

**Title: A *GAMYB* homolog *CsGAMYB1* regulates sex expression of cucumber via an ethylene-independent pathway**

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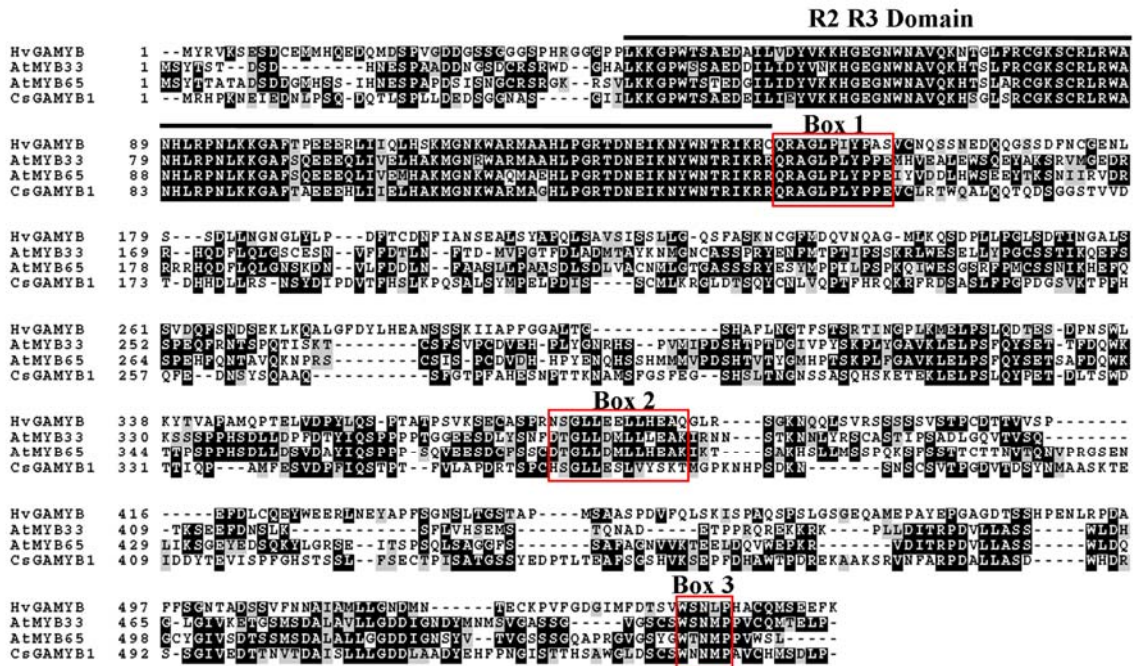
**Supplementary material**

Figure S1. Sequence alignment of amino acid residues of *CsGAMYB1* with other *GAMYB* proteins.

Table S1. *CsGAMYB1* can partially rescue the fertility of *myb33myb65* in *Arabidopsis*.

Table S2. Overexpression of *CsGAMYB1* resulted in partial sterility in *Arabidopsis*

Table S3. List of primers and their uses.



**Figure S1. Sequence alignment of amino acid residues of CsGAMYB1 with other GAMYB proteins.** The identical and similar residues are shown in black and gray, respectively. The highly conserved R2R3 domain and Boxes 1, 2, 3 are indicated in the black line and red boxes, respectively. Hv, *Hordeum vulgare*.

**Table S1 *CsGAMYB1* can partially rescue the fertility of *myb33myb65* in *Arabidopsis***

| Genotype                       |        | Total Number of<br>Siliques/Plant | Number of Filled<br>Siliques/Plant | Percentage of<br>Filled Siliques (%) |
|--------------------------------|--------|-----------------------------------|------------------------------------|--------------------------------------|
| <i>myb33myb65</i>              |        | 228.1±17.4                        | 6.3±1.1                            | 2.8±0.5                              |
| <i>35S:CsGAMYB1 myb33myb65</i> | Line 1 | 220                               | 60                                 | 27.3                                 |
|                                | Line 2 | 227                               | 70                                 | 30.8                                 |
|                                | Line 3 | 220                               | 63                                 | 28.6                                 |
|                                | Line 4 | 255                               | 84                                 | 32.9                                 |
|                                | Line 5 | 208                               | 54                                 | 26.0                                 |
|                                | Line 6 | 228                               | 60                                 | 26.3                                 |
|                                | Line 7 | 261                               | 120                                | 46.0                                 |
|                                | Line 8 | 233                               | 180                                | 77.3                                 |

The values shown are the means ± SE of more than 10 plants from *myb33myb65*.

**Table S2 Overexpression of *CsGAMYB1* resulted in partial sterility in *Arabidopsis***

| Genotype                |        | Total Number of<br>Siliques/Plant | Number of Filled<br>Siliques/Plant | Percentage of<br>Filled Siliques (%) |
|-------------------------|--------|-----------------------------------|------------------------------------|--------------------------------------|
| <i>Col</i>              |        | 114.8±9.9                         | 105.9 ±9.8                         | 92.2±1.7                             |
| <i>35S:CsGAMYB1 Col</i> | Line 1 | 127                               | 65                                 | 51.2                                 |
|                         | Line 2 | 106                               | 23                                 | 21.7                                 |
|                         | Line 3 | 111                               | 26                                 | 23.4                                 |
|                         | Line 4 | 118                               | 5                                  | 4.2                                  |
|                         | Line 5 | 105                               | 5                                  | 4.8                                  |
|                         | Line 6 | 97                                | 2                                  | 2.1                                  |
|                         | Line 7 | 114                               | 3                                  | 2.6                                  |
|                         | Line 8 | 103                               | 2                                  | 1.9                                  |

The values shown are the means ± SE of more than 10 plants from *Col*.

**Table S3 List of primers and their uses**

| <b>Primer</b> | <b>Sequence</b>                                       | <b>Used for</b>                             |
|---------------|---|---|
| CsGAMYB1-F    | 5'-ATGCGGCATCCGAAAAATGAGA-3'                          | Cloning of <i>CsGAMYB1</i>                  |
| CsGAMYB1-R    | 5'-TCAAGGAAGATCAGACATATGACATACT-3'                    |   |
| q-CsGAMYB1-F  | 5'-TCTAACCTACCACAAAGAACGC-3'                          | qRT-PCR of <i>CsGAMYB1</i>                  |
| q-CsGAMYB1-R  | 5'-TCTATCTGGTGCCAACACAAAAGT-3'                        |   |
| CsGAMYB1-SP6  | 5'-GATTTAGGTGACACTATAGAATGCTTAACCCTACCACAAAGAACGCC-3' | In situ probes of<br><i>CsGAMYB1</i>        |
| CsGAMYB1-T7   | 5'-TGTAATACGACTCACTATAGGGGCTGAATGTGTTGTTGAAATCCC-3'   |   |
| G-CsGAMYB1-F  | 5'-GGAATTCATGCGGCATCCGAAAAATGAGA-3'                   | CsGAMYB1-GFP<br>construct                   |
| G-CsGAMYB1-R  | 5'-CGGGATCCCAGGAAGATCAGACATATGACATACT-3'              |   |
| O-CsGAMYB1-F  | 5'-GAAGATCTGATGCGGCATCCGAAAAATGAGA-3'                 | <i>CsGAMYB1</i><br>overexpression construct |
| O-CsGAMYB1-R  | 5'-GGACTAGTTCAAGGAAGATCAGACATATGACATACT-3'            |   |
| I-CsGAMYB1-F1 | 5'-AGGCGCGCCTACCGAGGTTATTTCTCCATTTG-3'                | <i>CsGAMYB1</i> -RNAi<br>construct          |
| I-CsGAMYB1-R1 | 5'-CGATTTAAATTGACATACTGCTGGCATGTTATT-3'               |   |
| I-CsGAMYB1-F2 | 5'-GACTAGTTACCGAGGTTATTTCTCCATTTG-3'                  |   |
| I-CsGAMYB1-R2 | 5'-CGGGATCCTGACATACTGCTGGCATGTTATT-3'                 |   |
| CsACS1G-F     | 5'-AGAGGAGTAACGAAGACGAGGAAGT-3'                       | qRT-PCR of CsACS1G                          |
| CsACS1G-R     | 5'-CCACTCACCAACATTTTCTGTCTTT-3'                       |   |
| CsACS2-F      | 5'-GAAAACCTGTGAGGGAGAAGGGAA-3'                        | qRT-PCR of CsACS2                           |
| CsACS2-R      | 5'-GATAGTAAGGAGTGGGGACAAGCA-3'                        |   |
| TUA-F         | 5'-ACGCTGTTGGTGGTGGTAC-3'                             | Internal controls in<br>cucumber            |
| TUA-R         | 5'-GAGAGGGGTAAACAGTGAATC-3'                           |   |
| Actin2-F      | 5'-CCTTCGTCTTGATCTTGCGG-3'                            | Internal controls in<br><i>Arabidopsis</i>  |
| Actin2-R      | 5'-AGCGATGGCTGGAACAGAAC-3'                            |   |