doi:10.1017/S143192761300250X
Microsc. Microanal. 19 (Suppl 2), 2013

Chile Pepper Carotenoids: The Link Between Color and Sub-Cellular Morphology

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Chile pepper (*Capsicum annuum*) fruit accumulate very high concentrations and complex mixtures of carotenoids. This accumulation in the pericarp tissue results in pepper fruit colors such as red, yellow and orange [1]. In photosynthetically active tissues, carotenoids are antenna pigments associated with the light-harvesting and reaction centers on the thylakoid membranes in chloroplasts [2]. In flowers and especially fruit, carotenoids accumulate to even higher levels within differentiated plastids known as chromoplasts. Carotenoids function as antioxidants, and specific carotenoids (β-carotene and β-cryptoxanthin) are essential dietary components that function as vitamin A precursors [3]. We hypothesize that chromoplast architecture is different in fruits of different colors due to the accumulation of specific carotenoids. Therefore the objective of this research is to compare chromoplast shape and intra-organellar morphology in pericarp tissues of fruit that mature to different terminal colors and correlate distinct classes of chromoplast morphologies with specific carotenoid abundances in cultivars of chile peppers.

Laser scanning confocal microscope composite images (5 μm z-stack, Leica TCS SP5, excitation 488 nm argon laser, 670-700 nm chlorophyll autofluorescence emission, 515-590 nm carotenoid autofluorescence emission) were acquired using fresh pericarp tissue from *C. annuum* cultivars that mature to different terminal colors (Fig. 1). These images demonstrate unique chromoplast shapes in cultivars that accumulate different carotenoid profiles (Fig 1 D,H), final fruit color red for LB-25 (Fig. 1 A-D) and orange for Costeño Amarillo (Fig. 1 E-H). Mature chromoplasts in Costeño Amarillo pericarp tissue have a "sickle" or elongated morphology whereas mature chromoplasts in LB-25 are spherical. The information acquired via autofluorescence of carotenoids made this 'wet-prep' method for imaging fresh tissue very time efficient and effective in detecting structural differences in chromoplasts. Modified chromatography methods [1] are in use to quantify specific carotene and xanthophyll levels in extracts from these pericarp samples. Costeño Amarillo has very high violaxanthin content with over 50% of total identified carotenoids accumulating to this step in the pathway. In contrast, LB-25 has a much lower percentage of violaxanthin while accumulating a greater abundance of capsanthin. Additional examples of cultivar specific chromoplast shapes and details of carotenoid abundances will be presented.

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) methods are in use to investigate intra-organellar chromoplast architecture. Sections were fixed, embedded into Spurrs' resin, thin sectioned with a DiATOME histo knife, and visualized using TEM. A distinct composite of internal membrane rearrangement which gives rise to unique carotenoid structures can be seen in fully differentiated mature chromoplasts (Fig. 2). For the SEM panels (Fig. 1 B,F), a freeze-fracture method was utilized in conjunction with critical point drying and a final sputter coating to obtain a profile of the outer structural shape for chromoplasts from pepper cultivars with orange and red terminal colors. In orange cultivars, chromoplasts were observed as sickle shaped structures affixed to the inner surfaces of the cell membrane while red cultivars exhibited plastid morphologies that were more spherical in nature.

Unique chromoplast morphology of chile peppers with different terminal color is successfully observed using multiple microscopy methods with different preparation techniques. Statistical analysis of the dimensions of these organelles determined by SEM, TEM and LSCM is underway. Remarkable differences are noted in the arrangement, shape, and intra-organellar morphology of chromoplasts in cultivars known to accumulate different carotenoid profiles (Fig 1, 3). Further studies including inheritance of unique plastid morphologies are in progress that are likely to reveal more information about carotenoid packaging, architecture, and genetic control [4].

References:

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[4] Acknowledgements: P. Bosland for providing seed, plants and field space; funding by NM Agricultural Experiment Station, USDA NIFA 2010-34604-20886; NSF # MRI DBI-0520956

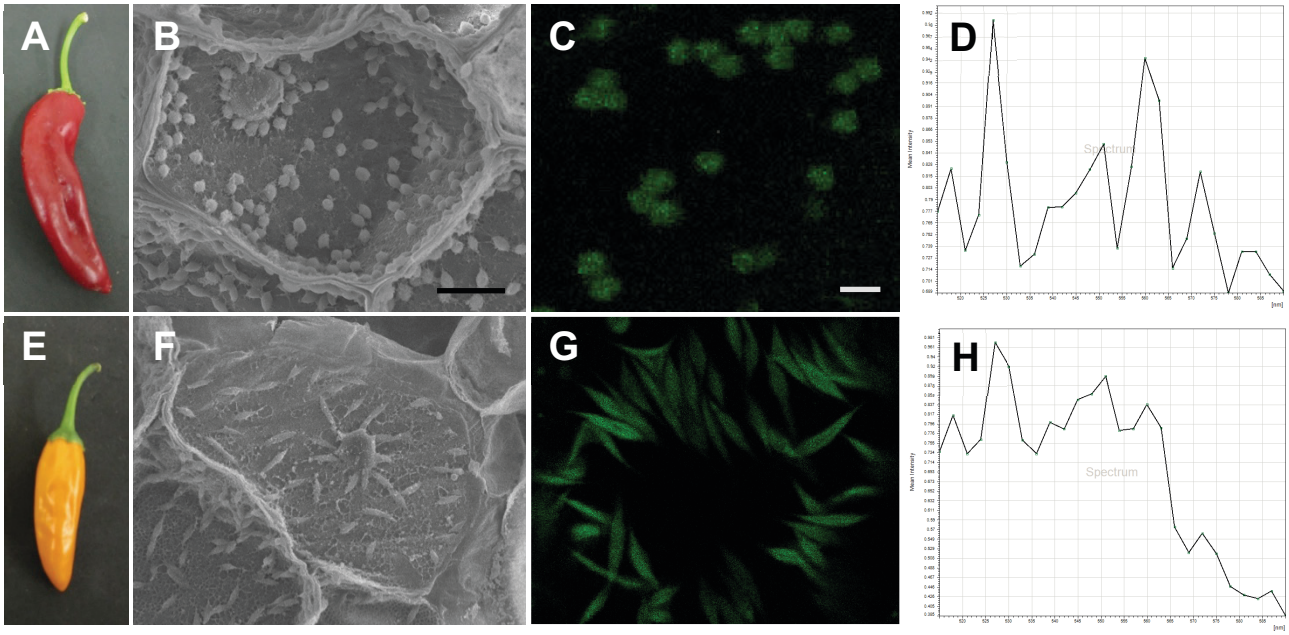


Fig. 1. Correlative microscopy methods for *Capsicum annuum* cv. LB-25 (A-D) and Costeño Amarillo (E-H). Macro imaging of mature pepper fruit (A,E); freeze-fracture SEM of pericarp cells containing mature chromoplasts (B,F); LSCM of mature chromoplasts with excitation at 488 nm and emission window of 515-590 nm (C,G); lambda scan for carotenoid autofluorescence emission window of 515-590 nm with excitation at 488 nm (D,H). Scale bars = 10 μm.

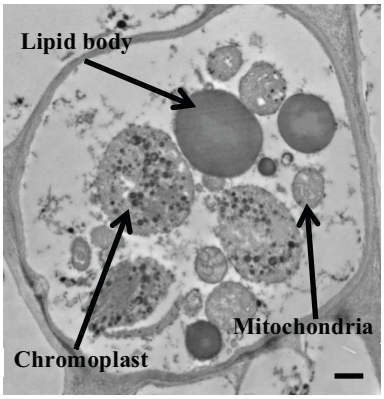
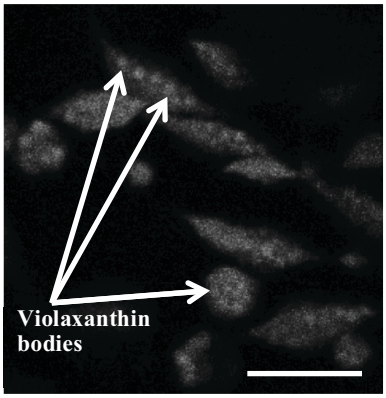


Fig. 2. (Left) TEM of Costeño Amarillo mature pericarp cell. Bilateral cross section containing chromoplasts, lipid bodies, and mitochondria. Scale bar, 500 nm.

Fig. 3. (Right) LSCM of Costeño Amarillo mature chromoplasts. Punctate areas of increased signal represent violaxanthin carotenoid bodies. Scale bar, 10 μm.



https://doi.org/10.1017/S143192761300250X Published online by Cambridge University Press