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The relationships between ethylene production and germination of *Cicer arietinum* seeds

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Abstract

The germination percentage of chick-pea (*Cicer arietinum*) seeds was greatly reduced by temperatures of 30 °C and 35 °C. This thermoinhibition was overcome by ethylene (ethrel). Both ABA and PEG diminished ethylene production and germination percentage in a parallel way. FC, MGBG and CHA stimulated both ethylene production and germination. AVG reduced ethylene production to some extent but did not inhibit germination. $CoCl_2$ and PG completely prevented both ethylene production and germination; this effect was reversed by ethylene but not by its immediate precursor ACC. NBE prevented both germination and ethylene production. Our results suggest that high ethylene production rates are not essential for germination of chick-pea seeds but that certain quantities of ethylene may be required.

Introduction

Radicle emergence, the first visible morphological sign of germination in chick-pea (*Cicer arietinum* L.) seeds, coincides with an increase in ethylene production (Gallardo *et al.* 1991). In previous papers we have reported the relationship between production of ethylene and cell elongation (Sánchez-Calle *et al.* 1989) or radicle emergence (Rodriguez *et al.* 1983) and the quantification of ethylene production in different parts of this seed (Gallardo *et al.* 1991). In this paper we describe the role of ethylene in the germination of chick-pea seeds. One way of examining this was to

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Abbreviations: ABA - abscisic acid; ACC - 1-aminocyclopropane-1-carboxylic acid; AVG - aminoethoxyvinylglycine; CHA - cyclohexylamine; EFE - ethylene-forming enzyme; ethrel - 2-chloroethylphosphonic acid; FC - fusicoccin; MGBG - methyl-glyoxal-bis-guanylhydrazone; NBE - norbornadiene; PEG - polyethyleneglycol; PG - propylgallate; SAM - S-adenosylmethionine.

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observe the effect of inhibitors of both biosynthesis and the action of endogenous ethylene and the effect of exogenous ethylene on radicle emergence.

Materials and methods

Commercially available seeds of *Cicer arietinum* L. c.v. Castellana, harvested in 1989, were placed in plastic trays with filter paper moistened either with 175 cm^3 of sterile distilled water or an aqueous solution of the corresponding treatments. The experiments were conducted in darkness at 25 °C, 30 °C and 35 °C. Three replicates of 50 seeds each were used and each experimental variant was repeated. The number of germinated seeds was determined after the times specified for each experiment, taking the emergence of the radicle as the significant criterion.

For analysis with NBE, triplicate samples of 10 seeds, uniform in size, were placed on 2 disks of filter paper moistened with 15 cm^3 of sterile distilled water in a 365-cm^3 flask. The flasks were sealed with a rubber stopper and were then injected with 0.1 cm^3 of liquid NBE.

In a previous paper we observed that of all the parts of the *Cicer arietinum* L. seeds studied, the embryonic axis produces the greatest amount of ethylene. Thus, to quantify ethylene production we took 0.5 g of embryonic axes of germinated chick-pea seeds (except to 6 h) from each experimental batch. After 1 h incubation a 1 cm^3 gas sample was withdrawn from the sealed flask (20 cm^3) with a hypodermic syringe and C_2H_4 was assayed with a gas chromatograph, as described previously (Gallardo *et al.* 1991).

Results and discussion

The optimum germination temperature for *Cicer arietinum* seeds is 25 °C; temperatures of 30 and 35 °C inhibit germination (Fig. 1), an effect known as thermoinhibition. Transferring thermoinhibited seeds to optimum temperatures resulted in a revival of their germinative capacity (data not shown). Supraoptimal temperatures brought about a decrease in ethylene production (Fig. 2) concomitantly with the inhibition of germination. We have recently demonstrated that high temperatures inhibit ethylene production by causing a decrease in EFE activity and an increase in MACC level (Gallardo *et al.* 1991). The application of ethrel (7 or 70 μM) increased the percentage of radicle emergence and overcame the inhibitory effect of high temperatures (Fig. 1). Thus, after 24 h at 35 °C, 93 % germination was achieved with 70 μM ethrel as opposed to only 20 % with water (Fig. 1). Similar results have been described with other species (Abeles 1986, Corbineau *et al.* 1988).

In *Cicer arietinum* seeds high temperatures reduce the cell size of embryonic axes (Rodriguez *et al.* 1983). Cell elongation is a signal event just before the emergence of the radicle axis, *i.e.* germination (Rodriguez *et al.* 1983), and there is a clear relationship between cell elongation and ethylene production (Sánchez-Calle *et al.*

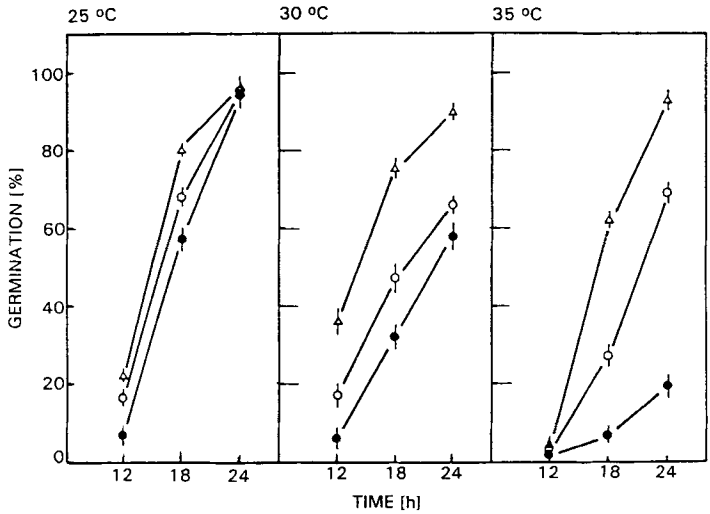

Fig. 1. Germination percentage of *Cicer arietinum* seeds at different temperatures in water (*closed circles*), 7 μM ethrel (*open circles*) or 70 μM ethrel (*triangles*). Each point is the mean of three replicates ± SE (vertical bars).

Table 1. Effects of several treatments on germination percentage and ethylene production in embryonic axes of chick-pea seeds germinated for 24 h at 25 °C. Data are the mean values of three replicates ± SE. N.D. - not detected

	Germination [%]	C_2H_4 [nl kg^{-1} s^{-1}]
Control	95 ± 3	13.89 ± 0.19
ACC (200 μM)	100 ± 0	28.39 ± 0.31
PEG-6000 (-0.15 MPa)	58 ± 2	10.42 ± 0.08
PEG-6000 (-0.30 MPa)	0	N.D.
ABA (25 μM)	35 ± 1	3.25 ± 0.14
ABA (25 μM) + Ethrel (70 μM)	95 ± 3	17.94 ± 0.22
AVG (1 mM)	80 ± 2	0.47 ± 0.06
NBE (100 μl)	0	N.D.
NBE (100 μl) + AVG (1 mM)	0	N.D.
$CoCl_2$ (50 mM)	0	N.D.
$CoCl_2$ (50 mM) + ACC (200 μM)	0	N.D.
PG (50 mM)	0	N.D.
PG (50 mM) + ACC (200 μM)	0	N.D.
MGBG (40 μM)	96 ± 2	32.92 ± 0.56
CHA (1 mM)	100 ± 0	36.22 ± 0.56
CHA (1 mM) + MGBG (40 μM)	98 ± 2	35.19 ± 0.56
MGBG (40 μM) + NBE (100 μl)	0	N.D.
CHA (1 mM) + NBE (100 μl)	0	N.D.
CHA + MGBG + NBE (100 μl)	0	N.D.

1989). High temperatures could inhibit germination by reducing ethylene production or by directly affecting cell elongation. When FC, a substance known to simulate cell elongation by accelerating the uptake of potassium (Hernandez-Nistal *et al.* 1983), was introduced into the germination medium this resulted in a sharpe increase in germination to the extent that not only did the percentage of radicle emergence rise

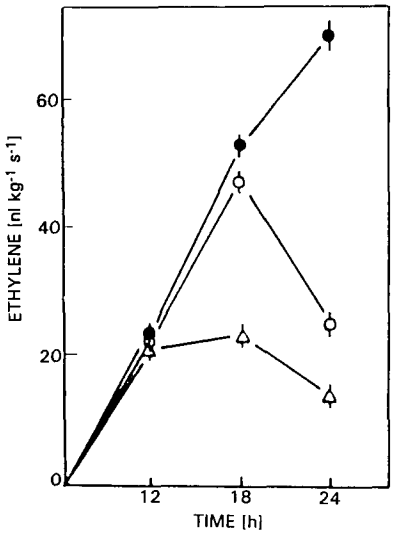

Fig. 2. Ethylene production by embryonic axes of chick-pea seeds germinated in water during the indicated times at 25 °C (*closed circles*), 30 °C (*open circles*) and 35 °C (*triangles*). Each point is the mean of three replicates ± SE (vertical bars).

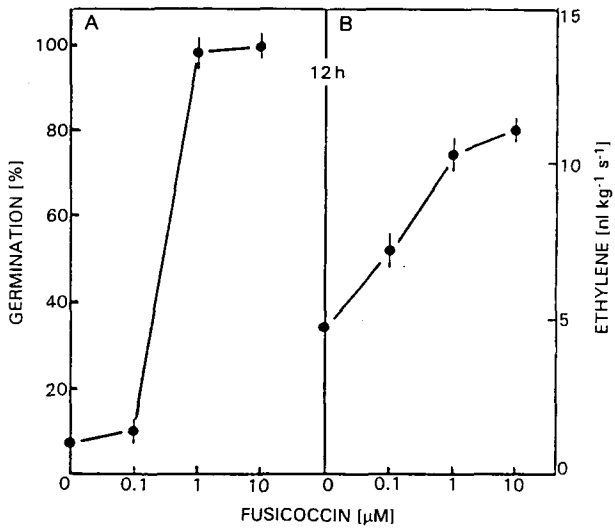

Fig. 3. Effect of different concentrations of fusicoccin on the germination percentage of chick-pea seeds at 25 °C (A) and ethylene production by the embryonic axes (B). Each point is the mean of three replicates ± SE (vertical bars).

but also germination time was brought forward. Thus, with FC (1 and 10 μM), 98 % and 100 % germinations were reached after only 12 h at 25 °C. FC caused a 1.5 - 2.4-fold increase in ethylene production after 12 h at 25 °C as compared to the controls (Fig. 3).

Cell elongation is inhibited by water deficiency caused by an osmotic agent such as PEG-6000 (Kepczynski 1986). In our experiments, the addition of PEG-6000 (-0.15 MPa) to the medium caused a reduction in the amount of ethylene produced and a concomitant fall in the germination percentage (Table 1). Higher concentrations of PEG (-0.30 MPa) completely stopped both germination and ethylene production. This effect could be reversed, however, by the application of exogenous ethylene (Table 2). This suggests that ethylene may play an active part in the germination of chick-pea seeds.

The phytohormone ABA reduces H^+/K^+ exchange, thus diminishing the water content of the cells (Walton 1980), and also diminishes EFE activity in *Cicer arietinum* (Gallardo *et al.* 1992). With 25 μM ABA in the medium, after 24 h at 25 °C, germination was only 35 % and ethylene production 4.3-fold lower than in the controls (Table 1). Ethylene (ethrel) reversed both these effects (Table 1).

On the other hand, polyamines are expected to be of antiethylene nature, for both these and ethylene have a common precursor, SAM and their biosynthetic pathways are thought to be competitive (Biasi *et al.* 1991). We tested the effects of inhibitors of polyamine biosynthesis on ethylene production and the germination of chick-pea seeds. The presence of MGBG or CHA, which are inhibitors of the SAM-decarboxylase (Suresh and Adiga 1977) and spermidine synthase (Barbieri *et al.* 1983) activities, respectively, increased both ethylene production and slightly also the percentage of radicle emergence (Table 1) and, like FC, the germination time was brought forward (data not shown).

Table 2. Effect of indicated treatments over 24 h on the germination percentage of *Cicer arietinum* seeds at 25 °C in the absence (-) or presence (+) of exogenous ethylene (25 μl l^{-1}) during the indicated time. The germination percentages at 12 h were: control - 7 ± 1 and remaining treatments - 0. Data are the mean values of three replicates ± SE.

Treatment	12 h (-) + 12 h (+)	24 h (-)	24 h (+)
Control	100	95 ± 3	100
$CoCl_2$ (50 mM)	100	0	100
PEG-6000 (-0.30 MPa)	100	0	100
PG (50 mM)	100	0	100

In spite of the significant fall in ethylene production caused by AVG (1 mM), germination remained uninhibited. Similar observations have been reported for *Phaseolus vulgaris*, *Arachis hypogea* and *Amaranthus caudatus* seeds (De Greef *et al.* 1980, Hoffman *et al.* 1983, Kepczynski and Karssen 1985). In this context, Fu and Yang (1983) reached the conclusion that ethylene is a by-product of germination in seeds rather than a requirement. It should be emphasised, however, that in our experiments germinating seeds still produced some ethylene (0.48 nl kg^{-1} s^{-1}) in the

presence of 1 mM AVG. It may well be then, as Saini *et al.* (1986) suggest, that AVG fails to inhibit germination completely because the seeds continue to produce sufficient ethylene to start the germination process, or that ethylene regulation of germination does not act at ACC-synthase level.

When we supplied 100 µl of NBE, an inhibitor of ethylene action, to the flask containing the seeds, emergence was stopped (Table 1), regardless of the time at which NBE was added (data not shown). The addition of AVG (1 mM) together with NBE cancelled seed germination. In the same way, when inhibitors of polyamine synthesis (MGBG, CHA or MGBG + CHA) were applied together with NBE, both the germination percentage and ethylene production were completely stopped (Table 1). The results with NBE seem to indicate that the action of ethylene is necessary for the germination process in chick-pea seeds.

EFE inhibitors such as $CoCl_2$ and PG (Yu and Yang 1979) at concentrations of 50 mM completely prevent both ethylene production and seed germination. This inhibitory effect was not reversed by ACC (Table 1). The application of ethylene did, however, counteract the effect and caused the seeds to germinate (Table 2). The results offered in Tables 1 and 2 suggest that one point in the regulation of the germination of *Cicer arietinum* seeds by ethylene is at the level of EFE.

Most of our experimental data point to the notion that a certain amount of ethylene production is essential for the germination process. When ethylene production was measured in non-germinated seeds only insignificant amounts of ethylene were detected, it being necessary to collect at least 100 embryonic axes (*ca.* 1 g) of this seeds for detecting 0.03 nl kg^{-1} s^{-1} (data not shown). It could be this ethylene which, when accumulated in sufficient amounts, provokes the germination, although the ethylene production rate increases concomitantly with the radicle emergence. Furthermore, when ethylene synthesis is completely blocked, either by osmotic agents or EFE-activity inhibitors, germination is prevented and is only renewed when the seeds are exposed to exogenous ethylene. This would seem to rule out the possibility that ethylene could be produced as a consequence of germination but rather that its presence would be necessary for germination to take place in *Cicer arietinum* seeds.

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