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Full Length Research Paper

Genome-wide examination of chlorophyll metabolic genes in maize and phylogenetic analysis among different photosynthetic organisms

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Chlorophyll (Chl) is the key pigment involved in photosynthesis. Analysis of the expression pattern of Chl metabolic genes will contribute to our understanding of photosynthesis. Also, the genes coding for Chl metabolism are ideal targets for revealing the evolution relationships of photosynthetic organisms. In this study, we summarized the Chl metabolic pathway in higher plants and conducted *in silico* expression analysis of related genes in maize. Phylogenetic analysis revealed that the evolution of Chl metabolic genes proceeded in a certain direction. Moreover, the diversity of some rate-limiting enzymes might have played a positive role in the evolution of Chl metabolism.

Kew words: Chlorophyll, maize, metabolism, phylogeny, photosynthesis.

INTRODUCTION

Chlorophyll (Chl) is the key pigment involved in photosynthesis and its metabolic activity has great impact on photosynthesis (Tanaka and Tanaka, 2007; Chen et al., 2010; Allahverdiyev et al., 2011). In higher plants, there are a total of 16 steps in Chl biosynthesis, from the precursor Glu-tRNA to the final product Chl *b*, during which 16 enzymes coded by more than 20 genes participate in the whole process (Matsumoto et al., 2004; Eckhardt et al., 2004; Beale, 1999, 2005; Masuda and Fujita, 2008). Within this pathway, HEMA (glutamyl-tRNA reductase) and POR (NADPH: protochlorophyllide oxidoreductase) are the two rate-limiting enzymes (McCormac et al., 2001; Schoefs and Franck, 2003). As for Chl catabolism, our knowledge has mainly come from the research on senescent leaves. The 5-step reactions of Chl breakdown during the early stage is common with all plants, which are accomplished by 4 enzymes and a metal chelating substance (Hörtensteiner, 2006).

As an important crop, maize (*Zea mays ssp. mays* L) is an ideal model for investigating Chl metabolism (Schnable et al., 2009). The availability of a complete genome sequence and large collections of ESTs in maize allow us to detect the expression profile and evolutionary pattern of Chl metabolic genes (Raymond et al., 2002; Yang and Cheng, 2004; Lohr et al., 2005; Schnable et al., 2009; Wang et al., 2009).

MATERIALS AND METHODS

Chl metabolic genes in *Arabidopsis thaliana* (von Wettstein et al., 1995; Matile et al., 1999; Eckhardt et al., 2004; Beale, 1999, 2005; Hörtensteiner, 2006; Masuda and Fujita, 2008) were used to search maize homologous cDNA sequences (Li et al, 2010). A total of 2,021,116 maize ESTs available in NCBI were downloaded. Then using MLE (maximum likelyhood estimation) (Stekel et al., 2000) in combination with double *in silico* hybridization strategy (Varuzza et al., 2008), we profiled the expression patterns of Chl metabolic genes in maize (Stekel et al., 2000). In addition, the neighbour-

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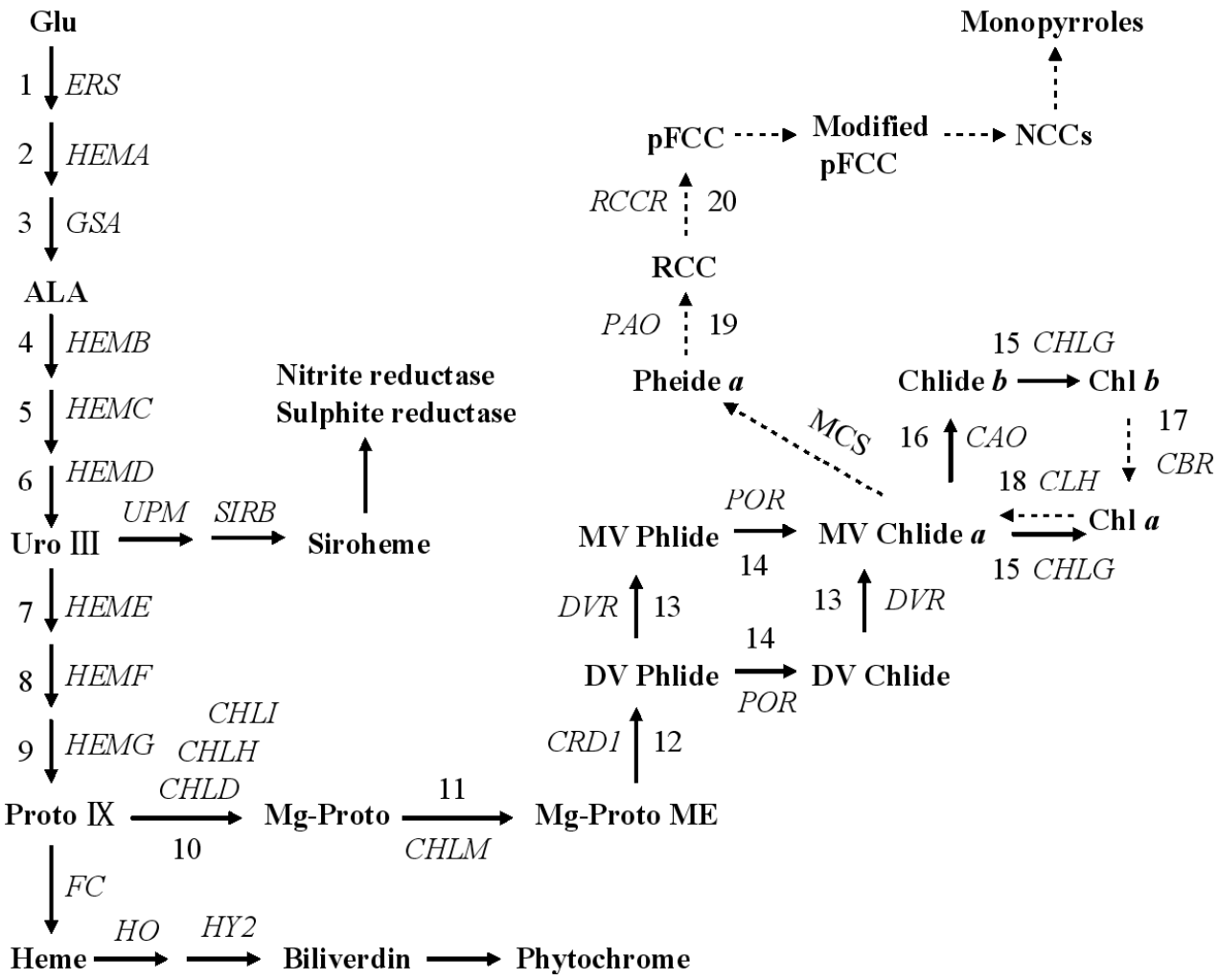

Figure 1. Chl metabolic pathway in higher plants. The ideogram of Chl metabolic pathway was adapted from Hörtensteiner (2006), Lohr et al. (2005) and Masuda and Fujita (2008). Solid arrows represent Chl biosynthesis, and dashed arrows represent Chl degradation. Intermediates: Glu, glutamate; ALA, 5-aminolevulinic acid; Uro III, uroporphyrin III; Proto IX, protoporphyrin IX; Mg-proto, Mg-protoporphyrin; Mg-proto ME, Mg-protoporphyrin monomethyl ester; DV Pchlide, divinyl protochlorophyllide; MV Chlide, monovinyl chlorophyllide; MCS, metal chelating substance; Pheide *a*, pheophorbide *a*; RCC, red Chl catabolite; pFCC, blue-fluorescing intermediate; NCCs, nonfluorescent Chl catabolites.

joining method in MEGA 4.0 program was chosen (Tamura et al., 2007) to construct the phylogenetic trees.

RESULTS AND DISCUSSION

The Chl metabolic pathway in higher plants are summarized and illustrated in Figure 1. The *In silico* expression analysis showed that Chl metabolism in maize had tissue specificity (Table 1); according to previous research that showed that different homologs within the same species may have distinct tissue-specific expression (Ilag et al., 1994; Kumar et al., 1996). In the leaf, *ZmCHLD* had R values over 8 which might indicate high level expression. As a comparison, *ZmHEMA1* and *ZmPOR1* were highly expressed in the root (Table 1), which made us wonder if the maize root might be able to synthesize Chl. By analyzing the D subunit of Mg^{2+}-chelatase that firstly entered the chlorophyll branch, we found that its R value in leaf was 17.8, while it was only 4.2 in the root (Table 1). The status of another subunit *CHLI* was the opposite, with R value of 12.5 in root and only 1.4 in the leaf. It suggested that the end tetrapyrrole in the root was different from that in the leaf. In contrast to the expression of genes in heme branch, we found that the R value of *ZmFC2* and *ZmHO1* in the root reached 9.7 and 10.9, respectively, but was 0 in the leaf (Table 1). We suggested that it might be related to feedback regulation of Chl biosynthesis, and that the active metabolism of tetrapyrrole in root was more likely related

Table 1. *In silico* expression of Chl metabolic genes in leaf and root. The R values of Chl metabolic genes *ZmHEMA1*, EU957870; *ZmCHLD*, AY109815; *ZmCHLI*, EU962417; *ZmPOR1*, NM_001174209; *ZmFC2*, NM_001157005; and *ZmHO1*, EU962994 in the leaf and root were listed. R value beyond 8 indicates significant expression.

Gene	*ZmHEMA1*	*ZmCHLD*	*ZmCHLI*	*ZmPOR1*	*ZmFC2*	*ZmHO1*
Leaf	6.7	17.8	1.4	0	0	0
Root	108.7	4.2	12.5	18.7	9.7	10.9

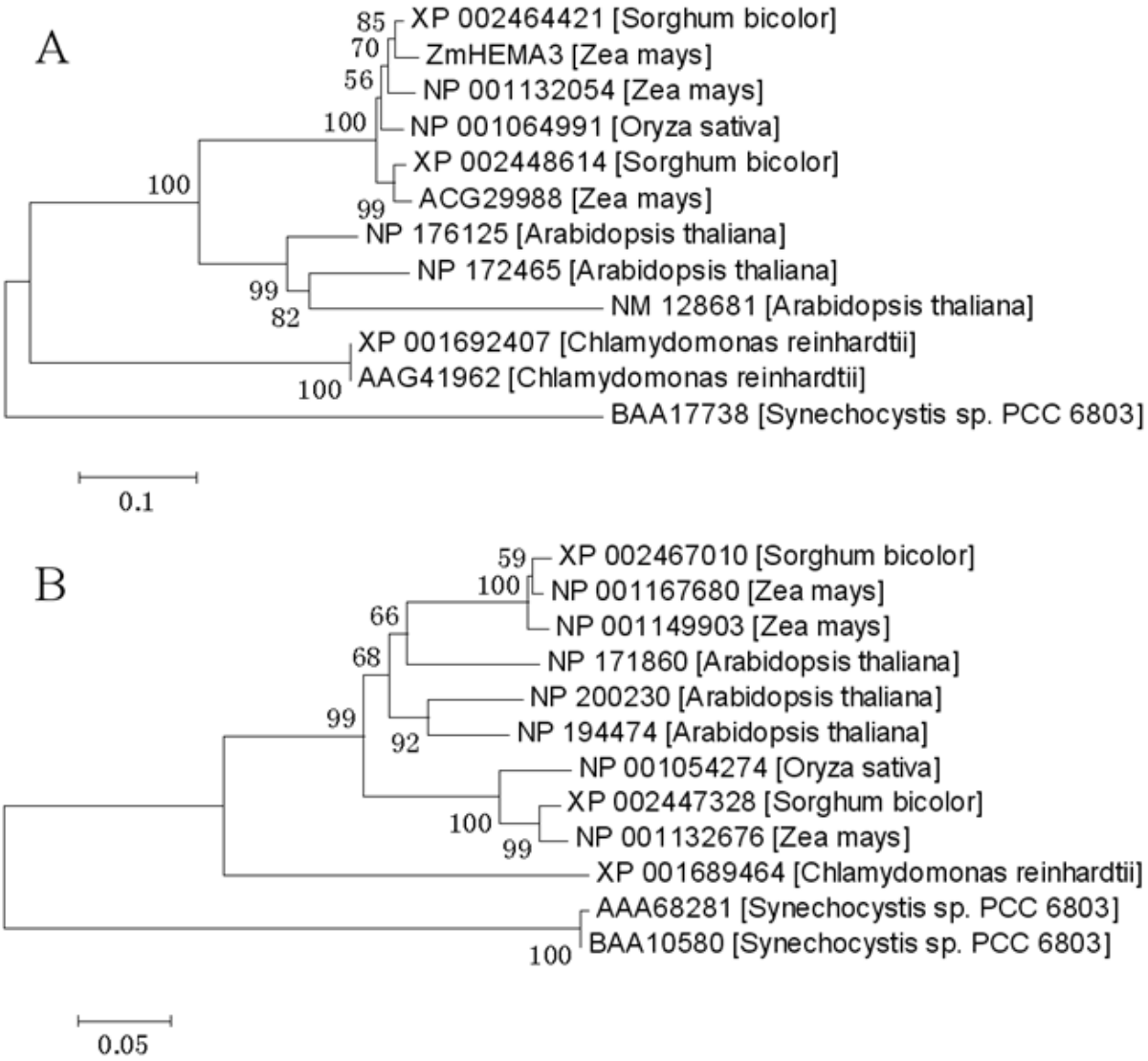

Figure 2. Phylogenetic analysis of HEMA and POR. HEMA (glutamyl-tRNA reductase) and POR (NADPH: protochlorophyllide oxidoreductase) are the two rate-limiting enzymes for Chl biosynthesis. The neighbour-joining method designed in MEGA 4.0 program (Tamura et al., 2007) was used to construct the phylogenetic trees. The length of branch lines indicates the extent of divergence according to the scale at the bottom. A, HEMA; B, POR

to heme or siroheme, rather than to Chl.

Phylogenetic analyses of the Chl metabolic enzymes could provide ideal genetic information for revealing evolutionary relationships (Xiong et al., 2000; Blankenship, 2001; Raymond et al., 2002; Lohr, 2005). Our data revealed that the evolution of Chl metabolic genes

proceeded in a certain direction, starting from *Synechocystis* sp. PCC 6803 and followed in the order: *Chlamydomonas reinhardtii*, Arabidopsis and grasses (Figure 2). In a detailed comparison of HEMA and POR, the two rate-limiting enzymes for Chl biosynthesis showed that maize and sorghum evolved in a more complicated way. Noticeable diversity appeared in the subfamilies of maize and sorghum (Figure 2). Although Chl metabolic genes were not subjected to selective pressure as C_4 genes (Yang, 1997, 2007; Wang et al., 2009), many homologous copies of Chl metabolic genes were lost due to possible gene dosage (Papp et al., 2003). In contrast, the homologs of HEMA and POR in maize and sorghum were retained (Figure 2). We suspected that the diversity between homologs might play a positive role in the evolution of Chl metabolism.

In summary, Chl metabolism had direct impact on photosynthesis. We performed a preliminary research on its expression and evolution in maize, which might help to further understand the genetic mechanism of photosynthesis, so as to improve crops' yield performance in the future.

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