

## Evolution of the Waxy Phenotype in Grain Amaranth

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### [Introduction]

Knowledge of the genetic variations in grain amaranths is important for studies on crop evolution. In this study, polymorphisms in a large gene pool of the *Waxy* locus from samples of *A. caudatus* collected from diverse regions were investigated to clarify the evolution of waxy phenotypes.

### [Materials and Methods]

A total of 58 accessions of grain amaranth (*A. caudatus*) were used here. We first investigated the nucleotide diversity and mutation sites in the *Waxy* locus by using the diverse genetic resources of *A. caudatus*. We then investigated the evolution of waxy phenotype from three species of grain amaranth in detail. Finally, we examined the coding sequence of the *Waxy* locus of low-amylose phenotype of *A. caudatus*.

### [Results and Discussions]

Polymorphisms at the *Waxy* locus of *Amaranthus caudatus* collected from wide range of regions have been used to investigate genetic diversity and mutation sites. A comparison of the *Waxy* locus revealed a very high level of sequence conservation. This result showed low environmental and evolutionary variability in their *Waxy* gene well. We also performed screening to confirm the mutation sites in the coding sequences of all accessions. The results indicate that one insertion in the coding region of *Waxy* genes was responsible for the change in perisperm starch leading to the waxy phenotype in all accessions of this species, and thus that a single mutation event altered the regulation of the *Waxy* gene during the domestication of this crop. In addition, phylogenetic analysis showed that waxy phenotypes within each of three species, *A. caudatus*, *A. cruentus* and *A. hypochondriacus*, originated separately or differentiated from nonwaxy phenotypes of each species through a single mutational event (i.e., a frame shift or base substitution). We also compared obvious structural features of the coding sequence of waxy and nonwaxy phenotypes with those of low-amylose phenotypes in *A. caudatus*. The *Waxy* coding sequences of low-amylose phenotypes do not show polymorphisms and are identical with those of waxy phenotypes. This could mean that there is another gene that encodes a key enzyme responsible for amylose synthesis as the elementary quantity in tissues other than perisperm in *A. caudatus*.

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