a number of ions as well as organic solutes across the plasmalemma [11, 12, 14, 18]; *b*) the activation of the proton-extruding capacity together with correlated phenomena such as hyperpolarization of the negative transmembrane potential and K^+ uptake play an important role in the onset of germination [2, 6, 13]. Of course, the loss of seed viability with ageing may be due to damage at the plasmalemma level that affects the activity of the proton extrusion mechanism. In this work we studied *a*) the correlation between seed viability and proton extrusion in wheat seeds and *b*) the effects of FC and K^+, that are known to stimulate the energy-dependent proton extrusion activity [12], in recovering germinability in aged seeds.

(*) Pervenuta all'Accademia il 5 ottobre 1981.
(**) Istituto del Germoplasma, Bari (Italia).
(***) Istituto di Chimica Agraria dell'Università di Milano, Milano (Italia).
Abbreviations: FC, fusicoccin; PD, electrical potential difference.

Materials and methods

Batches of wheat (*Triticum durum* L., cv. Appulo) seeds harvested in 1977 and 1979 were stored under laboratory conditions (temperature 20 ± 3 °C; relative humidity 50–70 %), the former for 24 months (aged seeds) and the latter for 6 months (unaged seeds). Both batches were subsequently stored in air-tight glass jars at 4 °C until used.

For germination tests, 10 samples of 10 seeds were germinated over two sheets of filter paper in 10 cm Petri dishes with 10 ml of the test solution at 20 °C. Germination was determined at the seventh day when the process was complete. Seeds were considered germinated when radicles and coleoptile were evident. The pH of solutions was adjusted to 6.5.

For evaluating the proton extrusion capacity isolated embryos were used. Embryos were separated from dry seeds by the method of Johnston and Stern [7]. Batches of 30 embryos, previously washed at 0 °C for 2 h in distilled water, were incubated in flasks containing 2 ml of the different test solutions (pH 6.5) to which 2 % sucrose had been added (since embryos grow very poorly without sucrose) and germinated in constant agitation (80 oscillations/min). Germination and pH were determined at 6 h intervals over a period of 42 h. Embryos were considered germinated when radicles and coleoptile were evident; at the end of the experiments, as a growth parameter, fresh weights were evaluated. pH was measured with an Orion Research pH meter model 001.

FC was dissolved in absolute ethanol to obtain a 1 mM stock solution containing 10^{-1} M ethanol.

Results

A) *Effect of* FC *and* K^+ *on viability of unaged and aged seeds.*

Table I shows the effect of FC and K^+ on the percentage of germination and on the elongation of radicles and coleoptile of unaged and aged wheat seeds. Ageing decreased seed viability from 82 % to 50 %. FC and K^+ restored the viability of the aged seeds up to the level of about 82 %, being ineffective in restoring the viability of the remaining seeds (20 %) from both the unaged and aged batches. This finding suggests that the process of ageing leads to a reversible damage of a mechanism sensitive to FC and K^+, and that the loss of viability in the remaining 20 % of seeds in both batches may be due to the damage of different mechanisms. The data of Table I also show that FC slightly inhibited radicle growth in both aged and unaged seeds, without affecting coleoptile growth; K^+ had a very slight inhibitory effect on radicle growth whereas it stimulated the growth of coleoptiles.

TABLE I.

Effect of FC *and* K^+ *on the percentage of germination and on the elongation of radicles and coleoptile of unaged and aged wheat seeds.*

Treatment	Unaged seeds			Aged seeds		
	Germination %	Radicles (mm/seed)	Coleoptile (mm/seed)	Germination %	Radicles (mm/seed)	Coleoptile (mm/seed)
Control	82±3	363.3±15.0	102.7±3.0	50±4	294.2±41.8	84.2±9.4
FC 5×10^{-6} M	83±2	306.7±14.9	99.6±4.1	86±2	210.4±19.4	81.8±3.3
K_2SO_4 2×10^{-3} M . . .	84±2	322.9±14.8	111.9±3.0	80±3	227.1±13.5	104.7±4.5

Germination and elongation were evaluated on batches of 10 seeds each in quintuplicate after seven days of incubation. The values are the means of measurements performed after three separate experiments ± S.E.

B) *Viability and proton extrusion capacity of isolated embryos from unaged and aged seeds. Effect of* FC *and* K^+.

For evaluating the proton extrusion capacity isolated embryos from unaged and aged seeds were used; in fact, the experiments performed with whole seeds gave unreliable results because of the high amounts of different materials leaked out into the incubation medium by the seeds incubated in flasks. We studied the behaviour of germination, together with the changes in the acidity of the incubation medium, in embryos isolated from unaged wheat seeds (unaged embryos) or from aged seeds (aged embryos) and incubated with or without FC and K^+. The results concerning the behaviour of germination (Fig. 1 A)

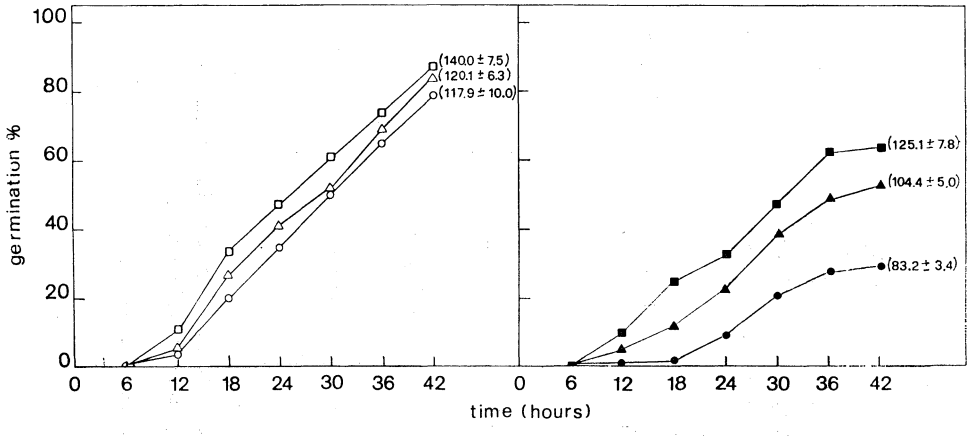

Fig. 1 A. - Effect of FC and K^+ on the percentage of germination of isolated wheat embryos. Open symbols: unaged embryos; filled symbols: aged embryos. (○ ,●), Control; (△ , ▲), KCl, 0.5 mM; (□ , ■), FC, 5×10^{-6} M. All the values are the means of three experiments with three replicates of 30 embryos each performed as described in Materials and Methods. The least significant difference at the 5% level was 6.6. The fresh weights ± S.E. (in mg) of the embryos after 42 h of germination are reported in brackets.

confirm the ones obtained from whole seeds: germinability was 78 % for the unaged embryos, 29 % for the aged ones. FC and K^+ recovered the viability of aged embryos up to 64 % and 53 %, respectively. These results seem to exclude a possible involvement of other parts of the seed in promoting the effects of fusicoccin and K^+. The decrease in germinability of isolated embryos compared to whole seeds is due to the different germination procedure (incubation in flasks); in fact, the percentage of germination observed when the embryos were germinated on Petri dishes was the same as that observed in the whole seeds. Figure 1 B shows that the unaged embryos appeared to be more effective in acidifying the medium than the aged ones; moreover, the effect of FC and K^+ on the proton extrusion capacity in aged embryos appeared to be correlated

with their effect on the recovery of viability. In the unaged embryos, FC and K^+ slightly affected germinability and proton extrusion, suggesting again that the loss of viability in the remaining seeds may be due to the damage of different mechanisms.

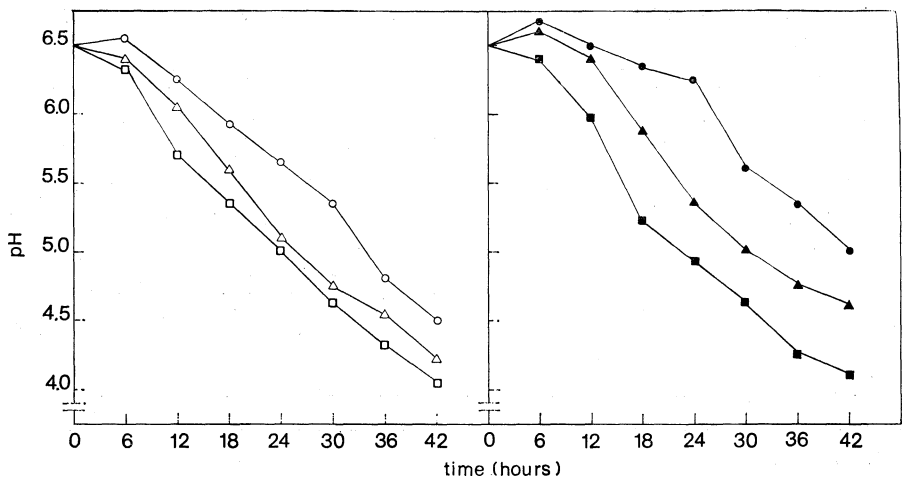

Fig. 1 B. – Effect of FC and K^+ on the pH of the incubation medium during germination of isolated wheat embryos. Open symbols: unaged embryos; filled symbols: aged embryos. (○ , ●), Control; (△ , ▲), KCl, 0.5 mM; (□ , ■), FC, 5×10^{-6} M. All the values are the means of three experiments, run in triplicate, and performed as described in Materials and Methods.

DISCUSSION

The loss of viability of wheat seeds stored for 2 years under laboratory conditions (Germoplasm Institute, Bari, Italy) appears to be due to damage of a mechanism at the plasmalemma level which is sensitive to FC and K^+. In the aged seeds FC increases the percentage of germination by restoring the typical condition of the unaged seeds, and this effect is accompanied by an increase of the capacity to acidify the incubation medium. In seeds FC stimulates germination by activating proton extrusion, hyperpolarization of the transmembrane electrical potential [2] and K^+ uptake [10]; moreover, various lines of evidence suggest that in seeds as well as in different plant materials a K^+—Mg^{++}–dependent plasmalemma ATPase mediates the utilization of metabolic energy for H^+ extrusion [6]. The activity of this transport mechanism during the early phases of germination is linked to the composition in polar lipids of the membrane [5]. The decrease in viability with ageing might be linked to the inactivation of the FC-sensitive plasmalemma ATPase, perhaps also through the degradation of one or more polar lipids. This hypothesis is also suggested by data from literature showing that in non-viable embryos a decrease in the level of certain phospholipids is detectable [15], together with a loss of activity of plasmalemma

ATPases measured with cytochemical tests [8]. The fact that FC, though stimulating the capacity to acidify the incubation medium, only slightly increases the rate of germination of unaged seeds (in contrast to what happen in other materials: see [6, 9, 12]), suggests that in unaged wheat seeds the proton extrusions activity already operates, in the controls, at a rate which allows the maximum activation of the mechanism responsible for the onset of germination.

The effect of K^+ appears to be more difficult to explain. In fact, K^+ normally leaks out from seed cells and, to a larger extent, from the cells of aged seeds. This observation suggests that the effect of the presence of K^+ in the medium in restoring the viability of aged seeds must be linked to its action during the very early phase of incubation when K^+ is still almost absent from the medium. During the earliest hours of germination an increase in the negative transmembrane electrical potential has been previously observed in radish seeds [2], probably due to the beginning of the release of K^+ (together with other positively charged ions) into the incubation medium.

This hyperpolarization of PD might during the first hours of germination, exert a negative effect on the activity of the mechanism which is linked to proton extrusion [11].

The positive effect on viability and germination of increasing K^+ concentrations during the early hours of incubation might consist in a favouring of ion uptake into embryo cells, thus leading to depolarization of PD and proton extrusion activation. In the aged seeds this effect triggers the subsequent events of germination before further degradative processes affect the organization of the plasmalemma and the functionality of the proton extrusion mechanism. In conclusion, the effects of FC and K^+ in restoring seed viability could be due to the activation, through two different mechanisms, of proton extrusion.

Acknowledgements. — Part of this research (Istituto di Chimica Agraria, Università di Milano) was supported by a special program of the Consiglio Nazionale delle Ricerche, Roma, on the " Biology of Reproduction ". The authors wish to thank Mr. G. Carella for skillful technical assistance.

References

[1] A. A. Abdul-Baki and J. D. Anderson (1970) - « Crop Sci. », *10*, 31–34.
[2] A. Ballarin-Denti and M. Cocucci (1979) - « Planta », *146*, 19–23.
[3] P. Berjak and T. A. Villiers (1972*a*) - « New Phytol. », *71*, 1069–1074.
[4] P. Berjak and T. A. Villiers (1972*b*) - « New Phytol. », *71*, 1075–1079.
[5] M. Cocucci and A. Ballarin-Denti (1981) - « Plant Physiol. », *68*.
[6] S. Cocucci and M. Cocucci (1977) - « Plant Sci. Lett. », *10*, 85–95.
[7] F. B. Johnston and H. Stern (1957) - « Nature », *179*, 160–161.
[8] N. D. Hallam (1973) - In « Seed Ecology » (W. Heydecker, ed.), p. 134. Butterworths, London.
[9] P. Lado, F. Rasi-Caldogno and R. Colombo (1975) - « Physiol. Plant .», *34*, 359–364.

[10] E. MARRÈ, P. LADO, F. RASI-CALDOGNO, R. COLOMBO, M. COCUCCI and M. I. DE MICHELIS (1975) – « Physiol. Vég. », *13* (4), 797–811.
[11] E. MARRÈ (1979*a*) – *Integration of solute transport in cereals.* In « Recent Advances in the Biochemistry of Cereals, eds. D. L. Laidman, R. G. Wyn Jones, London, New York: Academic Press, p. 3–25.
[12] E. MARRÈ (1979*b*) – « Annu. Rev. Plant. Physiol. », *30*, 273–288.
[13] D. PARDI, S. MORGUTTI and S. COCUCCI (1980) – « Physiol. Plant », *48*, 379–384.
[14] R. J. POOLE (1978) – « Annu. Rev. Plant Physiol. », *29*, 437–460.
[15] D. A. PRIESTLEY and A. C. LEOPOLD (1979) – « Plant Physiol. », *63*, 726–729.
[16] E. H. ROBERTS (1972) – In « Viability of Seeds ». Ed. E. H. Roberts. Chapman and Hall, London, p. 253–306.
[17] D. J. SCOTT and R. C. CLOSE (1976) – « Seed Sci. Technol. », *4*, 287–300.
[18] C. L. SLAYMAN (1974) – In « Membrane Transport in Plants », eds. U. Zimmermann and J. Dainty, Berlin, Heidelberg, New York: Springer-Verlag, p. 107–119.