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ABA LEVELS IN CHICK-PEA SEEDS DURING THE FIRST TWENTY-FOUR HOURS OF GERMINATION. EFFECT OF POLYETHYLENE-GLYCOL

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Key Word Index—*Cicer arietinum*; Leguminosae; chick-pea; abscisic acid; embryonic axis; germination; polyethylene-glycol.

Abstract—The abscisic acid (ABA) in chick-pea seeds germinated in water and stressed by 60 mM polyethylene-glycol (PEG) was studied. ABA in embryonic axes increased slightly at 3 hr, but underwent a higher increase at 18 hr, when elongation of the embryonic axes had started. PEG affected both growth and germination, diminishing the fresh weight of embryonic axes and inhibiting seed germination. The ABA level did not increase in the presence of PEG. The results suggest that abscisic acid is metabolized during the first 24 hours of germination. PEG treatment induced water stress and inhibited germination, without modifying significantly ABA levels in relation to those found in dry seeds.

INTRODUCTION

Mature dry seeds normally contain 5–10% water and under these conditions their metabolic activity is zero. At the onset of seed imbibition, water uptake takes place and cell membranes begin to reorganize.

In general, mature seeds contain very low levels of endogenous abscisic acid (ABA) and these are probably remanents of the ABA produced during development [1]. Furthermore, physiological studies have shown that ABA increases in vegetative tissues under osmotic stress induced by NaCl, polyethylene-glycol (PEG), drying and cold [2]. Proposed models suggest that cells recognize osmotic stress as plasmalemma perturbations resulting from a loss of turgor pressure [3]. There are several reports indicating that the application of osmotic stress to tomato seeds does not result in an increase of ABA levels [4, 5]. However, a correlation between the amount of endogenous ABA and germination has never been found.

We have studied changes in the content of ABA in embryonic axes of chick-pea seeds during normal conditions of germination and in seeds imbibed in PEG, which induced water stress and inhibited germination.

RESULTS AND DISCUSSION

In the course of the first 24 hours of germination of chick-pea seeds under normal conditions (H_2O, 25°), we found that the fresh weight of embryonic axes increased slowly until 14 hr and then, when radicle elongation took place, underwent a sharper rise (Fig. 1).

Endogenous ABA levels under these conditions (Fig. 2) showed a 37% increase at 3 hr in relation to 0 hr and after that it diminished until 14 hr. At 18 hr the ABA content increased (400% in relation to 0 hr), decreasing subsequently until 24 hr of germination to a very low level (less than 60% in relation to embryonic axes at 0 hr). In *Phellodendron wilsonii*, it has recently been suggested [6] that the changes in the ABA levels can reflect some combined effects of the remanent and *de novo* synthesized ABA, during the onset of germination. The increase observed between 14–18 hr could be due to a *de novo* synthesis of the hormone. In addition, ABA seems to be necessary for the growth of the embryonic axis, demonstrated by the increase undergone when elongation starts. Similar results have been obtained for the germination of barley and rice embryos [7, 8]. Yamada [7] and Qin [8] detected a decrease in ABA concentration after the onset of germination and a later increase when elongation of the embryonic axis took place.

Germination of chick-pea seeds imbibed with 60 mM PEG was inhibited and the fresh weight did not change as the time of germination proceeded (Fig. 1). This PEG concentration (−1 MPa) caused a strong water stress, preventing water uptake. Embryos of fenugreek [9], germinated under water stress, showed little growth, and similar results were obtained during chick-pea germination in the presence of PEG [10].

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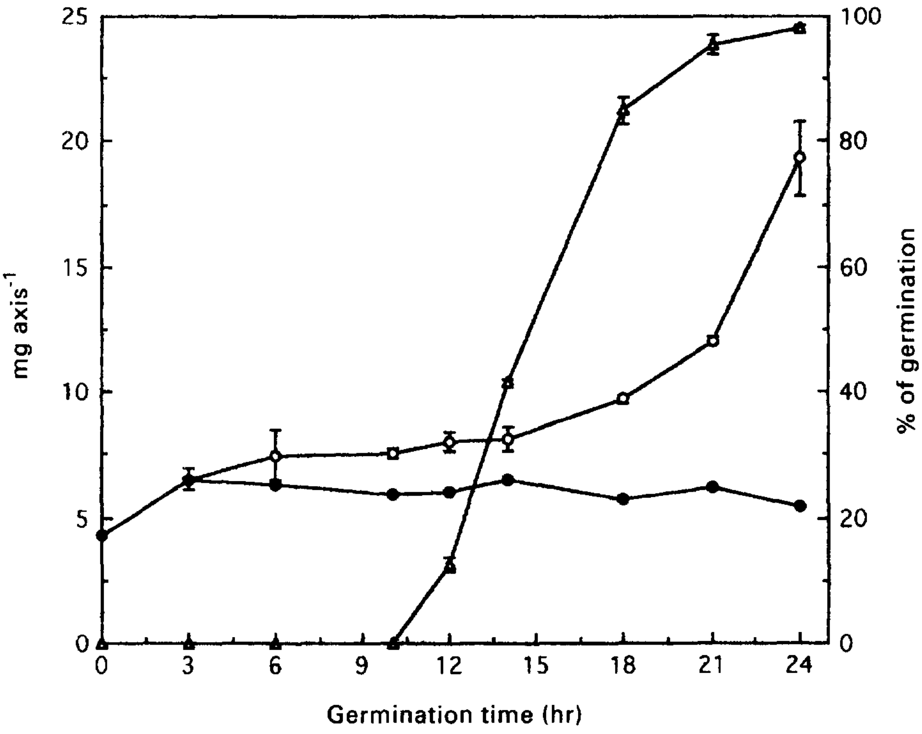

Fig. 1. Fresh weight and percentage germination chick-pea seeds under normal conditions of germination (H_2O, 25°) or in the presence of PEG (60 mM). ○ and ●, fr. wt, and △, percentage germination in normal (H_2O, 25°) (open symbols) and PEG-treated seeds (closed symbols). Experiments were performed in triplicate.

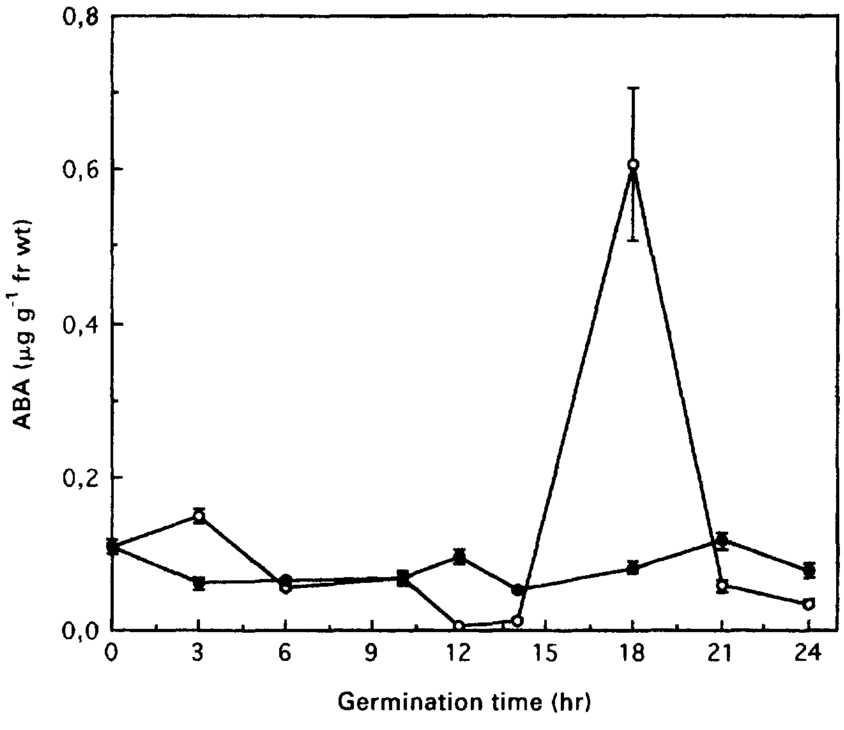

Fig. 2. ABA content in embryonic axis of chick-pea seeds. ○, normal conditions of germination (H_2O, 25°); ●, PEG-treated. Experiments were performed in triplicate.

In addition, ABA levels under PEG treatment did not undergo significant variations, being very similar (see Fig. 2). Thus, PEG did not induce ABA synthesis in contrast to the results found in other plant tissues subjected to water stress [2, 11]. It seems that the seeds imbibed in PEG cannot absorb water and reactivate the metabolic processes that lead to germination (e.g. radicle emergence; radicle elongation) as well as the metabolism of ABA. However, a direct correlation between the onset of germination and ABA synthesis has to be established, although it has been suggested that the osmotic potential surrounding the inside of the embryo cells plays a much more important role in regulating germination than that of ABA [12].

EXPERIMENTAL

Plant material. Seeds of *Cicer arietinum* L. (cv. Castellana) were sown as described previously [13]. The filter paper was moistened with H_2O or 60 mM PEG.

ABA analysis. Once harvested, plant material was either extracted immediately or deep-frozen in liquid N_2 and stored at −20°. Tissue was extracted with 80% MeOH in 0.1 M HOAc containing BHT (100 mg l^{-1}) as an antioxidant. 1.48 kBq of (±)-[^{3}H]ABA (2.36 MBq μmol^{-1}) was added to each sample to monitor losses during purification procedure (all results reported are corrected for recovery losses) and the extract filtered. The residue was washed with a further 100% MeOH, the filtrates pooled and evapd to dryness under red. pres. at 40°. The extract was dissolved in 25 ml 0.1 M HOAc and partitioned (×3) with equal vols Et_2O. The Et_2O was dried over $MgSO_4$, filtered and evapd to dryness. The extract was dissolved in 20% MeOH containing 0.1 M HOAc, filtered and fractionated by RP-HPLC, using an ODS- Spherisorb (150 mm long, 10 mm i.d.) column eluted with a linear gradient of 20–100% MeOH in 0.1 M HOAc over 40 min at 5 ml min^{-1}. The eluate was monitored at 265 nm. The fr. corresponding to ABA (16.3–16.9 min) was collected, evapd to dryness, and methylated with CH_2N_2. The Me ester was further purified by RP-HPLC using a Nova-Pak (150 mm long, 3.9 mm i.d.) column eluted with a linear gradient of 40–80% MeOH in 0.1 M HOAc over 20 min at 1.2 ml min^{-1}. Absorbance was monitored at 265 nm. The fr. corresponding to ABA (11.46–11.50 min) was evapd to dryness. Samples were quantified by ECD-EC. Samples were dissolved in EtOAc with the Me ester of *trans*-ABA as int. standard, and the analysis was performed on a gas semi-capillary column (30 m × 0.75 mm i.d.). The temp. of the injector, detector and column were 210, 300 and 190°, respectively. The carrier gas was He (60 ml min^{-1}) and N_2 was used as make-up gas and had a flow rate at the detector of 20 ml min^{-1}. Overall recovery of [^{3}H]ABA added to the samples was between 50 and 70%.

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