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## Supplementary Material

### ***Brachypodium distachyon*: a model species for aluminium tolerance in Poaceae**

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**Supplementary File S1.** Genomic DNA of the *BdALMT1* gene: The five exons are indicated in blue color and the four introns without color. The three pair of primers designed to amplify the genomic DNA of *BdALMT1* gene are indicated using three different colors: green (pair P1), yellow (pair P2) and red (pair P3).

>5 Brachy1.0:5:12998907:13001124:-1

ATGGAGGTTGATCACCAGATCAGAGT GAGCAACGACGGCGCCGATGGAGACCGTCTCTGGCAGCGGCTC  
CGCTCGTTGGTCTCGGGCTCGGGTGCCTGTGCGCGGGTTCGCGAGGAAAGTGGGGAGGATCGCTCGG  
GAGGACCCGAGGCGAGTGGCACACTCCTTCAAGGTGGGGCTGGCGCTCACGCTGGTGTCCGTGCTCTAC  
TACGTACGCCCCTCTTCAAGGGCTTCGGGGTCTCCACGCTCTGGGCCGTGCTCACCGTCTGCTCGTCTAC  
ATGGAGTACACCGTCGGCGGCACGCTCAGCAAAGGCTTGAACAGGGCCTTCGCCACGCTCGTGGCTGGG  
TTCATCGCCGTCGGAGCTCATCAGGTGGCTAACCGCTGTGGCACGCAGGGGGAACCCATAAATACTCGCC  
GCCTTCGTCTTCTTGCTAG GTAAGCCAGACGCACATATTAATACGGCATATATTTTGGAGACGGAGAGAG  
TATATAAGATGCGCCTACATTACCAAACCTCGAATTGAACTGGTGTACGTTTCATCTCTGTGGCAGCGTCC  
GCGGCGACGTTCTCACGCTTCATCCCGGAGATCAAGGCCAAGTACGACTACGGGGTGACCATATTCATA  
CTCACCTTTAGCCTGGTGGCCGTGTGCGAGCTACCGC GTGGAGGAGCTCATCCAGCTCGCACACCAGCGG  
TTCTCCACCATCGTGTGGGGGCTTTCACATGCCTCTGCACCACTA TCTTCGTCTTCCCGTCTGGGCA  
GGCGAGGACCTCCACAAGCTCTCCGCCGTAACCTCGACAAGCTGGCCAGTTCCTTGAAG GTACGTAC  
GTAGTACTTGTAGGGCCATGTGTTTCTGCATGTACTCCAATATTTGGTTATAAAGATTCCTGCACCTT  
TTTTGTGCCATACTGGTTCAAGTCATGATATTATAACTTTGTTAGACTATACTAATGCAACTTGAAGCG  
GGGTAAGACTCAAATACAAGTTAGCCTTGTGAAAGTTCTGCGGGGGGAATGTGGGGCAACAAATAATAGC  
AGACTCTTCTGACCAGAGTCATCAGATATACTCTGTTTTATAGTTCTTCCAGCCTCTTTGTAGCAGC  
TTCATATTGATAGGCTTTTTCGCTTCATATTGGTACACTGTACATGAATCTGGGCCAAAAAATTGCAGA  
CAAACATTTGTTGATATCATAGGAGACCAATAGACTGCCTTGGAAATGGCACCGACCTACTCTGCAAACA  
AAATTAGGGATCCAAAAAATTATTCGGAAAAGAGGGACATTTGAAGTGAAAAAAGTAAACTTTTCATG  
TACTTGTAGAGTTAGTTACTCACGTAGACTTTCACACGTTACTCCAGAGTTTGTGAGGTTGAAAACTGTG  
CACATGCATGCAGG GATGGAATCTGAATGCTTTGGAGA AAACTCTACAATTGAGAATTTG GAGAGTAAA  
ACCTTTCTCCAAG TGTACAAGAGCGTCTCAATTCCAAGGCCACTGAGGACTCTCTG GTAAGCATCCTT  
TACAAATGATACACCGTATGCTGAGTGCGAAAACCTCCAGTAAACATCTGCAGAGAAGGAAAGCTTGTAT  
ACACAGCAGTTCCTTTTTGTAATGGTATTCCAAAACCTGCAG TGCAATTTTGCCAAGTGGGAGCCAGGTC  
ATGGTAAATTCGGCTTCCGACACCCATGGAGCCAATACCAGAAGATCGGAGCTCTTTGTGCGCCAGTGTG  
CGTCTTCCATGGAGGCTCTTGCTTCCATATGTCATCACACTCCAAAAATCTCAG GTAATTGGAACCTTCTC  
CCCTTTTCTGCCAAAAAGACCATGGAGCCAGGTTTTGACCAACATCATTTTCTGTATCAACAGTACCC  
TGAAGCAAATCCAGAGCTATCCTTGAAGGTTTCGAACAGCATGCAGTGAAATGAGCTCGGACTCTGCCAA  
GGCGCTCAGGGAGCTCTCGACAGCAATTCGAACGATGACTGTACCATCCCCAGCCAACATCACCATGTC  
TGCAGCCATCACAGTTGCGAAAGGCCTCAGAAGTGAATTATCACAGGATATGGCTCTGCTGCAAGTGAT  
GCATGTAGCTGTTACTGCAACACTTCTCTCGGACTTGGTTACAACAATAAAGAAAAATGCAGAATCTGT  
TGATAATCTAGCGGACTTGCCCACTTCAAAAACCCCTGAAAAAATCCAAAA AGATGTTGTTATCAACAT  
TGCGAGTTGA

The three pair of primers designed to amplify the *BdALMT1* genomic DNA:

	Forward	Reverse	Temp °C
P1	ATGGAGGTTGATCACCAGATCAGAGT	TTCACATGCCTCTGCACCACTA	60.0
P2	GTGGAGGAGCTCATCCAGCTCG	GAGAGTAAAACCTTTCTCCAAG	53.3
P3	GATGGAATCTGAATGCTTTGGAGA	AGATGTTGTTATCAACATTGCGAG	55.5

**Supplementary File S2.** Alignment of the *Brachypodium ALMT1* nucleotide sequences from sensitive Bd21, ABR1 and E66 (*Bs*) and tolerant ABR8 diploid lines. The five exons of the *BdALMT1* genes are indicated in grey colour.

Bd21 1 ATGGAGGTTGATCACCAGAT---CAGAGTGAGCAACGACGGCGCCGATGGAGACC----

ABR1 1 ATGGAGGTTGATCACCAGAT---CAGAGTGAGCAACGACGGCGCCGATGGAGACC----

ABR8 1 ATGGAGGTTGATCACCAGAT---CAGAGTGAGCAACGACGGCGCCGTTGGAGACC----

E66 1 ATGGAGGTTGATCACCAGATATCCAGCGTGAGCAGCGAAGGCGCCGATGGAGACCACACG

Bd21 53 -----

GTCTCTGGCAGCGGCTCCGCTCG

ABR1 53 -----

GTCTCTGGCAGCGGCTCCGCTCG

ABR8 53 -----

GTCTCTGGCAGCGGCTCCGCTCG

E66 61 GCCGGGCATCATGGCGCTGTCGGCGTCTTCTTCGCGGTCTCTCTGGCTGATGCTCCGCTCG

Bd21 76 TTGGTCCTCGGGCTCGGGTGCCGTGTGCGCGGGTTCGCGAGGAAAGTGGGGAGGATCGCT

ABR1 76 TTGGTCCTCGGGCTCGGGTGCCGTGTGCGCGGGTTCGCGAGGAAAGTGGGGAGGATCGCT

ABR8 76 TTGGTCCTCGGGCTCGGGTGCCGTGTGCGCGGGTTCGCGAGGAAAGTGGGGAGGATCGCT

E66 121 TTGATCCTCGGGCTCTGGTGCCGTGTGCGCGGGTTCGCGAGGAAAGTGGGGAGGATCGCG

Bd21 136 CGGGAGGACCCGAGGCGAGTGGCACACTCCTTCAAGGTGGGGCTGGCGCTCACGCTGGTG

ABR1 136 CGGGAGGACCCGAGGCGAGTGGCACACTCCTTCAAGGTGGGGCTGGCGCTCACGCTGGTG

ABR8 136 CGGGAGGACCCGAGGCGAGTGGCACACTCCTTCAAGGTGGGGCTGGCGCTCACGCTGGTG

E66 181 AGGGAGGACCCGAGGCGAGTGGCGCACTCCTTCAAGGTGGGGCTGGCGCTCACGCTGGTG

Bd21 196 TCCGTGCTCTACTACGTACGCCGCTCTTCAAGGGCTTCGGGGTCTCCACGCTCTGGGCC

ABR1 196 TCCGTGCTCTACTACGTACGCCGCTCTTCAAGGGCTTCGGGGTCTCCACGCTCTGGGCC

ABR8 196 TCCGTGCTCTACTACGTACGCCGCTCTTCAAGGGCTTCGGGGTCTCCACGCTCTGGGCC

E66 241 TCCGTGCTCTACTACGTGACGCCGCTCTTCAAGGGCTTCGGGGTGTCCACCCTGTGGGCC

Bd21 256 GTGCTCACCGTCGTCGTCATGGAGTACACCGTCGGCGGCACGCTCAGCAAAGGCTTG

ABR1 256 GTGCTCACCGTCGTCGTCATGGAGTACACCGTCGGCGGCACGCTCAGCAAAGGCTTG

ABR8 256 GTGCTCACCGTCGTCGTCATGGAGTACACCGTCGGCGGCACGCTCAGCAAAGGCTTG

GTGCTCACCGTCGTTCGTTCATGGAGTACACCGTCGGCGGCACGCTCAGCAAAGGCTTG  
E66 301  
GTGCTCACCGTCGTTCGTTCATGGAGTACACCGTGGCGGCACGCTCAGCAAAGGCTTG

Bd21 316  
AACAGGGCCTTCGCCACGCTCGTGGCTGGGTTTCATCGCCGTCGGAGCTCATCAGGTGGCT  
ABR1 316  
AACAGGGCCTTCGCCACGCTCGTGGCTGGGTTTCATCGCCGTCGGAGCTCATCAGGTGGCT  
ABR8 316  
AACAGGGCCTTCGCCACGCTCGTGGCTGGGTTTCATCGCCGTCGGAGCTCATCAGGTGGCT  
E66 361  
AACAGGGCCTTCGCCACGCTCGTGGCTGGGTTTCATCGCCGTCGGAGCTCATCAGGTGGCT

Bd21 376  
AACCGCTGTGGCACGCAGGGGGAACCCATAATACTCGCCGCTTCGTCTTCTTGCTAGGT  
ABR1 376  
AACCGCTGTGGCACGCAGGGGGAACCCATAATACTCGCCGCTTCGTCTTCTTGCTAGGT  
ABR8 376  
AACCGCTGTGGCACGCAGGGGGAACCCATAATACTCGCCGCTTCGTCTTCTTGCTAGGT  
E66 421  
AACCGCTGCGGCGCGCAGGGGAGCCCATAAATGCTCGCCGCTTCGTCTTCTTGCTAGGT

Bd21 436 AAGCCA-GACGCACATATTAATACGGCATATATTTTGAGACGGAGAGAGTATAT-----  
-  
ABR1 436 AAGCCA-GACGCACATATTAATAC-----TGAGACGGAGAGAGTATAT-----  
-  
ABR8 436 AAGCCA-GACGCACATATTAATACGGCATATATTTTGAGACGGAGAGAGCATAT-----  
-  
E66 481 A--CTATGAAGCTCATGCCA-TGCAAAA-----  
GCAAAATTAAGGGAGTTGATTAAGCC

Bd21 489 AAGATGCGCCTACATTACCAAACCTCGAATT--GA--ACTGGTGTACGTTTCATC--  
TCTGT  
ABR1 489 AAGATGCGCCTACATTACCAAACCTCGAATT--GA--ACTGGTGTACGTTTCATC--  
TCTGT  
ABR8 478 AAGATGCGCCTACATTACCAAACCTCGAATT--GA--ACTGGTGTACGTTTCATC--  
TCTGT  
E66 531 AACACGCGCC--  
AGACTTTCAAACCTGGATTTTGATCACTGGTGTGCGTTCCTTCATTCAT

Bd21 543  
GGCAGCGTCCGCGGCACGTTCTCACGTTTCATCCCGGAGATCAAGGCCAAGTACGACTA  
ABR1 543  
GGCAGCGTCCGCGGCACGTTCTCACGTTTCATCCCGGAGATCAAGGCCAAGTACGACTA  
ABR8 532  
GGCAGCGTCCGCGGCACGTTCTCACGTTTCATCCCGGAGATCAAGGCCAAGTACGACTA  
E66 590  
GGCAGCGTCCGCGGCACGTTCTTCGCTTCATCCCGGAGATCAAGGCCAAGTACGACTA

Bd21 602  
CGGGGTGACCATATTCATACTCACCTTTAGCCTGGTGGCCGTGTCGAGCTACCGCGTGGA  
ABR1 602  
CGGGGTGACCATATTCATACTCACCTTTAGCCTGGTGGCCGTGTCGAGCTACCGCGTGGA  
ABR8 591  
CGGGGTGACCATATTCATACTCACCTTTAGCCTGGTGGCCGTGTCGAGCTACCGCGTGGA

E66 650  
CGGGGTGACCATATTCATACTCACCTTTAGCCTGGTGGCCGTGTCGAGCTACCGCGTGA

Bd21 662  
GGAGCTCATCCAGCTCGCACACCAGCGTTCTCCACCATCGTCGTGGGGTCTTCACATG  
ABR1 662  
GGAGCTCATCCAGCTCGCACACCAGCGTTCTCCACCATCGTCGTGGGGTCTTCACATG  
ABR8 651  
GGAGCTCATCCAGCTCGCACACCAGCGTTCTCCACCATCGTCGTGGGGTCTTCACATG  
E66 710  
GGAGCTCATCGAGCTCGCGCACACCAGCGATTCTCCACCATCGTCGTGGGCGTCTTCACATG

Bd21 722  
CCTCTGCACCACTATCTTCGTCTTCCCGTCTGGGCAGGCGAGGACCTCCACAAGCTCTC  
ABR1 722  
CCTCTGCACCACTATCTTCGTCTTCCCGTCTGGGCAGGCGAGGACCTCCACAAGCTCTC  
ABR8 711  
CCTCTGCACCACTATCTTCGTCTTCCCGTCTGGGCAGGCGAGGACCTCCACAAGCTCTC  
E66 770  
CCTCTGCACCACTATCTTCGTCTTCCCGTCTGGGCGGCGAGGACCTCCACAAGCTCTC

Bd21 782 CGCCGGTAACCTCGACAAGCTGGCCAGTTCCTTGAAGGTACGTACGTAGTAC-----  
-  
ABR1 782 CGCCGGTAACCTCGACAAGCTGGCCAGTTCCTTGAAGGTACGTACGTAGTAC-----  
-  
ABR8 771 CGCCGGTAACCTCGACAAGCTGGCCAGTTCCTTGAAGGTACGTACGTAGTAC-----  
-  
E66 830  
CGCCGGCAACCTCGACAAGCTGGCCAGTTCCTTGAAGGTGCGTACGTAAATATGCAAAAG

Bd21 835 -----TTGTTTAGGGCC-----ATGTGTTTCTGC-----  
ATGTA  
ABR1 835 -----TTGTTTAGGGCC-----ATGTGTTTCTGC-----  
ATGTA  
ABR8 824 -----TTGTTTAGGGCC-----ATGTGTTTCTGC-----  
ATGTA  
E66 890  
TCGACAGGAATCGGTTAGGGCTCTCACGTGATGTGTTTCTGCCGTCCATATGCATGCA

Bd21 864 CTCCAATATTTGGTTATAAAGATTCTGCACTTTTTTT-  
GTGCCATACTGGTTCAAGTCAT  
ABR1 864 CTCCAATATTTGGTGATAAAGATTCTGCACTTTTTTT-  
GTGCCATACTGGTTCAAGTCAT  
ABR8 853 CTCCAATATTTGGTGATAAAGATTCTGCACTTTTTTT-  
GTGCCATACTGGTTCAAGTCAT  
E66 950  
CTCCAATATTTGGTGACAAAGATTCTGCACTTTTTTTGTGTCATACTGATTAGGCTAAT

Bd21 923  
GATATTATAACTTTGTTAGACTATACTAATGCAACTTGAAGCGGGTAAGACTCAAATAC  
ABR1 923  
GATATTATAACTTTGTTAGACTATACTAATGCAACTTGAAGCGGGTAAGACTCAAATAC  
ABR8 912  
GATATTATAACTTTGTTAGACTATACTGATGCAACTTGAAGCGGGTAAGACTCAAATAC  
E66 1010

ACTATTATAACTTTGTTAGACTACACTAATGCAACTTGAAGTGAGGATAGCCTCAAATAC

Bd21 983

AAGTTAGCCTTGTGAAGTTCTGCGGGGGGAATGTGGGGCAACAAATAATAGCAGACTCTT

ABR1 983

AAGTTAGCCTCGTGAAGTTCTGCGGGGGGAATGTGGGGCAACAAATAATAGCAGACTCTT

ABR8 972

AAGTTAGCCTTGTGAAGTTCTGCGGGGGGAATGTGGGGCAACAAATAATAGCAGACTCTT

E66 1070 AAGACAGGCATGTGAACTT----

GGGAAAATGGGGAACAAGAAATAGTAGTGAACCTT

Bd21 1043 CCTGACCAGAGTCATCAGATATACTCTGTTTTATAGTTCTTCCCAG-----

CCTCTTTGT

ABR1 1043 CCTGACCAGAGTCACCAGATATACTCTGTTTTATAGTTCTTCCCAG-----

CCTCTTTGT

ABR8 1032 CCTGACCAGAGTCACCAGATATACTCTGTTTTATAGTTCTTCCCAG-----

CCTCTTTGT

E66 1126

CCTGACCAGAGTCACCACACATGCTCTGTTTTATAGCTCTTCCCAGTCCAGCCTCTCCGT

Bd21 1098 AGCAGCTTCATATTGATA--GGCTTTTCGCTTCATATTGGTACACTGTC-

ACATGAATCT

ABR1 1098 AGCAGCTTCATATTGATA--GGCTTTTCGCTTCATATTGGTACACTGTC-

ACATGAATCT

ABR8 1087 AGCAGCTTCATATTGATA--GGCTTTTCGCTTCATATTGGTACACTGTC-

ACATGAATCT

E66 1186

AGCAGTTTCATATTGATACAGTGTTTTCACTTCATACTGGTACACTGTCCACATGAAGCT

Bd21 1155

GGGCCAAAAAATTGCAGACAAACATTTGTTGATATCATAGGAGACCAATAGACTGCCTTG

ABR1 1155

GGGCCAAAAAATTGCAGACAAACATTTGTTGATATCATAGGAGACCAATAGACTGCCTTG

ABR8 1144

GGGCCAAAAAATTGCAGACAAACATTTGTTGATATCATAGGAGACCAATAGACTGCCTTG

E66 1246 GG--CATAAAAATGCAGACAA-CATTTGTT-----CATAA-----AATAGA-----

-

Bd21 1215

GAATGGCACCGACCTACTCTGCAAACAAAATTAGGGATCCCAAAAAATTATTCGAAAAAG

ABR1 1215

GAATGGCACCGACCTACTCTGCAAACAAAATTAGGGATCCCAAAAAATTATTCGAAAAAT

ABR8 1204

GAATGGCACCGACCTACTCTGCAAACAAAATTAGGGATCCCAAAAAATTATTCGAAAAAG

E66 1284 -----

GCACCGACCTACTCTGCAAACAAAATTAGGGAACCCAAAAAAATATTCGAAAAAG

Bd21 1275 AGGGACATTTGAAGTGAAAAT-

AAGTAACTTTTCATGTACTTGTAGAGTTAGTTACTCAC

ABR1 1275 AGGGACATTTGAAGTGAAAAT-

AAGTAACTTTTCATGTACTTGTAGAGTTAGTTACTCAC

ABR8 1264 AGGGACATTTGAAGTGAAAAT-

AAGTAACTTTTCATGTACTTGTAGAGTTAGTTACTCAC

E66 1339 AGGAACTTTTGAAGTGAAAATTAAGTAACTTTTCATGTACTTCTAGAGTTAGTTACT-

T-

Bd21 1334  
GTAGACTTCACACGTTACTCCAGAGTTTGTGAGGTTGAAAACCTGTGCACATGCATGCAGG  
ABR1 1334  
GTAGACTTCACACGTTACTCCAGAGTTTGTGAGGTTGAAAACCTGTGCACATGCATGCAGG  
ABR8 1323  
GTAGACTTCACACGTTACTCCAGAGTTTGTGAGGTTGAAAACCTGTGCACATGCATGCAGG  
E66 1397  
GTAGACTTCACCTTACTCCAGTGTGTTTGTGAGGTTGAAAACCTGTACACATGGATGCAGG

Bd21 1394  
GATGGAATCTGAATGCTTTGGAGAAAACCTCTACAATTGAGAATTTGGAGAGTAAAACCTT  
ABR1 1394  
GATGGAATCTGAATGCTTTGGAGAAAACCTCTACAATTGAGAATTTGGAGAGTAAAACCTT  
ABR8 1383  
GATGGAATCTGAATGCTTTGGAGAAAACCTCTACAATTGAGAATTTGGAGAGTAAAACCTT  
E66 1457  
GATGGAATCTGAATGCTTTGGAGAAAACCTCTGCAATTGAGAATTTGGAGAGTAAAACCTT

Bd21 1454  
TCTCCAAGTGTACAAGAGCGTCTCAATTCCAAGGCCACTGAGGACTCTCTGGTAAGCAT  
ABR1 1454  
TCTCCAAGTGTACAAGAGCGTCTCAATTCCAAGGCCACTGAGGACTCTCTGGTAAGCAT  
ABR8 1443  
TCTCCAAGTGTACAAGAGCGTCTCAATTCCAAGGCCACTGAGGACTCTCTGGTAAGCAT  
E66 1517  
TCTCCAAGTGTACAAGAGCGTCTCAATTCCAAGGCCACTGAGGACTCTCTGGTAAGCTT

Bd21 1514  
CCTTTACAAATGATACACCGTATGCTGAGTGCGAAAACCTCCAGTAAACATCTGCAGAGAA  
ABR1 1514  
CCTTTACAAATGATACACCGTATGCTGAGTGCGAAAACCTCCAGTAAACATCTGCAGAGAA  
ABR8 1503  
ACTTTACAAATGATACACCGTATGCTGAGTGCGAAAACCTCCAGTAAACATCTGCAGAGAA  
E66 1577  
CCTTTACAAATGATACACCGTATGCTGAGTGTGAAAAGCTCCAATAAACATCAGCAGAAAA

Bd21 1574 GGAAAGCTTGTATACACAGCAGTTCCTTTTTGTAATGGT--  
ATTCCAAAACCTGCAGTGCA  
ABR1 1574 GGAAAGCTTGTATACACAGCAGTTCCTTTTTGTAATGGT--  
ATTCCAAAACCTGCAGTGCA  
ABR8 1563 GGAAAGCTTGTATACACAGCAGTTCCTTTTTGTAATGGT--  
ATTCCAAAACCTGCAGTGCA  
E66 1637  
AGAAAGCTTTTATACACAGCAGTTCCTTTTTCGTCACGGTTTATTCCAAAACCTGCATGCA

Bd21 1632  
ATTTTGCCAAGTGGGAGCCAGGTCATGGTAAATTCGGCTTCCGACACCCATGGAGCCAAT  
ABR1 1632  
ATTTTGCCAAGTGGGAGCCAGGTCATGGTAAATTCGGCTTCCGACACCCATGGAGCCAAT  
ABR8 1621  
ATTTTGCCAAGTGGGAGCCAGGTCATGGTAAATTCGGCTTCCGACACCCATGGAGCCAAT  
E66 1697  
ATTTTGCCAAGTGGGAGCCAGGTCATGGTAAATTCGGCTTCCGACACCCATGGAGCCAAT

Bd21 1692

ACCAGAAGATCGGAGCTCTTTGTCGCCAGTGTGCGTCTTCCATGGAGGCTCTTGCTTCCT

ABR1 1692

ACCAGAAGATCGGAGCTCTTTGTCGCCAGTGTGCGTCTTCCATGGAGGCTCTTGCTTCCT

ABR8 1681

ACCAGAAGATCGGAGCTCTTTGTCGCCAGTGTGCGTCTTCCATGGAGGCTCTTGCTTCCT

E66 1757

ACCAGAAGCTTGGAGCTCTTTGTCGCCAGTGC~~CGCA~~TCTTCCATGGAGGCTCTCGCTTCCT

Bd21 1752

ATGTCATCACACTCCAAAAATCTCAGGTAATTGGA~~ACTTCTCCCTTTTCTGGCCAAAA~~

ABR1 1752

ATGTCATCACACTCCAAAAATCTCAGGTAATTGGA~~ACTTCTCCCTTTTCTGGCCAAAA~~

ABR8 1741

ATGTCATCACACTCCAAAAATCTCAGGTAATTGGA~~ACTTCTCCCTTTTCTGGCCAAAA~~

E66 1817

ATGTCATCACACTCCAAAAATCTCAGGTAATTGGA~~ATTCTTCCCTTTTCATGA~~CAAAA

Bd21 1812

GACCATGGAGCCAGGTTTTGACCAACATCATTTT~~CCTGATCAACAGTACCCTGAAGCAAA~~

ABR1 1812

GACCATGGAGCCAGGTTTTGACCAACATCATTTT~~CCTGATCAACAGTACCCTGAAGCAAA~~

ABR8 1801

GACCATGGAGCCAGGTTTTGACCAACATCATTTT~~CCTGATCAACAGTACCCTGAAGCAAA~~

E66 1877 GACCATGGAGCCAGAT----

ACCAACAACATTTT~~CCTGATCAACAGTACCCTGAAGCAAA~~

Bd21 1872

TCCAGAGCTATCCTTGAAGGTTTGAACAGCATGCAGT~~GAAATGAGCTCGGACTCTGCCAA~~

ABR1 1872

TCCAGAGCTATCCTTGAAGGTTTGAACAGCATGCAGT~~GAAATGAGCTCGGACTCTGCCAA~~

ABR8 1861

TCCAGAGCTATCCTTGAAGGTTTGAACAGCATGCAGT~~GAAATGAGCTCGGACTCTGCCAA~~

E66 1933

TCCAGAGCTATCCTTGAAGGTTTGAAC~~GCATGCG~~GAGAAATGAGCTCGGACTCTGCTAA

Bd21 1932

GGCGCTCAGGGAGCTCTCGACAGCAATTGGAACGATGACTGT~~ACCATCCCAGCCAACAT~~

ABR1 1932

GGCGCTCAGGGAGCTCTCGACAGCAATTGGAACGATGACTGT~~ACCATCCCAGCCAACAT~~

ABR8 1921

GGCGCTCAGGGAGCTCTCGACAGCAATTGGAACGATGACTGT~~ACCATCCCAGCCAACAT~~

E66 1993

GGCGCTCAGGGAGCTCTCGACAGCAATTGGAACGATGACTGT~~ACCATCCCAGCCAACAT~~

Bd21 1992

CACCATGTCTGCAGCCATCACAGTTGCGAAAGGCCTCAGAAGTGAATTATCACAGGATAT

ABR1 1992

CACCATGTCTGCAGCCATCACAGTTGCGAAAGGCCTCAGAAGTGAATTATCACAGGATAT

ABR8 1981

CACCATGTCTGCAGCCATCACAGTTGCGAAAGGCCTCAGAAGTGAATTATCACAGGATAT

E66 2053

CACCATGTCTGCAGCCATCA~~A~~AGTTGCGAAAGGCCTCAGAACTGAATTATCACAGGATAT



Bd21 2052  
GGCTCTGCTGCAAGTGATGCATGTAGCTGTTACTGCAACACTTCTCTCGGACTTGGTTAC  
ABR1 2052  
GGCTCTGCTGCAAGTGATGCATGTAGCTGTTACTGCAACACTTCTCTCGGACTTGGTTAC  
ABR8 2041  
GGCTCTGCTGCAAGTGATGCATGTAGCTGTTACTGCAACACTTCTCTCGGACTTGGTTAC  
E66 2113  
GGCTCTCCTGCAAGTGATGCATGTAGCTGTTACTGCAACACTTCTCTCGGACTTGGTTAC

Bd21 2112  
AACAAATAAGAAAATTGCAGAATCTGTTGATAATCTAGCGCGACTTGCCCACTTCAAAAC  
ABR1 2112  
AACAAATAAGAAAATTGCAGAATCTGTTGATAATCTAGCGCGACTTGCCCACTTCAAAAC  
ABR8 2101  
AACAAATAAGAAAATTGCAGAATCTGTTGATAATCTAGCGCGACTTGCCCACTTCAAAAC  
E66 2173  
AACAAATAAGAAAGATTGCAGAATCCGTTGATAATCTAGCGCTACTTGCGCACTTCAAAAC

Bd21 2172 CCCTGAAAAAATCCAAAAAGATGTTGTTATCAACATTGCGAGTTGA  
ABR1 2172 CCCTGAAAAAATCCAAAAAGATGTTGTTATCAACATTGCGAGTTGA  
ABR8 2161 CCCTGAAAAAATCCAAAAAGATGTTGTTATCAACATTGCGAGTTGA  
E66 2233 CCCTGAAAAAACA AAAAAGATGTTGTTATCAACATTGCGAGATGA

Alignment of the amino acid sequences of *Brachypodium* ALMT1  
hypothetic proteins from sensitive Bd21, ABR1 and E66 and tolerant  
ABR8 diploid lines.

Bd21 1 MEVDHQR--VSNDGADGD-----  
LWQRLRSLV LGLGCRVAGFARKVGR I A  
ABR1 1 MEVDHQR--VSNDGADGD-----  
LWQRLRSLV LGLGCRVAGFARKVGR I A  
ABR8 1 MEVDHQR--VSNDGAVGD-----  
LWQRLRSLV LGLGCRVAGFARKVGR I A  
E66 1  
MEVDHQISSVSEEGADGDHTAGHHGAVGVFFAGLWLM LRS L I LGLWCRVAGFARKVGR I A

Bd21 44  
REDPRRVAHSFKVGLALTLVSVLYYVTPLFKGFGVSTLWAVLTVVVVMEYTVGGT LSKGL  
ABR1 44  
REDPRRVAHSFKVGLALTLVSVLYYVTPLFKGFGVSTLWAVLTVVVVMEYTVGGT LSKGL  
ABR8 44  
REDPRRVAHSFKVGLALTLVSVLYYVTPLFKGFGVSTLWAVLTVVVVMEYTVGGT LSKGL  
E66 61  
REDPRRVAHSFKVGLALTLVSVLYYVTPLFKGFGVSTLWAVLTVVVVMEYTVGGT LSKGL

Bd21 101  
NRAFATLVAGFIAVGAHQVANRCGTQGEPI I LAAFVFL LASAATFSRF I PE I KAKYDYGV  
ABR1 101  
NRAFATLVAGFIAVGAHQVANRCGTQGEPI I LAAFVFL LASAATFSRF I PE I KAKYDYGV  
ABR8 101  
NRAFATLVAGFIAVGAHQVANRCGTQGEPI I LAAFVFL LASAATFSRF I PE I KAKYDYGV  
E66 118  
NRAFATLVAGFIAVGAHQVANRCGAQGEPI M LAAFVFL LASAATFSRF I PE I KAR YDYGV

Bd21 157  
TIFILTFSLVAVSSYRVEELIQLAHQRFSTIVGVFTCLCTTIFVFPVWAGEDLHKLSAG  
ABR1 157  
TIFILTFSLVAVSSYRVEELIQLAHQRFSTIVGVFTCLCTTIFVFPVWAGEDLHKLSAG  
ABR8 157  
TIFILTFSLVAVSSYRVEELIQLAHQRFSTIVGVFTCLCTTIFVFPVWAGEDLHKLSAG  
E66 174  
TIFILTFSLVAVSSYRVEELIELAHQRFSTIVGVFTCLCTTIFVFPVWAGEDLHKLSAG

Bd21 213  
NLDKLAQFLEGMSESECFGENSTIENLESKTFLQVYKSVLNSKATEDSLCNFAKWEPGHGK  
ABR1 213  
NLDKLAQFLEGMSESECFGENSTIENLESKTFLQVYKSVLNSKATEDSLCNFAKWEPGHGK  
ABR8 213  
NLDKLAQFLEGMSESECFGENSTIENLESKTFLQVYKSVLNSKATEDSLCNFAKWEPGHGK  
E66 230  
NLDKLAQFLEGMSESECFGENSAIENLESKTFLQVYKSVLNSKATEDSLCNFAKWEPGHGK

Bd21 269  
FGFRHPWSQYQKIGALCRQCASSMEALASYVITLQKSQYPEANPELSLKVRTACSEMSSD  
ABR1 269  
FGFRHPWSQYQKIGALCRQCASSMEALASYVITLQKSQYPEANPELSLKVRTACSEMSSD  
ABR8 269  
FGFRHPWSQYQKIGALCRQCASSMEALASYVITLQKSQYPEANPELSLKVRTACSEMSSD  
E66 286  
FGFRHPWSQYQKLGALCRQCASSMEALASYVITLQKSQYPEANPELSLKVRTACGEMSSD

Bd21 325  
SAKALRELSTAIRTMTVPSPANITMSAAITVAKGLRSELSQDMALLQVMHVAVTATLLSD  
ABR1 325  
SAKALRELSTAIRTMTVPSPANITMSAAITVAKGLRSELSQDMALLQVMHVAVTATLLSD  
ABR8 325  
SAKALRELSTAIRTMTVPSPANITMSAAITVAKGLRSELSQDMALLQVMHVAVTATLLSD  
E66 342  
SAKALRELSTAIRTMTVPSPANITMSAAIKVAKGLRTSELSQDMALLQVMHVAVTATLLSD

Bd21 381 LVTTIKKIAESVDNLARLAHFKTPEKIQKDVVINIAS  
ABR1 381 LVTTIKKIAESVDNLARLAHFKAPEKIQKDVVINIAS  
ABR8 381 LVTTIKKIAESVDNLARLAHFKTPEKIQKDVVINIAS  
E66 398 LVTTIKKIAESVDNLALLAHFKTPEKNKQKDVVINIAR

Alignment of the *BdALMT1* 5' upstream (B promoter) nucleotide sequences from sensitive Bd21, ABR1 and E66 and tolerant ABR8 diploid lines.

Bd21 1  
TGTCGGGGAATAGATCGGAGAAAGGCGAAATAAACGGCGCCGGGGCCTTTGCTAGTGCT-  
ABR1 1  
TGTCGGGGAATAGATCGGAGAAAGGCGAAATAAACGACGCGCCGGGGCCTTTGCTAGTGCT-  
ABR8 1  
TGTCGGGGAATAGATCGGAGAAAGGCGAAATAAACGGAGCCGGGGCCTTTGCTAGATCT-  
E66 1  
TGTCGGGGAATAGATCGGAGAAAGGCGAAATAAACGGCGCCGGGGCCTTTGCTAGTGCTG

Bd21 60 -----CTGTTGTTGTACA---  
TGCGTGCAAGCTGTTTCACGAAG  
ABR1 60 -----CTGTTGTTGTACA---  
TGCGTGCAAGCTGTTTCACGAAG  
ABR8 60 -----CTGTTGTTGTACA---  
TGCGTGCAAGCTGTTTCACGAAG  
E66 61  
CTCCTTACTGTACTGCTTGTGCAGTTGTTGTACAATGCGTGTGCAAGCTGTTTCACGAAG

Bd21 96  
GTCCTAGTGGCTGAGCAATAGGGTGTGGAAACTGTACTTCGGTTCACTTGCAGAGTTGCA  
ABR1 96  
GTCCTAGTGGCTGAGCAATAGGGTGTGGAAACTGTACTTCGGTTCACTTGCAGAGTTGCA  
ABR8 96  
GTCCTAGTGGCTGAGCAATAGGGTGTGGAAACTGTACTTCGGTTCACTTGCAGAGTTGCA  
E66 121  
GTCCTAGTGGCTGAGCAATAGGGTGTGGAAACTGTACTTCGGTTCACTTGCAGAGTTGCA

Bd21 156 GTGATTCTTGCTAGCATCTAGCTGCATGCACGAA--GAAT-----  
CAGTCATGAATT  
ABR1 156 GTGATTCTTGCTAGCATCTAGCTGCATGCACGAA--GAAT-----  
CAGTCATGAATT  
ABR8 156 GTGATTCTAGCTAGCATCTAGCTGCATGCACGAA--GAAT-----  
CAGTCATGAATT  
E66 181  
GTGATTCTAGCTAGCATCTAGCTGCATGCACGAAATTGAAGAAGCTTAGCAGTCGTGAATT

Bd21 206 GAGCGAGAGAACCGCTGTAGTGCACTCAGCCGGATAAATACATAGTGCTGCGTCGT----  
-  
ABR1 206 GAGCGAGAGAACCGCTGTAGTGCACTCAGCCGGATAAATACATAGTGCTGCGTCGT----  
-  
ABR8 206 GAGCGAGAGAACCGCTGTAGTGCACTCGCCGGATAAATACATAGTGCTGCGTCGT----  
-  
E66 241  
GAGTGAGAGAACCGCTGTAGTGCAATCAGCCGAAATGAGAGTGCTGCGTCGTGAAGC

Bd21 261 -----CTCTGAACTCTGAA--  
CCATTGC  
ABR1 261 -----  
CTCTGAACTCTGAAAACCATTGC  
ABR8 261 -----  
TTCTGAAATCTGAAAACCATTGC  
E66 301  
TGTGCTGCAGTGTTCAGAGGTCTCCCGGCTTCTCGTCTCCGAAATCTGAAAACCATTGC

Bd21 282 AATCAATCTGTGCATAC--  
ACGGGTCTTCTCCACCTGAGACGGTGACTGGTAATTAAC  
ABR1 284 AATCAATCTGTGCATAC--  
ACGGGTCTTCTCCACCTGAGACGGTGACTGGTAATTAAC  
ABR8 284 AATCAATCTGTGCATAC--  
ACGGGTCTTCTCCACCTGAGACGGTGACTGGTAATTAAC  
E66 361 AATCAATCTGTGGATACGTACGGATCTTCTTCCACCTGAGACGGTGACTGGTAATTAAC  
T

Bd21 340  
GGTTGAGTACATGAGATGTGCGGGAGATTCCGTGCAGAAACAAACGAAATCAAAGCAGCG  
ABR1 342  
GGTTGAGTACATGAGATGTGCGGGAGATTCCGTGCAGAAACAAACGAAATCAAAGCAGCG  
ABR8 342  
GGTTGAGTACATGAGATGTGCGGGAGATTCCGTGCAGAAACAAACGAAATCAAAGCAGCG  
E66 420 GGTTGAGTACATGAGATGCGTGGGA~~A~~ATTTCGTGCAGAAACAAAC-  
AAATCAAAGCAGCG

Bd21 400  
GTCAGCTGCAGTTGAACCCCTTGAACGGTGAAGGAAGAAGTTTGGACTAGGGATGACAAGA  
ABR1 402  
GTCAGCTGCAGTTGAACCCCTTGAACGGTGAAGGAAGAAGTTTGGACTAGGGATGACAAGA  
ABR8 402  
GTCAGCTGCAGTTGAACCCCTTGAACGGTGAAGGAAGAAGTTTGGACTAGGGATGACAAGA  
E66 479 GTCAGCTGCAGGTGAACC-  
TTGAACGGTGAAGCAAGGAGTTTGGACTAGGGATGACAAGA

Bd21 460  
CGATATGTAGATCCGTCCACATGCATTGAACAAGCATTTCATAACATTTTGGGCTGCGAAA  
ABR1 462  
CGATATGTAGATCCGTCCACATGCATTGAACAAGCATTTCATAACATTTTGGGCTGCGAAA  
ABR8 462  
CGATATGTAGATCCGTCCACATGCATTGAACAAGCATTTCATAACATTTTGGGCTGCGAAA  
E66 538  
CGATATATAGATCCGTCCACATACATTGAACAAGCATTTCATAACATTTTGGTTTGGCGAAA

Bd21 520 AAATAGAAGTGCGTGGATGCTTGGAGACTTGG-  
GTAGAAGAGGACAAGAGCTTCTCATGC  
ABR1 522 AAATAGAAGTGCGTGGATGCTTGGAGACTTGG-  
GTAGAAGAGGACAAGAGCTTCTCATGC  
ABR8 522  
AAATAGAAGTGCGTGGATGCTTGGAGACTTGGAGTAGAAGAGGACAAGATCTTCTCATGC  
E66 598 AC-  
TAGAAGTACGTGGATGCTTGGAGACTTGGAGTAGAAGAGGACAAGAGCTTCTCATGC

Bd21 579  
ATGCATGCATGGTACGCAATGTGATAAGCTGTAGTTGAGATCCACCTAAAAAATTCAGA  
ABR1 581  
ATGCATGCATGGTACGCCATGTGATAAGCTGTAGTTGAGATCCACCTAAAAAATTCAGA  
ABR8 582  
ATGCATGCATGGTACGCAATGTGATAAGCTGTAGTTGAGATCCACCTAAAATTTTTCAGA  
E66 657 ATGCATG---GG--CGCGG~~T~~ATGATAAGCTGTAGTTGAGATCCACCTAAAA---  
TTCAGA

Bd21 639  
AAAGCGGCCGAATTGATAAGTCAGGCCACATCTTTGATGATAGCTAGCCGGCCAGTAGAG  
ABR1 641  
AAAGCGGCCGAATTGATAAGTCAGGCCACATCTTTGATGATAGCTAGCCGGCCAGTAGAG  
ABR8 642  
AAAGCGGCCGAATTGATAAGTCAGGCCACATCTTTGATGATAGCTAGCCGGCCAGTAGAG  
E66 709  
AAAGCGGCCG~~G~~ATTGATAAGTCAGGCCACATCTTTGATGATAGCTAGCCGGCCAGCAGAG

Bd21 699  
AGTAGAGACAACAGGTGGTGTATATACAGCAAGTCATCAAATTGCTTTTGTGTTTTCAA  
ABR1 701  
AGTAGAGACAACAGGTGGTGTATATACAGCAAGTCATCAAATTGCTTTTGTGTTTTCAA  
ABR8 702  
AGTAGAGACAACAGGTGGTGTATATACAGCAAATCATCAAATTGCTTTTGTGTTTTCAA  
E66 769 AGTAGAGACAACAGATGGTGTAT--  
ACAGCAAGTCATCAAATTGCTTTTGTGTTTTGAAA

Bd21 759  
TGAAGCAACGAGTTCCTTTAGATATCACTAACGTGAGTAGTACGAAGTAGAAGAATTTTC  
ABR1 761  
TGAAGCAACGAGTTCCTTTAGATATCACTAACGTGAGTAGTACGAAGTAGAAGAATTTTC  
ABR8 762  
TGAAGCAACGAGTTCCTTTAGATATCACTAACGTGAGTAGTACGAAGTAGAAGAATTTTC  
E66 827 TGAAGCAACGAGTTCCTTTAGATATCAGTAACGTGA-----  
TAGAAGAATTTTC

Bd21 819 TAGCGATAAAATATGGAGTACAACGCTTT-  
GGCGCGTTGCTCAGCTTTGTCGTCCGTTCA  
ABR1 821 TAGCGATAAAATATGGAGTACAACGCTTT-  
GGCGCGTTGCTCAGCTTTGTCGTCCGTTCA  
ABR8 822  
TAGCGATAAAATATGGAGTACAACGCTTTTGGCGGTTGCTCAGCTTTGTCGTCCGTTCA  
E66 876 TAGCAATAAAATAT-----  
ACAGCACTTTTGGCGCCTTGCTCGGCTTTGTCGTCCGTTCA

Bd21 878  
TCAAAAGTCAGACGTGGCCACTTCTAACTTTTCGCGTAACAGAAAAAGAGAGTGAGATCAT  
ABR1 880  
TCAAAAGTCAGACGTGGCCACTTCTAACTTTTCGCGTAACAGAAAAAGAGAGTGAGATCAT  
ABR8 882  
TCAAAAGTCAGCGGTGGCCACTTCTGACTTTTCGCGTAACAGGAAAAGAGAGTGAGATTAT  
E66 931  
TCAAAAGTCAGACGTGGCCACTTCTAACTTTTCGCGTAACATAAAAAGAGAGTCGGGCCAT

Bd21 938 TTTACAGAGATATCATGGCTGGG--  
TCAGCACGTACGGTAGAGTAATTAAGGCGTACATT  
ABR1 940 TTTACAGAGATATCATGGCTGGG--  
TCAGCACGTACGGTAGAGTAATTAAGGCGTACATT  
ABR8 942 TTTACAGAGATATCATGGCTGGG--  
TCAGCACGTACGGTAGAGTAATTAAGGCGTACATT  
E66 991 TT---  
ACAGATATCATGGCTGGGGGTCAGCACGTATGGTAGAGTAATTAAGGCGTACATT

Bd21 996  
TGTAGGGCGGCAAAGAACAAGTTGCGTTTTACTTTCATCCGACAGTAATCTCAGAGAAATA  
ABR1 998  
TGTAGGGCGGCAAAGAACAAGTTGCGTTTTACTTTCATCCGACAGTAATCTCAGAGAAATA  
ABR8 1000  
TGTAGGGCGGCAAAGAACAAGATGCGTTTTACTTTCATCCGACAGTAATCTCAGAGAAATA  
E66 1048 TGTAGGGCAAACAA-GAACAAAGTTGCGTTTTACTTTCATCCGACAGTAACCTCAGAGAA--  
-

Bd21 1056  
GAATGAATATGTAAGTCAAAGAAGGCGACTAACTAATTACCGACACAAGTGATTTGTC  
ABR1 1058  
GAATGAATATGTAAGTCAAAGAAGGCGACTAACTAATTACCGACACAAGTGATTTGTC  
ABR8 1060  
GAATGAATATGTAAGTCAAAGAAGGCGACTAACTAATTACCGACACAAGTGATTTGTC  
E66 1104 ---  
TGAATATGCAAGTCAAAGCAGGCGACTAACTAATTACCGACACAAGTGATTTGTC

Bd21 1116  
GAGCGGTGGTACGGAAGTACGGAACCTTTATGAACAACACAACAAGGCATGCTGATCAA  
ABR1 1118  
GAGCGGTGGTACGGAAGTACGGAACCTTTATGAACAACACAACAAGGCACGCTGATCAA  
ABR8 1120  
GAGCGGTGGTACGGAAGTACGGAACCTTTATGAACAACACAACAAGGCAAGCTGATCAA  
E66 1161 GATCTCGTGGTACGGAAGTACGGAACCTTTATGAACAACACAACGAGGCAACC---  
TCAA

Bd21 1176 TCTAGCGTCTCTCTAGAACAAACACGCC-  
AGTGACACGTTGGTACAAGAACACAAAA  
ABR1 1178 TCTAGCGTCTCTCTAGAACAAACACGCC-  
AGTGACACGTTGGTACAAGAACACAAAA  
ABR8 1180 TCTAGCGTCTCTCTAGAACAAACACGCC-  
AGTGACACGTTGGTACAACAACACAAAA  
E66 1218 TCTAGCGTATC--TAGAACAAACACGCCCAGTGGACACGTTGCTACAACAA----  
AAAA

Bd21 1235  
TGTGTGTACGACTTATATATCCAACATGTAAGTTCGTCCGAAAAAATCATTA AAAACTG  
ABR1 1237  
TGTGTGTACGACTTATATATCCAACATGTAAGTTCGTCCGAAAAAATCATTA AAAACTG  
ABR8 1239  
TGTGTGTACGACTTATATATCCAACATGTAAGTTCGTCCGAAAAAATCATTA AAAACTG  
E66 1272  
AATGTATACGACTTACATGTCCAATATGTAAGTTCGTCCGAAAAAATCATTA AAAACTG

Bd21 1295  
AATCCAGCAGCACGTGTTAGGTAGAGAGAGTTCTCCTTGTATTCCAAATAGCATGACGCA  
ABR1 1297  
AATCCAGCAGCACGTGTTAGGTAGAGAGAGTTCTCCTTGTATTCCAAATAGCATGACGCA  
ABR8 1299  
AATCCAGCAGCACGTGTTAGGTAGAGAGAGTTCTCCTTGTATTCCAAATAGCATGACGCA  
E66 1332 AATCCAGCAGCACGTGTTAGAGAGAGAGATCTCCTTGTATTCAA-AGCATGACGCA

Bd21 1355  
GAGGCTAAAACGTAGGCAACCGAATACACATCGACCTCCCATATCAGAATGATCAAGAT  
ABR1 1357  
GAGGCTAAAACGTAGGCAACCGAATACACATCGACCTCCCATATCAGAATGATCAAGAT  
ABR8 1359  
GAGGCTAAAACGTAGGCAACCGAATACACATCGACCTCCCATATCAGAATGATCAAGAT  
E66 1390 GAG-CTAAAACGTAGGCAAGCCGAATACACATCGACCTCCTCATATCAGAATGATCA-  
GAT

Bd21 1415 CACACAC-----  
TCTTGATTTGAGTTTCCTGTG  
ABR1 1417 CACACAC-----  
TCTTGATTTGAGTTTCCTGTG  
ABR8 1419 CACACAC-----  
TCTTGATTTGAGTTTCCTGTG  
E66 1448  
CACACACGTACTTGCCGTCCGACGCTACTATACATGTCCCTTTAATTTCGATTTTCCAGTG

Bd21 1443 GAGCAGAGCAAAAACG-----  
TACGTACGTACGAGAACACGAGACGAAACAAAACAAGA  
ABR1 1445 GAGCAGAGCAAAAACG-----  
TACGTACGTACGAGAACACGAGACGAAACAAAACACGA  
ABR8 1447 GAGCAGAGCAAAAACG-----  
TACGTACGTACGAGAACACGAGACGAAACAAAACACGA  
E66 1508 GAGCAGAAAAAAAGCGACCGAGTACGTACGTTCGAGA-  
CACGAGACGAAACAAAACACAA

Bd21 1497 CAAGAGAGCAAGCAATATCTGGTGTC-AT--ATTCTCCGAGAAAGTGGACGGC-----  
-  
ABR1 1499 CAAGAGAGCAAGCAATATCTGGTGTC-AT--ATTCTCCGAGAAAGTGGACGGC-----  
-  
ABR8 1501 CAAGAGAGCAAGCAATATCTGGTGTC-AT--ATTCTCCGAGAAAGTGGACGGC-----  
-  
E66 1567 CAAGAG--CGAGCAATATC-  
GATCTCGATCGGTCTCCGAGAAAGTGGACGGCGCCATGC

Bd21 1547 ---CTTGATGCTTT-  
TGTTCCACTACCGATCGACCCTTGTCTGTGTCGCATCACCAGTGTC-  
ABR1 1549 ---CTTGATGCTTT-  
TGTTCCACTACCGATCGACCCTTGTCTGTGTCGCATCACCAGTGTC-  
ABR8 1551 ---CTTGATGCTTT-  
TGTTCCACTACCGATCGACCCTTGTCTGTGTCGCATCACCAGTGTC-  
E66 1624 ATGCTTGATGCTTTCTGTTCCACTAGCGATCGACCCT-  
GTCTGTGTCGCATCACCAGTGCA

Bd21 1602 -  
CCGATCCAAAAAAGCGCCGCATGCATGCAGCAGCTGGCGGTTCCCTCCGGCCGCTTATA  
ABR1 1604 -  
CCGATCCAAAAAAGCGCCGCATGCATGCAGCAGCTGGCGGTTCCCTCCGGCCACTTATA  
ABR8 1606 -  
CCGATCCAAAAAAGCGCCGCATGCATGCAGCAGCTGGCGGTTCCCTCCGGCCGCTTATA  
E66 1683 CCCGATCCAAAAAAGCGCCGCATGCACAT-  
GCAGCTGGCGGTTCTCGAGCTCCGCTTATA

Bd21 1661 TAAGCCCGGCCCCGCGGTCATAGCC-  
TGCATTCCCAAATTCGAGTCCCACACACTACT  
ABR1 1663 TAAGCCCGGCCCCGCGGTCATAGCC-  
TGCCTTCCCAAATTCGAGTCCCACACACTACT  
ABR8 1665 TAAGCCCGGCCCCGCGGTCATAGCC-  
TGCATTCCCAAATTCGAGTCCCACACACTACT  
E66 1742 TAAGCC---CTCCCGCGC--  
CACAGCCATGCTTCCCAAATTCGAGTCCCACACATTTACT

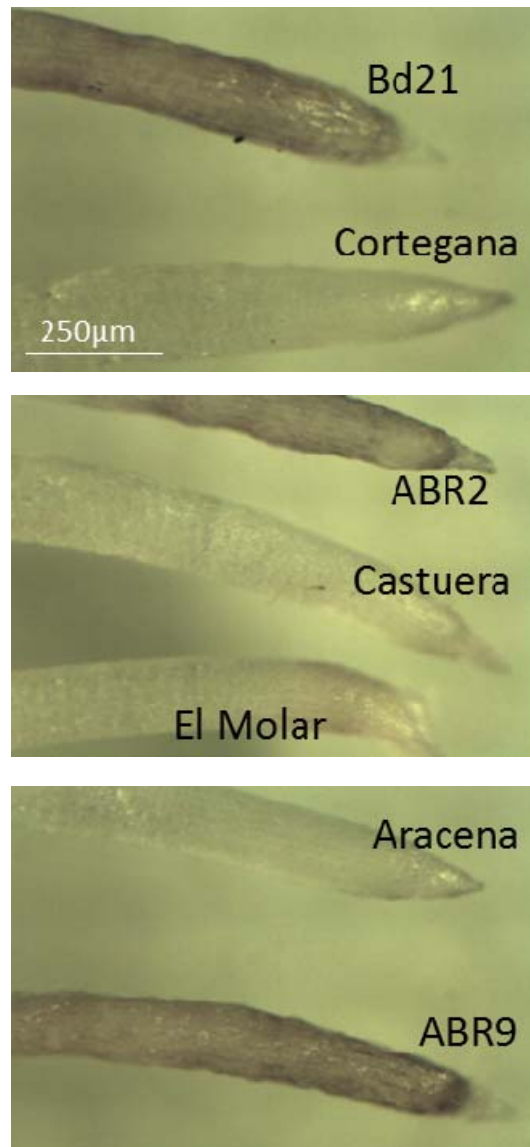
Bd21 1720 AGCTAGTACCAG-----TGCCTAGGTGTTTCACGGAACACAG--  
CACGTACGGC  
ABR1 1722 AGCTAGTACCAG-----TGCCTAGGTGTTTCACGGAACACAG--  
CACGTACGGC  
ABR8 1724 AGCTAGTACCAG-----TGCCTAGGTGTTTCACGGAACACAG--  
CACGTACGGC  
E66 1797  
AGTGAGTACCTGCAGGCACTGGCTAGCTAGGTGCTTCACAGAACACAAGACGCGTACGGC

Bd21 1767 GGCGTATAGA--TAGA-----TCAAAG-----ATG  
ABR1 1769 GGCGTATAGA--TAGA-----TCAAAG-----ATG  
ABR8 1771 GGCGTATAGA--TAGA-----TCAAAG-----ATG  
E66 1857 GACGCATAGAACTAGAAATCCATCGAAATTAATTCGCCATG

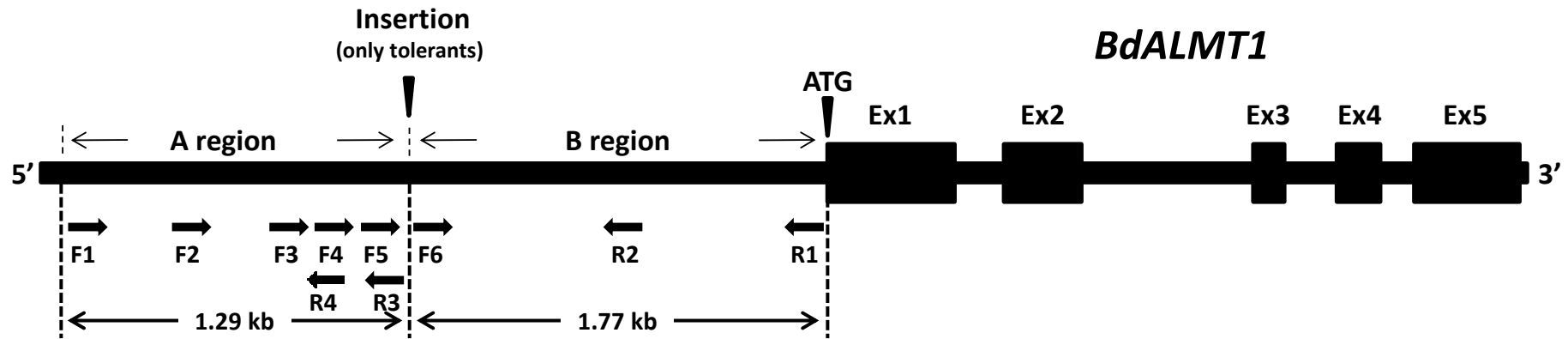




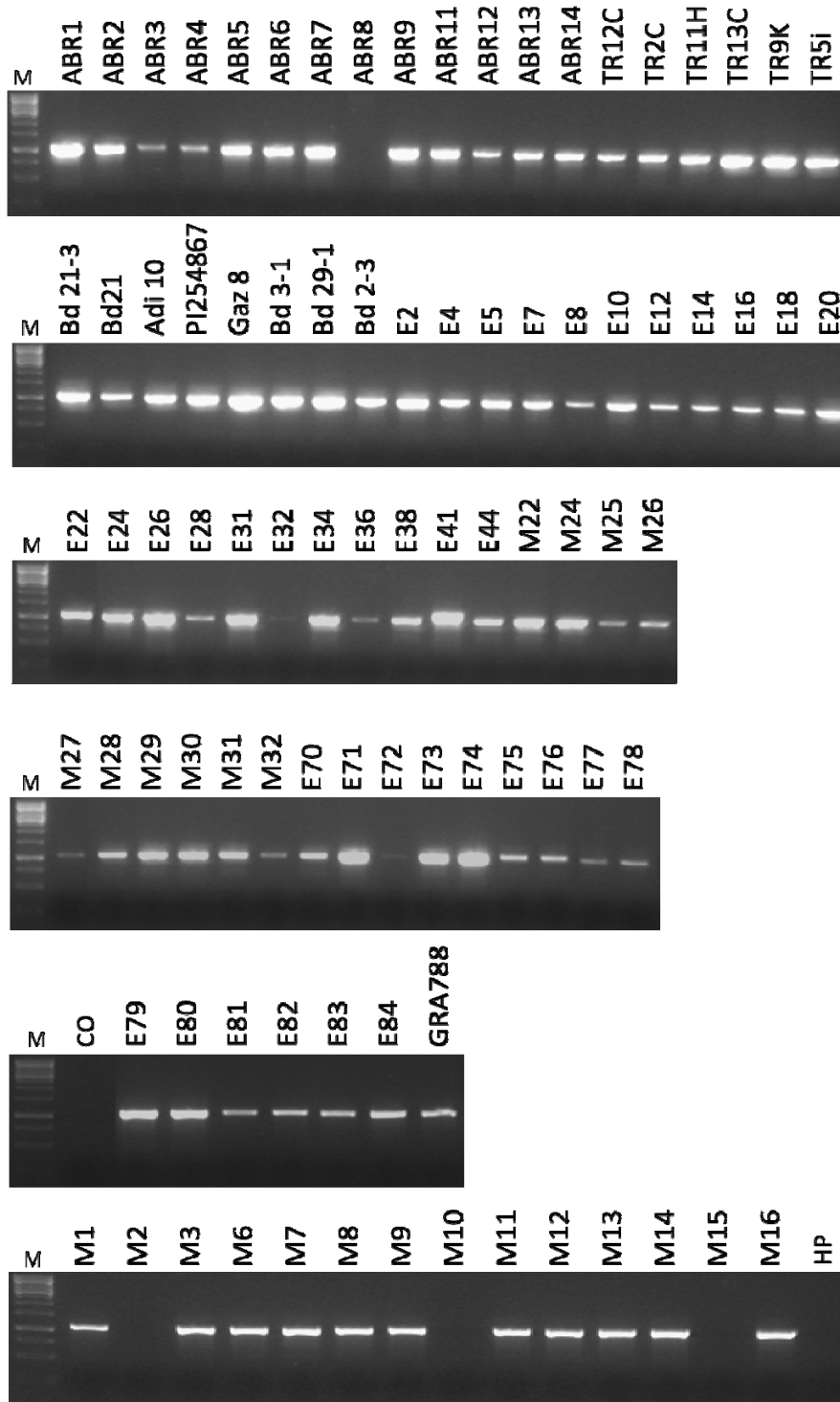
**Fig. S1.** Treatment with Schiff's reagent in *Brachypodium*. Schiff's reagent staining of root apices from several *Brachypodium* lines and ecotypes exposed to 20 $\mu$ M  $\text{AlK}(\text{SO}_4)_2$  for 24 h. There are three or two different root apices for line or ecotype. Diploid tolerant line: ABR8. Allotetraploid tolerant ecotypes: Castuera, Aracena, Serradilla, Cabeza de Buey, El Molar, Hoyo de Pinares and Cortegana. Allotetraploid sensitive line: GRA 788. The remainder lines are diploid sensitive.



**Fig. S2.** Roots without any stain after Al treatment at 10  $\mu\text{M}$   $\text{AlK}(\text{SO}_4)_2$ . Sensitive (dark colour) diploid lines Bd21, ABR2 and ABR9 and tolerant (without dark colour) allotetraploid ecotypes Cortegana, Castuera, El Molar and Aracena.



**Fig. S3.** Genetic map of the *BdALMT1* region. The *BdALMT1* gene has five exons (Ex1 to Ex5) and four introns. The 5' upstream region (promoter) is subdivided into two zones (A and B regions). The first zone, “A region” (1,768 bp), is present in sensitive and tolerant diploids and allotetraploid plants. The second zone, “B region” (1,290 bp), has been amplified in sensitive and tolerant diploids and allotetraploid samples. The site of the probable insertion (among A and B region) and the first codon translated (ATG) have been indicated. The different pairs of primers used to amplify these different regions are shown below the map.



**Fig. S4.** PCR amplification products generated from *BdALMT1* upstream region in 59 diploids and 32 allotetraploid plants using primer pair F3-R2 (see Table 2). This pair of primers was used to detect the presence (without amplification product) and the absence (with amplification product) of one insertion in the promoter region of *BdALMT1* gene. The amplification products were separated on agarose gels electrophoresis and stained with ethidium bromide. The tolerant diploid line ABR8 and tolerant allotetraploid plants (CO, M2, M10, M15 and HP) have the insertion. The remaining sensitive diploid lines and allotetraploid plants have not the insertion.

**Table S1**

<b>Population/Line*</b>	<b>Locality</b>	<b>Chromosome number</b>	<b>Ploidy level</b>	<b>Reference</b>
Bd2-3	Iraq	2n=10	2x	D. Garvin Lab
Bd3-1	Iraq	2n=10	2x	D. Garvin Lab
Bd21	Salakudin (Iraq)	2n=10	2x	D. Garvin Lab
PI254867 (Bd21)	Salakudin (Iraq)	2n=10	2x	Vogel et al. 2006
Bd21-3	Salakudin (Iraq)	2n=10	2x	Vogel et al. 2008
Bd29-1	Ukraine	2n=10	2x	D. Garvin Lab
ABR1	Kaman, Kiresehir (Turkey)	2n=10	2x	Jenkins et al. 2003
ABR2	Octon, Herault (France)	2n=10	2x	Jenkins et al. 2003
ABR3	Aisa, Huesca (Spain)	2n=10	2x	Jenkins et al. 2003
ABR4	Arén, Huesca (Spain)	2n=10	2x	Jenkins et al. 2003
ABR5	Jaca, Huesca (Spain)	2n=10	2x	Jenkins et al. 2003
ABR6	Los Arcos, Navarra (Spain)	2n=10	2x	Jenkins et al. 2003
ABR7	Otero, Valladolid (Spain)	2n=10	2x	Jenkins et al. 2003
<u>ABR8</u>	Siena (Italy)	2n=10	2x	Jenkins et al. 2003
ABR9	Ljubljana (Croatia)	2n=10	2x	Jenkins et al. 2003
ABR11	Manisa, Soma (Turkey)	2n=10	2x	Jenkins et al. 2003
ABR12	Iraq	2n=10	2x	Jenkins et al. 2003
ABR13	Iraq	2n=10	2x	Jenkins et al. 2003
ABR14	Turkey	2n=10	2x	Jenkins et al. 2003
BdTR2C	Near Kaşören (Turkey)	2n=10	2x	Vogel et al. 2009
BdTR12C	Near Beyvelioğlu (Turkey)	2n=10	2x	Vogel et al. 2009
BdTR13C	Near Afşar (Turkey)	2n=10	2x	Vogel et al. 2009
BdTR11H	Near Cambazdere (Turkey)	2n=10	2x	Vogel et al. 2009
BdTR5I	Near Yukarı Ç. (Turkey)	2n=10	2x	Vogel et al. 2009
BdTR9K	Near Kireçköyü (Turkey)	2n=10	2x	Vogel et al. 2009
Adi 10	Adiyaman (Turkey)	2n=10	2x	Vogel et al. 2009
GAZ-8	Gaziantep (Turkey)	2n=10	2x	Vogel et al. 2009
M22	Roncal, Navarra (Spain)	2n=10	2x	Manzaneda et al. 2012
M24	Cádiz (Spain)	2n=10	2x	Manzaneda et al. 2012
M25	Valladolid (Spain)	2n=10	2x	Manzaneda et al. 2012
M26	Valencia (Spain)	2n=10	2x	Manzaneda et al. 2012
M27	Valencia (Spain)	2n=10	2x	Manzaneda et al. 2012
M28	Lugo (Spain)	2n=10	2x	Manzaneda et al. 2012
M29	Lérida (Spain)	2n=10	2x	Manzaneda et al. 2012
M30	Jaén (Spain)	2n=10	2x	Manzaneda et al. 2012
M31	Palencia (Spain)	2n=10	2x	Manzaneda et al. 2012
M32	Murcia (Spain)	2n=10	2x	Manzaneda et al. 2012
E2	V. Guadalimar, Albacete (Spain)	2n=10	2x	E. Benavente
E4	Albacete (Spain)	2n=10	2x	E. Benavente
E5	Albacete (Spain)	2n=10	2x	E. Benavente
E7	Fregenal, Badajoz (Spain)	2n=10	2x	E. Benavente
E8	Gabaldón, Cuenca (Spain)	2n=10	2x	E. Benavente
E10	Tórdiga, Cuenca (Spain)	2n=10	2x	E. Benavente
E12	Rocho, Cuenca (Spain)	2n=10	2x	E. Benavente
E14	Huerta Marquesado, Cuenca (Spain)	2n=10	2x	E. Benavente
E16	Cuenca (Spain)	2n=10	2x	E. Benavente
E18	Río Cuervo, Cuenca (Spain)	2n=10	2x	E. Benavente
E20	Onitar, Granada (Spain)	2n=10	2x	E. Benavente
E22	Paso de Zegri, Granada (Spain)	2n=10	2x	E. Benavente
E24	Iznalloz, Granada (Spain)	2n=10	2x	E. Benavente
E26	Baza, Granada (Spain)	2n=10	2x	E. Benavente
E28	Guadalajara (Spain)	2n=10	2x	E. Benavente
E31	Guadalajara (Spain)	2n=10	2x	E. Benavente
E32	Guadalajara (Spain)	2n=10	2x	E. Benavente
E34	Guadalajara (Spain)	2n=10	2x	E. Benavente
E36	Guadalajara (Spain)	2n=10	2x	E. Benavente
E38	Guadalajara (Spain)	2n=10	2x	E. Benavente
E41	Huesca (Spain)	2n=10	2x	E. Benavente
E44	Huesca (Spain)	2n=10	2x	E. Benavente
E66	Cala, Almería (Spain)	2n=20	2x	E. Benavente
E70	Albacete (Spain)	2n=30	4x	E. Benavente
E71	Cala, Almería (Spain)	2n=30	4x	E. Benavente

E72	San José, Almería (Spain)	2n=30	4x	E. Benavente
E73	Badajoz (Spain)	2n=30	4x	E. Benavente
E74	Cáceres (Spain)	2n=30	4x	E. Benavente
E75	Granada (Spain)	2n=30	4x	E. Benavente
E76	Huesca (Spain)	2n=30	4x	E. Benavente
E77	Jaén (Spain)	2n=30	4x	E. Benavente
E78	Jaén (Spain)	2n=30	4x	E. Benavente
E79	Madrid (Spain)	2n=30	4x	E. Benavente
E80	Sant Lluís, Mallorca (Spain)	2n=30	4x	E. Benavente
E81	Zaragoza (Spain)	2n=30	4x	E. Benavente
E82	Las Palmas (Spain)	2n=30	4x	E. Benavente
E83	Buenaventura, Tenerife (Spain)	2n=30	4x	E. Benavente
E84	Gomera (Spain)	2n=30	4x	E. Benavente
GRA 788	Iran	2n=30	4x	IPK Gatersleben
M1	Cazorla, Jaén (Spain)	2n=30	4x	Manzaneda et al. 2012
<u>M2</u>	Córdoba (Spain)	2n=30	4x	Manzaneda et al. 2012
M3	Ciudad Real (Spain)	2n=30	4x	Manzaneda et al. 2012
M6	Algeciras, Cádiz (Spain)	2n=30	4x	Manzaneda et al. 2012
M7	Mogadour (Portugal)	2n=30	4x	Manzaneda et al. 2012
M8	Rosas, Gerona (Spain)	2n=30	4x	Manzaneda et al. 2012
M9	Monegros, Cáceres (Spain)	2n=30	4x	Manzaneda et al. 2012
<u>M10</u>	Río Águeda, Salamanca (Spain)	2n=30	4x	Manzaneda et al. 2012
M11	Jaén (Spain)	2n=30	4x	Manzaneda et al. 2012
M12	Nerio, Albacete (Spain)	2n=30	4x	Manzaneda et al. 2012
M13	Cubillas, Granada (Spain)	2n=30	4x	Manzaneda et al. 2012
M14	Murcia (Spain)	2n=30	4x	Manzaneda et al., 2012
<u>M15</u>	Lepe, Huelva (Spain)	2n=30	4x	Manzaneda et al. 2012
M16	Baza, Granada (Spain)	2n=30	4x	Manzaneda et al. 2012
<u>Aracena (AR)</u>	Huelva (Spain)	2n=30	4x	M.A. Casado
<u>Cabeza de Buey (CB)</u>	Badajoz (Spain)	2n=30	4x	M.A. Casado
<u>Castuera (CA)</u>	Badajoz (Spain)	2n=30	4x	M.A. Casado
<u>Cortegana (CO)</u>	Huelva (Spain)	2n=30	4x	M.A. Casado
<u>El Molar (EM)</u>	Madrid (Spain)	2n=30	4x	M.A. Casado
<u>Hoyo de Pinares (HP)</u>	Ávila (Spain)	2n=30	4x	M.A. Casado
<u>Serradilla (SE)</u>	Cáceres (Spain)	2n=30	4x	M.A. Casado

(\*) The Populations/Lines underlined were classified as AI tolerant by relative root growth and different roots histochemical staining methods. The Populations/Lines not underlined were classified as AI sensitive.

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**Table S2.** Sequences of the primer pairs used in this study to amplify the encoding and the promoter regions of the *BdALMT1* (Bd) and *BsALMT1* (Bs) genes.

<i>ALMT</i> gene region	Sequence (5'→3')	Size (pb)	T (°C)
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Bd/coding	Forward	ATGGAGGTTGATCACCAGATCAG	736	60
	Reverse	TAGTGGTGCAGAGGCATGTGAA		
Bd/coding	Forward	GTGGAGGAGCTCATCCAGCTCG	805	53
	Reverse	CTTGAGAAAGGTTTTACTCTC		
Bd/coding	Forward	GATGGAATCTGAATGCTTTGGAG	820	55
	Reverse	CTCGCAATGTTGATAACAACATC		
Bs/coding	Forward	ATGGAGGTTGATCACCAGATATCC	783	60
	Reverse	TAGTGGTGCAGAGGCATGTGAA		
Bs/coding	Forward	GTGGAGGAGCTCATCCAGCTCG	820	53
	Reverse	CTTGAGAAAGGTTTTACTCTC		
Bs/coding	Forward	GATGGAATCTGAATGCTTTGGAG	818	55
	Reverse	CTCGCAATGTTGATAACAACATC		
Bd/upstream	F6	TGTCGGGGAATAGATCGGAGA	1,789	56
	R1	CATCTTTGATCTATCTATACGCCGCC		
Bd/upstream	F6	TGTCGGGGAATAGATCGGAGA	679	56
	R2	ATCATCAAAGATGTGGCCTGACTTAT		
Bd/upstream	F1	ATCAAATGAAAGCGGTTCCAT	1,046	57
	R4	CGGTGGGGTGACGGCAACGCAGC		
Bd/upstream	F2	CCACTAAAGATGTACTGCGATAATCA	596	57
	R4	CGGTGGGGTGACGGCAACGCAGC		
Bd/upstream	F2	CCACTAAAGATGTACTGCGATAATCA	674	57
	R3	AGACGGCGGGGGAGCGAC		
Bd/upstream	F1	ATCAAATGAAAGCGGTTCCAT	1,969	56
	R2	ATCATCAAAGATGTGGCCTGACTTAT		
Bd/upstream	F2	CCACTAAAGATGTACTGCGATAATCA	1,519	56
	R2	ATCATCAAAGATGTGGCCTGACTTAT		
Bd/upstream	F3	GCCCGGATCTGACGCGCCCTAAT	1,058	58
	R2	ATCATCAAAGATGTGGCCTGACTTAT		
Bd/upstream	F4	CGAGATTCGCTGCGTTGCCGTCACCC	954	57
	R2	ATCATCAAAGATGTGGCCTGACTTAT		
Bd/upstream	F5	TGTCTCTCTCGGCCCTACC	1,864	56
	R1	CATCTTTGATCTATCTATACGCCGCC		

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**Table S3. Mean lengths of root growth (mm) at different treatments without Al and with Al (10  $\mu$ M)**

Values are means of 16 plants from three independent replicates

Source	Ploidy	Root growth		Relative root growth inhibition (%) (*)
		0 $\mu$ M Al	10 $\mu$ M Al	
		Mean (mm)	Mean (mm)	
ABR8	2n=2x=10	15 $\pm$ 1	11 $\pm$ 3	26 (T)
ABR1	2n=2x=10	17 $\pm$ 2	2 $\pm$ 2	88 (S)
ABR9	2n=2x=10	5 $\pm$ 1	0.7 $\pm$ 0.7	86 (S)
Bd21	2n=2x=10	15 $\pm$ 3	1 $\pm$ 2	93 (S)
E10	2n=2x=10	8 $\pm$ 2	0.1 $\pm$ 0.1	98 (S)
ABR6	2n=2x=10	23 $\pm$ 2	1 $\pm$ 1	95 (S)
M2	2n=4x=30	14 $\pm$ 2	14 $\pm$ 1	0 (T)
M15	2n=4x=30	23 $\pm$ 4	19 $\pm$ 4	17 (T)
M10	2n=4x=30	16 $\pm$ 3	12 $\pm$ 4	25 (T)
HP	2n=4x=30	20 $\pm$ 4	17 $\pm$ 4	15 (T)
CB	2n=4x=30	23 $\pm$ 5	18 $\pm$ 5	21 (T)
M12	2n=4x=30	25 $\pm$ 3	6 $\pm$ 1	77 (S)
M11	2n=4x=30	27 $\pm$ 5	5 $\pm$ 2	81 (S)
E82	2n=4x=30	10 $\pm$ 2	1.5 $\pm$ 1.5	85 (S)
GRA788	2n=4x=30	12 $\pm$ 3	0.2 $\pm$ 0.2	98 (S)
E80	2n=4x=30	9 $\pm$ 02	0.1 $\pm$ 0.1	98 (S)
E78	2n=4x=30	6 $\pm$ 1	0.6 $\pm$ 0.6	90 (S)

(\*) Relative root growth inhibition= [(Mean without Al – Mean with Al)/ Mean without Al] x 100. S = Al-sensitive. T = Al-tolerant.