On the inhibitory action of endosperm on germination of *Cercis siliquastrum* seeds

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Summary. The endosperm inhibits the growth of embryos isolated from *Cercis siliquastrum* seeds. The inhibitory activity is present in autoclaved homogenates as well as in homogenates remaining after an extraction with chloroform or isobutanol.

Previous work has shown that in *Cercis siliquastrum* seeds the dormancy removed by either gibberellic acid (GA_3), or fusicoccin (FC), or by pre-treatment at low temperature, is connected with the presence of the thick endosperm[2-4].

In the present paper we have investigated: a) whether the inhibition exercised by the endosperm is mechanical or chemical, or both[5,6]; b) whether a cold temperature, which breaks the dormancy, affects only the endosperm, removing its inhibitory effect, or also the embryo, increasing its 'growth potential', namely its growth capacity when isolated from the seed[7].

Material and methods. Seeds of *Cercis siliquastrum*, collected in mid-October 1977, were sterilized with sodium hypochlorite and repeatedly washed with deionized water. Some seeds were immediately used, and endosperms and embryos were given various treatments at ±24 °C (non-prerefrigerated endosperms and embryos), while other seeds were immersed in deionized water for about 40 days at 6 °C to obtain prerefrigerated endosperms and embryos. The pH value of the water in which the seeds were plunged during the cold-pretreatment was constant (6.7±0.1).

To isolate the embryo the integument was removed from the seed, thus baring the endosperm, consisting of thick stiff-walled cells. Separation of the embryo from the endosperm was obtained by a gentle pressure on both sides of the decoated seed. The isolated endosperms were either left intact or used to obtain homogenates or extracts.

Treatment of isolated embryos. In the 1st series of experiments the isolated embryos, arranged in Petri dishes on filter paper soaked in deionized water, were brought into contact with 1 or both halves of endosperms, while in a 2nd series the embryos were put in Petri dishes on filter paper wetted with homogenates or extracts obtained from variously treated endosperms. The dishes were kept in darkness at ±24 °C.

1. Preparation of the homogenate. The isolated endosperms (1 endosperm = mg 28 f.w.) were ground in a mortar with washed and calcined quartz sand and with deionized water in the ratio 1 ml/4 endosperms. A homogeneous suspension was thus obtained. The pH value of the homogenate was 6.4±0.1.

2. Incubation of the homogenate. The endosperm homogenate, prepared as described above, was incubated at 25 °C for 6 days. During the incubation (in an aseptic room) the pH maintained a uniform value of about 6.4±0.1. Before and after the incubation, the homogenate was autoclaved for 45 min at 0.5 atmosphere.

3. Separation of the aqueous extract. This was directly obtained from the homogenate, before or after incubation, by centrifugation at 21.800×g for 10 min. Both the supernatant and the precipitate thus obtained were used for treatment of the embryos.

4. Extraction with solvents. 80 endosperms were ground in a mortar with quartz and treated with 60 ml of solvent containing 20 ml of petroleum ether, 20 ml of chloroform and 20 ml of iso-butanol at pH values of 7.4 and 2. The smashed endosperms in the solvents were incubated for 48 h at 25 °C. The suspension was then filtered on What-

Conditions of endosperm		Cold pre-treatment of the seed	Percent increase in length of embryonic axis at 7th day
	control: no endosperm	–	100
	radical pole portion removed	–	0
	longitudinally slit	–	0
	divided into two halves with embryo insertion	–	9.2
	lower half removed	–	23
	upper half replaced with inert substance	–	90.4
	divided into two halves with embryo insertion	+	67.6
	lower half removed	+	80

endosperm embryo

Fig. 1. Growth of embryos isolated from *Cercis* seeds with variously treated endosperm. In all cases embryos were obtained from non-refrigerated seeds. SD±2.8%.

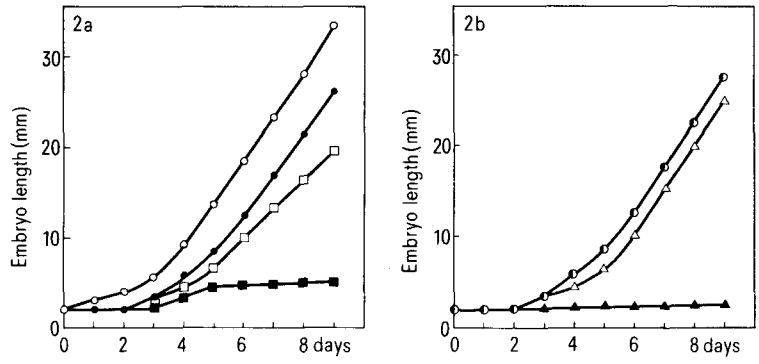

Fig. 2. Effects of endosperm homogenates or extracts on the growth of isolated *Cercis* embryos. *a* – ○ –, R embryos on water; – ● –, NR embryos on water; – □ –, R embryos on homogenate of endosperms from NR seeds; – ■ –, NR embryos on homogenate of endosperms from NR seeds. *b* – ◐ –, NR embryos on fresh aqueous extract of endosperms from NR seeds; – △ – NR embryos on homogenate of endosperms from R seeds; – ▲ – NR embryos on aqueous extract of homogenate from NR seeds incubated for 7 days. SD did non exceed ± 2.8%. R refrigerated; NR non-refrigerated.

man No. 1 paper and the insoluble residue was washed with 60 ml of petroleum ether, 60 ml of chloroform and 60 ml of iso-butanol. The combined filtrates were then concentrated under vacuum at 40 °C to a final volume of 20 ml. This solution was spread on 4 filter paper dishes (5 ml on each dish) and evaporated under vacuum at room temperature to obtain dishes each corresponding to 20 endosperms. The insoluble residues were used for the preparation of homogenates or subjected to extraction after incubation in water.

5. All the experiments were repeated at last 5 times under sterile conditions, and no relevant presence of bacteria or fungi was shown during the time of the observations.

Results. Effects of the endosperms on embryo growth. a) Non-refrigerated endosperms. Embryo growth was severely inhibited when the embryo was inserted between the 2 endosperm halves, or simply set underneath a half endosperm. This inhibition was not observed in the absence of the upper half endosperm, even when the upper half endosperm was replaced by a piece of aluminium foil of equal weight and shape (figure 1). b) Refrigerated endosperms. Endosperm halves isolated from pre-refrigerated seeds did not inhibit the growth of embryos under any of the experimental conditions investigated (figure 1).

The finding that embryo growth is not inhibited by the endosperm from refrigerated seed or by the replacement of the upper endosperm half with an equivalent weight of inert material seems to rule out the possibility that hindrance of gas (O_2) diffusion or mechanical factors play a role in the phenomenon. The simplest explanation of our results seems thus to be that of a chemical inhibitor moving from the endosperm to the embryo. This hypothesis is confirmed by the experiments with homogenates and water extracts of endosperm.

Effects of endosperm homogenates or extracts on embryo growth. Figure 2 shows the growth curves of isolated embryos from non-refrigerated and from refrigerated seeds, together with the effect of homogenates of endosperms isolated from either refrigerated or non-refrigerated seeds on the growth of embryos. The data show that in the absence of endosperm homogenates, the growth of refrigerated embryos starts earlier, and proceeds at a somewhat faster rate for about 8 days than for the non-refrigerated embryos (figure 2, a), thus indicating that pre-refrigeration induces some change in the embryo, independently of other effects mediated by other parts of the seed.

The data also show that the freshly prepared homogenates of endosperms isolated from non-refrigerated seeds inhibit the growth of embryos from non-refrigerated seeds, and also, but to a lesser extent, that of embryos from refrigerated seeds (figure 2, a). In contrast, no inhibition of embryo growth is induced by the fresh homogenate from refrigerated endosperms (figure 2, b).

Furthermore the aqueous extract (21.800 × g for 10 min centrifugation) of the freshly prepared endosperm homogenate from non-refrigerated seeds shows no inhibitory activity, while that from the autoclaved homogenates incubated for 7 days at 25 °C in sterile conditions is even more inhibitory than the whole, fresh homogenate (figure 2, b). This could suggest that the inhibitory principle(s) is slowly released in a soluble form during the incubation.

No inhibition of embryo growth was induced by the endosperm extracts obtained with organic solvents such as petroleum ether, iso-butanol or chloroform, while the homogenates obtained by resuspending in water the insoluble fraction of endosperms extracted with the above-mentioned solvents maintain their inhibitory activity unchanged.

Conclusions. The results reported above suggest a chemical component in the inhibitory action of the endosperm on the germination of *Cercis siliquastrum* seeds. The inhibitory action of the endosperm on the growth in length of the embryo becomes detectable only after a time lag of 4–5 days, both in the case of the embryo placed in contact with portions of the endosperms or treated with homogenates. This time lag is no longer detectable either with the homogenate which has been incubated for seven days or with the water extract obtained from this homogenate. This suggests that the endosperm contains the inhibitor(s) in some inactive, insoluble form, the activation of which requires a relatively slow process. The effect of the cold pretreatment on the endosperm could thus be explained as due to a lowering of the level of the 'precursor' of the inhibitor(s) and/or to an inhibiting effect on the activation of the precursor. As regards the embryo, the action of the cold pre-treatment in stimulating its growth could depend on a decrease of the level of inhibitor(s) or on an increase of stimulatory substances, as suggested in the literature for other material[8–15].

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