

Down-regulation of the *IbEXP1* gene enhanced storage root development in sweetpotato

Seol Ah Noh, Haeng-Soon Lee, Youn-Sung Kim, Kyung-Hee Paek, Jeong Sheop Shin,
Jung Myung Bae*

SRD2 : CAT CCTCTACCAATTCACACGCGCAATAA CAATGGCGGTCTTGGAGCTCTCTCTGGTGGAGTTCTTGCCAGCTTCTCCGGTG CATGCTACTGGG : 100
IbEXP2 : ----->----- : -
IbEXPL1 : ---TTCTCTCAAGATCAAGACGGCCACATTTATA-----TCCTCTCCCCTGATTTCTAGTTCG--ATCTCCAATTCAAACCTA--AGG : 79

SRD2 : GCTGGAGCAGCGCTCGGCCACCTTCTACGGCGGGGTGATGCTTCTGGAACAATGGCGGAGCCTGCGGGTATGGGAACCTGTATAGCTCAGGCTATGG : 200
IbEXP2 : -----GGAACAATGGG----- : 11
IbEXPL1 : GCGCGG-----TTGAACAAT----- : 94

SRD2 : CACCAACACTGCGCACCTTAGCAACGCGCTCTTCAACAATGGGCTCAGCTCGGGTCTCTGTTTCAGATAGGCTGTGGAACGACCGCTCTGCTCCGCG : 300
IbEXP2 : -----GCGTTGAGCAGCGGCGCTCTTCAACAATGGGCTCAGCTCTGAGCTCTGTTTCAGATCAGAGTGTGTAAGGACAAAGTCTGCTATGCC : 98
IbEXPL1 : -----ATAATAAATGGCTCTCTCTGATCATCATCTG---TGCGCGCTCTGTTTCT-----TGTGAGCAGCATC---CTCATCTGCTC : 166

SRD2 : GGCG-----AAATCACCGTCAACGCCACCAA-----TTTCTGCCGCGCC-----GGCGGCTGGTGGAGCGCCCAAC : 363
IbEXP2 : GGCGCCCGCTATCTTTATTAACGCCACCAA-----TTTCTGCCCTCTTAATATGCTTTGCCTAACGACAATGGCGGCTGGTGAACCCCTCCCGC : 191
IbEXPL1 : TGCATCCGCTTGGCATCGCGCTCCACCAAAACCAAAAGTCTCTTTCTTTTC-----CAAACTTCCC-----CCCCAGTCC : 238

SRD2 : CCACACTTT--GATCT--CTCTCAGCTGTTTTCTTGAAGATTGGCCAGTAAFAAGCGGAGTGTCTCCGTTCTTAC-----CGAGGGGTGC--- : 448
IbEXP2 : TCGCATTTT--GATCT--CCGCATGCTATGCTGCTGAAGATCGGAGAGTACCCCGCGGGAATTGTCCCGTCTGTTAC-----GGCGAAGTGC--- : 276
IbEXPL1 : TGGCGCTTCCGATATGGCTCCATGGCTATTTGCTTTCAA-----CGAGGACATCTTCTGCCSCAGTCCCTACCGTTTACAAGGATGGTGAAGCTTCCGCT : 335

SRD2 : -----CTTGACAGGAGGAGTGGAGCAATCAGGTTG-----AC-CATTAA-----CGGCCAATGCTTTCT-----TCAAC-CTGGTA-----CTAG : 514
IbEXP2 : -----CTTGCAAAAAGAGGCGGGATCCGGTTC-----AC-RAACAA-----CGGCCACCGCTACT-----TCAAC-CTGATTT-----TTGA : 342
IbEXPL1 : GCATGTTTTCAGATGAGATGCAAGACCCAAATCTGTGTACTTAAGCAAGGACCACATCATCGTTACTGATCTCAACACAAATTAACCAACAGATTTTG : 435

SRD2 : TAACCAACCTGGGAGGCTCCCGCGACCTAACACCGCG-TGTACATCA-ANGGTCMAAAGC-----CGG--TGGCA--AATGA--TGCACAGAAACTGG : 601
IbEXP2 : TCAGGAACGTGGGCGGACGACGGGACAT-CTGAGGGCGAGCGTGA-AGGGCTCGAAGC-----CGGG-TGG-----ATGGACTTGAAGCCGGAACCTGGG : 429
IbEXPL1 : TCATCAGCAGCAGAGCTTTCAGAGCCAT-----GGTACTCAGGGCAAGGATCAAGACCTCCCTCAAGCTTGGCATTGCCTGATCTCGACTTTAAAGGGT : 529

SRD2 : GCC--AAAACCTGGGAGCAACAAAGCCAACTCTCAAGGGCCAAAGCCTCTGATFCCGGGGTGGTACAGGGCGAC--AGCCGGAGGCTCGTC---TCCTACAAAC : 693
IbEXP2 : GAC--AAAACCTGGGAAAGAAAGCCGAATTGATGGGCCAATCGCTCTGCTFCCCGGTACAGGGCAGCGAT--CGCCGCTCTCTACT---TCCTGGAAAC : 521
IbEXPL1 : GCCCTGTGTAATATAAAACCAA---GAATTGGGGTGC-----TTGTGCGAAAGAACAGCCAGAAACCTATTACTTGCATCAAAATTCCTGTACC : 618

SRD2 : GCCTGTCGCCCGGCTGCTCTCTCGGCCA--CACCTA--TCCGGCGCCAGTTCCGCTAGGCGCGAA-ATCAACAAACACCCCATTTTTCCTCCGCCATA : 790
IbEXP2 : ATCGCCCTCCACTGCGCATTCGGCCA--AACTTGGCTCGGAAAGCACTCCG---AGCTGAA-ATAAGGAAGAGGCACTA-----GCATA : 608
IbEXPL1 : AAGCTGG--TCASACTGAAATGTTGCTGTTGAGTTCGCTCAGTACAGGCTCTCA--AACTGGAACCTTATGAGCAAGAAATCAAGGA-----GCTATT : 707

SRD2 : ---ATAATATCCTCAACCTATACATAACTAAAGCCTACACCATTTTACAGTTTGAATTCATTTAAAGTCATGGGGATGGGAAA--AGTTGAT : 885
IbEXP2 : ---ATAA---TCCAGATTT-----CCCG---CTTTTCAGATCTAATTTGCT---AGAAAC--AGTACCC : 665
IbEXPL1 : TGGGACAGCA---CASAGTC-----CCTAGCGGGGCTTTCGAAATTCGATTTGTTTCTC-----ACCCAGGGTACGACGGC : 776

SRD2 : CAA-CTTCCGGGCGCCCTCTCTCACTTTTCTTCTTAAAGGGATTGGTTTGTATCGAAA GCCCTTTGGGCTTAAAATTGGCCATFCAAT-CAACAG : 983
IbEXP2 : AGA-TCTTTGGGC---TCTTTTACGCTCTCTCT-----TCTA---CTTCTGTCGCTGG---TTCAGT-EGGC--- : 724
IbEXPL1 : AAATGCTATTGGGCTAAGAACTAATCTCTCCGAT-----TGGAAA---ACGAGTCATA-----TATGATCGGGCT-- : 842

SRD2 : AATTGAACACAGASTGAAAGTGCTAGTATGTTATATCAAGATTGTGCTACCCATGACTAGCTTAAATTGATAC---TG--CATTATT-CA--TGTSAITA : 1074
IbEXP2 : --TTGGG---AGT---GTGAAGCTGCA-----AAATTGCTTGGTGGATGG--AACTT--TTAG-AC---CA--TTTATT- AAGGTGTSTGTG : 797
IbEXPL1 : --TTGCAATGACT---GACATTCCTCAAG---AAGGTGCTCT--CCATGAGCAGCATC--TTGG-ACATCTTAATTTTATTCTACCTTTATTTA : 929

SRD2 : TT---TTATTA-TGCAGCAAAANTGTGCTGCAACCTACCTG--GGAAC---AACATTA-ATTTT---TTTTCTCGTCTTTCTTCTG--CGTCTT-T : 1161
IbEXP2 : TT---GGGATT--TGTCTCAA--GTGTATTAGACCCCAAC---AGAGC---AGCTGAA-GTCTGCGCAGAAATGCTAGCCCGC-AGCTGTT-C : 876
IbEXPL1 : CTTTACAAATTAAGTTCGACAAAC---CCATTGATACCATCACCTTTTAACTTTTACCATATATAGTGCACAAAGATACTTATGATATTATGC : 1026

SRD2 : GTAATTAGTATAGCATTAAGCTTAAACAGCTA-ANG-CTGAGCTGGA-CACAGT----- : 1213
IbEXP2 : GTATTTA-CA-AGTAGTCAAGTACTAATAATTAATGAATTATATATATGACTGTGTTTC : 935
IbEXPL1 : ACATTTTACAAA-ACTTAAT--AATAGTAAAGA---TATAATTCAAAAATAA----- : 1076

Figure S1. Nucleotide positions of *IbEXP1*-, *IbEXP2*-, and *IbEXPL1*-specific primers. Red, green, and blue arrows indicate the nucleotide positions of *IbEXP1*-, *IbEXP2*-, and *IbEXPL1*-specific primers, respectively, used in RT-PCR and qRT-PCR. The full-length *IbEXP1* cDNA was amplified using the forward primer indicated by black arrow and the reverse primer indicated by red arrow and used in construction of the binary vector containing *IbEXP1* cDNA in an antisense orientation.

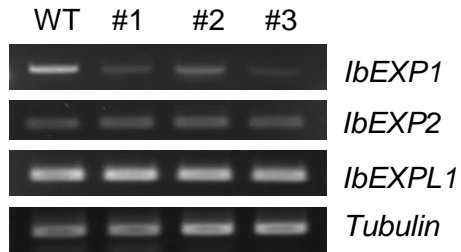
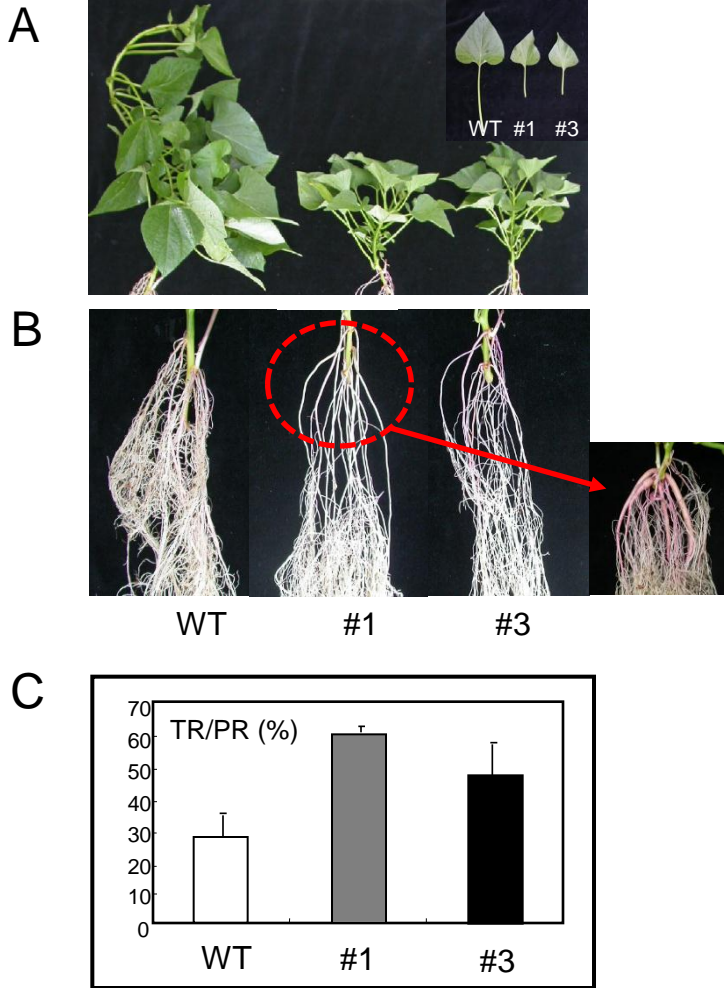


Figure S2. Transcript levels of *IbEXP2* and *IbEXPL1* in *IbEXP1*-antisense plants. Total RNA was isolated from fibrous root tissue of the sweetpotato plants cultured in vitro. Semi-quantitative RT-PCR analysis was conducted with *IbEXP1*-, *IbEXP2*-, or *IbEXPL1*-specific primers. Numbers #1-#3 represent *IbEXP1*-antisense sweetpotato lines #1-#3. Sweetpotato β -*tubulin* was used as an equal loading internal control.

6 weeks after planting



10 weeks after planting

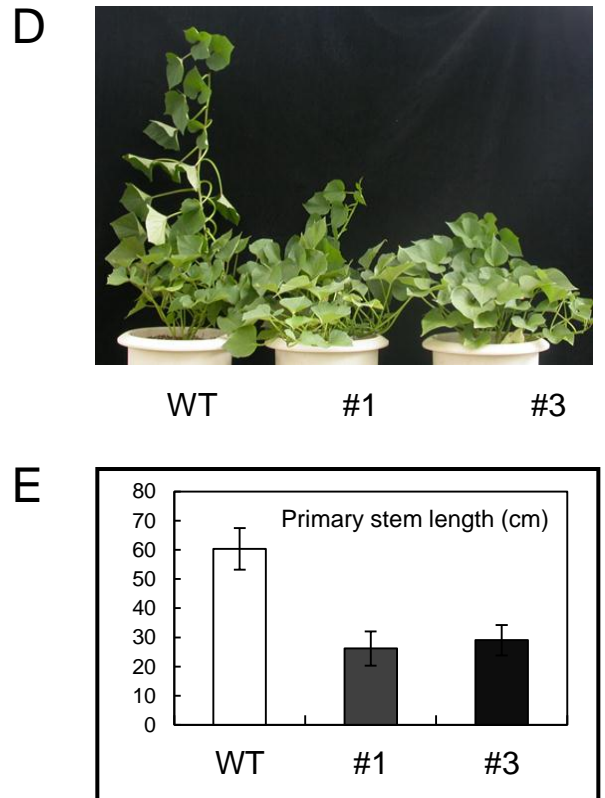


Figure S3. Growth of *IbEXP1*-antisense sweetpotato plants. (A) Shoot growth of *IbEXP1*-antisense plants at 6 weeks after planting. (B) Root growth of *IbEXP1*-antisense plants at 6 weeks after planting. Red arrow indicates young storage roots observed at 8 weeks after planting that developed from the thick roots in the red dotted circle. (C) Thick root development in *IbEXP1*-antisense plants at 6 weeks after planting. TR, thick root; PR, primary root. (D) Shoot growth of *IbEXP1*-antisense plants at 10 weeks after planting. (E) Growth of primary stem in *IbEXP1*-antisense plants at 10 weeks after planting. (C and E) Data were collected from the sweetpotato plants at 6 or 10 weeks after planting and are the means \pm SD from three separate measurements of three individual plants. (A-E) Numbers #1 and #3 represent *IbEXP1*-antisense sweetpotato lines #1 and #3, respectively. Sweetpotato plants were grown in large cylindrical pots [25 (top diameter) \times 23 (bottom diameter) \times 23 (height) cm] containing commercial horticultural potting soil (Baroker; Seoul Bio, Chungcheongbuk-do, Korea) in the greenhouse at 25–30°C under a long-day photoperiod (16/8 h, light/dark). No additional fertilizer was added. WT, wild-type.

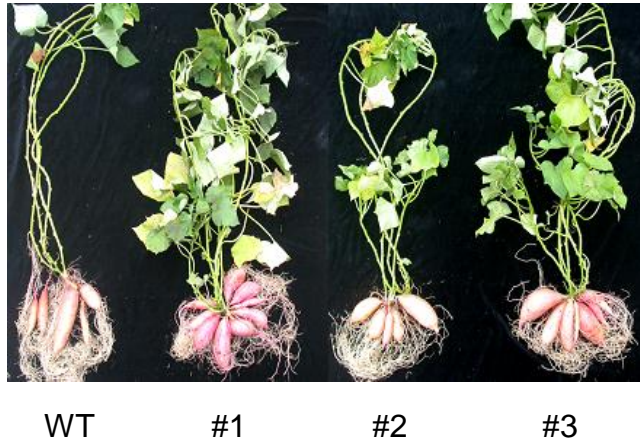


Figure S4. Phenotypic alteration in *IbEXP1*-antisense sweetpotato plants grown in soil. Sweetpotato plants were grown in large [27 x 27 x 24 (height) cm] pots containing commercial horticultural potting soil (Baroker; Seoul Bio, Chungcheongbuk-do, Korea) in the greenhouse at 25–30°C under a long-day photoperiod (16/8 h, light/dark) and harvested at 5 months after planting. No additional fertilizer was added. Numbers #1-#3 represent *IbEXP1*-antisense sweetpotato lines #1-#3, respectively. WT, wild-type.