G3 Genes | Genomes | Genetics

The *ben1-1* brassinosteroid-catabolism mutation is unstable due to epigenetic modifications of the intronic T-DNA insertion

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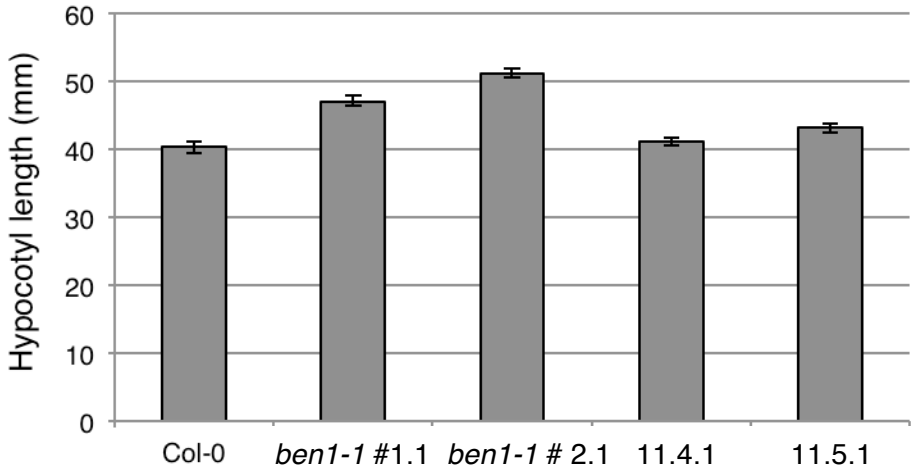

Figure S1 The attenuated hypocotyl-elongation phenotype of the re-isolated *ben1-1* lines is stably inherited to the next generation. The hypocotyl-elongation phenotype of progeny from the re-isolated *ben1-1* lines is significantly different from the original *ben1-1* lines ($p < 0.05$). Seedlings were grown in darkness or 9 µmol m^{-2} sec^{-1} of white-light for five days before being digitized and measured. Hypocotyl lengths were then normalized to seedlings grown in the dark and expressed as a percentage change. Error bars represent standard error (SE). To calculate SE, each seedling value in light was normalized to the average of the genotype in dark. The resulting group of values was used to calculate standard error (SE).

```
ben1_O_NPTII-F1  NNNNNNNACNTNNNNGAGCCACTCAGCCGCGGGTTTCTGGAGTTTAATGAGCTAAGCACA
ben1_R_NPTII-F1  NNNNNNNACNTNNANGANCCNCTCAGCCGCGGGTTTCTGGAGTTTAATGAGCTAAGCACA
                 ************* *** ** ************************************

ben1_O_NPTII-F1  TACGTCAGAAACCATTATTGCGCGTTCAAAAGTCGCCTAAGGTCACTATCAGCTAGCAAA
ben1_R_NPTII-F1  TACGTCAGAAACCATTATTGCGCGTTCAAAAGTCGCCTAAGGTCACTATCAGCTAGCAAA
                 ************************************************************

ben1_O_NPTII-F1  TATTTCTTGTCAAAAATGCTCCACTGACGTTCCATAAATTCCCCTCGGTATCCAATTAGA
ben1_R_NPTII-F1  TATTTCTTGTCAAAAATGCTCCACTGACGTTCCATAAATTCCCCTCGGTATCCAATTAGA
                 ************************************************************

ben1_O_NPTII-F1  GTCTCATATTCACTCTCAATCCAAATAATCTGCACCGGATCTGGATCGTTTCGCATGATT
ben1_R_NPTII-F1  GTCTCATATTCACTCTCAATCCAAATAATCTGCACCGGATCTGGATCGTTTCGCATGATT
                 ************************************************************

ben1_O_NPTII-F1  GAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTAT
ben1_R_NPTII-F1  GAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTAT
                 ************************************************************

ben1_O_NPTII-F1  GACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAG
ben1_R_NPTII-F1  GACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAG
                 ************************************************************

ben1_O_NPTII-F1  GGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGAC
ben1_R_NPTII-F1  GGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGAC
                 ************************************************************

ben1_O_NPTII-F1  GAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGAC
ben1_R_NPTII-F1  GAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGAC
                 ************************************************************

ben1_O_NPTII-F1  GTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTC
ben1_R_NPTII-F1  GTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTC
                 ************************************************************

ben1_O_NPTII-F1  CTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGG
ben1_R_NPTII-F1  CTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGG
                 ************************************************************

ben1_O_NPTII-F1  CTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAG
ben1_R_NPTII-F1  CTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAG
                 ************************************************************

ben1_O_NPTII-F1  CGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCAT
ben1_R_NPTII-F1  CGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCAT
                 ************************************************************
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ben1_O_NPTII-F2  ACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTG-TCGAT
ben1_R_NPTII-F2  ACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTNNGTCGAT
                 **************************************************   *****

ben1_O_NPTII-F2  CAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTC
ben1_R_NPTII-F2  CAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTC
                 ************************************************************

ben1_O_NPTII-F2  AAGGCGCGCATGCCCGACGGCGATGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCG
ben1_R_NPTII-F2  AAGGCGCGCATGCCCGACGGCGATGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCG
                 ************************************************************

ben1_O_NPTII-F2  AATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTG
ben1_R_NPTII-F2  AATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTG
                 ************************************************************

ben1_O_NPTII-F2  GCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGC
ben1_R_NPTII-F2  GCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGC
                 ************************************************************

ben1_O_NPTII-F2  GAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATC
ben1_R_NPTII-F2  GAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATC
                 ************************************************************

ben1_O_NPTII-F2  GCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGNN-GAAATGAAAG
ben1_R_NPTII-F2  GCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGNNCNAAATGAANN
                 ************************************************  *******
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Figure S2 ***NPTII*** **gene sequence did not change in the pre-triple and post-triple** ***ben1-1*** **mutants.** The *NPTII* gene was amplified using NPT-Fv1, NPT-Fv2 and NPT-Rv from the pre-triple and post-triple *ben1-1* lines. Primers NPT-Fv1 and NPT-Fv2 were used for sequencing *NPTII* gene (Table S1). The color coded yellow nucleotides show the complete *NPTII* coding region in the sequence.

(A)

```
ben1_O_pBEN1_BST  GGAATTTAATAATAATGTTTGTAATAAATGGATAAAGAATGTGAATTATTTATATAAATG
ben1_T_pBEN1_BST  GGAATTTAATAATAATGTTTGTAATAAATGGATAAAGAATGTGAATTATTTATATAAATG
Col-0_BST         GGAATTTAATAATAATGTTTGTAATAAATGGATAAAGAATGTGAATTATTTATATAAATG
Col-0_no_BST      GGAATCCAATAACAATGTTTGTAATAAACGGACAAAGAATGTGAATTATTCACATAAATG
                  *****  ***** *************** *** ***************** * *******

ben1_O_pBEN1_BST  ATTTGTAAAATGTGAGTATTTGATTATAATGGATATAATAAAATAAAAATTGTAATAAAG
ben1_T_pBEN1_BST  ATTTGTAAAATGTGAGTATTTGATTATAATGGATATAATAAAATAAAAATTGTAATAAAG
Col-0_BST         ATTTGTAAAATGTGAGTATTTGATTATAATGGATATAATAAAATAAAAATTGTAATAAAG
Col-0_no_BST      ATCTGTAAAATGTGAGCATTTGATTATAACGGATATAATAAAACAAAAATTGTAATAAAG
                  ** ************* ************ ************* ****************

ben1_O_pBEN1_BST  AGAGAAGAGGGTAAAATGGGGAATATGTTATGTGGATAGGTGTGAGAGAGTAAATGATGT
ben1_T_pBEN1_BST  AGAGAAGAGGGTAAAATGGGGAATATGTTATGTGGATAGGTGTGAGAGAGTAAATGATGT
Col-0_BST         AGAGAAGAGGGTAAAATGGGGAATATGTTATGTGGATAGGTGTGAGAGAGTAAATGATGT
Col-0_no_BST      AGAGAAGAGGGCAAAACGGGGAACATGTCACGCGGATAGGTGTGAGAGAGTAAATGACGT
                  *********** **** ****** **** * * ************************ **

ben1_O_pBEN1_BST  AGGTTTTGTTATTTAAGAATTTTTTTAGAGAGGTTTTTATATAT-AGNNG----------
ben1_T_pBEN1_BST  AGGTTTTGTTATTTAAGAATTTTTTTAGAGAGGTTTTTATATATTAGAAGAGAGAGAAAG
Col-0_BST         AGGTTTTGTTATTTAAGAATTTTTTTAGAGAGGTTTTTATATATTAGAAGAGAGAGAAAG
Col-0_no_BST      AGGTTCCGTTATTTAAGAACCCTTCTAGAGAGGTCCC-ACACACCAGAAGAG--------
                  *****  ************   ** *********    * * *  **  *

ben1_O_pBEN1_BST  --------------------
ben1_T_pBEN1_BST  AGATAGAGAGAAAATGGTG
Col-0_BST         AGATAGAGAGAAAATGGTG
Col-0_no_BST      --------------------
```

(B)

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ben1_O_Ex2_BST  GGTGGTGTTTGAGTTTGGTGGGAAGAATGGGTTAGAGGTTGTGATGTTTGTTATTTTTTT
ben1_T_Ex2_BST  GGTGGTGTTTGAGTTTGGTGGGAAGAATGGGTTAGAGGTTGTGATGTTTGTTATTTTTTT
Col-0_BST       GGTGGTGTTTGAGTTTGGTGGGAAGAATGGGTTAGAGGTTGTGATGTTTGTTATTTTTTT
Col-0_no_BST    GGCGGCGCTTGAGTTCGGTGGGAAGAATGGGTTAGAGGTTGTGACGCTCGTTATTCCTCT
                ** ** * ******* ***************************** * * ******  * *

ben1_O_Ex2_BST  TGTTGTTGGATTTTTTATATTTTTGTTGTTGTTTTTTTTTGTTTTTATATTTTTTGTTAT
ben1_T_Ex2_BST  TGTTGTTGGATTTTTTATATTTTTGTTGTTGTTTTTTTTTGTTTTTATATTTTTTGTTAT
Col-0_BST       TGTTGTTGGATTTTTTATATTTTTGTTGTTGTTTTTTTTTGTTTTTATATTTTTTGTTAT
Col-0_no_BST    CGTCGTCGGACCTTTTATATCTTCGTCGTTGCCTTCCTCCGTCTTCATATCTCTCGCCAT
                ** ** ***  ******** ** ** ****  **  *  ** ** **** * * *  **

ben1_O_Ex2_BST  GTTTTTTG/GTATTTAA
ben1_T_Ex2_BST  GTTTTTTG/GTATTTAA
Col-0_BST       GTTTTTTG/GTATTTAA
Col-0_no_BST    GCTCTTTG/GTACTTAA
                * * **** *** ****
```

Figure S3 Genomic DNA in the *BEN1* Promoter and *BEN1* 2nd Exon-Intron junction does not show any methylation in both the pre-triple *ben1-1* and the *bas1-2 sob7-1 ben1-1* triple-mutant lines. (A) *BEN1* promoter DNA was amplified using primers pBEN1-BS-F and pBEN1-BS-R from the bisulfite converted genomic DNA from the Col-0, pre-triple *ben1-1* and the triple-mutant lines. (B) *BEN1* DNA from the 2nd Exon-Intron junction was amplified using primers BEN1-E2-BS-F and BEN1-E2-BS-R from the bisulfite converted genomic DNA from the Col-0, pre-triple *ben1-1* and the triple-mutant lines. BST= bisulfite treatment. ben1_O= pre-triple *ben1-1*, and ben1_T= bas1-*2 sob7-1 ben1-1* triple-mutant.

File S1

Bisulfite *BEN1* 2nd exon raw sequences

ACCAGAAGAGAGAGAAAGAGACAGAGAGAAAATGGTGAGAGAAGAACAAGAAGAAGATGACAACAACAACAACAACAACGGAGG
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TGGTACTTAATTCTCATATCTAATAAGTTTTTTATTTTATTTTTCTCCATGATTAAACTAAAGCTGAAAATTAATAATTTTAAAAACTTAA
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CGATGATATTTCTATTAGAAAAGCCAGTTGCAAAAGGTAGATATATTTGTTCGTCGGTGGAGATGAAGATCGATGAGGTCTTTGAGT
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TTTCATCGAAGAAGCTGAAAAGTGCCGGGTTCGAATTCAAGTATGGAGCCGAGGAAATATTCAGTGGAGCTATAAGGAGCTGTCAA
GCAAGGGGATTTCTTTAAATTCTTATGGGATTTTTGTGTTAAAATTTGTATATGAATTGAAAATATGTTTTTTCTTTATTGGCCATTTCC
GTTTTGTAACATGTTCTAGTTACTTGTTGACGAAAAAAAAAGAAAGTGTTTTGATTCAG

BLUE=Exons; Black=Introns

Primers:

BEN1-E2-BS-F: TTAGGAATTAAAAGGAGAAGAG

BEN1-E2-BS-R: AATTAAATACCAAAAAACATAAC

Replicate 1

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ben1_T_Ex2_BST  GGTGGTGTTTGAGTTTGGTGGGAAGAATGGGTTAGAGGTTGTGATGTTTGTTATTTTTTT
Col-0_BST       GGTGGTGTTTGAGTTTGGTGGGAAGAATGGGTTAGAGGTTGTGATGTTTGTTATTTTTTT
Col-0_no_BST    GGCGGCGCTTGAGTTCGGTGGGAAGAATGGGTTAGAGGTTGTGACGCTCGTTATTCCTCT
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ben1_T_Ex2_BST  TGTTGTTGGATTTTTTATATTTTTGTTGTTGTTTTTTTTTTGTTTTTATATTTTTTGTTAT
Col-0_BST       TGTTGTTGGATTTTTTATATTTTTGTTGTTGTTTTTTTTTTGTTTTTATATTTTTTGTTAT
Col-0_no_BST    CGTCGTCGGACCTTTTATATCTTCGTCGTTGCCTTCCTCCGTCTTCATATCTCTCGCCAT
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ben1_O_Ex2_BST  GTTTTTTG/GTATTTAA
ben1_T_Ex2_BST  GTTTTTTG/GTATTTAA
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Replicate 2

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Bisulfite sequences of the *BEN1* promoter

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AGAACAAGAAGAAGATGACAACAACAACAACAAC

Primers:

pBEN1-BS-Fv: 5'-GTATAGTAGTAAAAAAAAGAAGAAGAAG-3'

pBEN1-BS-Rv: 5'-CTTRTTCTTCTCTCACCATTTTCTCTCT-3'

(RC=reverse compliment)

Replicate 1

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CAAATGCTCACATTTTACAGATCATTTATGTGAATAATTCACATTCTTTGTCCGTTTATTACAAACATTGTTATTGGATT
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>RC_328409-528_5-R_Primer-D_A02.ab1/>Col-0_no_BST
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>PK_328409-530_6-R_Primer-D_C02.ab1/>Col-0_BST
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Sequence alignment:

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ben1_T_pBEN1_BST  GGAATTTAATAATAATGTTTGTAATAAATGGATAAAGAATGTGAATTATTTATATAAATG
Col-0_BST         GGAATTTAATAATAATGTTTGTAATAAATGGATAAAGAATGTGAATTATTTATATAAATG
Col-0_no_BST      GGAATCCAATAACAATGTTTGTAATAAACGGACAAAGAATGTGAATTATTCACATAAATG
                  *****  ***** *************** *** ***************** * *******

ben1_O_pBEN1_BST  ATTTGTAAAATGTGAGTATTTGATTATAATGGATATAATAAAATAAAAATTGTAATAAAG
ben1_T_pBEN1_BST  ATTTGTAAAATGTGAGTATTTGATTATAATGGATATAATAAAATAAAAATTGTAATAAAG
Col-0_BST         ATTTGTAAAATGTGAGTATTTGATTATAATGGATATAATAAAATAAAAATTGTAATAAAG
Col-0_no_BST      ATCTGTAAAATGTGAGCATTTGATTATAACGGATATAATAAAACAAAAATTGTAATAAAG
                  ** ************* ************ ************* ****************

ben1_O_pBEN1_BST  AGAGAAGAGGGTAAAATGGGGAATATGTTATGTGGATAGGTGTGAGAGAGTAAATGATGT
ben1_T_pBEN1_BST  AGAGAAGAGGGTAAAATGGGGAATATGTTATGTGGATAGGTGTGAGAGAGTAAATGATGT
Col-0_BST         AGAGAAGAGGGTAAAATGGGGAATATGTTATGTGGATAGGTGTGAGAGAGTAAATGATGT
Col-0_no_BST      AGAGAAGAGGGCAAAACGGGGAACATGTCACGCGGATAGGTGTGAGAGAGTAAATGACGT
                  *********** **** ****** **** * * ************************ **
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ben1_O_pBEN1_BST  AGGTTTTGTTATTTAAGAATTTTTTTAGAGAGGTTTTTATATAT-AGNNG----------
ben1_T_pBEN1_BST  AGGTTTTGTTATTTAAGAATTTTTTTAGAGAGGTTTTTATATATTAGAAGAGAGAGAAAG
Col-0_BST         AGGTTTTGTTATTTAAGAATTTTTTTAGAGAGGTTTTTATATATTAGAAGAGAGAGAAAG
Col-0_no_BST      AGGTTCCGTTATTTAAGAACCCTTCTAGAGAGGTCCC-ACACACCAGAAGAG--------
                  *****  ************   ** *********    * * *  **  *

ben1_O_pBEN1_BST  --------------------
ben1_T_pBEN1_BST  AGATAGAGAGAAAATGGTG
Col-0_BST         AGATAGAGAGAAAATGGTG
Col-0_no_BST      --------------------
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Replicate 2

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File S3

Bisulfite pNOS raw sequences

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TCTATCGCCTTCTTGACGAGTTCTTCTGA

Primers:

pNOS-BS-F (Primer A/pROK2-Fv): 5'-GGGTTTYTGGAGTTTAATGAGYTAAG-3'
pNOS-BS-R (Primer B/pROK2-Rv): 5'-CACTTCRCCCAATARCARCCARTCCCTTCC-3'

Replicate 1

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>RC_328409-504_2-R_Primer-B_D01.ab1/>*ben1*_O_pNOS-BST
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>PK_328409-505_3-F_Primer-A_E01.ab1/>*ben1*_R_pNOS_BST
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>RC_328409-506_3-R_Primer-B_F01.ab1/>*ben1*_R_pNOS-BST

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Sequence alignment:

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ben1_O_pNOS_no-BST  AAATATTTCTTGTCAAAAATGCTCCACTGACGTTCCATAAATTCCCCTCGGTATCCAATT
ben1_R_pNOS_BST     AAATATTTTTTGTCAAAAATGTTTTACTGACGTTCCATAAATTTTTCTCGGTATTCAATT
ben1_O_pNOS_BST     AAATATTTCTTGTCAAAAATGCTCCACTGATGTTCCATAAATTCCCCTTGGTATCCAATT
                    ******** ************ *  ***** ************   ** ***** *****

ben1_O_pNOS_no-BST  AGAGTCTCATATTCACTCTCAATCCAAATAATCTGCACCGGATCTGGATCGTTTCGCATG
ben1_R_pNOS_BST     AGAGTCTCATATTTATTCTCAATTCAAATAATCTGTACCGGATCTGGATCGTTTCGTATG
ben1_O_pNOS_BST     AGAGTCTCATATTCACTCTCAATCCAAATAATCTGCACTGGATCTGGATTGTTTTGCATG
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ben1_O_pNOS_no-BST  ATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGC
ben1_R_pNOS_BST     ATTGAACAAGATGGATTGTACGCANGTTTTCCGGTCGTTTGGGTGGAGAGGCTATTCGGC
ben1_O_pNOS_BST     ATTGAACAAGATGGATTGCATGCAGGTTCTTTGGTTGTTTGGGTGGAGAGGTTATTTGGT
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ben1_O_pNOS_no-BST  TATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTC
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ben1_O_pNOS_BST     TATGANTGGGCACAACANATAATTGGCTGCTCTGATGTCGTTGTGTTT
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Replicate 2

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File S4

***NPTII* gene sequencing V2**

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ben1_R_NPTII-F1  TACGTCAGAAACCATTATTGCGCGTTCAAAAGTCGCCTAAGGTCACTATCAGCTAGCAAA
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ben1_O_NPTII-F1  TATTTCTTGTCAAAAATGCTCCACTGACGTTCCATAAATTCCCCTCGGTATCCAATTAGA
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ben1_O_NPTII-F1  GTCTCATATTCACTCTCAATCCAAATAATCTGCACCGGATCTGGATCGTTTCGCATGATT
ben1_R_NPTII-F1  GTCTCATATTCACTCTCAATCCAAATAATCTGCACCGGATCTGGATCGTTTCGCATGATT
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ben1_O_NPTII-F1  GAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTAT
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ben1_O_NPTII-F1   GAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGAC
ben1_R_NPTII-F1   GAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGAC
                  ************************************************************

ben1_O_NPTII-F1   GTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTC
ben1_R_NPTII-F1   GTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTC
                  ************************************************************

ben1_O_NPTII-F1   CTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGG
ben1_R_NPTII-F1   CTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGG
                  ************************************************************

ben1_O_NPTII-F1   CTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAG
ben1_R_NPTII-F1   CTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAG
                  ************************************************************

ben1_O_NPTII-F1   CGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCAT
ben1_R_NPTII-F1   CGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCAT
                  ************************************************************

ben1_O_NPTII-F1   CAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAT
ben1_R_NPTII-F1   CAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAT
                  ************************************************************

ben1_O_NPTII-F1   GATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGC
ben1_R_NPTII-F1   GATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGC
                  ************************************************************

ben1_O_NPTII-F1   TTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCNNNCATAGCGT
ben1_R_NPTII-F1   TTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCNGACATAGCGT
                  ************************************************  ********

ben1_O_NPTII-F1   TGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGC
ben1_R_NPTII-F1   TGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGC
                  ************************************************************

ben1_O_NPTII-F1   TTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCNCCTTCTATCGCCTNCTTGACNAGT
ben1_R_NPTII-F1   TTTACGGTATCGCCGCTCCCGATTCNCAGCGCATCGCCTTCTATCGCCTTCTTGACGANT
                  ************************* ********* ************* ****** * *

ben1_O_NPTII-F1   TCTTCTGANCGGNNNNNNNNNGGGGNNNNNNNNNNNNNNNAAAAAGNAN
ben1_R_NPTII-F1   TCTTCTGANCNGGNNNNNNNNNNNNGGNNNGNNNANNNNNAA--------
                  ********** * *********      *** *** ***** *


ben1_O_NPTII-F2   -NNNNNNNNGNGGCGGNNNGCNNNNNNCNNNNNTCCGGCTNACCTNNCCCATT--CGACC
ben1_R_NPTII-F2   NNNNNNNNNGCGGNNNNNNNNNNNNNNCNNNNNNCCGNNTNACNTNNCCCATTTNCGACC
                   ********* **   ***  ************ ***  **** *********  *****
```

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ben1_O_NPTII-F2  ACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTG-TCGAT
ben1_R_NPTII-F2  ACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTNNGTCGAT
                 **************************************************   *****

ben1_O_NPTII-F2  CAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTC
ben1_R_NPTII-F2  CAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTC
                 ************************************************************

ben1_O_NPTII-F2  AAGGCGCGCATGCCCGACGGCGATGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCG
ben1_R_NPTII-F2  AAGGCGCGCATGCCCGACGGCGATGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCG
                 ************************************************************

ben1_O_NPTII-F2  AATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTG
ben1_R_NPTII-F2  AATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTG
                 ************************************************************

ben1_O_NPTII-F2  GCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGC
ben1_R_NPTII-F2  GCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGC
                 ************************************************************

ben1_O_NPTII-F2  GAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATC
ben1_R_NPTII-F2  GAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATC
                 ************************************************************

ben1_O_NPTII-F2  GCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGNN-GAAATGAAAG
ben1_R_NPTII-F2  GCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGNNCNAAATGAANN
                 ************************************************  *******
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File S5
BEN1 BL-100 analysis

File S6
Fluence rate analysis

File S7
Next generation unstable *ben1-1* Hypocotyl data

File S8
Real time RT-PCR Expression data

Files S5-S8 are available for download at http://www.g3journal.org/lookup/suppl/doi:10.1534/g3.113.006353/-/DC1.

Table S1 Table of PCR primer sequences used in the study.

Primer Name	Primer Sequences
GSP1	5'-TTTATATCTTCGTCGTTGCCT-3'
GSP2	5'-ACCTTTTGCAACTGGCTTTT-3'
LBb1.3	5'-ATTTTGCCGATTTCGGAAC-3'
PRT1	5'-ATGGTGAGAGAAGAACAAGA-3'
PRT2	5'-TTAAAGAAATCCCCTTGCTTG-3'
PQ1	5'-ATCTCTCGCCATGCTCTTTG-3'
PQ2	5'-CTTCATCTCCACCGACGAAC-3'
UBQ1	5'-GAAATGCATGGAGACGGATT-3'
UBQ2	5'-TTGGTCTCTGCTCCCACTCT-3'
ACTIN2 RT-F	5'-GGTCGTACAACCGGTATTGTGCTGG-3'
ACTIN2 RT-R	5'-CTGTGAACGATTCCTGGACCTGCC-3'
PQ3	5'-TCTAAACAAGGAATTCGAGGTC-3'
PQ4	5'-AACCAGAAATGTAGCACCGT-3'
NPT-Fv1/MT1	5'-ACAAGCCGTTTTACGTTTGG-3'
NPT-Rv	5'-TCATTTCGAACCCCAGAGTC-3'
MT2	5'-ATACTTTCTCGGCAGGAGCA-3'
MT3	5'-TTCGCAAGACCCTTCCTCTA-3'
MT4	5'-GTTTTCCCAGTCACGACGTT-3'
NPT-Fv2	5'-TGCTCCTGCCGAGAAAGTAT-3'
pNOS-BS-F	5'-GGGTTTYTGGAGTTTAATGAGYTAAG-3'
pNOS-BS-R	5'-CACTTCRCCCAATARCARCCARTCCCTTCC-3'
pBEN1-BS-F	5'-GTATAGTAGTAAAAAAAAGAAGAAGAAG-3'
pBEN1-BS-R	5'-CTTRTTCTTCTCTCACCATTTTCTCTCT-3'
BEN1-E2-BS-F	5'-TTAGGAATTAAAAGGAGAAGAG-3'
BEN1-E2-BS-R	5'-AATTAAATACCAAAAAACATAAC-3'