Changes in ultrastructure of *Phaseolus vulgaris* L. cotyledons associated with their modulated life span

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Abstract

French bean (*Phaseolus vulgaris* L.) cotyledons lost most of their reserve substances during several early days of germination and turned green. In cotyledon mesophyll cells of one-week-old seedlings, plastids were represented predominantly by amyloplasts (starch grains) and chloroamyloplasts, and the cells appeared to be metabolically highly active. Cell heterogeneity associated with distance of the cells from cotyledon vascular bundles was evident. Only mesophyll cells near to the bundles were rich in plastids. In two-weeks-old intact bean plants, the cotyledons were yellow and shrunken, and their cells were nearly "empty". The plastids in them were represented by senescent plastids (gerontoplasts) only. In the gerontoplasts as well as freely in cytosol, fluorescent lipoid inclusions were accumulated. This cotyledon development was more or less independent of irradiance. In "decapitated" bean plants, senescence of mesophyll cells and plastids was slowed down considerably, and the life span of the cotyledons was prolonged.

Additional key words: cell ultrastructure; chloroamyloplasts; chloroplasts; electron microscopy; fluorescence microscopy; French bean; gerontoplasts; senescence.

Introduction

The development of photosynthetic apparatus (including chloroplast or, more generally, plastid development) during leaf ontogeny is worth of attention both from theoretical and practical point of view (Kutík 1985, 1997). The cotyledons of plants

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with epigeic germination are suitable for such studies (Wilhelmová *et al.* 1997). They function temporarily as photosynthesizing organs having a short (days) and easily manipulated life span. This is why the development of the cotyledons of cucumber (Butler 1967, Harnischfeger 1973), radish (Arai and Bonnett 1972), mustard (Hudák 1981), soybean (Huber and Newman 1976), and French bean and other plants (Rascio *et al.* 1990) was studied. The development of French bean "primary" (first after cotyledons) leaves was followed in a series of papers, see Kutík *et al.* (1988).

In the present work, we continued in our study of development of French bean cotyledons (Wilhelmová *et al.* 1997). We followed ultrastructural development of cotyledon mesophyll cells and their plastids influenced by irradiance or seedling decapitation. Not only "young" and senescent cotyledons but cotyledons in several developmental stages were analysed. Electron and light microscopic studies of structural development of the cotyledons were supplemented by fluorescence microscopy.

Materials and methods

Control plants of *Phaseolus vulgaris* L. cv. Jantar were grown in sand in a growth chamber at ambient temperature 18/16 °C, air humidity 60/80 %, and irradiance of 200/0 μmol(photosynthetically active radiation, PAR) m^{-2} s^{-1} day/night, respectively. The plants were supplied twice a week with the International Biological Programme nutrient solution. The "low irradiance" (LI) and "high irradiance" (HI) plants received 2-5/0 and 1 000-1 500/0 μmol m^{-2} s^{-1} (PAR), respectively. In the "decapitated" plants, whole shoot above cotyledons was excised, firstly at the seventh day of plant germination and then always when needed.

Samples for microscopic study were always taken from three plants (*i.e.*, three cotyledons), from interior of the middle part of a cotyledon. They were double fixed with glutaraldehyde and osmic acid, then embedded into Spurr's low viscosity resin *via* propylene oxide after dehydration in graded ethanol series (for details of the procedure see Kutík *et al.* 1984). Ultrathin sections of embedded material were contrasted in saturated solution of uranyl acetate in 70 % ethanol and in lead citrate solution after Reynolds, in both for 20 min. The cell ultrastructure was followed using transmission electron microscope *Philips EM 300*. Anatomical structure of the samples was checked using light microscope on semithin sections (thick about 1 μm) from the material prepared for electron microscopy, after staining in 1 % solution of toluidine blue in 1 % sodium tetraborate. For fluorescence microscopy, free hand sections from fresh cotyledons of one- or two-weeks-old control bean plants were used. The autofluorescence of them was excited by short-wavelength part of visible radiation spectrum plus near ultraviolet radiation.

Results and discussion

French bean cotyledons turned green after raising over soil surface, and functioned temporarily as photosynthesizing organs. In preliminary experiments, their structure was found fairly complex and conspicuously changing during ontogeny, in accordance with description given by Rascio *et al.* (1990). Under current conditions of germination, their life span (beginning from seed imbibition) took about two weeks. During first three to four days of germination, the cotyledons, originally yellowish, became more or less green for five to six next days. During that time, they lose very quickly reserve starch and proteins. Usually at the end of the second week of germination, they became shrunken, yellow, dry, and they fell finally. The most pronounced cell heterogeneity in mesophyll tissue of the cotyledons was connected with the distance of respective mesophyll cells from the vascular bundles. In young cotyledons, some stripes of mesophyll tissue in proximity of the bundles were greener than more distant cells. The cells of these stripes contained several large amyloplasts (containing huge grains of reserve starch) and many chloroamyloplasts (chloroplasts with assimilation starch inclusions of different size), see Fig. 1*A*. The cells more distant from vascular bundles contained markedly less plastids of both above types. In senescent cotyledons, the cells distant from the vascular bundles seemed to be almost "empty" (without organelles) and shrunken whereas the cells adjacent to the bundles contained senescent plastids (gerontoplasts, see Sitte 1977), mitochondria, and other organelles.

In 8-d-old control plants, the cotyledons functioned as photosynthesizing organs. Their mesophyll cells were metabolically highly active (Wilhelmová *et al.* 1997). The most prominent type of plastids in them—leaving aside the huge amyloplasts—were chloroamyloplasts of various size and proportion of starch inclusions (Fig. 1*B*). In the experimental series evaluated here, proplastids with slightly developed membrane system or small chloroplasts without starch (Rascio *et al.* 1990, Wilhelmová *et al.* 1997) were only rarely seen. However, these plastids were probably precursors of the chloroamyloplasts observed (Rascio *et al.* 1990). Probably, the physiological age of our "young" cotyledons was more advanced. In free hand sections from unfixed cotyledons of 8-d-old control plants intensive red fluorescence of chlorophylls in the chloroamyloplasts was seen. No blue fluorescence was apparent in mesophyll cells. Mesophyll cells of 8-d-old LI and HI bean plants had the ultrastructure very similar to that in control plants (Fig. 1*C,D*).

During further ontogeny, cell and plastid ultrastructure of the cotyledon mesophyll tissue changed rapidly. In 14-d-old control plants, only senescent plastids (gerontoplasts) were found in mesophyll cells adjacent to the vascular bundles. Very altered, slightly contrast thylakoid system and large, relatively electron transparent plastoglobuli formed their main structural parts (Fig. 2*C*). Alongside with the gerontoplasts, many plastoglobuli-like, probably lipoid inclusions were seen freely in cytosol. The groups of them were in some cases hardly distinguishable from degraded gerontoplasts. A corresponding picture was seen using fluorescence microscopy. Beside more or less intensive red fluorescence of remaining chloro-

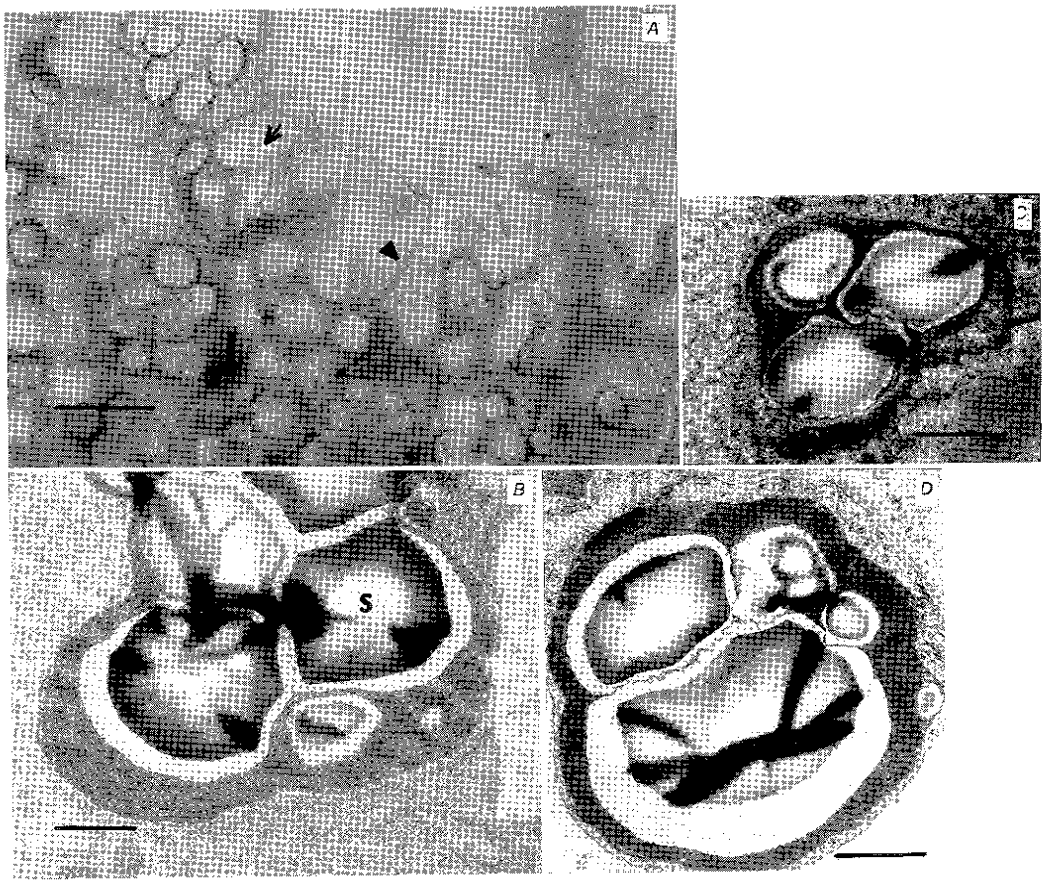

Fig. 1. *A*: Cotyledons of one-week-old control French bean plant, light microscopy, free hand section mesophyll cells with large starch grains (*arrow*) and chloroamyloplasts (*arrowhead*). Bar = 50 μm. *B* to *D*: Cotyledons of 8-d-old control (*B*), low irradiance (*C*), or high irradiance (*D*) plants studied by transmission electron microscopy show chloroamyloplasts. S - starch inclusion. Bar = 1 μm.

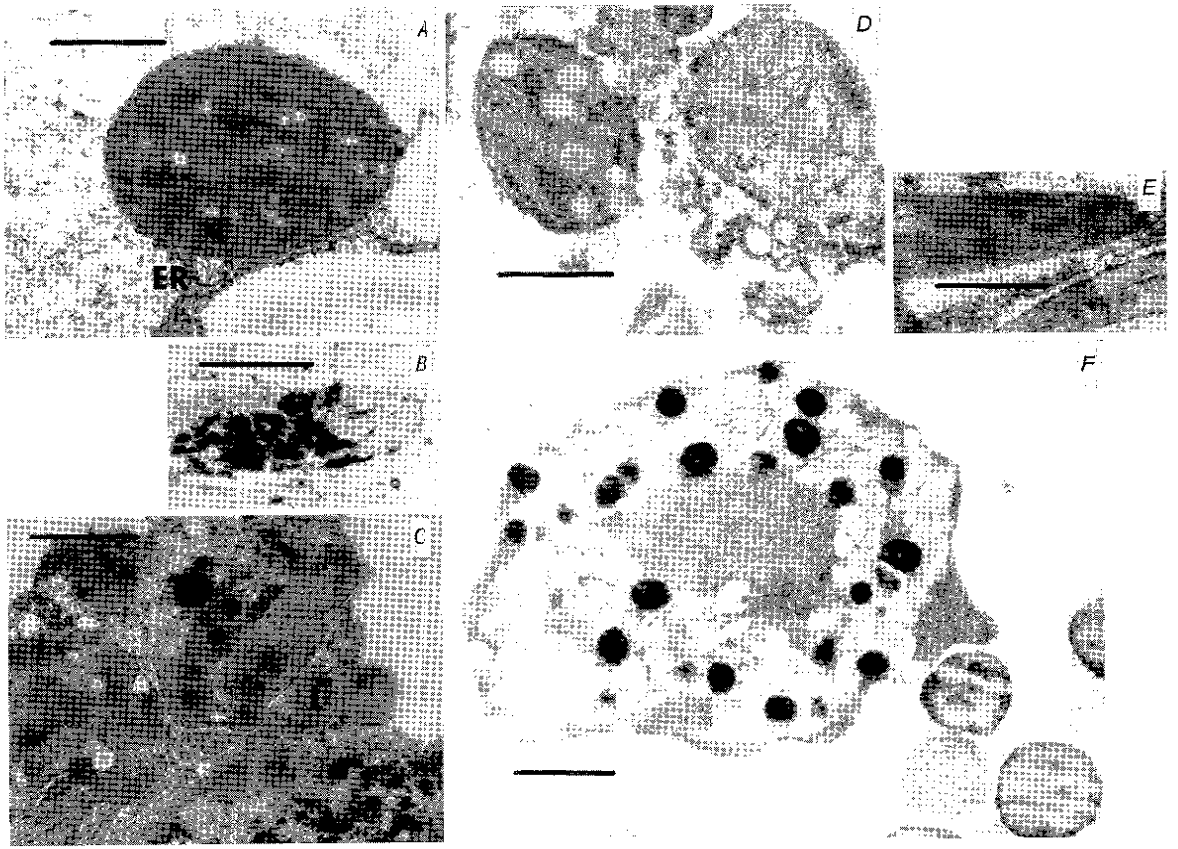

Fig. 2. Cotyledons of 12-d- (*A,B*) or 14-d- (*C*) old control French bean plants, 14-d-old high irradiance plant (*D*), and 26-d-old decapitated (*E*) or high-irradiance decapitated (*F*) plants studied by transmission electron microscopy show mostly senescent chloroplast (*A*) or gerontoplasts (*B,C,D,F*), only rarely a chloroplast (*E*). *Asterisk* – granum, ER – rough endoplasmic reticulum, L - lipoid inclusion in cytosol, P - plastoglobulus. Bar = 1 μm.

phylls in the gerontoplasts, a weak blue fluorescence of plastoglobuli in the gerontoplasts and lipoid inclusions in the cytosol was apparent. The mesophyll cells distant from the vascular bundles were nearly "empty", without any organelles, in 14-d-old control plants. The senescence phase of French bean cotyledons' development was not studied by Rascio *et al.* (1990) nor, as far as we know, by other authors.

The ultrastructure of mesophyll cells and plastids in 14-d-old LI and HI bean plants was again similar to that in control plants of the same age (Fig. 2*D*). Unlike in our previous study (Wilhelmová *et al.* 1997), the preservation of gerontoplasts and other cellular structures under LI was even worse, not better than in the control plants. In contrast, in "decapitated" bean plants, the senescence of the cotyledons was slowed down considerably. In 14-d-old "decapitated" plants, the ultrastructure of the mesophyll cells was similar to that in 8-d-old control plants. The chloroamyloplasts, mitochondria, and other organelles were well preserved here. Even in 26-d-old "decapitated" plants, a complicated system of thylakoids was still present in mesophyll cells' chloroplasts, usually devoid of starch (Fig. 2*E*). The cotyledon mesophyll cells of decapitated bean plants of the same age (26 d) cultivated under HI contained, however, gerontoplasts with dilated disorganized thylakoids and many plastoglobuli. Lipoid inclusions in cytosol were also apparent here (Fig. 2*F*). The slowing down of cotyledon or leaf senescence owing to decapitation of a plant above the respective leaves was demonstrated by Huber and Newman (1976), Hudák (1981), Kutík *et al.* (1984), and Wilhelmová *et al.* (1997). Accumulation of cytokinins (synthesized in roots) in these cotyledons or leaves seems to be the reason of the retardation of ageing.

In the control bean plants, we followed the development of cotyledon mesophyll cells in two-day intervals, from the 8th to the 14th d of plant age. Whereas at the 10th d, the chloroamyloplasts and other cell organelles were very similar to those at the 8th d (in "young" plants), at the 12th d, very altered gerontoplasts were present in almost "empty" cells (as in "old" plants, see Fig. 2*B*) as well as preserved senescent chloroplasts with clearly discernible thylakoids, surrounded by other cell organelles, *e.g.*, endoplasmic reticulum with ribosomes (Fig. 2*A*). Moreover, there were even macroscopically evident differences between cotyledons of individual bean plants of the same age. The heterogeneity in mesophyll cell ultrastructure in the same cotyledon mainly owing to their different distance from the cells of vascular bundles has been mentioned earlier. Concentration gradients of cytokinins and other substances transported through the bundles may be the reason of this heterogeneity.

The decapitation of French bean plants is a reliable method for prolongation of the life of their cotyledons, including plastid integrity in them. Even very low irradiance was not effective in this direction. *Vice versa*, HI did not shorten the life span of cotyledons. However, gerontoplasts rather than chloro(amylo)plasts were present in cotyledon mesophyll cells of very old decapitated HI plants. There were fairly large differences in physiological age of the cotyledons between individual seedlings.

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