# THE LIGHT PROMOTED GERMINATION OF THE SEEDS OF CHENOPODIUM ALBUM L.; II. EFFECTS OF (RS) - ABSCISIC ACID

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SUMMARY

The visible germination phenomena of *Chenopodium album* seeds can be divided into two stages.

The first stage, being light-dependent in these seeds, consists of the splitting of the outer testa layer only and the extending of the radicle from within the seed. This stage is not prevented by incubation in (RS)-abscisic acid (ABA). Only the protrusion of the radicle through the inner testa layer and the underlying endosperm layer (the second stage) is prevented by ABA. This effect is more pronounced in darkness than in light.

After 2 weeks of incubation the inhibition can be released by transfer to water. After 4 weeks in most of the seeds only scarification of the inner layers enables the start of rootlet growth. It is assumed that ABA inhibits one or some components of the cell expansion of the embryo.

## 1. INTRODUCTION

To the number of naturally occurring substances, regulating plant growth and development, most recently a new one has been added. About the name of this substance some confusion arose owing to the use of "abscisin II" as well as "dormin". As a compromis ADDICOTT *c.s.* (1968) proposed a new name: abscisic acid (ABA), which name will be used in this paper.

The enantiomorph (S)-abscisic acid has been isolated and subsequently identified in a large number of plants and organs, see for instance MILBORROW (1967), who in a literature review also gives some good evidence for its important function in the regulation of bud dormancy, the induction of abscission and seed dormancy.

Regarding seed dormancy, ABA has been found in the fruits and seeds of avocado, coconut, linden, maize (MILBORROW 1967) and peach (LIPE & CRANE 1966). In field rose *(Rosa arvensis)* there is an inverse relation between inhibitor content, identified as ABA by CORNFORTH *c.s.* (1966), and the ability of the seeds to germinate; removing of the pericarp and testa, being the major sources of ABA, or leaching in water favours germination (JACKSON & BLUNDELL 1963, 1965). Among other things the germination of rose seeds (MILBORROW 1967, referring to unpublished results of JACKSON & BLUNDELL), hazel seeds (BRADBEER 1968) and four species of grasses (SUMNER & LYON 1967) were inhibited by application of synthetic (RS)-ABA.

WENTLAND (1965) isolated some inhibiting substances from the embryo-endosperm complex of *Chenopodium album* seeds. One of them was found at an Rf of 0.7 to 0.9, on chromatograms run in systems comparable to those in

which the inhibitor-β complex had been located in dormant buds of *Acer pseudoplatanus* by ROBINSON & WAREING (1964).

MILBORROW (1967), comparing several reported Rf values of inhibitor-β from a number of plants and in a number of paper chromatographic systems, with the Rf values of synthetic ABA, revealed that those of the latter fell within the range of values reported for inhibitor-β. WENTLAND (1965) also reported that this substance was present in a higher concentration, based on the inhibition of the root growth of *C. album*, in dormant seeds, grown under LD conditions, than in the non-dormant SD seeds. The germination of the seeds, however, was not inhibited by application of these substances.

This paper describes some preliminary experiments about the effects of synthetic (RS)-ABA on the germination of the seeds of *C. album*.

## 2. MATERIALS AND METHODS

The seeds (code number 147) used in this study were harvested in 1966 from a group of plants on a waste lot near Utrecht. The storing-conditions and the germination methods employed have been described before (KARSSEN 1967). The only change in the method was that during the present experiments the seeds were spread on only one layer of filterpaper, wetted with 4 ml of de-ionised water or test solution.

Three white fluorescent lamps (Philips, Eindhoven, TLF 40 W/33) were used for continuous irradiation. Green light was used for the determination of the germination-time-course and for some manipulations with dark incubated seeds. It was obtained in the same way as described previously.

In some experiments the time-course of the incomplete as well as the complete germination stage (see 3. for definitions) was determined. When the seeds had reached the latter stage they were removed from the dishes.

A stock solution of ABA was prepared by dissolving 2 mg in 50 ml de-ionised water. This 40 ppm solution was stored at 4°C and diluted to the desired concentration just before use (1 ppm $= 3.8 \times 10^{-6}$ M).

## 3. ANATOMY OF THE SEED AND STAGES OF VISIBLE GERMINATION

The anatomy of the seeds of the *Chenopodiaceae* has been described by NETOLITZKY (1926). For some species detailed studies of the development and morphology of the seed are available: for *C. album* by BHARGAVA (1937) and for *Beta vulgaris* by ARTSCHWAGER (1927).

The seed *(fig 1)* is biconvex, black, glossy, has an obtuse margin and is generally smooth. The size of the seeds varies from 1.0 to 2.0 mm. In the mature seed the peripheral embryo forms a complete ring around the perisperm, a storage tissue of nucellar origin. A thin lining of crushed nucellar cells separates the embryo at the proximal side from the perisperm and at the distal side from the testa, except in the region of the radicle where a single endosperm layer encloses the last part of the embryo like the fingers of a glove.

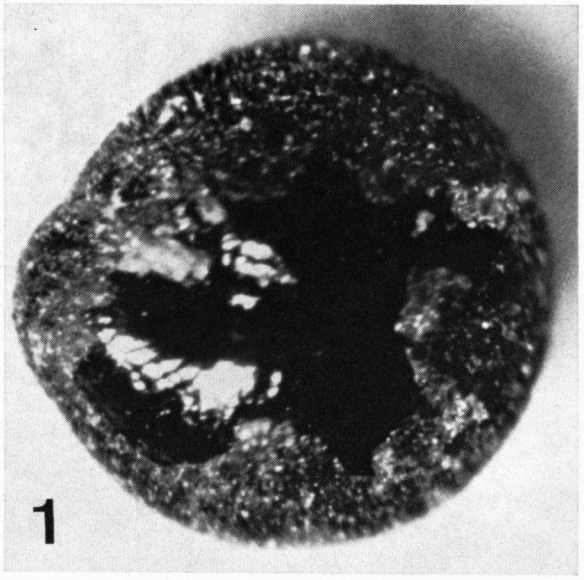


Fig. 1.
The seed of *C. album* before any visible germination phenomenon occurs. The pericarp is only partly removed.

The mature embryo is surrounded by a testa consisting of two layers derived from the two integuments of the ovule, each two cell layers thick. Of the original four cell layers in the mature testa only three remain, since the outer layer of the inner integument develops very delicately striated cell wall thickenings. Those of the side walls rise above the distal surface, forming a reticulate structure. The cell content gets hardened and brownish. The mature outer testa layer*) separates easily from the inner one. It consists nearly only of the dark thickened outer wall (from 10–40μ thick). The remaining walls of the outer cell layer and the complete inner cell layer are compressed.

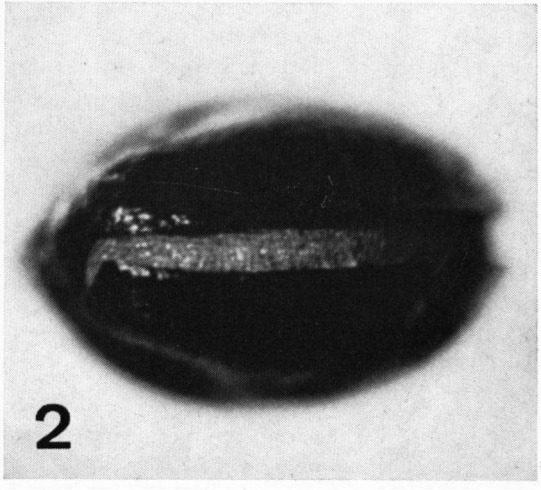


Fig. 2.
The first visible phenomenon of incomplete germination: the splitting of the outer testa layer in the area overlying the radicle.

* In the previous paper of this series (KARSSEN 1967) this layer was erroneously called "fruitwall".

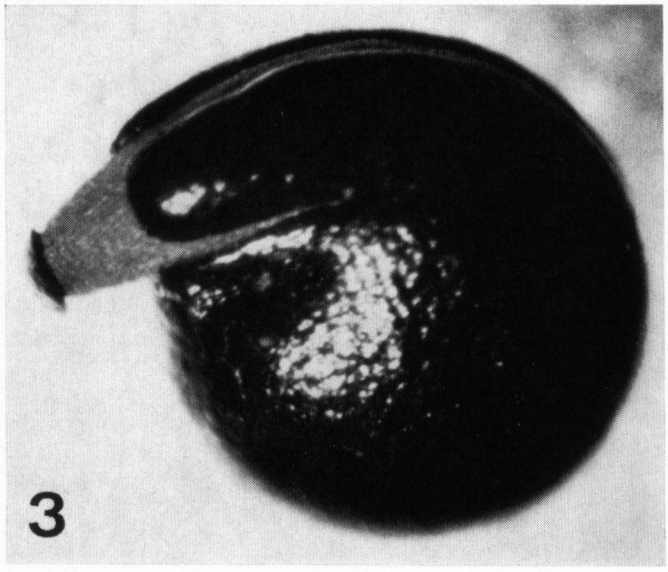


Fig. 3. The radicle still enclosed within the inner testa layer, with reticulate surface, and the underlying endosperm layer, extending from within the seed. A cap of outer testa layer material is on the top of the radicle.

The pericarp is somewhat adherent *(fig 1)*. During the cleaning of the seeds, before sowing in the petri dishes, in most cases the pericarp is fully removed, together with the perianth.

The first indication of visible germination is the splitting of the outer testa layer in the area overlying the radicle *(fig. 2)*, after that the radicle extends from within the split outer testa layer, but it remains at first enclosed within the inner testa layer and the single endosperm layer *(fig. 3)*. The last stage consists of the protrusion of the radicle through these inner layers *(fig. 4)*. After CUMMING (1963) we will refer to the first stage as "incomplete germination" *(figs. 2* and *3)*, to the second one as "complete germination" *(fig. 4)*.

## 4. RESULTS

One year after harvest most of the seeds of selection 147 were light-dependent, 85% of the seeds germinated under continuous irradiation with white light, 8% in continuous darkness. To make possible an investigation into the effect of ABA on light-dependent as well as on light-independent seeds, a method was employed to increase the light-independent fraction. The seeds were stored in an desiccator above granulous $CaCl_2$ (FUJII & YOKOHAMA 1965). In the course of some months the germination in darkness increased gradually, reaching a constant level of about 60%.

The effects of a range of concentrations ABA on the germination of *C. album*

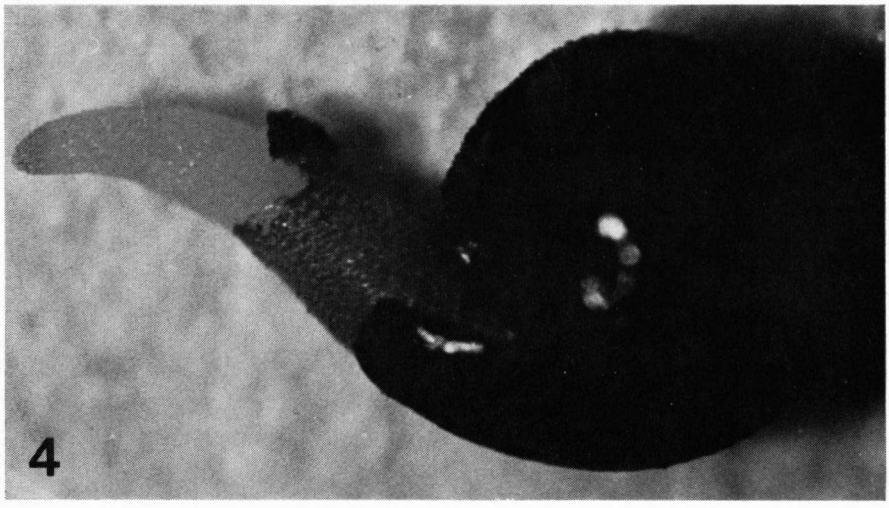


Fig. 4. The seed is completely germinated, the radicle has protruded through the inner layers.

seeds are presented in *table 1*; each value is the average of two experiments, with two dishes per concentration each. Quite evidently incubation in ABA does not prevent the incomplete germination stage. But whereas in water the percentages of incomplete and complete germination are always identical, as was observed before (KARSSEN 1967), a decreasing part of the incompletely germinated seeds reached the complete stage with increasing concentration ABA of the medium.

Table 1. Effect of ABA in a range of concentrations on the percentages incomplete and complete germination in light and in darkness

| Incubation medium | Incomplete germination | | Complete germination | |
|---|---|---|---|---|
| | Light | Dark | Light | Dark |
| $H_2O$ | 96 | 57 | 96 | 57 |
| 1 ppm ABA | 92 | 55 | 92 | 52 |
| 2½ ppm ABA | 95 | 57 | 69 | 26 |
| 5 ppm ABA | 94 | 58 | 37 | 10 |
| 10 ppm ABA | 94 | 61 | 9 | 1 |
| 20 ppm ABA | 92 | 56 | 3 | 1 |

Expression of the complete germination in percentages of the incomplete germination *(fig. 7)* reveals that the inhibiting effect of ABA is not identical in light and darkness. A seed that has reached the incomplete stage has, at a certain ABA concentration, a better chance in light to complete the germination process than in darkness. It is important to note here that the primary effect of the irradiation – the induction of the incomplete stage – is not influenced by ABA.

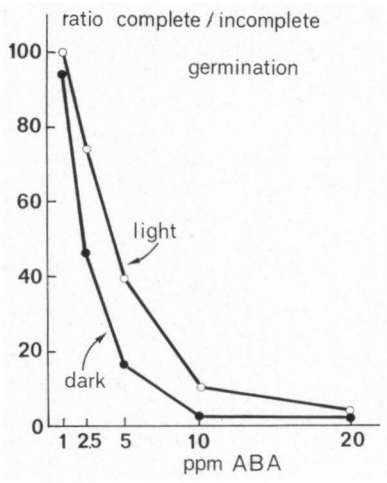


Fig. 7. The complete germination expressed as percentages of the incomplete germination Data of *table 1*.

The determination of the germination-time-course showed that the rate of the complete germination was decreased with increasing ABA concentration. It was, however, very difficult to determine exactly the moment whereupon the first sign of germination appeared. The only preliminary conclusion of these determinations is that the rate of the incomplete germination was hardly influenced by ABA, if it was influenced at all.

The inhibiting effect of ABA only on the germination stage after the splitting of the outer testa layer might be due to the impermeability of this layer to ABA. To test this hypothesis the outer testa layer of dry seeds was removed in a small area overlying the extreme point of the radicle ("half-operated seeds"). This treatment was possible without damaging the inner testa layer. In spite of this treatment ABA still did not inhibit the incomplete germination *(table 2)*, in this case only visible by the elongation of the radicle, still surrounded by the inner testa layer.

Table 2. Effect of ABA in a range of concentrations on the percentages incomplete and complete germination of "half-operated" seeds, in light and in darkness

| Incubation medium | Incomplete germination | | Complete germination | |
|---|---|---|---|---|
| | Light | Dark | Light | Dark |
| $H_2O$ | 97 | 100 | 97 | 100 |
| 2½ ppm ABA | 93 | 88 | 57 | 51 |
| 5 ppm ABA | 89 | 90 | 46 | 35 |
| 10 ppm ABA | 79 | 88 | 26 | 33 |

The percentages complete germination in light in this single experiment are in fair agreement with those of the non-operated seeds *(table 1)*. The different sensitivity of light- and dark-incubated seeds, which was shown in *fig. 1*, was, however, absent here. It seems reasonable to relate the light-insensitive inhibi-

tion of the half-operated seeds to their light-independent incomplete germination, which is shown by the identical percentages in light and darkness. If the outer testa layer was removed over the complete margin of the seed, we observed the same effect.

Both preceding experiments make it plausible that ABA inhibits only the processes that ultimately lead to the protrusion of the radicle through the inner testa layer and the endosperm layer.

The course of the germination in an experiment in which the seeds after an incubation (in light) for 72 hours in water were transferred to 5 ppm ABA *(fig. 8)* show that in water at that moment only 30 seeds had not yet reached the complete stage. When these seeds were transferred to ABA only a small number of them completed their germination, in contrast to the seeds that stayed in water. These results reveal that the inhibition of the complete germination does not need a preparation during the preceding processes.

The reverse treatment of that in the previous experiment *(fig. 9)* shows that the inhibition of the complete germination, which is already clearly present after 72 hours of incubation in 5 ppm ABA, can be easily released by transfer of the seeds to water. These results indicate that the incubation in ABA had not induced any unreleasable inhibition, and moreover, that the protrusion through the inner layers, in spite of the presence of ABA, had been prepared already, since the transferred seeds within 24 hours nearly completely worked off their arrear to the seeds continuously incubated in water.

Both these transfer-experiments underline our previous conclusion.

The question arose whether this fast release of the inhibition was still possible after longer incubation periods in ABA. Therefore the first experiment *(table 1)*

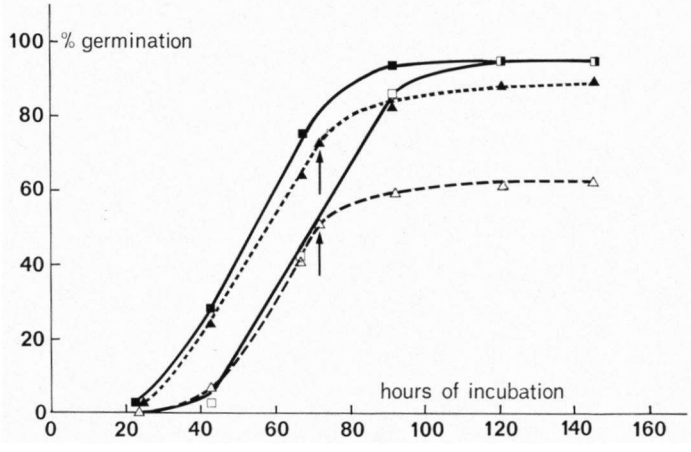


Fig. 8. Effect of transfer to ABA (5 ppm) after an incubation for 72 hours in water, under continuous irradiation.
□: water; △: transferred seeds; open symbols: complete germination; closed symbols: incomplete germination; →: moment of transfer.

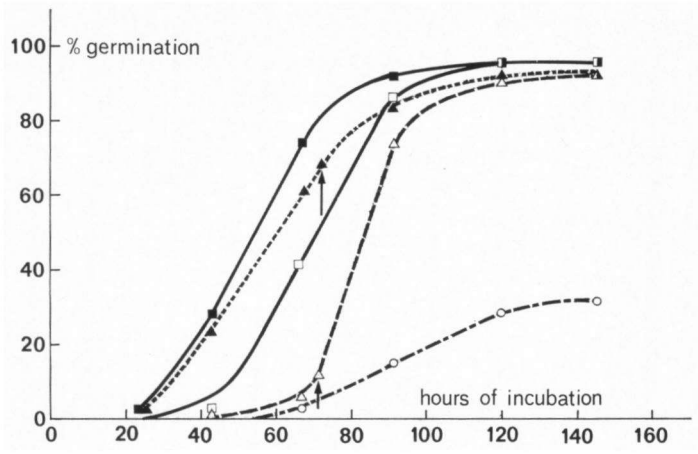


Fig. 9. Effect of transfer to water after an incubation for 72 hours in 5 ppm ABA, under continuous irradiation.
□: water; △: transferred seeds; ○: 5 ppm ABA; open symbols: complete germination; closed symbols: incomplete germination; →: moment of transfer.

was prolonged. After the first 14 days of incubation the seeds were either transferred to water or to a fresh incubation medium containing the same ABA concentration as before. After a second 14 days of incubation the latter seeds were also transferred to water.

The results of these treatments *(table 3)*, represented separately for two experiments, show somewhat controversial effects of the refreshment of the ABA medium of the light-incubated seeds after the first 14 days. While in the first experiment the percentage complete germination increased to some extent by this treatment this increase is negligible in the second experiment. A possible explanation of this difference may be the more or less desiccated state of the seeds after this period in experiment 1. In experiment 2 these light-incubated seeds were still in an imbibed state. The dark-incubated seeds never showed any increase in the percentage complete germination after refreshing of the ABA medium, they were all still in an imbibed state.

The results show, however, very clearly that a transfer after 14 days of the still incompletely germinated seeds to water, released the inhibition in nearly all the seeds, except after incubation in the highest tested concentration of 20 ppm. In these seeds also a second transfer to fresh incubation-water after 4 weeks did not release the inhibition in all the seeds.

The situation was completely changed after 4 weeks of incubation in the various ABA-concentrations. Now only the inhibition of the light-incubated seeds in $2\frac{1}{2}$ ppm (exp. 1 and 2) and in 5 ppm (exp. 1) could fully be released. After all the other preceding incubation treatments only some of the seeds were able to complete their germination, the number of them dependent on the concentration of the preceding ABA medium.

Table 3. Effect of transfer to water after 14 or 28 days of incubation in ABA

| Concentration ABA | Condition | Percentage germination complete (incomplete) after: | | | |
|---|---|---|---|---|---|
| | | First 14 days in ABA | Second in ABA | 14 days in water | Third 14 days in water |
| *Experiment 1* | | | | | |
| | Light | 3 (92) | | 66 (94) | 79 (92) |
| | | 4 (93) | 20 (95) | | 32 (94) |
| 20 ppm | Dark | 2 (55) | | 50 (57) | 52 (57) |
| | | 0 (58) | 2 (61) | | 8 (61) |
| | Light | 11 (94) | | 93 (94) | |
| | | 7 (95) | 34 (96) | | 45 (96) |
| 10 ppm | Dark | 1 (54) | | 54 (56) | |
| | | 2 (69) | 6 (70) | | 34 (70) |
| | Light | 39 (92) | | 94 (94) | |
| | | 31 (96) | 51 (97) | | 95 (97) |
| 5 ppm | Dark | 17 (59) | | 60 (60) | |
| | | 7 (53) | 14 (56) | | 35 (56) |
| | Light | 71 (94) | | 96 (96) | |
| | | 61 (97) | 89 (97) | | 94 (97) |
| 2½ ppm | Dark | 16 (49) | | – (–) | |
| | | 28 (59) | 36 (60) | | 55 (60) |
| *Experiment 2* | | | | | |
| | Light | 50 (96) | | 96 (96) | |
| | | 39 (91) | 42 (93) | | 66 (93) |
| 5 ppm | Dark | 23 (59) | | 59 (59) | |
| | | 12 (62) | 21 (64) | | 52 (64) |
| | Light | 73 (94) | | 94 (94) | |
| | | 76 (95) | 84 (95) | | 95 (95) |
| 2½ ppm | Dark | 33 (65) | | 65 (65) | |
| | | 26 (63) | 32 (64) | | 49 (66) |

The seeds that could escape from the inhibition did so at both transfer moments within about 24 hours, except again the seeds transferred from 20 ppm after 14 days, which started their renewed complete germination at first after 24 hours and at a much slower rate.

This type of experiment needs some refinement, *e.g.* by refreshing the incubation medium at short intervals to prevent the effect of desiccation. It is, however, clearly shown that ABA in *Chenopodium* seeds can induce an inhibition of the last stage of the germination process and that after longer incubination periods this inhibition can not be released by water.

To gather some information about this inhibition the seeds that after 4 weeks in ABA and 2 weeks in water were still in the inhibited state, were subjected to one of the following treatments: (1) refreshing of the incubation water; (2) rinsing for one hour in running tapwater; (3) scarification of the distal side of the inner testa layer and the underlying endosperm layer in the area where they enclose the extending radicle.

The results of these treatments, only presented for the seeds previously incu-

Table 4. Effects of refreshing of the incubation water, rinsing for 1 hour by running tapwater or scarification of the distal sides of the inner testa layer and the endosperm layer on seeds which were only incompletely germinated after incubation for 4 weeks in 10 or 20 ppm ABA and for 2 weeks in water.

| Concentration of ABA during first 4 weeks | Condition | Number of incompletely germinated seeds | Treatment | Number | Total number of completely germinated seeds after: 1 day | 2 days | 6 days |
|---|---|---|---|---|---|---|---|
| 20 | Light | 61 | Refreshed water | 21 | 1 | 1 | 2 |
| | | | Rinsed | 21 | 1 | 4 | 5 |
| | | | Scarified | 20 | 20 | – | – |
| 20 | Dark | 55 | Refreshed water | 17 | 0 | 1 | 2 |
| | | | Rinsed | 17 | 0 | 5 | 5 |
| | | | Scarified | 21 | 21 | – | – |
| 10 | Light | 51 | Refreshed water | 17 | 1 | 2 | 4 |
| | | | Rinsed | 17 | 3 | 8 | 8 |
| | | | Scarified | 17 | 17 | – | – |
| 10 | Dark | 36 | Refreshed water | – | – | – | – |
| | | | Rinsed | 18 | 1 | 2 | 3 |
| | | | Scarified | 18 | 17 | 17 | 17 |

bated in 10 or 20 ppm *(table 4)*, show that refreshing of the water still had no effect. In some seeds rinsing released the inhibition, but scarification of the inner layers had the most dramatic effect. It is at least evident that the embryos were still living and that an incision in the inner layers released the inhibition.

During the scarification of these layers we made two important observations. When the incision was made in the area where the layers enclose the extending part of the radicle ("outside incision"), the embryo within half an hour tore up the remaining non-scarified tissue. The radicle stretched after that first half hour at least twice as far out of the seed as before the incision was made ("typical germination", *fig. 5)*.

When the incision in these layers was made in an area where the embryo was still partly enclosed by the split outer testa layer ("inside incision") – this layer had to be removed for this manipulation – the embryo could in most cases not tear up the connection between the layers around its top and the layers inside the seed.

The elongation that anyhow took place, pressed the embryo through the incision in the inner testa layer out of the seed ("atypical germination", *fig. 6)*, indicating a certain mechanical restraint of the inner layers.

These observations were checked among other things in an experiment in which seeds, after 2 weeks of dark-incubation in $2\frac{1}{2}$ ppm ABA still incompletely germinated, were all transferred to water and immediately afterwards were subjected to one of the following treatments: (1) controls; (2) removing of the brown cell material of the inner testa layer without damaging the underlying endosperm layer; (3) an "inside incision"; and (4) an "outside incision".

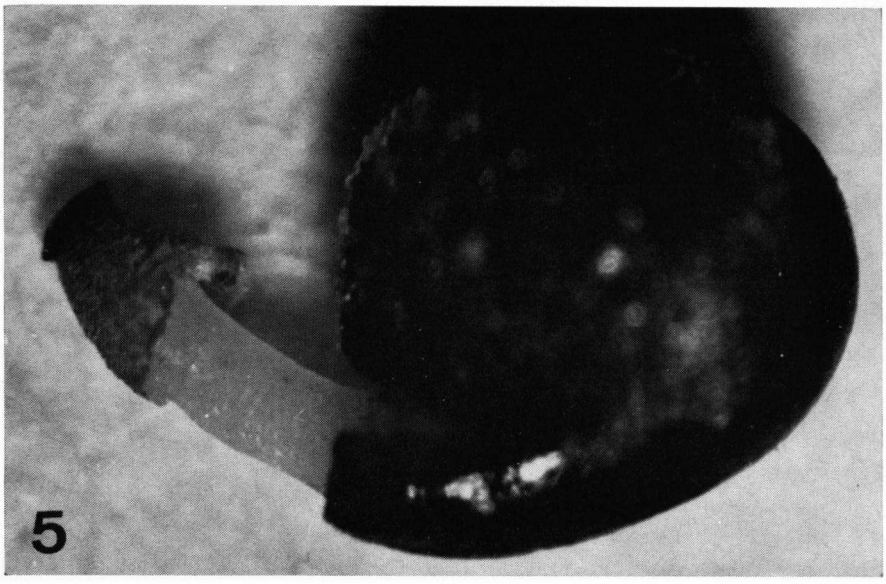


Fig. 5. The result of an "outside incision", made half-an-hour before. The radicle has been elongated at least twice ("typical germination").

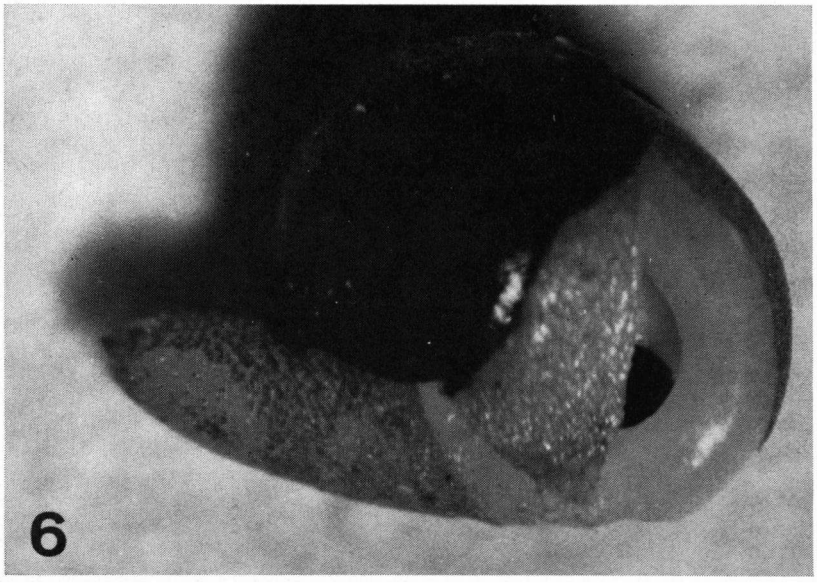


Fig. 6. The result of an "inside incision", made half-an-hour before. The stretching embryo cannot tear up the remaining layers round its top ("atypical germination").

Table 5. The release of the inhibition caused by incubation for two weeks in 2½ ppm ABA by different treatments. The seeds were all transferred to water.

| Treatment | Number of incompletely germinated seeds | Number of elongated embryos after | | | | |
|---|---|---|---|---|---|---|
| | | ½ hr | 2 hr | 4 hr | 7 hr | 20 hr |
| Undamaged control | 40 | 0 | 1 | 2 | 8 | 34 |
| Inner testa layer removed | 25 | 0 | 1 | 3 | 6 | 23 |
| "inside" scarification | 40 | at[1]) 24 | 25 | 26 | 27 | 26 |
| | | t[1]) 4 | 6 | 6 | 7 | 14 |
| "outside" scarification | 40 | at 4 | 6 | 7 | 7 | 8 |
| | | t 29 | 30 | 30 | 31 | 32 |

[1]) at = atypical germination (*fig.* 6)
t = typical germination (*fig.* 5)

*Table 5* shows the effects of these treatments on the start of the rootlet growth, – complete germination would be a confusing term in this case.

As the seeds had incubated for only 2 weeks in 2½ ppm ABA a transfer to water was sufficient to release them from the inhibition (treatment 1). Most of them needed, however, at least some hours before they could protrude and start the second elongation. After treatment 2 the seeds behaved in the same way. After treatment 3 and 4 the elongation started immediately, in agreement with the previously described observations. Comparison of the effects of treatment 2 with those of 3 and 4 reveals that the fast start of the second elongation only occurs after damaging of the endosperm layer and most likely of the cuticle at the distal side of these cells.

*Fig. 10* shows the course of the elongation of the rootlet in water during the first 6 hours after the "outside incision" was made. It is evident from this curve that the fast elongation during the first half hour slows down soon afterwards.

Would the same fast elongation after an "outside incision" also occur when

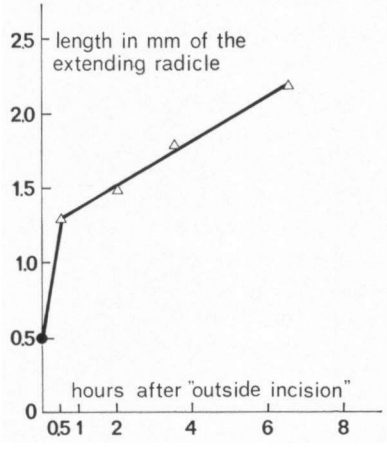


Fig. 10. The course of the elongation of the rootlet in water when an "outside incision" had been made. The seeds had incubated in 2½ ppm ABA for 14 days in darkness (see *table 5* ). The point at the ordinate indicates the length of the extending part of the enclosed radicle.

the seeds were not transferred to water but remained in the ABA medium?

100 Seeds that showed incomplete germination after incubation for 2 weeks in 10 ppm ABA were distributed equally over four new dishes containing 20, 10 or 5 ppm ABA or water respectively. In all cases the embryo elongated very fast, immediately after the incision had been made. A very important difference between the seeds in the various ABA concentrations and the water-controls presented itself however in het following days.

Whereas the elongation in water still continued, resulting in normal seedlings after 2 to 3 days, in 10 and 20 ppm ABA the elongation stopped in all embryos with a radicle-length of about 2 mm, in 5 ppm ABA this happened in 20 out of the 25 individuals.

We obtained similar results with "fully operated" seeds, of which before the start of the incubation all the surrounding layers in the area overlying the radicle had been removed. When after this treatment the seeds were incubated in ABA solutions of 20, 10 or 5 ppm or in water, all the radicles extended within 24 hours 1 mm from within the surrounding structures. After 2 days in water the rootlets had reached a length of about 5 mm, in all the ABA solutions they were still only 1 mm long. Here too all the rootlets in water and a small part of them in 5 ppm ABA continued normal growth, but none in 10 and 20 ppm.

## 5. DISCUSSION

The present results allow the general conclusion that ABA effects in some way the elongation of the embryo and so prevents its protrusion through the inner surrounding layers.

To interpret these results it is necessary to understand the nature of the elongation processes and hence the mechanism of the protrusion phenomenon.

HABER & LUIPPOLD (1960a) clearly demonstrated that in lettuce seeds rootlet protrusion results from cellular expansion, whereas cell division plays little or no part. In the seeds of corn, barley and broadbean (see the last mentioned authors for references) rootlet protrusion even starts before mitosis.

Although the nature of the elongation processes in the seeds of *C. album* has so far not been investigated it seems reasonable to assume that it is also in this seed largely a question of cell expansion.

Regarding the effects of ABA it has been shown by MILBORROW (1966) that synthetic (RS)-ABA inhibits the growth of *Avena* mesocotyls with as well as without IAA or GA. THOMAS *c.s.* (1965) demonstrated the same effect of ABA in *Avena* coleoptile sections. Both growth processes consist mainly of cellular expansion.

The question arises whether the nature of the inhibiting effect of ABA on the embryo elongation in the seeds of *C. album* is also an inhibition of the cell expansion capacity or an increase in the mechanical properties of the surrounding layers.

The experiments with the "fully operated" seeds and also the experiments in which the inner layers were scarified during incubation in ABA show that also

when the inner layers had been removed ABA is still able to prevent real root growth. So it is assumed that ABA indeed inhibits the cell expansion of the embryo.

The same experiments, however, show also that during incubation in ABA a certain part of the overall elongation process can still proceed. This observation is supported by some other experimental results. *Table 1* shows that ABA in none of the tested concentrations prevents the start of the elongation visible in the splitting of the outer layer of the testa. It was also concluded that some parts of the reactions that lead to the protrusion through the inner layers can still proceed during incubation in ABA *(fig. 9)*. And lastly the fast increase in the length of the radicle after an "outside incision" in water *(figs. 5* and *10)* indicates that the osmotic value of the cells has been increased during incubation in ABA and moreover that the cell walls are prepared for such a sudden stretch.

A possible explanation for these results is that the inner layers form a manyfold tougher barrier than the outer testa layer. An embryo with a decreased expansion capacity might thus still be able to overcome the first barrier and so to germinate incompletely. Its growth will however be stopped by the second one. The anatomy of the different layers makes this explanation at first sight unreasonable. The more so, because this difference in mechanical restraint should have to be very great. Even in the highest tested concentration of 20 ppm the embryo is still able to split the outer layer in every seed, whereas the protrusion through the inner layers is completely prevented.

Another explanation, more attractive at the moment, is that ABA does not inhibit all the components of the expansion process, but only one or some of them. The components of the process that occur at the start and the first expansion afterwards can still proceed in ABA. It is assumed that these uninhibited components alone are not able to overcome the mechanical restraint of the inner layers. The components inhibited by ABA are necessary to allow the protrusion and at the same moment the start of the seedling growth. The present results do not elucidate which are these inhibited and uninhibited components.

These results agree with those of SUMNER & LYON (1967). They showed that synthetic ABA inhibited the germination of the seeds of four species of grasses. Remarkable in their results is that the growth of the primary root was reduced with even the lowest tested concentration (0.1 ppm), that, however, dit not prevent the germination i.e. the elongation, but only caused a slight retardation of the onset of it.

The effects of light on the germination of *C. album* seeds seem to be twofold. In the light-dependent seeds light is required to allow the start of the elongation. The lower sensitivity to ABA in light *(fig. 7)* indicates that irradiation also increases the rate of the ABA-inhibited components of the cell expansion process.

These conclusions confirm those by HABER & LUIPPOLD (1960b) who observed that in lettuce seeds red light and other germination stimulating factors initiate the cellular expansion of the embryo. SCHEIBE & LANG (1965, 1967) have shown that a short irradiation with red light increased the growth rate of

halved lettuce seeds, whereas far-red light had an inverse effect. These radicle portions of halved seeds could always start growth. The differences between red and far-red irradiated radicles were enhanced by applying a water stress by imbibing the half seeds in 0.46 M mannitol which served as an osmoticum.

There seems to be a relation between these two effects of light as is shown in *table 2.* Light-indepency of the start of the elongation seems to be related to the absence of a light stimulation of the succeeding growth.

The inhibition releasing effect of a transfer to water after an incubation time of 14 days indicates that the inhibition needs the constant presence of the inhibitor. It is very remarkable, however, that after 4 weeks of incubation – in 20 ppm already after 2 weeks – transfer to water does not release the inhibition *(table 3).* It is possible that after these longer incubation times a second effect comes in, for instance an increase in the impermeability of the endosperm layer to ABA or to ABA-induced inhibiting metabolites. Anyhow it is evident that the embryos have not lost their growth capacity, because scarification in water immediately enables them to grow.

Before these hypotheses can be accepted as valid, several aspects of the effects of ABA on the germination of *C. album* seeds will have to be investigated in more detail.

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