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Control of Seed Germination by Abscisic Acid

I. TIME COURSE OF ACTION IN *SINAPIS ALBA* L.[1]

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ABSTRACT

The germination process of mustard seeds (*Sinapis alba* L.) has been characterized by the time courses of water uptake, rupturing of the seed coat (12 hours after sowing), onset of axis growth (18 hours after sowing), and the point of no return, where the seeds lose the ability to survive redesiccation (12 to 24 hours after sowing, depending on embryo part). Abscisic acid (ABA) reversibly arrests embryo development at the brink of radicle growth initiation, inhibiting the water uptake which accompanies embryo growth. Seeds which have been kept dormant by ABA for several days will, after removal of the hormone, rapidly take up water and continue the germination process. Seeds which have been preincubated in water lose the sensitivity to be arrested by ABA after about 12 hours after sowing. This escape from ABA-mediated dormancy is not due to an inactivation of the hormone but to a loss of competence to respond to ABA during the course of germination. The sensitivity to ABA can be restored in these seeds by redrying. It is concluded that a primary action of ABA in inhibiting seed germination is the control of water uptake of the embryo tissues rather than the control of DNA, RNA, or protein syntheses.

When a quiescent, nondormant seed (13) is supplied with water and O_2 at favorable temperatures the embryo rapidly takes up water and continues its temporarily suspended development. After building up a certain threshold hydrostatic pressure the seed coat is ruptured and visible protrusion of the elongating radicle indicates the onset of elongation of the embryonic axis. Some time later the release from quiescence becomes irreversible, *i.e.* the embryo will no longer survive redesiccation. The developmental period from the increase of metabolic activity after imbibition up to this point of no return, logically separating the embryo from the seedling stage, can be referred to as germination. At 25 C germination lasts not more than about 1 day in many seeds.

The dormancy hormone ABA can inhibit continuation of embryo development during germination and related developmental processes (*e.g.* the growth of *Lemna* turions or tree buds, 19, 20). Exogenously applied ABA is rapidly taken up by the embryo even through the intact seed coat (3, 16). The investigation of dormancy imposed by supplying germinable seeds with ABA provides an opportunity to study a physiologically relevant phenomenon (5) which, in contrast to most cases of natural dormancy, can be precisely controlled. This approach may provide information not only on the regulation of seed dormancy but also on the molecular mechanism of ABA action in plants.

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It has been shown that fundamental processes such as cell division and the synthesis of DNA, RNA, and protein become inhibited in plant systems treated with ABA (6, 10, 11, 19, 21). In barley aleurone tissue and cotton embryos exogenous ABA prevents the formation of enzymes which are involved in mobilizing storage materials during germination (5, 12). Although the molecular nature of the physiological block, by which ABA prevents the completion of germination, is still unknown, it is generally believed that ABA functions by interfering with mRNA synthesis, processing, or translation (*e.g.* 5–7, 10, 11, 24). In a previous study with mustard (1) we have observed that seedling development is surprisingly insensitive to ABA if the hormone is applied after germination. In particular it has been established that seedling photomorphogenesis including the induction of several enzyme activities is essentially unaffected by ABA concentrations which prevent the completion of germination. Here, we characterize the physiological action of ABA and the disappearance of sensitivity toward this hormone during the germination of mustard seeds.

MATERIALS AND METHODS

± Abscisic acid (puriss.) was a product of Fluka AG (Buchs, Switzerland). Seeds of *Sinapis alba* L. (harvest 1972, obtained from Asgrow Company, Freiburg-Ebnet) were selected for uniformity of color, size, shape, and sown using standard conditions: 50 seeds were rinsed briefly with distilled H_2O (or ABA solution) and placed on five layers of Schleicher and Schüll chromatography paper (No. 2043 b Mgl, presoaked with the appropriate solution for 12 h) in covered plastic boxes (10 × 10 × 8 cm). After adding 5 ml excess water (or ABA solution) a small meniscus formed around the seed which was covered by a thin film of liquid due to the mucilaginous testa surface. The boxes were kept in darkness at 25.0 ± 0.3 C if not indicated otherwise. Before transferring to a different medium the seeds were thoroughly washed by incubation for 10 min in an abundance of new medium. All manipulations were done under green safelight. For redrying, pregerminated seeds were transferred to Petri dishes containing silica drying beads (Merck, Darmstadt). The seeds lost 50% of their moisture within 1 h and reached their original dry weight within 3 h. These seeds were left on the silica beads for 24 to 72 h and were then resown on water. Their further development was observed in white fluorescent light (600 lx) at 20 C.

The percentage of seeds with broken testa (tip of radicle just visible) and of seeds with growing radicle (radicle protruding for more than 2 mm) was determined at suitable intervals under safelight. Data of the figures represent the means of at least four independent experiments (200 seeds).

RESULTS

Time Course of Germination Parameters. The conventional criterion for discriminating germinated from nongerminated seeds is the protrusion of the radicle tip through the seed coat (2, 9). This purely operational criterion for germination appears to be

inadequate in studying ABA-imposed dormancy of mustard seeds. Low concentrations of ABA (*e.g.* 5 mg/l, 19 μmol/l) completely and permanently inhibit the further growth of the embryo. However, rupturing of the testa and stretching of the previously curved embryo axis is not, or, only slightly, impaired, thus leading to apparently "germinated" seeds according to the above criterion. Similar observations with seeds of *Chenopodium album* have led Karssen (15, 16) to discriminate between incomplete germination (which is independent of ABA) and complete germination (which is inhibited by ABA). For the investigation of the germination process it is necessary to avoid any arbitrary discrimination between germinated and ungerminated seeds based on a single selected criterion. We prefer to use the term "germination" (period) as defined in the introductory section. Consequently, "rupturing of the testa" (operationally: tip of the straightened embryo axis just visible) and "onset of axis growth" (operationally: radicle protruding more than 2 mm through the seed coat) are regarded as two subsequent stages of embryo development in the course of germination which is terminated at the "point of no return." The time courses of these germination parameters are shown in Figure 1. The seed population reaches half final numbers of rupturing of the testa and onset of axis growth at about 12 and 18 h after sowing (onset of imbibition), respectively. Termination of the germination period, defined by the disappearance of the ability to survive redrying, takes place at different times in the three seedling organs (Fig. 2). The point of no return, based on the ability to resume growth after desiccation and reimbibition, was passed after about 12, 17, and 24 h in the radicle, hypocotyl, and cotyledons, respectively. The ability to synthesize visible amounts of anthocyanin and Chl in the light disappears at similar, but significantly later times, indicating that even individual organ functions differ with respect to the point of no return. The resumable growth capacity of the radicle and hypocotyl becomes reduced after about 8 and 12 h of preincubation. In seedlings which were obtained from seeds preincubated for less than 8 h before drying there was no detectable deviation from seeds not pretreated.

Figure 2 indicates that the germination period of mustard seeds at 25 C lasts about 12 h with respect to radicle growth. This developmental stage coincides with the rupturing of the testa (Fig. 1). The hypocotyl and the cotyledons retain the embryonic stage for a significantly longer period of time.

Action of ABA during Germination. Figure 3 shows the time course of testa breaking and initiation of axis growth at various ambient ABA concentrations. Axis growth is much more sensitive to the hormone than testa breaking. In the range of concentrations tested, ABA appears to prevent testa breaking permanently in part

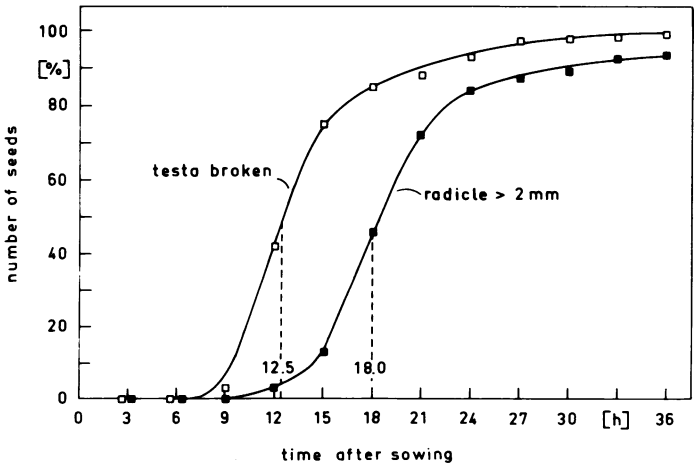

FIG. 1. Time course of germination as measured by two different parameters (rupturing of the testa, onset of [radicle + hypocotyl] growth) in mustard seeds incubated on water. (- - -): Half final values.

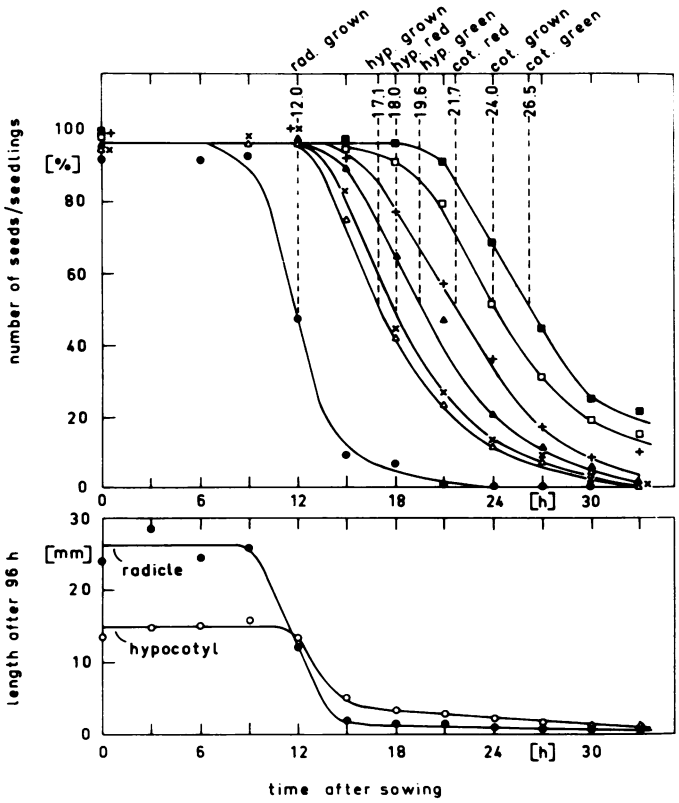

FIG. 2. Time course of disappearance of various developmental abilities in seeds which have been incubated on water for various times (indicated on the abscissa) and then redried to their original dry weight within 3 h. Upper panel: Percentage of embryos still able to increase in radicle length (●), hypocotyl length (△), cotyledon area (□), and to produce anthocyanin (×, +) and Chl (▲, ■) in the hypocotyl and cotyledons, respectively, was determined after a 96-h reincubation period in the light at 20 C. (- - -): Half final values. Lower panel: final length of radicle (●) and hypocotyl (○) after reincubation period.

of the seed population, while axis growth is more or less severely delayed by the hormone (similar curves have been reported for lettuce seeds; see ref. 3). The ABA concentration response curve for testa breaking is virtually independent of time after 3 days of incubation (Fig. 3).

Figure 4 provides evidence that ABA can indeed block embryo development before completion of the germination period, *i.e.* increase the time until the point of no return. Seeds incubated in ABA up to 3 days continue the germination process after redrying and reimbibition in the absence of ABA. However, if the ABA treatment lasts longer than 3 days an increasing number of the seeds lose the capacity to continue germination after the redrying/reimbibition treatment, especially when they have been previously treated with low ABA concentrations. Imbibed seeds can be kept dormant in ≧ 5 mg ABA/l for at least 30 days at 25 C; during this time the seeds remain fully viable as demonstrated by a rapid continuation of germination and normal seedling development immediately after transfer to water.

The uptake of water is indistinguishable in ABA-treated and water control seedlings during the 8-h imbibition period following sowing. The temperature-dependent water uptake which is related to embryo growth can be almost completely prevented by ≧ 2.5 mg ABA/l (Fig. 5). These results indicate that ABA is effective in inhibiting further germination processes at least 10 to 12 h after imbibition. Furthermore, Figure 5 shows that ABA has no effect on the imbibition rate, but stops the water influx which accompanies (temperature-dependent) growth. This result is in accord with the observation that ABA has no effect on O_2 uptake and

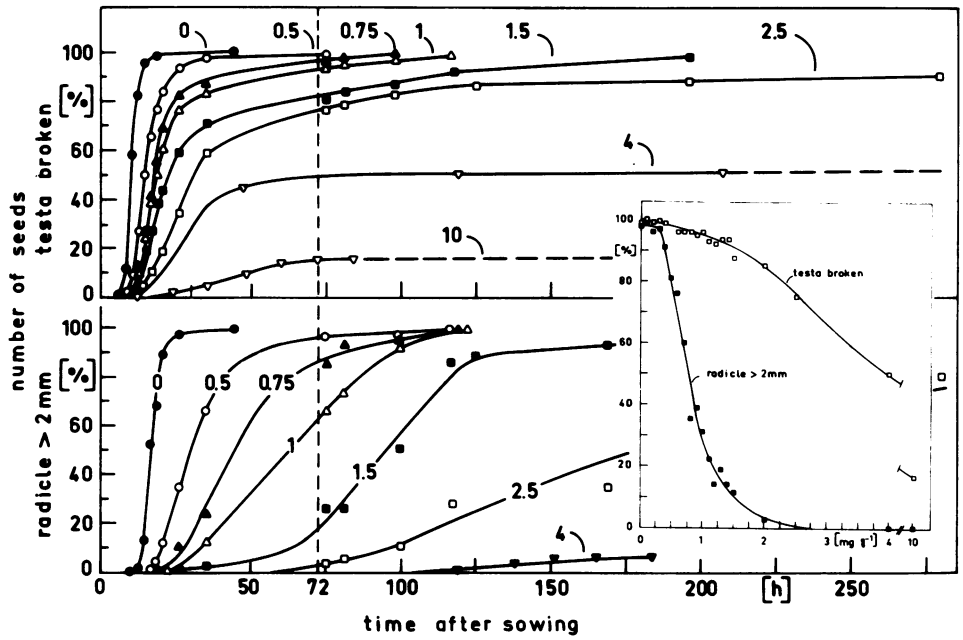

FIG. 3. Time course of the rupturing of the testa and the initiation of (radicle + hypocotyl) growth in the presence of various concentrations of ABA (numbers represent mg/l). Dry seeds were imbibed in ABA solutions. Inset shows ABA concentration effect curves for the two germination parameters 72 h after sowing.

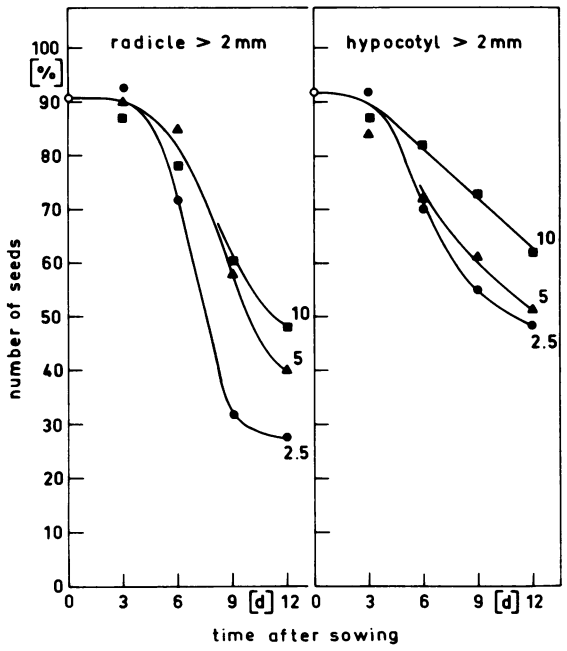

FIG. 4. Preservation of capacity in ABA-preincubated and redried seeds to continue germination. The seeds were incubated in ABA solutions for various times (indicated on abscissa) and then redried to their original dry weight within 3 h. After 2 to 10 days of storage in the dry state the seeds were soaked in water for 2 h, thoroughly washed, and the percentage of embryos still able to increase in radicle length (left) and hypocotyl length (right) was determined after a 72-h reincubation period on water in the light at 20 C. Embryos showing a positive growth response generally developed into normal seedlings during the reincubation period. Numbers represent mg ABA/l.

RNA synthesis of germinating lettuce seeds up to the point where the radicle protrudes through the seed coat (3) but contrasts with the finding that ABA inhibits polysome formation during the imbibition period in isolated lettuce embryos (6). Mustard seeds which have been kept dormant by ABA for several days rapidly take up water and continue the germination process after removal of the hormone, indicating that the influx of water can be rapidly modulated by ABA at this stage of germination (Fig. 6).

Escape from Responsiveness to ABA. The potential of ABA to inhibit the completion of germination disappears during incubation of seeds on water. The time course of the apparent escape from inhibition by ABA in the seed population is shown in Figure 7. Half final values are reached after 11 to 13 h at 2.5 to 10 mg ABA/l, indicating that the average water-imbibed seed loses the competence to respond to ABA by assuming the dormant state

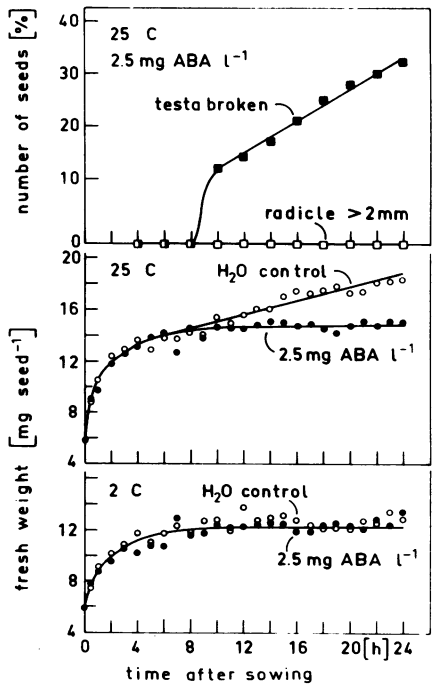

FIG. 5. Effect of ABA on germination parameters and water uptake. Dry seeds were imbibed in 2.5 mg ABA/l (or water) at 25 C (or 2 C).

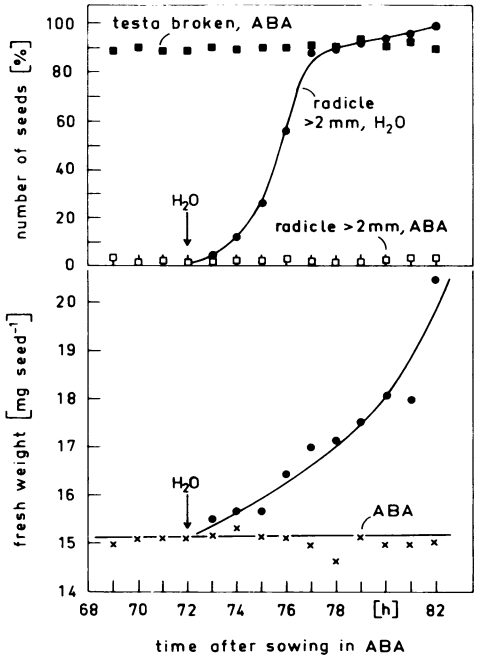

FIG. 6. Time course of onset of (radicle + hypocotyl) growth and water uptake in seeds kept on 5 mg ABA/l from sowing and transferred to water after 3 days.

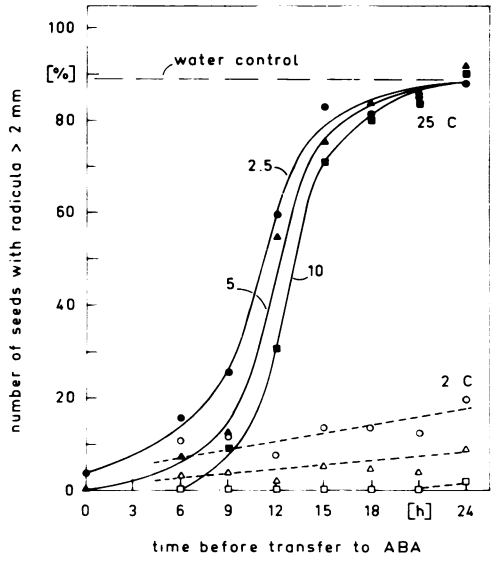

FIG. 7. Time course of escape from responsiveness to ABA during germination on water. Seeds were incubated on water at 25 or 2 C for various times as indicated on the abscissa and transferred to solutions containing 2.5 (●, ○), 5 (▲, △), or 10 (■, □) mg ABA/l at 25 C. Evaluation of (final) numbers of seeds with growing (radicle + hypocotyl) was at 72 h after transfer to ABA.

shortly after active extension growth (rupturing of the testa, Fig. 1) and temperature-dependent water uptake (Fig. 5) have commenced. Low temperature (2 C) inhibits the disappearance of responsiveness to ABA (Fig. 7).

Seeds which escaped ABA inhibition in the experiments of Figure 7 invariably developed into healthy seedlings. The only visible effect of ABA treatment was a reduction in radicle and hypocotyl lengths. The effect of ABA on the growth of fully germinated seedlings is shown in Figure 8. The continuation of the expansion growth of cotyledons, hypocotyl, and radicle is reduced by low ABA concentrations (1) indicating that these organs are not insensitive to ABA inhibition. However, this inhibition is incomplete even in the presence of high concentrations of ABA. The ABA-insensitive portion of growth may be responsible for the escape from ABA sensitivity during germination on water (Fig. 7).

In the experiments of Figure 9 we tested whether the disappearance of ABA inhibition of germination could be explained by

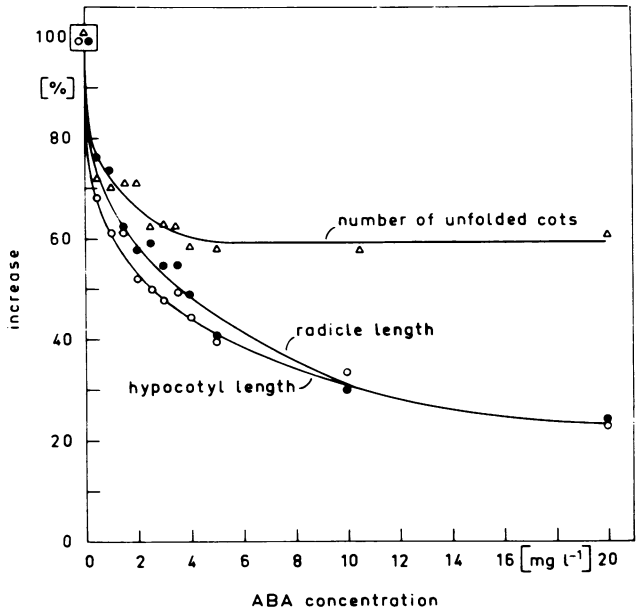

FIG. 8. Concentration effect curves for ABA-mediated inhibition of radicle lengthening (●), hypocotyl lengthening (○), and cotyledon unfolding (△) in seedlings grown for 36 h on water and then transferred for further 24 h to ABA. The changes of the three growth parameters during the 24-h period on ABA are plotted as percentages of the water controls (100% equals 20.8 to 5.0 mm and 28.3 to 7.5 mm difference of hypocotyl and radicle lengths, and 96 to 17% unfolded cotyledons, respectively).

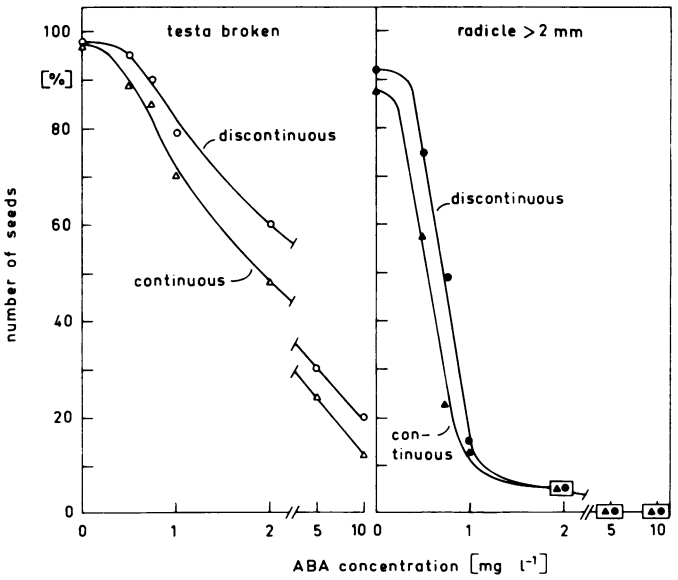

FIG. 9. Concentration effect curves for ABA-mediated inhibition of rupturing of testa and onset of (radicle + hypocotyl) growth during discontinuous and continuous incubation. Seeds were transferred to fresh medium every 24 h (discontinuous; ○, ●) or left on the original medium from sowing (continuous; △, ▲). Evaluation of germination parameters was at 72 h after sowing. Essentially the same results were obtained at 48 and 84 h after sowing.

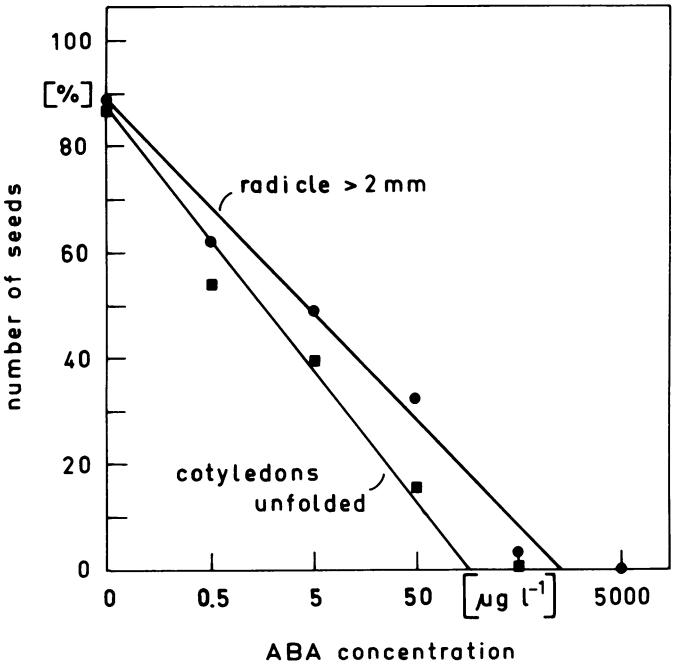

FIG. 10. Concentration effect curves for ABA-mediated inhibition of germination process (●, initiation of [radicle + hypocotyl] growth; ■, initiation of cotyledon growth) in seeds which were redried and reimbibed on ABA solutions after 15 h of incubation on water (final values, reached after 72 h on ABA).

the appearance of an ABA-inactivating system during germination. Seeds were either continuously kept on the same ABA solution or transferred to fresh solution every 24 h. If the hormone becomes inactivated in the medium during germination, the discontinuous ABA treatment should result in a stronger inhibition than the continuous treatment, especially at nonsaturating ABA concentrations. This expectation was not borne out. In the range of 0.5 to 10 mg/l the hormone was either equally or less effective if the medium was changed at regular intervals (Fig. 9). The positive effect of medium replacement which was most obvious in the initiation of axis growth may be explained by the removal of growth-inhibiting substances produced by the ABA-inhibited seeds. These results make it unlikely that the escape from ABA inhibition (Fig. 7) is due to the development of the ability to inactivate the hormone during germination.

When seeds pregerminated on water were redried they lost the ability to continue the germination process in the presence of ABA. Figure 10 shows that seeds incubated on water for 15 h (where about 80% of the population will continue germination if transferred directly to ABA; Fig. 7) are even more effectively inhibited by ABA if the germination process is interrupted at this point by redrying and resowing. Thus, the reestablishment of the desiccated state abolishes the ability to overcome the ABA inhibition of germination.

DISCUSSION

The data of this paper provide a first description of the time course of mustard seed germination and some characteristics of ABA action in this developmental period. During germination of the average mustard seed the embryo ruptures the testa after 12 h. At 18 h the growing axis has passed the 2-mm stage and at about 24 h the embryo has completely reached the seedling stage (Figs. 1 and 2). With this background the action of ABA can be characterized as follows. The physiological block imposed by ABA becomes effective before 12 h after sowing, *i.e.* before the onset of visible embryo axis growth. However, water uptake during the imbibition period (up to 8 h after sowing) and rupturing of the testa is not, or incompletely, inhibited by concentrations of ABA (1–5 mg/l) which impose dormancy on the embryo at a subsequent stage (Figs. 3 and 5). These results support the proposal that low concentrations of ABA do not interfere with the initial germination processes but arrest (or delay) the progress of embryo development at the brink of radicle growth initiation (3, 16). Consequently, in seeds incubated in ABA for 3 days radicle growth starts immediately upon removal of the hormone (unpublished results).

Seeds germinating on water lose the ability to become arrested by ABA at about 12 h after imbibition, *i.e.* at the same time when the seedling axis starts to pass the point of no return terminating the germination period in this organ (Figs. 2 and 7). This escape from ABA-dependent dormancy appears to be due neither to the development of an ABA-inactivating activity (Fig. 9) nor to any other stable quality of the embryo since it can be abolished by reestablishment of the desiccated state in pregerminated seeds (Fig. 10).

These data are in agreement with the hypothesis that a primary action of ABA in inhibiting seed germination is the control of water uptake of the metabolically active embryo tissues rather than the control of DNA, RNA, or protein synthesis (23). Water uptake during the imbibition period (up to about 8 h after sowing) is not influenced by ABA. It thus seems unlikely that ABA acts in germination by impairing the water permeability of membranes (8). It rather appears appropriate to discuss the action of ABA in terms of the water potential equation ($\Psi_W = \Psi_P + \Psi_\pi + \Psi_\tau$). Although at present all three components of tissue water potential are possible candidates for ABA control the osmotic potential may assume a superior position. The idea of a potentially ABA-controlled accumulation of ions in the embryonic cells of germinating seeds is attractive because of the opposite effects of ABA and fusicoccin on germination, proton secretion, and potassium ion uptake in seeds (4, 18) and on phloem loading by sucrose/proton co-transport (17). Furthermore, ABA is an effector of osmoregulatory ion transport in hydroactive stomata control (19, 22) and in energy-dependent ion transport into the xylem of roots (14).

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