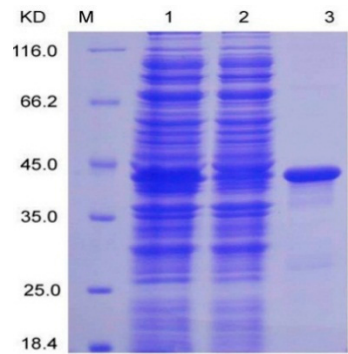
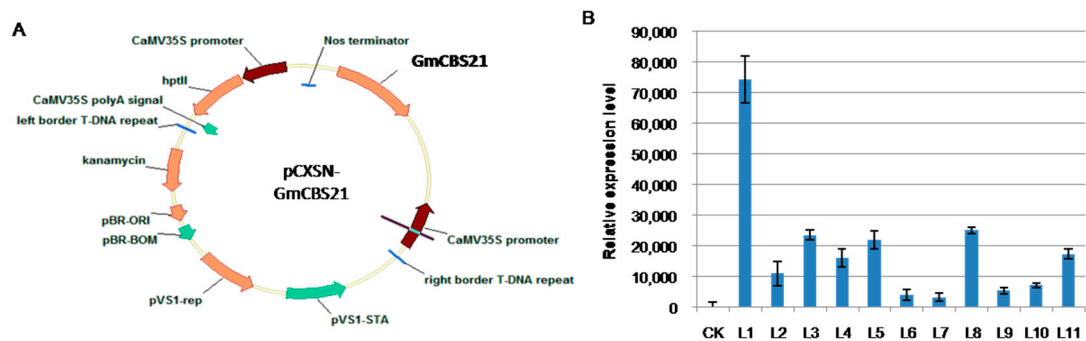


# Supplementary Materials: Identification and Comparative Analysis of CBS Domain-Containing Proteins in Soybean (*Glycine max*) and the Primary Function of *GmCBS21* in Enhanced Tolerance to Low Nitrogen Stress

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**Figure S1.** SDS-PAGE of pCZN1-GmCBS21-His fusion protein purification. M. Protein Marker; 1.Un-purified; 2. Flow through; 3 Elution.



**Figure S2.** Generation and RT-PCR testing of GmCBS21-OXP lines. (A) GmCBS21 overexpression plasmid profile; (B) The relative expression level of GmCBS21-OXP lines.

**Table S5.** The calculation method of cystathionine activities.

| Serial Number | S     | B     | A     | Cystathionine (U) [(S – B)/A] × 2.2                                   |
|---------------|-------|-------|-------|---|
| 1             | 2.112 | 1.937 | 0.124 | $[(S - B)/A] \times 2.2 = [(2.010 - 1.960)/0.119] \times 2.2 = 0.924$ |
| 2             | 1.948 | 1.970 | 0.112 |   |
| 3             | 1.916 | 1.979 | 0.125 |   |
| 4             | 1.952 | 1.917 | 0.120 |   |
| 5             | 2.123 | 1.995 | 0.113 |   |
| average       | 2.010 | 1.960 | 0.119 |   |

S: assay group absorbance; value: control group absorbance; value A: absorbance value of 1 μmol cystathionine.

**Table S6.** The information of genes involved in nitrogen uptake, assimilation, and remobilization of transgenic plants.

| Abbreviated Name | Full Name                                 | TAIR Coding Number |
|------------------|---|--------------------|
| <i>ATANR1</i>    | MADS-BOX TRANSCRIPTION FACTOR ANR1        | AT2G14210          |
| <i>AtNRT1.1</i>  | nitrate transporter 1.1                   | AT1G12110          |
| <i>AtCKX2</i>    | cytokinin oxidase 2                       | AT2G19500          |
| <i>AtIPT3</i>    | isopentenyltransferase 3                  | AT3G63110          |
| <i>AtNR1</i>     | nitrate reductase 1                       | AT1G77760          |
| <i>At14-3-3</i>  | 14-3-3 family protein                     | AT1G22290          |
| <i>ATAMT1</i>    | ammonium transporter 1                    | AT4G13510          |
| <i>AtAAP1</i>    | amino acid permease 1                     | AT1G58360          |
| <i>AtNiR1</i>    | nitrite reductase 1                       | AT2G15620          |
| <i>AtPII</i>     | Nitrogen regulatory PII-like              | AT2G33740          |
| <i>AtGS1</i>     | glutamate synthase 1                      | AT5G04140          |
| <i>AtNADPH1</i>  | NADH-dependent glutamate synthase 1       | AT5G53460          |
| <i>AtAlaAT</i>   | alanine aminotransferase                  | AT1G17290          |
| <i>AtAspAT1</i>  | aspartate aminotransferase 1              | AT2G30970          |
| <i>AtDof1</i>    | DOF zinc finger protein 1                 | AT1G51700          |
| <i>AtPEPC1</i>   | phosphoenolpyruvate carboxykinase 1       | AT4G37870          |
| <i>AtGDH2</i>    | glutamate dehydrogenase 2                 | AT5G07440          |
| <i>AtPPDK</i>    | pyruvate orthophosphate dikinase          | AT4G15530          |
| <i>AtPK1</i>     | pyruvate kinase family protein 1          | AT3G49160          |
| <i>AtASN1</i>    | glutamine-dependent asparagine synthase 1 | AT3G47340.1        |
| <i>AtGS2</i>     | glutamine synthetase 2                    | AT5G35630          |
| <i>AtGDH1</i>    | glutamate dehydrogenase 1                 | AT5G18170          |
| <i>AtAlaAT2</i>  | alanine aminotransferase 2                | AT1G72330          |
| <i>AtAspAT</i>   | aspartate aminotransferase                | AT2G22250          |
| <i>AtPK2</i>     | Pyruvate kinase family protein 2          | AT3G22960          |
| <i>AtAAP2</i>    | amino acid permease 2                     | AT5G09220          |