a

b


Supplementary Figure S1. Construction of expression vectors for plant transformation. (a) pBPFAA. (b) pBPOD.


Supplementary Figure S2. Typical photos demonstrating shoot regeneration under selection medium and then plant regeneration of transgenic Bidens pilosa plants by transforming two expression vectors and Agrobacterium-mediated method. Panels (a) and (c): transgenic FAA lines; panels (b) and (d): transgenic OD lines; panel (e): transgenic plants together with wild-type plants were grown in greenhouse. Mature flower from a transgenic plant is shown in a small panel at (e).


Supplementary Figure S3. Examination of PCR products between genomic DNA and cDNA for $F A A$ and $O D$ genes in Bidens pilosa. Plasmids pBPFAA and pBPOD were used as cDNA template, PCR was carried out using specific FAA (FAA-F and FAA-R) and OD (OD-F and OD-R) primers to amplify full-length regions of FAA (1134 bp) and OD (1152 bp), and compared with PCR products of genomic DNA isolated from wild-type B. pilosa var. radiata.


Supplementary Figure S4. Representative HPLC profiles of WT and a few randomly selected transformants. Chemical structures for seven polyacetylenic (PA) compounds have been determined by NMR spectroscopy as previous report [8]. The UV detection wave length was set at 245 nm . Retention times for PA compounds $1(11 \mathrm{~min}), 2 \& 3$ (co-eluted at 13 min$), 4(17$ min ), $5 \& 6$ (co-eluted at 18 min ) and $7(35 \mathrm{~min})$ were determined as previously described [4,8,48].

Supplementary Table S1. Primers used for this study.

| Primer | Sequence (5' $\rightarrow 3^{\prime}$ ) |
| :--- | :--- |
| 35S Pro-F1 | TGA TAT CTC CAC TGA CGT |
| FAA-r2 | CAA AGT GAA CAC TCG AC |
| FAA-F | ATG GGT GCA GGT GGC CGG |
| FAA-R | TTA AAA CTT ATG GTA CCA |
| OD-F | ATG GGT GCA GGC GGG CGA |
| OD-R | TCA TAT GTT ATT ACG GTA CCA A |
| Kan-F | GCA TGC TCG GTG TGC TTT AC |
| Kan-R | GCC CCT TTG ATC CAG TTC CA |
| qFAA-F2 | AAC TCT AAC CCT TGG CTG GC |
| qFAA-R2 | AAC CCA TTC ACC ACG AGC AA |
| qOD-F3 | CAT CAT GTG GTA AAG GTC GTA ATG |
| qOD-R3 | CGC TTA TGA CCT CCC CCT CTA |
| qL2-F | GGA |
| qL2-R | GAT CAT AGC CCA CGA GTG CGG |
| cOD-f1 | CTA TGA CCG CTT CGC ATG CCA |
| cOD-f2 | CFTG TTC GAG GTT GAG AAC AGA TGG TG |
| cOD-r1 | cOD-r2 |

