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LETTUCE ENDOSPERM STRUCTURAL CHANGES DURING GERMINATION UNDER DIFFERENT LIGHT, TEMPERATURE, AND HYDRATION CONDITIONS

K. GEORGHIOU, G. PSARAS, AND K. MITRAKOS

Institute of General Botany, University of Athens, Panepistimiopolis, Athens 621, Greece

The endosperm structure of dark-germinating Grand Rapids lettuce (*Lactuca sativa* L.) achenes was examined by light microscopy under different germination conditions. Achenes imbibed in darkness at 25 C show significant structural modifications in a restricted area of the endosperm, opposite the radicle tip, before radicle emergence. These preemergence changes, similar to those observed in light-requiring Grand Rapids lettuce achenes after phytochrome activation, are mainly characterized by the mobilization of storage materials and the vacuolation of the cytoplasm. Achenes imbibed under conditions not permitting the completion of germination (continuous far-red light, high temperature, and appropriate osmotic solution) do not show any structural change in their endosperm cells. We conclude that cytoplasmic modification of the endosperm cells in the micropylar region is necessary for the completion of germination in lettuce achenes, in addition to the necessity for decrease in embryo water potential.

Introduction

In the endosperm of light-requiring Grand Rapids lettuce achenes, changes in the surface appearance (PAVLISTA and VALDOVINOS 1978), localized and characteristic structural modifications in its cells (PSARAS, GEORGHIOU, and MITRAKOS 1981), and increase in α-galactosidase activity (LEUNG and BEWLEY 1981*a*, 1981*b*) were reported as preemergence events after red- or white-light irradiation. According to PAVLISTA and VALDOVINOS (1978), the inhibition of germination by high temperature (35 C) does not prevent the appearance of cracks and pits in the surface of the endosperm in the presence of continuous white light.

Phytochrome activation also induces the degradation of storage protein and lipid in the radicles of Grand Rapids (NABORS, KUGRENS, and ROSS 1974) and the accumulation of free amino acids in the embryonic axes of New York lettuce achenes (TAKEBA 1980*a*), even when germination is prevented with an osmotic solution. Differences in the amount of free amino acids in the embryonic axes were also observed between low- and high-temperature-treated achenes imbibed in darkness under osmotic stress (TAKEBA 1980*b*).

The estimated water potential decrease in the embryonic axis from the accumulation of osmotically active substances (CARPITA et al. 1979; TAKEBA 1980*a*) has been considered sufficient for radicle protrusion and embryonic axis elongation (NABORS and LANG 1971*a*, 1971*b*; BEWLEY and HALMER 1980–1981). However, the role of the endosperm as a mechanical barrier to radicle protrusion is still under debate (WERKER 1980–1981). According to our observations (PSARAS et al. 1981), the red-light-induced structural changes in the endosperm constitute a prerequisite for radicle protrusion since they are localized opposite the radicle tip and are observed before radicle protrusion.

We investigated the structural changes in dark-germinating achenes both in darkness (with a high level of the active form of phytochrome, P_{fr}) and under continuous far-red light (with a low P_{fr} level of phytochrome). We also studied the endosperm structure under high temperature, which inhibits germination. In addition, we investigated whether the degradation of proteins, observed in the radicles of lettuce achenes imbibed in osmoticum (NABORS et al. 1974), also occurs in the endosperm under similar conditions.

Material and methods

Dark-germinating achenes of *Lactuca sativa* L. 'Grand Rapids' (obtained from Carolina Biological Supply Co., Burlington, N.C., 1979 harvest) were imbibed in 3 ml of water or 0.35 M mannitol solution in 7-cm-diameter glass petri dishes lined with two sheets of Whatman no. 1 filter paper. Each dish contained 50 achenes. The achenes were incubated as follows: (1) in water in darkness at 25 C, (2) in water under continuous far-red light at 25 C, (3) in water in darkness at 35 C, and (4) in 0.35 M mannitol solution in darkness at 25 C. The final germination percentage under the first condition was nearly 100%; in all other conditions, germination was completely inhibited. The green safe light, under which all manipulations were conducted, and the far-red light source were described by PSARAS et al. (1981).

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Address for correspondence and reprints: K. GEORGHIOU, Institute of General Botany, University of Athens, Panepistimiopolis, Athens 621, Greece.

Achenes were removed from petri dishes after 1, 12, or 48 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 (PSARAS et al. 1981).

Observations

The time course of germination in darkness at 25 C of achenes imbibed in water showed that germination was completed in the first seeds after 10 h from the onset of imbibition and was fully completed by 18 h.

Light-microscopic examination of median longitudinal sections of dark-germinating achenes imbibed in water and darkness at 25 C for 1 h revealed that the tough endosperm consists of two layers of thick-walled cells characterized by dense cytoplasm, a few vacuoles, and abundant storage material (figs. 1, 2).

In achenes imbibed for 12 h in darkness at 25 C, the endosperm cells facing the radicle tip have a completely different appearance—a large part of their storage materials, especially proteins, having been mobilized (figs. 3, 4). Protein bodies develop into vacuoles (fig. 4). In ruptured endosperms (fig. 5), these cells exhibit marked features of mobilization; they lose their cytoplasmic density and are highly vacuolated as most of their protein bodies have developed into vacuoles (fig. 7). Lateral cells of the endosperm retain their storage materials even after the completion of germination (fig. 6).

Achenes held in osmoticum for 12 (fig. 8) or 48 h (fig. 9) have endosperm cells with dense cytoplasm, few vacuoles, and abundant storage materials.

In achenes imbibed in water in darkness at 35 C, and at 25 C under continuous far-red light, the endosperm cell structure remained unchanged after 12 (figs. 10, 12) and 48 h (figs. 11, 13) from the start of imbibition. These endosperm cells show the same structure as those imbibed in mannitol solution for 12 (fig. 8) and 48 h (fig. 9).

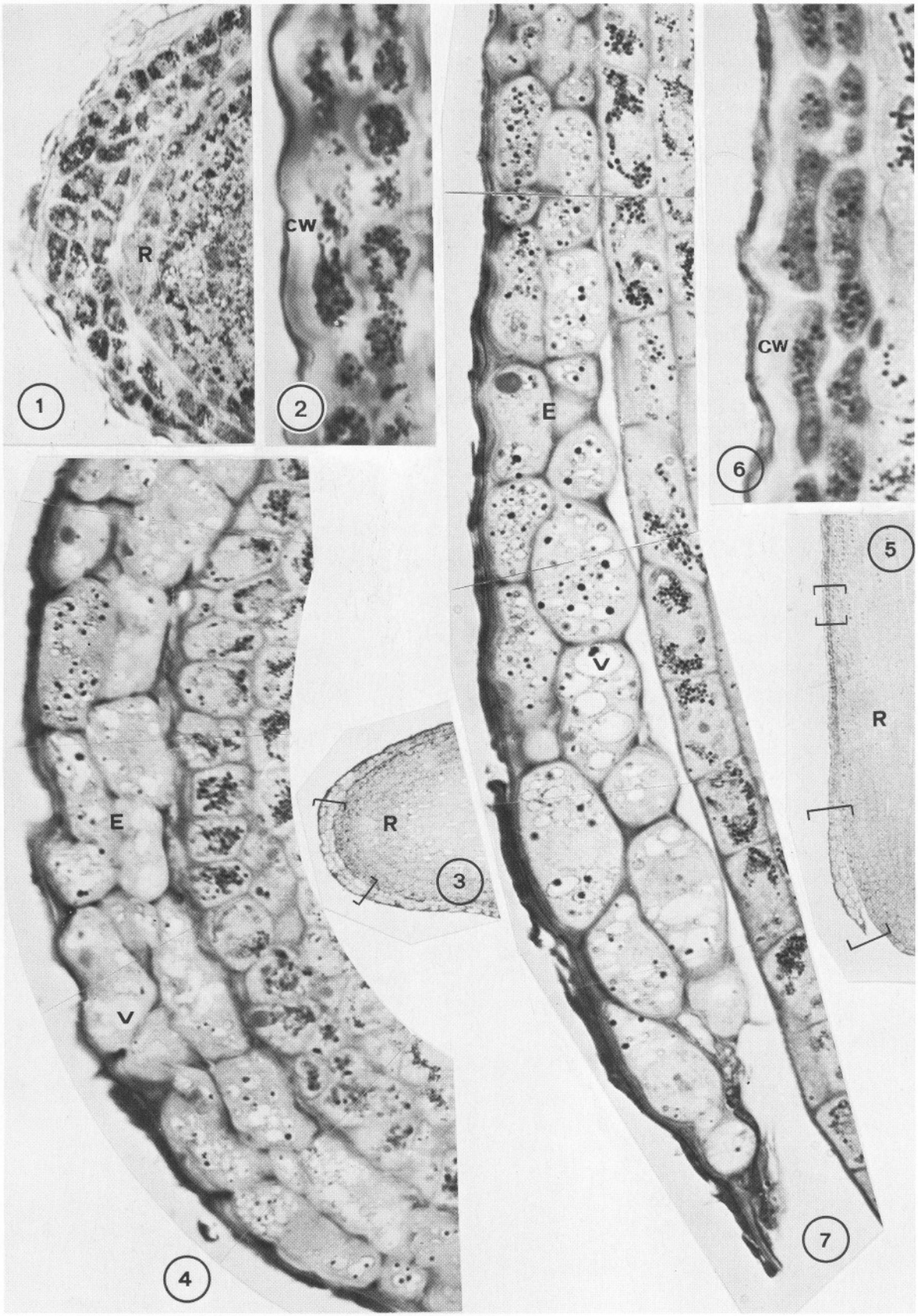
Discussion

The embryo of nondormant, dark-germinating Grand Rapids lettuce achenes is enclosed by the tough endosperm, as described for dormant, light-requiring achenes (JONES 1974; PSARAS et al. 1981). During germination, the embryo of the nondormant lettuce achene has to overcome the physical constraint of the endosperm. We conclude that the passage from the dormant to the nondormant stage (SUZUKI 1981) is not related to structural alterations in the endosperm cells but to physiological changes in the embryo.

The necessity for endosperm weakening during germination of lettuce achenes was proposed by IKUMA and THIMANN (1963). Many attempts have been made to elucidate the exact time and mode of endosperm weakening both microscopically and biochemically. Endosperm cell walls are degraded after germination, commencing at about the time of, but not discernibly before, radicle emergence (JONES 1974; HALMER, BEWLEY, and THORPE 1978). Of the hydrolytic enzymes studied, only α-galactosidase activity increased in the endosperm before radicle emergence (LEUNG, REID, and BEWLEY 1979; LEUNG and BEWLEY 1981*a*, 1981*b*). In our opinion, endosperm "weakening" should not be connected exclusively with cell wall degradation. The transformation of the micropylar endosperm cells, before radicle protrusion, from typically reserve cells to metabolically active ones, either induced by red light (PSARAS et al. 1981) or advanced without external stimulus as shown here, may contribute to endosperm strength reduction in the appropriate area and facilitate radicle protrusion.

Lettuce endosperm serves as a food reserve for the germinating embryo (PARK and CHEN 1974; HALMER et al. 1978; BEWLEY and HALMER 1980–1981), and our observations provide evidence that the endosperm is the early food source for the embryo. In view of both the timing and the region of mobilization of endosperm storage materials, osmotically active substances may be provided to the embryonic axis, thus contributing to an increase in its growth potential, the second essential step for germination.

Phytochrome inactivation by continuous far-red light not only inhibits germination but also prevents any structural change in the endosperm cells. This agrees both with the well-known phytochrome control of germination in dark-germinating lettuce achenes (MANCINELLI and BORTHWICK 1964) and with our report on phytochrome-controlled endosperm preparation in light-requiring achenes (PSARAS et al. 1981). Phytochrome destruction and/or reversion (TOOLE 1973), or the inactivation of a thermolabile factor (TAKEBA and MATSUBARA 1976), could explain the absence of structural changes in the endosperm of high-temperature-treated achenes. Red light is unable to increase appreciably the growth potential in the embryos of New York lettuce achenes incubated at high temperature (TAKEBA and MATSUBARA 1979). The disappearance of small lipid bodies from New York lettuce achenes, observed during the early stage of imbibition at 20 C, was completely suppressed after thermo-inhibition of germination at 35 C (TAKEBA and MATSUBARA 1977). Only high-



FIGS. 1–7.—Figs. 1, 2, Median longitudinal sections of micropylar (fig. 1, × 350) and lateral (fig. 2, × 1,000) area of the endosperm from an achene imbibed in water in darkness at 25 C for 1 h. Figs. 3–7, Median longitudinal sections of the endosperm from achenes imbibed in water in darkness at 25 C for 12 h. Fig. 3, Micropylar area of a nonruptured achene; × 90. Portion in brackets magnified in fig. 4 (× 900). Fig. 5, Radicle end endosperm of a ruptured achene; × 100. Upper brackets magnified in fig. 6 (× 1,000), lower bracket magnified in fig. 7 (× 1,000). Note the formation of vacuoles after mobilization of storage materials of micropylar endosperm cells only. *CW* = cell wall, *E* = endosperm, *R* = radicle, *V* = vacuole.

temperature inhibition of germination did not suppress totally the appearance of some changes in the surface of the endosperm (PAVLISTA and VALDOVINOS 1978). We could not find any structural modification of the endosperm cells that would be responsible for surface alterations.

Osmotic solutions as imbibition media have been used to dissociate the final event of germination (radicle emergence) from the underlying structural and biochemical processes (NABORS et al. 1974; TAKEBA 1980*b*). With this method, we did not observe any structural change in the endosperm even after 48 h of imbibition. It seems that the level of hydration does not permit the transfer of the "message" leading to germination from the embryonic axis to the endosperm. Also, the hydration level under osmotic stress may not be enough to mobilize storage materials and vacuolation of the endosperm cells.

The absence of any structural change in the endosperm cells of far-red-, high-temperature-, and osmoticum-treated achenes could provide the basis for the induction of secondary dormancy by these agents, as observed in different lettuce cultivars (MANCINELLI and BORTHWICK 1964; BLAAUW-JANSEN and BLAAUW 1975), including Grand Rapids (KAHN 1960; GEORGHIOU 1981).

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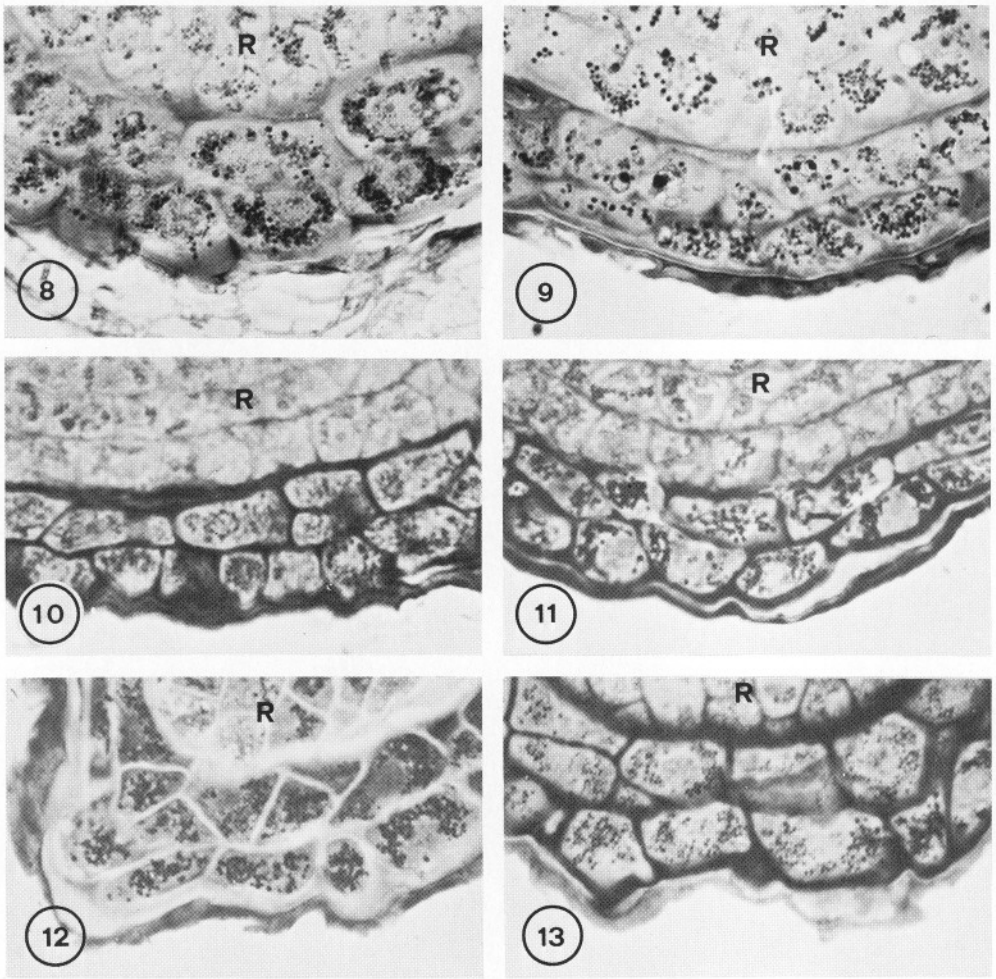

FIGS. 8–13.—Median longitudinal sections of the endosperm in the micropylar region from achenes imbibed under conditions not permitting the completion of germination. Figs. 8, 9, Achenes imbibed in 0.35 M mannitol solution for 12 and 48 h, respectively. Figs. 10, 11, Achenes imbibed at 35 C for 12 and 48 h, respectively. Figs. 12, 13, Achenes imbibed under continuous far-red light for 12 and 48 h, respectively. All × 700. Note the existence of storage materials. *R* = radicle.

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