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Assessing functional diversity in the soybean β-substituted alanine synthase enzyme family

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ABSTRACT

In plants, proteins of the β-substituted alanine synthase (BSAS) enzyme family perform a diverse range of reactions, including formation of cysteine from *O*-acetylserine and sulfide, detoxification of cyanide by its addition to cysteine, the breakdown of cysteine into pyruvate, ammonia, and sulfide, and the synthesis of *S*-sulfocysteine. With the completed genome sequence of soybean (*Glycine max* (L.) Merr. cv. Williams 82), the functional diversity of the BSAS in this highly duplicated plant species was examined to determine whether soybean BSAS enzymes catalyze the various reactions connected to cysteine metabolism. The 16 soybean BSAS can be grouped into clades that are similar to those observed in *Arabidopsis*. Biochemical analysis of soybean BSAS proteins demonstrate that enzymes of clades I and III function as *O*-acetylserine sulfhydrylases for cysteine synthesis, clade II encodes cysteine desulfhydrase activity, and that clade V proteins function as β-cyanoalanine synthase for cyanide detoxification. Although clade IV is similar to *Arabidopsis S*-sulfocysteine synthase, this activity was not detected in the soybean homolog. Overall, our results show that bioinformatics approach provides a useful method to assess the biochemical properties of BSAS enzymes in plant species.

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1. Introduction

Cysteine (**2**) (Fig. 1) biosynthesis in plants plays a central role in providing sulfur to diverse cellular components and metabolic compounds. Structurally, cysteine (**2**) is a building block of proteins, glutathione, and phytochelatins, in which its sulfhydryl group has biochemically important roles in macromolecular structure and function, the reduction of diverse target molecules, and chelation of cytotoxic heavy metals, respectively (Lewandowska and Sirko, 2008). It is also important for the synthesis of methionine, iron–sulfur clusters, glucosinolates, and vitamin cofactors such as thiamin and biotin. Cysteine (**2**)-derived production of methionine, an essential amino acid for livestock and human, emphasizes the agronomical importance of its metabolism in plants, not to mention other sulfur-containing compounds essential for basic metabolism and defense responses in plants (Amir and Hacham, 2008).

Cysteine (**2**) is produced by *O*-acetylserine sulfhydrylase (OASS, EC 2.5.1.47), which catalyzes a β-substitution reaction to replace the acetate group of *O*-acetylserine (**1**) (OAS) with sulfide (Fig. 1) (Bonner et al., 2005). Sulfide for this reaction is produced by the assimilatory reduction of sulfate and OAS is generated by serine acetyltransferase (SAT, EC 2.3.1.30) (Takahashi et al., 2011). These two enzymes form a multienzyme complex (i.e., cysteine regulatory complex; CRC) through physical interaction between the active site of OASS and the C-terminal tail of SAT (Kredich et al., 1969; Droux et al., 1998; Bonner et al., 2005; Francois et al., 2006; Kumaran and Jez, 2007). In plants, it has been shown that SAT activity is enhanced in the CRC compared to that in the free form while OASS activity is completely abolished in the complex (Droux et al., 1998; Kumaran et al., 2009). Based on the observations that the CRC can be dissociated by either OAS (**1**) or cysteine (**2**), but stabilized by sulfide, it was proposed that its formation has a role in flux control of cysteine (**2**) production (Droux et al., 1998; Hell and Hillebrand, 2001). CRC formation also protects SAT from feedback inhibition by cysteine (**2**) (Kumaran et al., 2009). Consistent with its importance in other metabolic processes, an array of control mechanisms affecting diverse steps leading to cysteine (**2**) production has been described (Yi et al., 2010a). In plants, multiple OASS and SAT are encoded by small gene families (Watanabe et al., 2008a,b; Wirtz et al., 2004). OASS is a member of pyridoxal-5′-phosphate (PLP)-dependent β-substituted alanine synthase

Abbreviations: 2-DE, two-dimensional gel electrophoresis; BSAS, β-substituted alanine synthase; CAS, β-cyanoalanine synthase; DES, cysteine desulfhydrase; DMPD, *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride; DTT, dithiothreitol; ESI, electron spray ionization; FPLC, fast protein liquid chromatography; OAS, *O*-acetylserine; OASS, *O*-acetylserine sulfhydrylase; PLP, pyridoxal-5′-phosphate; SAT, serine acetyltransferase; SSCS, *S*-sulfocysteine synthase.

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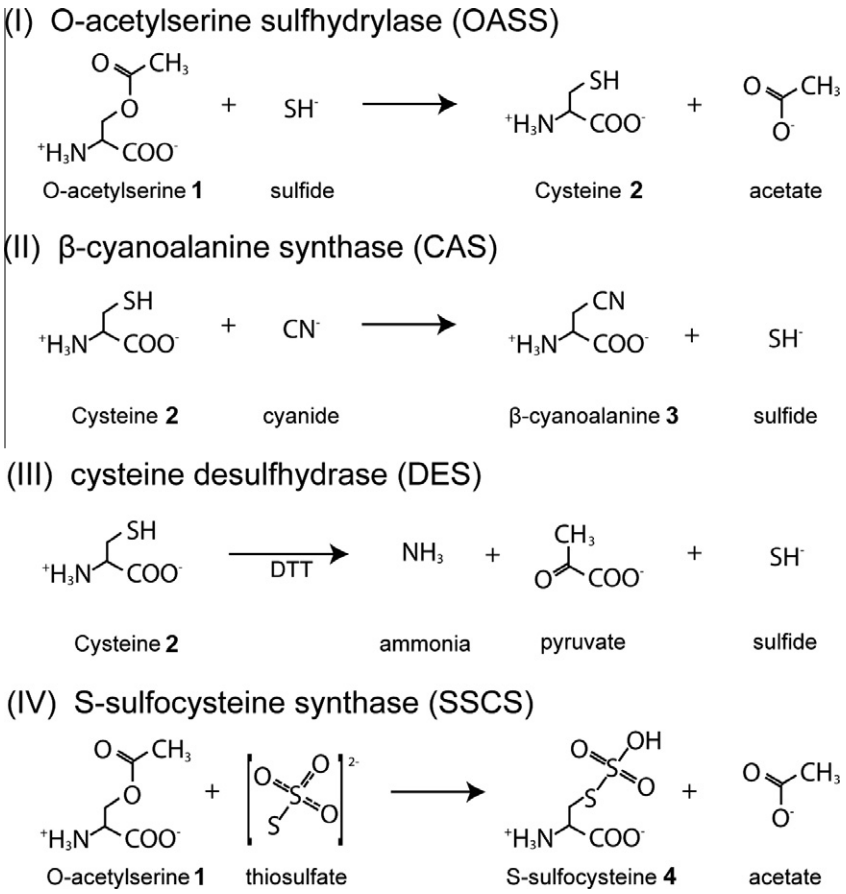

Fig. 1. Reactions in the BSAS family. Overall reactions catalyzed by OASS, CAS, DES, SSCS in the BSAS enzyme family are shown.

(BSAS) enzyme family (Hatzfeld et al., 2000). In addition to multiple isoforms of OASS, which are targeted to the cytosol, mitrochondria, and chloroplasts, the BSAS family includes enzymes with high sequence similarity to OASS, but with little activity for cysteine synthesis (Watanabe et al., 2008a).

Multiple studies demonstrate biosynthetic diversity in the plant BSAS family (Fig. 1). In diverse plant species, β-cyanoalanine synthase (CAS, EC 4.4.1.9), another member in BSAS enzyme family, uses cysteine (**2**) and cyanide as substrates to produce β-cyanoalanine (**3**) and sulfide (Wurtele et al., 1985; Hatzfeld et al., 2000; Warrilow and Hawkesford, 2000; Yamaguchi et al., 2000; Maruyama et al., 2001; Han et al., 2008; Lai et al., 2009). This reaction is important for the detoxification of cyanide generated as a byproduct during ethylene biosynthesis in plants (Yip and Yang, 1988). In *Arabidopsis* and spinach, CAS activity is mainly found in mitochondria where cyanide toxicity is most evident (Hatzfeld et al., 2000; Warrilow and Hawkesford, 2000; García et al., 2010).

A BSAS family member in *Arabidopsis* was recently shown to encode a cysteine desulfhydrase (DES, EC 4.4.1.1), which catalyzes the degradation of cysteine (**2**) to pyruvate, ammonia, and sulfide (Álvarez et al., 2010). Similarly, *S*-sulfocysteine synthase (SSCS, EC 2.5.1.47), yet another BSAS isoform in *Arabidopsis*, converts OAS and thiosulfate into acetate and *S*-sulfocysteine (**4**), which can be metabolized back to cysteine (**2**) and sulfate by reductive conversion (Bermúdez et al., 2010). In addition, other biosynthetic activities have been ascribed to BSAS proteins, including a possible role for OASS in the biosynthesis of pyrrolizidine alkaloids (Noji et al., 1993; Ikegami et al., 1996).

Phylogenetic analyses of BSAS sequences in various plant species have been informative in differentiating CAS from OASS (Hatzfeld et al., 2000; Jost et al., 2000; Han et al., 2008; Lai et al., 2009). Such groupings of BSAS enzymes can identify the major cytosolic and organellar OASS enzymes (Hatzfeld et al., 2000; Yi et al., 2010b). It still remains to be determined though whether BSAS enzymes in other plant species have the four different biochemical activities reported so far only in *Arabidopsis*.

Like other plant species, multiple BSAS-encoding genes have been identified in the soybean genome (Chronis and Krishnan, 2003; Zhang et al., 2008a,b; Yi et al., 2010b). Although OASS activity has been reported for one of these proteins (i.e., GmOASS1 or GLYMA11G00810; Chronis and Krishnan, 2003; Wirtz et al., 2010b), it remains unclear whether CAS, DES, and SSCS activities are also catalyzed by BSAS enzymes in this plant. Because soybean is an economically important cash crop, used for both human consumption and animal feed, and is the target of efforts to improve the content of sulfur–amino acid content, elaboration of the biochemical roles of various BSAS in this plant would help define possible targets for improving nutritional content (Krishnan, 2005; Kim et al., 2012). With the completed soybean genome and predictions based on phylogenetic analysis, the biochemical properties of representative BSAS-related proteins in soybean were characterized, whose collective activities may affect cysteine (**2**) levels in the cell.

2. Results and discussion

2.1. The BSAS family in soybean

The amino acid sequences of eight *Arabidopsis* BSAS with four different biochemical activities (i.e., OASS, CAS, DES, and SSCS),

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