

## Characterization of a new *granule-bound starch synthase* gene found in amaranth grains (*Amaranthus cruentus* L.)

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### [Introduction]

The genus *Amaranthus* includes approximately 60 species that grow in many areas of the world. This crop is one of the New World super grains and is gaining favor among health-conscious consumers. The amylose content of the seed and plant tissues in amaranth is thought to play important roles in palatability and starch quality as in cereals. In this study, we have attempted to identify a new isoforms of starch synthase responsible for amylose synthesis in leaves of the amaranth plant.

### [Materials and Methods]

We used an *A. cruentus* line (accession number: Ames 22004) obtained from the USDA-ARS. The spatio temporal starch accumulation patterns in developing seeds were observed by staining with I2/KI. A cDNA clones (designated *CrGBSSIb*) were isolated from amaranth leaves by reverse transcriptase-mediated PCR (RT-PCR), 5'-rapid amplification of cDNA end (RACE), and 3'-RACE. Expression analysis of amaranth *CrGBSSIb* genes in different tissues (seeds, leaves, petioles, stems and roots) were determined using quantitative real-time PCR (qRT-PCR) analysis.

### [Results and Discussions]

To clarify the mechanism underlying amylose synthesis in the amaranth pericarp, we attempted to identify new *GBSS* isoforms. A new *GBSS*-encoding gene (i.e., *CrGBSSIb*) was isolated from amaranth leaf tissue. The *CrGBSSIb* gene consists of 4699-bp, including a 1938-bp open reading frame encoding 606 amino acids. A comparison of the cDNA and genomic sequences suggested that contains 12 introns and 13 exons. Interestingly, a phylogenetic analysis revealed that the amaranth *GBSSIb* gene evolved independently from the other *GBSSI* isoforms within this crop (i.e., intraspecies) and differed from the other plant *GBSSII* genes. The expression patterns of two *GBSS* isoforms revealed that *CrGBSSIb* and *CrGBSSI* are expressed during the early and late phases of seed development, respectively. Additionally, a high *CrGBSSIb* transcript level was detected in leaf tissue. This result indicates that *CrGBSSI* and *CrGBSSIb*, which affect amylose synthesis in amaranth grains, are active in the perisperm and pericarp, respectively. Therefore, *CrGBSSIb* encodes an enzyme associated with amylose synthesis. The enzyme may be primarily responsible for amylose metabolism in pericarp tissue.

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