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Tissue- and organ specific plastid differentiation in various plant species

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ABSTRACT Plastid differentiation and the greening process are usually studied in etiolated leaves. In this work, we studied stems or stem related organs of various plant species. Electron microscopy studies combined with fluorescence spectroscopy showed the formation of protochlorophyllide forms similar to those of etiolated leaves but their ratio had a big variation, even more in different tissues of the same organ. In a series of measurements, we proved that etiolation conditions can occur in the nature in closed cabbage head or inside closed leaf buds.
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KEY WORDS

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POR(NADPH:protochlorophyllide oxidoreductase)
protochlotophyllide

The phototransformation of protochlorophyllide into chlorophyllide is a key regulatory step of chlorophyll biosynthesis; it is widely studied in plant biochemistry (Masuda and Takamiya 2004). In addition to the biochemical and biophysical aspects of this reaction, the molecular biology provided basically new information. For example, isoforms of the enzyme NADPH:protochlorophyllide oxidoreductase (POR, EC: 1.3.1.33) have been identified (named POR-A, POR-B and POR-C), the biosynthesis, regulation, activity and physiological role of which are different (Reinbothe et al. 1996; Oosawa et al. 2000). Most of the results in this research field originate from experiments on leaves of dark-grown (etiolated) seedlings of a few test-plants. However, the etioplast development, the formation of their inner membranes (prolamellar bodies and prothylakoids) and the arrangement of POR subunits and protochlorophyllide complexes in them have a great variation among species, organs and have a certain tissue-specificity. In this work, this heterogeneity is demonstrated and discussed on the basis of our results published in the last few years.

Results and Discussion

Pea epicotyl: the physiological role of the monomer protochlorophyllide (Pchlide) forms

In earlier works, we detected poorly developed etioplastids in the epicotyls of dark-germinated pea seedlings (Böddi et al. 1994). In this organ, two Pchlide forms are dominating with fluorescence emission maxima at 629 and 636 nm; both contain this pigment in monomer state (Böddi et al. 1998). Using low intensity white or 632.8 nm laser light illumination at room temperature or at – 15ºC, the flash-photoactivity of the 636 nm emitting form was found, it converted into a 678 nm emitting chlorophyllide (Chlide) form (Kósa et al. 2005). The 629 nm form was inactive at flash-illumination. Instead, during dark incubation following a short time illumination, it regenerated the flash-photoactive complexes. At illumination with strong light, this form sensibilized a photo-oxidation pathway, in which the production of O_2^-, lipid peroxidation, membrane damage, turgor loss and wilting of the epicotyl were observed (Erdei et al. 2005).

Plastid development in pea shoot cultures

Data have been published about the stimulating effect of cytokinin on the size of prolamellar bodies (PLBs) and the amounts of oligomer Pchlide complexes in leaf etioplasts (Seyedi et al. 2001). We studied this effect of cytokinin in etiolated pea stems of shoot cultures. Instead of the poorly developed etioplasts of stems, structures characteristic for leaves and the increase in the amounts of flash-photoactive oligomer Pchlide complexes were found. Our results prove that the organ-specific differentiation processes of etioplasts and thus the chlorophyll biosynthesis pathways can be modified.

Plastid development and Pchlide forms in sunflower cotyledons

The cotyledon of sunflower develops into true leaf after germination. When the seeds were germinated in the dark, a 628-631 nm emitting Pchlide form appeared at very early developmental stages, after1-1.5 days. Later, a shoulder appeared at 655 nm, which finally dominated the spectrum. In parallel, etioplasts with well-developed PLBs appeared, which proves that the 655 nm Pchlide form is an integral component of PLBs.

Distribution and properties of Pchlide forms in dark-forced stems of grapevine

Dark-forced shoots are used in grapevine propagation; the newly developed shoots often suffer from photodamage. The reason is the dominance of the short-wavelength emitting monomer Pchlide forms which are not flash-photoactive and

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so sensibilize photo-oxidation processes similar to those observed in pea epicotyls. However, the lignification of the dark-forced stems prevents the wilting. Thus a bleached shoot can totally regenerate. Interestingly, the 655 nm emitting Pchlide form appeared first when the totally bleached, pigment-free shoots were kept in the dark for 60 min. The detailed analyses of the tissue layers along with EM studies of oriented samples showed an unequal distribution of the Pchlide forms and etioplasts. The subepidermal cell layers were the most similar to leaves; they contained well developed PLBs and much 655 nm emitting Pchlide form. The cells of the pith, contained proplastid type plastids mainly with short-wavelength Pchlide forms (Böddi et al. 2004).

Plastids in potato tuber

Potato tubers have chlorenchyma tissue layers under their periderma. When the tubers were kept on light, chlorophyll (Chl) accumulated in fully developed chloroplasts the emission spectra of which were similar to those of green leaves. When the tubers were kept in the dark for long periods, the Chl-protein complexes of the photosynthetic apparatus decomposed and a special Chl form with emission maximum at 682 nm appeared and remained unchanged for several months. In the dark, however, Pchlide accumulated, too. The tuber contained various transitional plastids in different layers. Proplastids or starch storing amyloplasts were characteristic in the centre of the tuber. Etio-chloro and chloro-etioplasts were found in layers close to the surface. Potato tuber is a suitable experimental sample for studying the inter-conversions of the different plastid types.

Pchlide forms in etiolated leaves developed from storage organs

Etiolated shoots can be grown from storage organs of several plants. Thus the storage taproot of carrot develops etiolated leaves when kept in the dark under wet conditions. These leaves had fluorescence emission spectra and etioplasts similar to those of leaves of etiolated seedlings. However, the storaged material is metabolised and thus the etiolated leaves are vital for several weeks. A similar phenomenon was found in etiolated leaves developed from bulbs of onion. These samples are suitable for studying the senescence of etiolated tissues for a prolonged period.

Etiolated tissues in the nature

a) Plastids in the cabbage head

In white cabbage, the outer leaf layers behave as optical filters, thus a steep decreasing light gradient can be measured towards the centre of the head. The outermost leaves are fully developed and green, they contain usual chloroplasts in freshly harvested heads or senescing plastids when the head is stored for long period. In the centre of the head, however, leaf primordia were found with developing stoma apparatus and proplastid type plastids. These primordia contained Pchlide and no Chl. The fluorescence emission spectra were similar to those of very young leaves of seedlings or dark-forced stems. In other leaf layers a series of plastids in transitional stages were found (Solymosi et al. 2005).

b) Plastids in the leaf primordia of closed buds

Leaf buds of various tree species have thick, brown scales, which tightly close the developing leaf primordia. In case of horse chestnut, for example, etiolation conditions appear after closing the scales. The bud development starts at the end of summer of the previous year, when the primordia are exposed to light. Later, the scales develop and overlap with each other. The new leaf primordia thus develop in total darkness. Proplastid type etioplasts, etiochloroplasts and chloroetioplasts were found in primordia of buds collected in different months of the year.

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