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EFFECTS OF ABSCISIC ACID ON OXYGEN UPTAKE AND RNA SYNTHESIS IN GERMINATING LETTUCE SEEDS

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SUMMARY

Effects of abscisic acid on radicle protrusion, oxygen uptake and total RNA synthesis were investigated. It was found that ABA had no effect on oxygen uptake before radicle protrusion. RNA synthesis was only inhibited by ABA after 9 hours of imbibition. Extrapolation of radicle protrusion time curves from seeds treated with various concentrations of ABA suggested that ABA did not affect the initiation of cell extension, but inhibited growth of the radicle.

1. INTRODUCTION

Abscisic acid (ABA) is a naturally-occurring inhibitor in higher plants which, among other physiological effects, suppresses germination and also partially inhibits RNA synthesis (ADDICOTT & LYON 1969).

VILLIERS (1968) has suggested that inhibition of RNA synthesis might be the basis of its mode of action in *Fraxinus* embryos. On the other hand, many reports suggest that RNA synthesis does not appear to be necessary for the initial stages of seed germination. MARCUS & FEELEY (1964, 1966) and CHEN *et al.* (1968) reported strong evidence for the presence of messenger RNA in dry wheat seeds which becomes functional during imbibition. Similar observations were made by WATERS & DURE (1966) in cotton seeds.

Germination is often defined as radicle protrusion through the seed coat. However, before the radicle protrudes through the seed coat, growth will already have started. It is therefore not clear what stage of the germination process is affected by germination inhibitors.

Very few data are available on the changes which occur in lettuce seeds during the initial stages of germination. The inhibition of lettuce seed germination by nucleotide base analogues (KHAN 1966; SMITH & FRANKLAND 1966) indicates that, at least in lettuce seeds, RNA synthesis is an essential prerequisite for germination. ABA is also known to inhibit RNA synthesis as well as radicle protrusion in lettuce seeds (FRANKLAND *et al.* 1971).

In the work reported here an attempt was made to investigate whether ABA prolonged the lag-phase of the germination process, before growth of the radicle starts, or whether ABA inhibited growth of the radicle. Changes in total RNA synthesis and O_2 uptake during imbibition as well as the effect of ABA

on these processes were investigated to obtain an indication after how many hours of imbitition cell elongation was initiated and what effect ABA had on the initiation.

2. MATERIALS AND METHODS

2.1. General procedure for germination of seeds

Lettuce seeds (*Lactuca sativa* L.) of the cultivar 'Grand Rapids' were purchased from Dickson, Brown and Tait Ltd. Batches of 100 seeds were sown in 4 cm petri dishes on two layers of Whatman no. 1 filter paper moistened with 1.5 ml of ABA solution or 1.5 ml water. Seeds were germinated under continuous light at 25°C. The time course of radicle protrusion in water and the effects of different concentrations of ABA on radicle protrusion after 24 hours imbibition in ABA solution were determined.

2.2. Estimation of ABA uptake in lettuce seeds

Lettuce seeds were incubated in ^{14}C-ABA (4.7 mCi/mmol). The seeds were twice extracted with 80% methanol and an aliquot was counted for total uptake in a Beckman liquid scintillation counter.

2.3. Estimation of respiration

The respiration of seeds was measured by the Warburg manometric technique. Fifty seeds were placed in the main compartment to which 0.5 ml 0.01 M ammonium citrate (pH 6) was added. CO_2 was fixed by 0.2 ml of 10% KOH in the centre well.

2.4. Estimation of nucleic acid synthesis

In all nucleic acid studies seeds were sterilized as described by FRANKLAND *et al.* (1971). Seeds were imbibed either in water or in 1.5 ppm ABA solution. Prior to incubation in nucleotides the seed coat was removed. 25 embryos were incubated for 1 hour at 27° on a waterbath shaker in 25 ml conical flasks in 1 ml 0.01 M ammonium citrate buffer (pH 6) containing 5 μCi ^{14}C-cytidine (505 mCi/mmol). Total RNA synthesis was estimated according to the method of PILET & BRAUN (1967).

3. RESULTS

3.1. Characterization of radicle protrusion

To investigate effects of ABA on radicle protrusion, respiration, and RNA synthesis, it was important to have a population of seeds which was relatively insensitive to ABA and in which the radicle protrusion of the majority of the seeds was not spread over a long period of time, when imbibed in water.

Three batches of seeds were tested. In the batch of seeds which was chosen for the work reported here, radicle protrusion in the majority of seeds could be observed between 14 and 17 hours after onset of imbibition (*fig. 1A*).

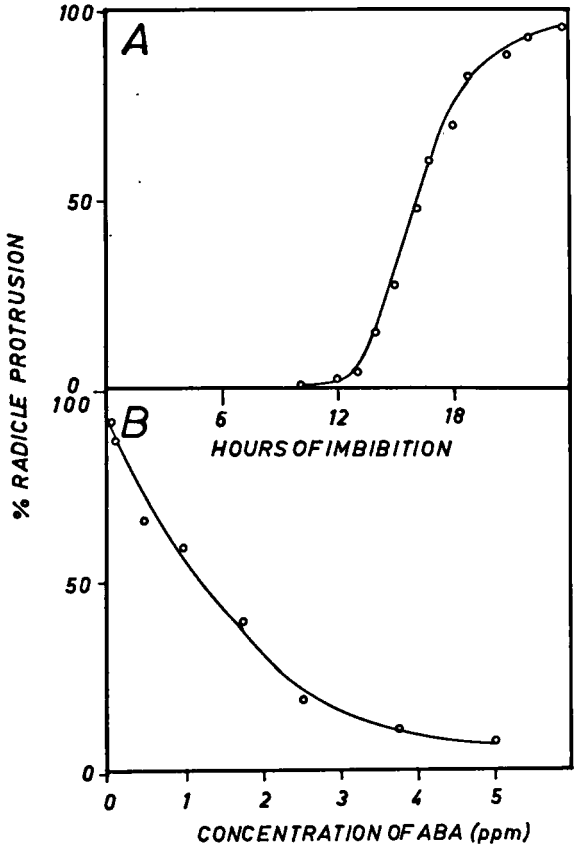

Fig. 1. Characterization of lettuce seeds.
A. time curve of radicle protrusion when lettuce seeds were imbibed in water.
B. effect of various concentrations of ABA on radicle protrusion after 24 hours imbibition in ABA solution.

In *fig. 1B* the effects of various concentrations of ABA on radicle protrusion after 24 hours imbibition in the ABA solutions are presented.

3.2. Uptake of ABA by the seed

The concentration in the seed of externally applied ABA was estimated during imbibition, since the failure of ABA to produce an effect on, for instance, RNA synthesis could be due to a low level of ABA during the early hours of imbibition. Seeds were imbibed in 1.5 ppm ^{14}C-ABA (4.7 μCi/mmol.) and the ABA extracted from the embryos after various times. A considerable amount of ABA was taken up during the first 6 hours of imbibition (*fig. 2*). In the seeds which did not germinate in the following 18 hours, no additional uptake of ABA could be found.

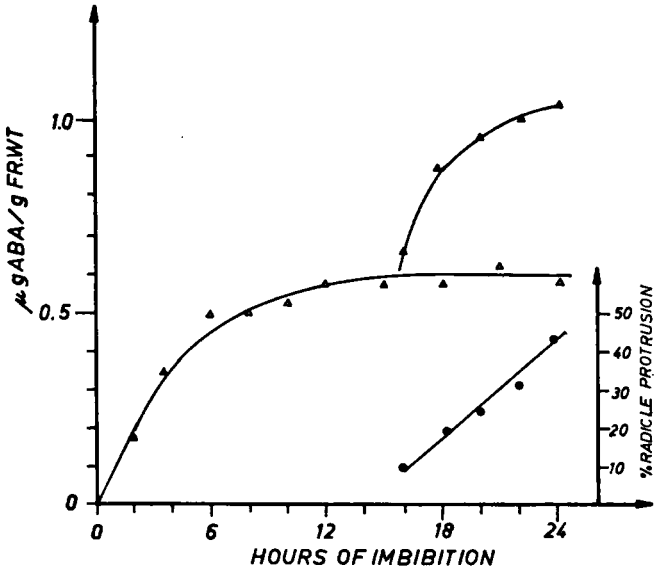

Fig. 2. Uptake of ^{14}C-ABA (4.7 mCi/mmol) from 1.5 ppm ABA solution.
△ time course of ABA uptake in seeds before radicle protrusion.
▲ time course of ABA uptake in seeds where the radicle had protruded.
● time course of radicle protrusion in 1.5 ppm ABA.

As soon as the radicle protruded through the seed coat, a further marked increase in ABA uptake could be observed.

3.3. Effects of ABA on O_2 uptake

Since it is known that ABA inhibits many metabolic processes (ADDICOTT & LYON 1969), effects of ABA on oxygen uptake were investigated. The respiration increased during imbibition and a plateau was reached after 3 hours (*fig. 3*). After 8 hours of imbibition a further increase in respiration took place, but a plateau was attained after which oxygen uptake did not increase until the radicle protruded through the seed coat. ABA had no effect on the oxygen uptake in these stages. Only after the radicle had protruded through the seed coat, could an inhibition of oxygen uptake by ABA be observed.

3.4. Effects of ABA on radicle protrusion

Germination is often defined as protrusion of the radicle through the seed coat. However, growth of the radicle must have already started within the seed coat. Before radicle protrusion occurs, two phases of germination can be observed:

1. Early imbibition phase; this is the phase when the development of the machinery for enzyme synthesis takes place.

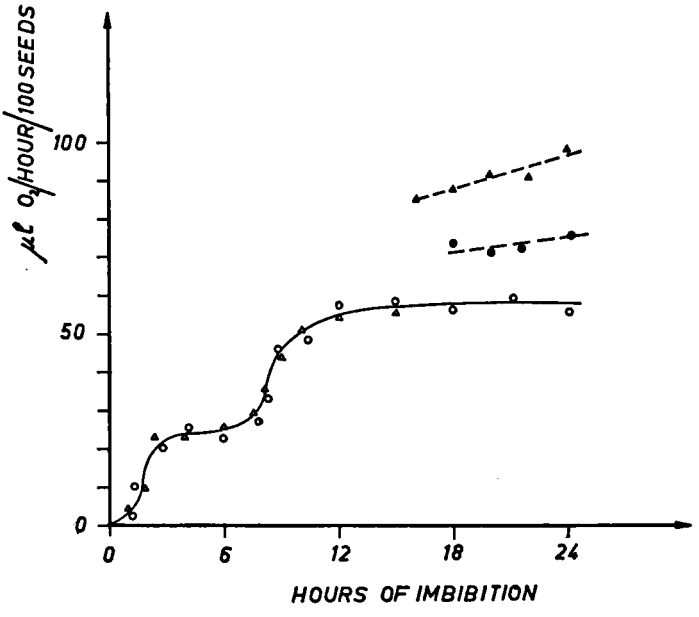

Fig. 3. Oxygen uptake in lettuce seeds.
△ O_2 uptake in seeds imbibed in water before radicle protrusion.
○ O_2 uptake in seeds imbibed in 1.5 ppm ABA before radicle protrusion.
▲ O_2 uptake in seeds imbibed in water after radicle protrusion.
● O_2 uptake in seeds imbibed in 1.5 ppm ABA after radicle protrusion.

2. Growth phase; this is the phase when growth of the radicle within the seed coat takes place.

Interpretation of inhibition of radicle protrusion by germination inhibitors must therefore be made with care since it is not known which stage of the germination is affected.

In *fig. 4* the effect of imbibition of lettuce seeds in various concentrations of ABA on the time course of radicle protrusion is presented.

It was observed that increasing concentrations of ABA resulted in an increase in time before the first radicle protruded in a population of 100 seeds. The slope of the germination curve decreased with increasing concentration of ABA. When lines were extrapolated from the linear parts of the germination curves, these lines all met in the same point.

3.5. Effects of ABA on RNA synthesis

Inhibition of RNA synthesis by ABA has been reported in many systems. In lettuce seeds a strong inhibition of RNA synthesis was observed after 18 hours ABA treatment (FRANKLAND *et al.* 1971). However, since ABA did not affect incorporation of either ^{32}P or ^{14}C-uracil into nucleic acids of embryonic bean

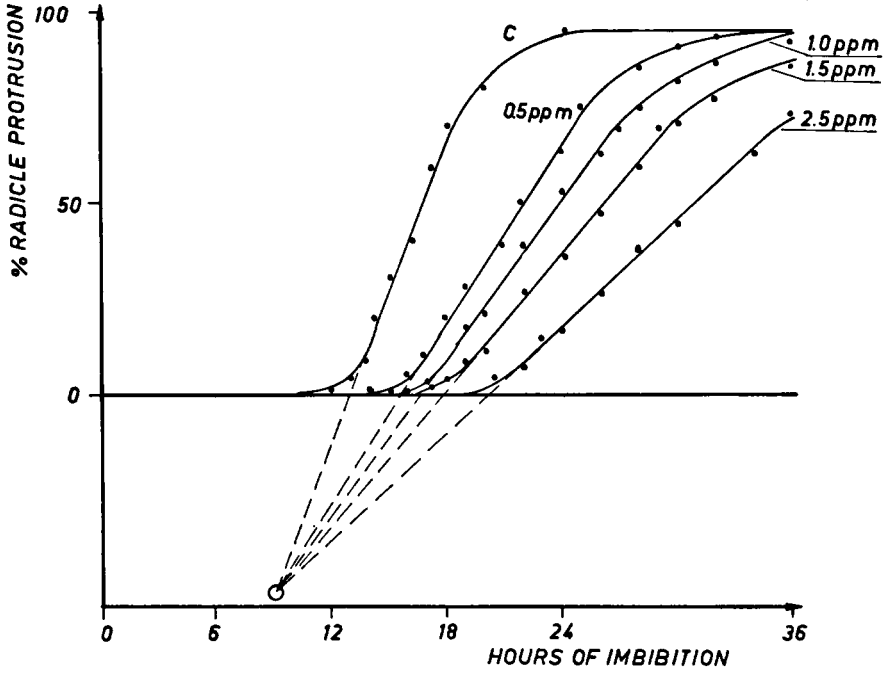

Fig. 4. The effect of various concentrations of ABA on the time course of radicle protrusion.

axes prior to the initiation of cell elongation (WALTON *et al.* 1970), it is not clear at what stage RNA synthesis in lettuce seeds is affected.

In *fig. 5* the time course of RNA synthesis during germination in seeds imbibed in 1.5 ppm ABA as well as those imbibed in water is presented. ABA did not affect RNA synthesis during the first 9 hours of imbibition. After 10 hours imbibition in 1.5 ppm ABA a marked inhibition of incorporation of ^{14}C-cytidine into RNA could be observed.

4. DISCUSSION

Little is known about the mechanism by which ABA delays radicle protrusion through the seed coat in lettuce seeds.

^{14}C-ABA uptake proceeded rapidly in lettuce seeds and reached a maximum after 6 hours. Since the level of total labelled ABA did not increase after 18 hours imbibition in the seeds where no radicle protrusion took place, it could be concluded that the total amount of applied ABA in those seeds where radicle protrusion occurred within 24 hours did not differ (until the moment of radicle protrusion) from that in the seeds where the radicle did not protrude within 24 hours.

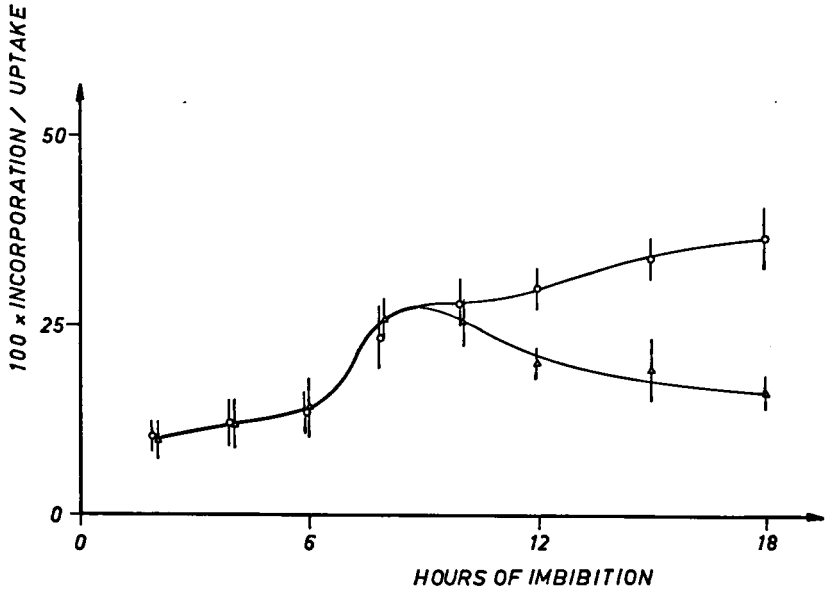

Fig. 5. The effect of ABA on total RNA synthesis.
△ seeds imbibed in 1.5 ppm ABA.
○ seeds imbibed in water.

After 8 hours imbibition a marked increase of O_2 uptake was observed. This indicates an increase in metabolic activity. O_2 uptake was inhibited in ABA treated seeds after radicle protrusion. Since the uptake of O_2 is much lower in seeds before radicle protrusion, transport of O_2 through the seed coat may have been the limiting factor, so that no effect of ABA on the O_2 uptake was observed before radicle protrusion. ABA delayed radicle protrusion; however, when the linear parts of the radicle protrusion time curves were extrapolated, they all met at the same point, after about 8 hours of imbibition.

This suggests that in a population of seeds the time after which growth of the radicle within the seed coat starts is not influenced by ABA. ABA most probably inhibits cell elongation after its initiation.

The fact that O_2 uptake and total RNA synthesis increased after about 8 hours of imbibition also supports the previous assumption.

No difference in total RNA synthesis was observed during the first 8 hours of imbibition between seeds imbibed in an ABA solution and those imbibed in water. The first real difference in the rate of total RNA synthesis between seeds imbibed in ABA solution and those imbibed in water was after 9 hours and this difference appeared after a marked increase had taken place in O_2 uptake and total RNA synthesis. These results agree with experiments described by WALTON *et al.* (1970), who also did not find any effect of ABA on RNA synthesis in embryonic bean axes prior to the initiation of cell elongation.

The present data suggest that ABA inhibits radicle protrusion of lettuce seeds by its effect on growth. It may well be that ABA has an early effect on the initiation of germination in seeds that require different treatment to induce germination, although we have no data to support this.

ACKNOWLEDGMENTS

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