

Immunocytochemical Studies of Chloroplast Changes in Black Bean Seeds

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Dedifferentiation of chloroplasts into proplastids found in yellowing cotyledons due to desiccation process during seeds maturation and redifferentiation of chloroplast in greening cotyledons after seed germination was found in non-evergreen embryo such as soybean, *Phaseolus vulgaris*, and *Spinapis alba* [1]. Stay-green embryos come from maple, clemetine and kumpart [2]. In the present study, the morphological changes of cotyledons of black beans were examined light and electronic microscopic levels. Based on ultrastructural changes of chloroplasts in seed formation and germination, immunolocalization of LHC IIb in chloroplasts at different stage of cotyledon development was examined.

Immunolocalization study followed the methods of Lin and Langenberg [3] with some modifications. Samples preparations up to infiltration in LR white resin were the same as the described in traditional methods except OsO_4 fixation. The resin was polymerized at 60 °C for 24 h. Sections in golden color were collected on 100-meshed grid were incubated in NET solution (0.16M NaCl, 5 mM Na-EDTA and 0.05% Tween-20) containing 0.25 % gelatin for 60 min to blocking the nonspecific binding, re-incubated in 1/100 dilution of antiserum with NET medium, washed five times of de-ionized water (each for 3 min), and finally brooded in gold-conjugated goat anti-rabbit antibody of LHCIIb protein for 60 min. After being thoroughly washing, the immuno-labeled sections were doubly stained with uranyl acetate and lead citrate, observed and photographed with a Hitachi H-600.

The LHCIIb is a major and high molar fraction protein in LHCII proteins that are related to lamellar thylakoid stackingness. The antibody of LHCIIb originated from *Ficus microcarpa* cv. *Golden leaf* was used for immunoblotting and immunolocalization. Western blotting with antibody of LHCIIb protein had proved the expression of LHCIIb protein in all developmental stages of cotyledons and mature leaves had higher expression than that of cotyledons at stages 3 to 8. Immunogold-labelling studies showed that the chloroplasts in all tested cotyledons at stages 3 to 8 were the only organelles labeled with 18 nm gold. The gold particles in chloroplast were preferentially distributed in the regions of lamellar thylakoid membrane (Figs. 1-6) and membrane stacking in cotyledonal chloroplasts was related to stages of seed maturation, seed germination and age of seedlings. The localization and distribution pattern of immunogold were reflected in the shape and structure of lamellar thylakoid (Figs. 2, 3, 4). No matter the dehydrated states of cotyledons during seed maturation, the gold particle distinctly favored in grana and no ones in intergranal thylakoid been found. After seed germination, chloroplasts in the extended cotyledons of seedlings at stages 7 to 8 were also preferentially labeled in stacking thylakoid membrane (Figs. 5, 6).

Phosphorylation and dephosphorylation of LHCII proteins resulted in the membrane stacking and destacking of thylakoid were suggested before [4] [5]. In the present studies, LHCIIb has been detected in cotyledonal chloroplast at stages 3 to 8 through immunolocalization. It indicated that lamellar thylakoid membranes were always kept in green cotyledonal chloroplast and was contrast to proplastids present in the yellow mature seeds of soybean. However, the persisted grana presented in cytoledonal chloroplasts were also observed in green mature soybean *d1d2* and *cyt-G1* mutants [6]. During leaf senescence Clark lost almost all of its thylakoid proteins, whereas *d1d2* and *cyt-G1* retained the LHCs. Similar observations also reported in chlorophyll-retention sid mutants of *Festuca pratensis* leaves and tomato fruits [7].

References

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Figures 1-6. Immunolocalization of LHCII b in plastids of green cotyledon at different stages of seed development. 1, Stage 3 with red seed coat. 2, Stage 4 with purple seed coat. 3, Stage 5 with black seed coat. 4, Stage 6 with seed coat imbibing water. 5, Stage 7 at 5 d after seed germination. 6, Stage 8 at 10 d after after seed germination. All bars in 1 μm . Notes: Ch, Chloroplast; O, Oil droplet; SG, Starch grain;

