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Gibberellic Acid-induced Structural Alterations in the Endosperm of Germinating *Lactuca sativa* L. Achenes

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Summary

Exogenously applied gibberellic acid induces structural alterations in the micropylar region of the endosperm in Grand Rapids lettuce (*Lactuca sativa* L.) achenes prior to radicle protrusion. Besides the pattern of storage material degradation and vacuole formation, both timing and localization of endosperm alterations are similar to the previously reported phytochrome-mediated ones. These data favor the concept that gibberellic acid and phytochrome promote lettuce achene germination, at least in the later stages, through a similar channel.

Key words: Lactuca sativa, endosperm structure, germination, gibberellic acid.

Introduction

The light requirement for lettuce achene germination can be substituted for by exogenously supplied gibberellic acid (Kahn, 1960). The physiological action of gibberellic acid on lettuce achene germination and probable interactions with phytochrome system have been a subject of argument (Black, 1969; Bewley and Halmer, 1980/81; Bewley and Black, 1982).

A considerable number of investigations carried out on biochemical events during lettuce achene germination support the hypothesis that major biochemical changes occurring after red light irradiation coincide more or less to the ones following the gibberellic acid induction of germination (Bewley et al., 1968; Vidaver and Hsiao, 1974; Halmer et al., 1976; Leung and Bewley, 1981; Carpita and Nabors, 1981).

A consideration of the events (mainly biochemical) so far known to take place during lettuce achene germination, proves that most of them start actually after the time of radicle protrusion (Bewley and Halmer, 1980/81; Fig. 2). Until now, only one enzyme activity (α-galactosidase; Leung and Bewley, 1981) has been shown to be clearly enhanced before radicle emergence.

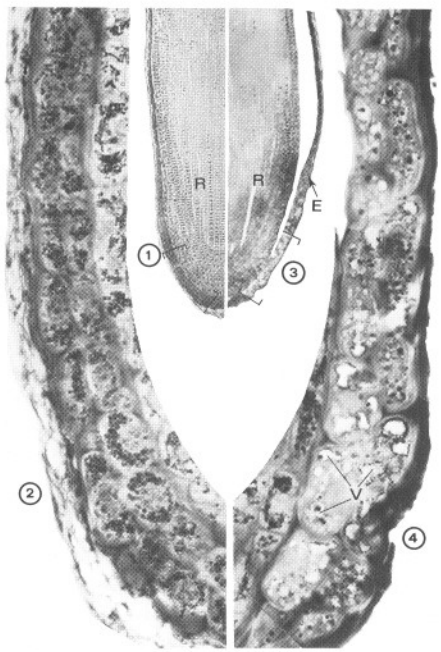
Furthermore, after the suggestion of the weakening of the entire endosperm cell walls (Jones, 1974), it has been reported that only the micropylar endosperm cells undergo significant structural alterations of their protoplast before radicle emergence, in both light-requiring (Psaras et al., 1981) and dark-germinating (Georghiou et al., 1983) lettuce achenes.

This work was undertaken to determine whether gibberellic acid-induced germination of lettuce achenes advances through analogous structural preparation of the endosperm.

Material and Methods

Light-requiring lettuce achenes (*Lactuca sativa* L. cv. Grand Rapids), 1978 harvest, were purchased from Carolina Biological Supply Co. (Burlington, NC, USA) and stored at 3 °C. The achenes were imbibed in 3 ml of water or $0.5\,g \cdot l^{-1}$ gibberellic acid solution (Sigma Chemical Company) in 7-cm diameter glass petri dishes lined with two sheets of filter paper. Each dish contained 50 achenes. Germination values are means of at least 5 replicates. The achenes were incubated in darkness at 25 °C. All manipulations were conducted under a green safe light.

Achenes were removed from petri dishes after 12 h from the start of imbibition and were fixed in 6 % glutaraldehyde in 0.025 M phosphate buffer at pH 7 for 3 h at room temperature. Postfixation, dehydration, infiltration in Durcupan ACM (Fluka), sectioning, and staining of plant material were carried out as described previously (Psaras et al., 1981).



Figs. 1–4: Median longitudinal sections of the radicle end (R) of the lettuce embryo with the two cell-layered endosperm (E). Fig. 1. Section from an achene imbibed in water for 12 h (×40). The area of the endosperm in brackets is magnified in Fig. 2 (×325). Note protoplast density and abundant storage materials. Fig. 3. Section from an achene imbibed in gibberellic acid for 12 h (×40). The portion of the endosperm indicated in brackets is magnified in Fig. 4 (×325). Note formation of vacuoles after mobilization of storage materials (V).

Results

The final germination percentage of lettuce achenes, scored after 48 h from the start of imbibition, in water and darkness at 25 °C was low (5 ± 1 %). Exogenously supplied gibberellic acid promoted germination to 98 ± 2 %. The time course of germination in gibberellic acid showed that radicle protrusion was completed in the first seeds 12 h from the onset of imbibition, and was fully completed by 20 h.

Achenes imbibed in water in darkness for 12 h at 25 °C exhibit endosperm cells characterized by a dense protoplast and abundant storage materials (Figs. 1, 2). In achenes imbibed in gibberellic acid in darkness for 12 h, the endosperm cells of the radicle end have a completely different structure (Figs. 3, 4). Before rupture of the tissue a large part of their storage materials have been mobilized. Protein bodies develop into vacuoles and their cytoplasmic density is reduced (Fig. 4). These protoplast alterations are similar to the ones described previously for both red-light requiring (Psaras et al., 1981) and dark-germinating lettuce achenes (Georghiou et al., 1983). On the contrary the cells of the lateral region of the endosperm retain their density (Fig. 3).

Discussion

Gibberellic acid, which stimulates the germination of lettuce achenes in darkness also induces protoplast modifications such as the degradation of protein bodies, and vacuole formation in micropylar endosperm cells. Thus, the action of gibberellic acid and red light during lettuce achene germination is similar not only to the end effect (radicle protrusion) but also to the pattern of endosperm structural preparation (Psaras et al., 1981). Results of our work further support the hypothesis that endosperm structural alterations occurring in front of the radicle tip before radicle emergence are necessary for typical germination of lettuce achenes (Psaras et al., 1981; Georghiou et al., 1983). Although after 12 hours some radicles already were protruded, the conclusion (viz., endosperm alteration as a prerequisite) is justified insofar, as endosperm alteration was observed only in seeds without any sign of protrusion yet. This also becomes obvious when comparing shape and size of the radicles in Figs. 1 and 2 (non germinating) and in Figs. 3 and 4 (germinating).

Comparably, a similarity of effects of red light and of gibberellic acid during lettuce seed germination has been reported already for protein synthesis (Bewley and Black, 1972), enzyme activation (Halmer et al., 1976; Leung and Bewley, 1981), and embryonic axis elongation (Carpita and Nabors, 1981). Based on these data and on our microscopic observations, it is proposed that the two mechanisms are closely linked and that their later stages, at least, advance through the same channel.

It must be noted that although achenes are uniformly imbibed in exogenously supplied gibberellic acid solution, endosperm mobilization occurs in the radicle end only. It has been suggested that endosperm breakdown, which occurs actually after the completion of radicle emergence, is dependent on a factor emanating from the

embryo, the release of which is light dependent (Bewley and Halmer, 1980/81). This has been suggested for celery seed also (Jacobsen and Pressman, 1979). According to our results, two possibilities concerning the mode of endosperm behaviour before radicle emergence may be considered. One is that the «message» for endosperm preparation emanates from the radicle end of the embryonic axis in response to an external stimulus such as red light or gibberellic acid (Fig. 5). The second possibility is that endosperm cells have been programmed to respond differentially to a uniformly applied triggering «message».

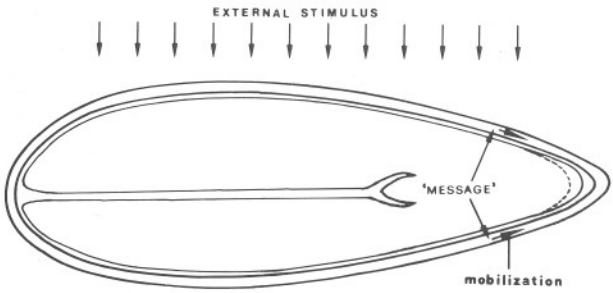


Fig. 5: A probable role of the embryo on the endosperm alterations before radicle emergence, following red light or gibberellic acid induction of germination in Grand Rapids lettuce achenes.

Acknowledgements

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