A-116

Molecular identification of magnesium chelatase subunit H in soybean [*Glycine max* (L.)Merr.]

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Chlorophyll is the main pigment absorbing light for photosynthesis, which converts solar energy to chemical energy to perpetuate most biological activities on earth. In chlorophyll biosynthesis, the first committed step is the formation of magnesium-protoporphyrin IX through a two-step reaction catalyzed by magnesium chelatase. Mg-chelatase is a highly conserved polymeric enzyme composed of three different subunits I (40KDa), D (M.W. 70KDa), and H (M.W. 140KDa), forming a three-tiered structure with a hexametric I ring, a hexametric D ring, and a monomer H. Among them, the subunit H (chlH) is the catalytic subunit and responsible for inserting magnesium into protoporphyrin. In addition, it has been reported to be involved in the plastid-to-nucleus retrograde signaling and ABA signaling pathways. In soybean genome, we found three genes at loci Glyma3g13700, Glyma19g139300, and Glyma10g097800encoding chlHs, and named them *GmchlH1*, *GmchlH2*, and *GmchlH3*, respectively, among which GmchlH1 (Glyma3g13700) has been reported having the Mg-chelatase activity. All three genes showed very similar with respect to the encoded polypeptide and expression level. GmchlH1 showed 99% amino acid sequence identity to GmchlH2, and 97% to GmchlH3.At the transcription level, all three genes expressed the most and at similar level in photosynthetic leaves, much less expressed in flowers, stems and seeds, and barely expressed in roots. Notably, *GmchlH3*'s expression level in cotyledons and seeds was less than half of the other two genes. BiFC assay showed that GmchlH1, 2, and 3 all can physically interact with AtchlI andAtchlD in *Nicotiana benthamiana* leaves, suggesting they can be assembled into Mg-chelatase complex *in vivo*. To further examine whether the proteins encoded by three *GmchlH*s had similar biological function, we transformed and expressed them under 35S promoter, respectively, in *Arabidopsis gun5* mutants in which *chlh* harbors a point mutation and leads plants pale green. The results showed expressing GmchlH3 could fully recover the *gun5* phenotype and lead *gun5* mutants producing green seedlings like wild types, whereas, expressing GmchlH2 can only partially recover the *gun5* phenotype; however, more experiments are needed to explain this phenomenon. Taken together, soybean genome encodes three chlH subunits with subtle differences on primary sequence and expression pattern, but they possibly already evolved different biological function *in vivo*.