

Overexpression of the aspartic protease *ASPG1* gene confers the drought avoidance in *Arabidopsis*. Xuan Yao, Wei Xiong, Tiantian Ye, and Yan Wu

SUPPLEMENTARY DATA

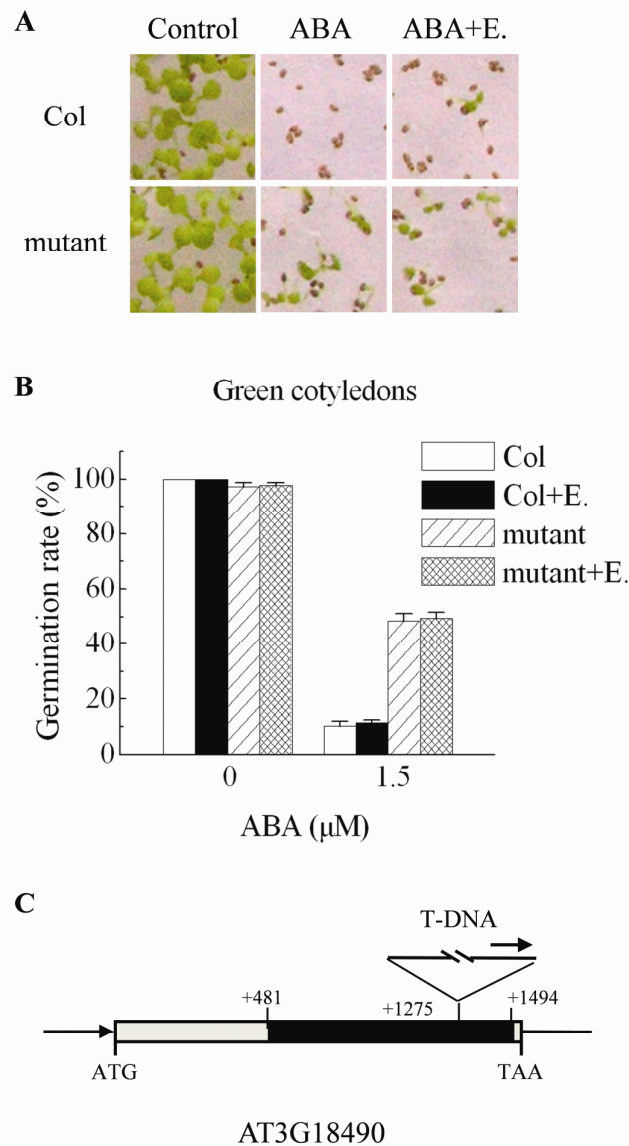


Fig. S1. The original screening with the XVE-tagging T-DNA insertion mutant lines. (A) Seeds of XVE T-DNA-tagging lines were screened on the MS plate containing 1% sucrose, 0.8% (w/v) agar, with or without 10 μM 17- β -estradiol (E.) and 1.5 μM ABA. Photographs were taken to show the germination phenotypes at the day 7 after stratification. (B) Germination rates (%) were analyzed at the day 7 after stratification by scoring the open green cotyledons. Values are means \pm SE from three independent experiments (n=100). (C) Schematic drawing (not in scale) to show T-DNA insertion site in gene AT3G18490 revealed in the original mutant screen. Putative aspartic protease domain: +481 bp to +1494 bp (black box). Arrows denote the orientation of gene transcription.

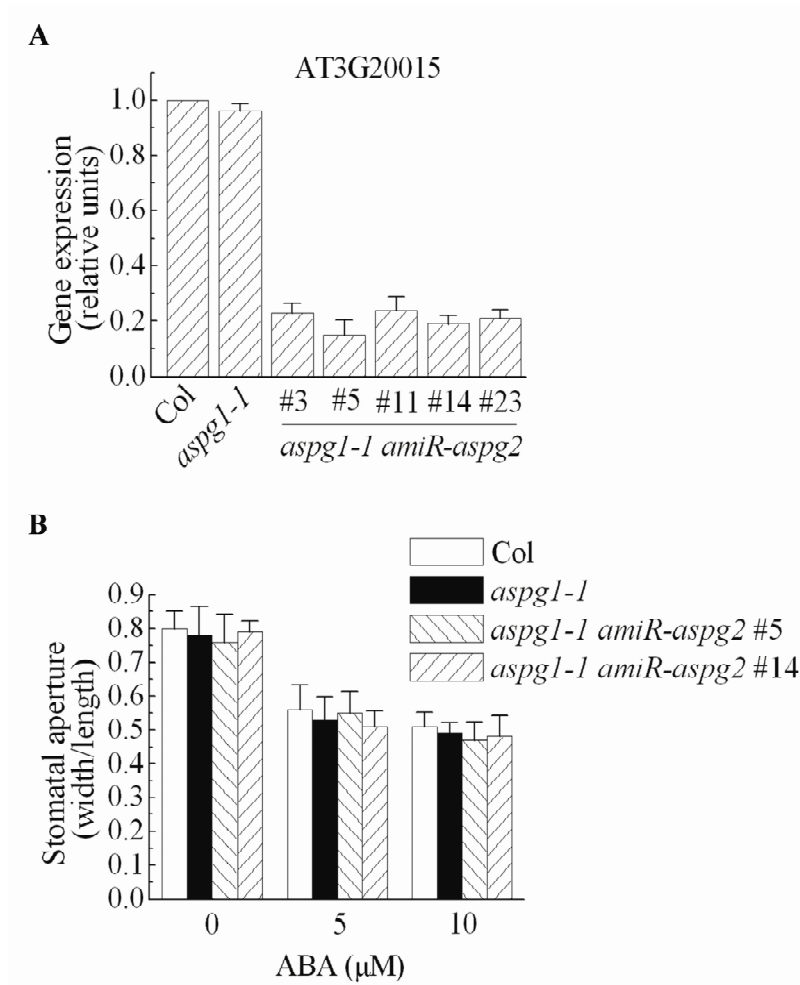


Fig. S2. The response to ABA of the artificial microRNA (amiRNA) lines. (A) Analyses of gene expression of *ASPG2* (AT3G20015) in Col, *aspg1-1* and *aspg1-1 amiR-aspg2* lines (#3, #5, #11, #14 and #23). The expression levels were analyzed as the relative unit against with the level of *ASPG2* in Col plants, which was taken as “1”. Each value is the mean \pm SE of three independent experiments. (B) ABA-induced stomatal closure. Values are means \pm SE from three independent experiments (n=50). The leaves from 4-week-old plants of Col, *aspg1-1* and *aspg1-1 amiR-aspg2* lines (#5 and #14) were first incubated in the light for 3 hours to induce stomatal opening and then treated with ABA (0, 5, and 10 μM ABA) for 3 hours.

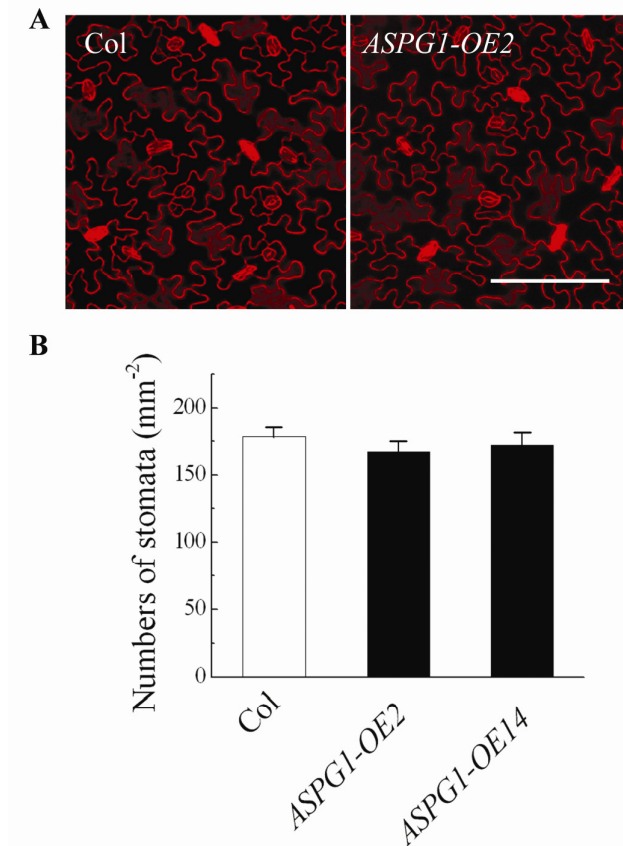


Fig. S3. Overexpression of *ASPGI* has no effect in the development of guard cells. (A) The epidermis of the abaxial surface of rosette leaves from Col and *ASPGI-OE2* plants (Bar = 80 μm). (B) Numbers of stomata per square millimeter in the epidermis of the abaxial surface of rosette leaves of Col, *ASPGI-OE2*, and *ASPGI-OE14* lines were determined. Values are the means \pm SE from leaves of three individual plants of Col and *ASPGI-OE* lines. Three independent counts were performed on each leaf.

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ASPG1 : MAFPRFLSLLAVVTLSLFLTTDASSRSLSTPPKTNVLDVSSLQOTQTLSLSDPTRSSLTTPKESLSDPVFFNSSSSPLSLELHSDRTFVASQHKDYKS : 100
CDR1 : -----MASLFSVVTLISCLLSLFLSN-----ANAKPKLGTADLHRDPSKS : 43
PCS1 : -----MFSRFHAFPLLVLSVRTYKCVS-----SSSSSSSFSSSFSSS : 41
CND41 : MEHSLMSTGSYFLLESFAFLLLLSFVSKSHALETRETIESHPTLQSSLLPSSSCNPAKTKG-----RRGASLEVVNRGCPCLLNQKAKAP : 92
cnb-1 : -----MUTYRSFRPAASNST-----SAFVSMHRRHGPCSPF-LQTPDDAP : 39
S5 : -----MVILEQPQLLLLLELVAATAATGATAADDELECP-----SIFDHAVNSQGAIQPFVFKHKHQLC : 61
Q3UKT5 : -----LLVLLLELLELQAQAGALHRVP-----LRRHOSLKKLRAOQQLS : 44
Q8WWD9 : -----MSPPELQLPQLLLELPHNVFPGGATLIRIP-----LHRVQPGRRLLN---LL : 44
Q9VLK3 : -----MFKTIAVVLAAALASAEHLHRVP-----LLKEQNFVKTRQN---V : 37

ASPG1 : LTLSLERDSSVAGIVAKIRFAVEGVDRSDLKPVYNEDTRYQEDLTTFVVSASQSGSEVFSRPLVCHPAKEMYVLDTPGSDVNHQCEPQADC---- : 196
CDR1 : PFYNPMETSSQVIRNAIHRSVNRVE-----HFTEKDNTPOPIIDLTNSGELMNVSHGCPFFPPIMAADTGGDLITQCAEPDDC---- : 124
PCS1 : SSQTLVLPKTRT-----PTDHRPTDKLPHHNVLTVPLVCHPQNISVLDTPGSELNIRCNRSS----- : 105
CND41 : TLTEILAHDOAVDSIQARITDQSYDLFKKKDKSSNKKKSVKDSKANLPAQSGPLGTGNIVNVSHGCEPKKDLSELDTPGSLDITQGOQPVKS---C : 189
cnb-1 : SDADLLEHDOAVDSIHRMIANET-----AVVGQDVSLPAERGISVGTGNIVNVSHGCEPARDLTVLDTPGSDLSNVQGGVPGSSGG--C : 121
S5 : RFWVRATQASSTGASGAGGGGLNN-----LQEBEITSSSSSTKIDVIEDSSINDFLVLMVSHGCEPQVNVLDVLDTPGSLSNVQGGVPAVHCHTQ : 152
Q3UKT5 : EFWRSHLMDTSLIS-----ESCNVYSSVNEPLINYLDMEVFGTSHGCEPQNFTVLDTPGSLSNLVPSVY---S-P : 113
Q8WWD9 : RGWRPE-ALPLHIG-----APSPGKPIFVPLSNYRDVQVFGELHGPCPQNFTVLDTPGSLSNLVPSRRHFHFS--V : 114
Q9VLK3 : LAEKSYLRKYQCP-----SLRSEVD---QLSNSNMMAVCAASVCTLAQSEKVLFDGSSSLNLDVSNITK--S-D : 103

ASPG1 : YQOQSDVFNHTSSVTKSITCSAPOCSLLETSAACRSNK-----CLVQVSYE-DGSEFVVEJATVTVFGNS-----GKINNVALGCGHNEG : 277
CDR1 : YTVNDFEDDKTSSTKDWSCSSSOCTALENQASCSTN-----DNTCSMSIYEDNSYTRCNNAVETLITGSS-----DTRPMOLKNIIIGCGHNAG : 212
PCS1 : NFMVFNVEDPTSSSSPHPCSSPTCRTRRDFLIASCDS--DKLCHAFISMA-DASSEPCNAAEIHFGNST-----NDSNLIFPCMGSVSG : 192
CND41 : YAQQDPEFDETSKTSNHSCTSAACSLSKATGNSPGC-S--SSNVVYCHQYD-DS-ETICFAKIKITQON-----DVFDGFMFGCGGNKKG : 275
cnb-1 : YHQDPEFAHSSSTSAVRCGEPCCPRARQSCSSPG-----DRCPEVYVYD-KKRTVCHIGNTITLITGTPSTNASNNSNKLPGVFPVCGGNNTG : 215
S5 : SAKAGPEFDEGRSISRRVRCSSVKCGELRYDLRQQANCKEKEDSCTVSVYVNGWAYSVKIVTITRIGDS-----FMDLMPGSMDBVKY : 240
Q3UKT5 : ACKAHFDEHSSQDTEVGNH-----SHTQYETGS-LTCLGAVQVSEGLT-----VDGQGEQSEVKEPGQT : 176
Q8WWD9 : PCWLHHPDEKASSVQANGTK-----ALQVYETGR-VDCHSEFVITGCIK-----GASVIEGALWEPSLV : 177
Q9VLK3 : ACLTHNVSSASSTVAVGES-----SHTQYETGS-LTCLGAVQVSEGLT-----IQSQTAEESTNEPGTN : 166

ASPG1 : ---LFT-GAAGLCLGGGVSLITNOMKATS---FSYCVVDR---DSGKSSLDNFNSVOLGGDAT---ABLLRNKIDTXYVIGISGFSVGGKVVV : 361
CDR1 : ---TENKKGSLVCLGGGVSLIKQLGDSIDGKFSYCVVPLTS---KKDQTSKINFNTAIVSGSGVVS---TPLIAKASQETXYTKSISVSGKQIQY : 304
PCS1 : SDPEEDTKTTLCLGNRGSSEFISOMGFP---KFSYCVSGTDDFPGLLGDSINFVLTPLNYTFLIR---ISTPLPYFDRAVATQITGKLVNGKLLPI : 286
CND41 : ---LFG-KTAGLCLGRDPELVVQTAQKFGKFSYCVPTSR---GSHGLTFGNGVGVKAKVNGITTFEFAS-SQGTAYFPHDLCLVSGGKALS : 367
cnb-1 : ---LFG-KADGFLCLGRKVSLSQAAGKYGEGFSYCVPS---SSNAHYLSLGTAPAPAHAR---FTPLNRSNTPSXYVILVGLRAGRAIKV : 303
S5 : ---SFEFAVFFFGSSSFFFEQLAGYF-DILSYKAFSYCLPTDETKPGVMILGRYDRAAMDGG---YTELFRSINRPTSLTMEMLANGORIVTS : 330
Q3UKT5 : ---FVNAHFS---FVNAHFS---GS---LNWIIVTKQAQVQANADGCLVQ--- : 207
Q8WWD9 : ---FAFAHFDLCLGFPILSVEGVVPPMDVLVROGLDKPVPFSFYLNRPPEEPDGGELVLLGSDPAHYIPLTFVVEVTPAQLQHIERKVG--- : 268
Q9VLK3 : ---FNDANFDLCLGAYESAVDGVAPPFYNNMVSOGLDQNSVFSFYLRADGTSTMGGELIFGSDASLYSGALTYVPISEQVQFTVAGSSIT--- : 256

ASPG1 : PDAIFDVASGSGGVLLCCLAVTRLOTQAYNSRDAFLKLTV---NLK-KGSSSISLFDTCYDFSSLS---TVKVFVAEHETGKSLDLEPA--- : 447
CDR1 : SSGDSSESE---GNILIDSGTITLLPTEFYSEEDAVASSID---AE---KKQDPQSGLSLCLCYSAAGDL---KVFVIMHEDH---ADVKLDS--- : 383
PCS1 : PKSVLVPDHTGAGQTMVDSGQPTFLGVPVYTAQRSHFLNRTNGILTVLDEPDEFVFGQTMDLCYRISFVIRIRSGILHRPFTVSLVECAEIAVSGQLLY : 386
CND41 : S---PMLFQN---AGTLIDSGVITRLPSTAYGSKSAFKQMS---KY---PTAPALSLDTCYDLNNTY---SISFIRISFNENGNANVELDPP--- : 447
cnb-1 : SSRPALWP---AGLVDSGVITRLAPRAYSARTAFLSAMG---KYGYKRAPRLSILDTCYDFVAHAN---ATVSEFAVAVLVEAGGATISVDF--- : 388
S5 : SSEMEVDSGAQRTSLMPSFALDKTITQAMSSCYHRTSRARQESYICYLSEHDYSGMNGTITPFSNWS---ADPVEIIEAGGAALALDPP--- : 419
Q3UKT5 : DTVMECSSEG---CAALVDTGSSITGPPDKIKQVBEAIG---ATP-IDGEYAVDCATLD---TENVVFLINEVSYTLNPT--- : 278
Q8WWD9 : EGLTICAKG---CAALVDTGSSITGPPDEIRAHAAIG---GIPLLAGYEIILCSEIP---KPEAVSFLLGAVVFNLTAAH--- : 340
Q9VLK3 : DGYSLCDD---CAALVDTGSSIVAVYNAITSEILN-----VGE---DGYLDCSVS---SPEVTFNIGCTNFVLKPS--- : 323

ASPG1 : KNYIIPVDSGTFEFAAPTSS---SLSLGNVQOQGTRETYDLS---KNVTLGSGNKC----- : 500
CDR1 : SNAFVQVS-EDLVFAERGSFS---FSYCVNAQMNELVGDVTV---SKTVSEKPTDCAKM----- : 437
PCS1 : RVPHLTVGSDSYVETFGNSDLMG---MEAYVCHHHQONMWEFDDQ---RSRLGLAPVECDVSGQRLGIGS----- : 453
CND41 : NGIITING-ASQVCFARAGNGDD---DSIGFCHIQOQTELVVVDVA---GGQLGFGYKGCSS----- : 502
cnb-1 : SCVIVYVAK-VAQCFARAPNGNG---RSAGLCHTQORTVAVVVDVG---ROKIFPAKGCSS----- : 443
S5 : -RMVFNYPHGRGLMTEAQNPAIR---SOLIGNRVTRSEGTEDDQ---GKQFSEKYAAC----- : 472
Q3UKT5 : DYTIPDLVGMQFCSGFGQLDIPPPAGPLWELGDVPIRQFYSVDRG---NNQVGLAPAVP----- : 337
Q8WWD9 : DYVQTRNGVRLCLSGFQALDVPVPPAGPFWELGDVPLGTYYAVVDRGDMKSSARVGLARARTGADLGMGETAQAAQFPG : 420
Q9VLK3 : AYTLQSDGN---MSAFEYMGV---DFWELGDFVFIGOXYEFDLG---NNRIGFAPVA----- : 372

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Fig. S4. Two conserved putative aspartic activation sites in ASPG1 protein. The alignments between predicted ASPG1 protein sequences containing 2 aspartic proteases in a number of organisms by using Clustalx and GeneDoc3.2 tools. CDR1 and PCS1 are from *Arabidopsis thaliana*. CND41 is from *Nicotiana tobacum*. CNB-1 is from *Brachypodium sylvaticum*. S5 is from *Oryza sativa*. Q3UKT5 is from *Mus musculus*. Q9VLK3 is from *Drosophila melanogaster*. Q8WWD9 is from *Homo sapiens*. Arrows indicate the two catalytic aspartic acid residues in ASPG1 protein.

Table S1 Primer sequences were used for plasmids constructions in this study.	
ProASPG1-GUS	F: 5'-TTTCCTGCAGGTTGTGGATGTTAGAGACG-3'
	R: 5'-TCCCCCGGGTATTTCCGGCGAAGGT-3'
p35S-CFP-ASPG1	F: 5'-GGGGTACCATGGCTTTCCCGCGATTT-3'
	R: 5'-TCCCCGCGGTTAGCATTATTCCT-3'
pET-30c-ASPG1	F: 5'-CGGGATATCTAATGGCTTTCCCGCG-3'
	R: 5'-ATAAGAATGCGGCCGCTTAGCATTATTCCTGAC-3'
pET-30c-ASPG1 _{D180N}	F: 5'-CTTGGTTCTCAACACCGGAAGCGACGTAAATTG-3'
	R: 5'-CGCTTCCGGTGTTGAGAACCAAGTACATCTCTT-3'
pET-30c-ASPG1 _{D379N}	F: 5'-AGTGATCTTGAATTGTGGAACCGCCGTGACTCG-3'
	R: 5'-CGGTTCCACAATTCAAGATCACTCCTCCGCTTC-3'
pET-30c-ASPG1 _{D180N/D379N}	See pET-30c-ASPG1 _{D180N} and pET-30c-ASPG1 _{D379N}
p35S-ASPG1	F: 5'-GGGGTACCATGGCTTTCCCGCGATTT-3'
	R: 5'-TCCCCGCGGTTAGCATTATTCCT-3'
p35S-ASPG1 _{D180N} p35S-ASPG1 _{D379N} p35S-ASPG1 _{D180N/D379N}	See p35S-ASPG1

Table S2 Primer Sequences were used for semi-quantitative and real-time RT-PCR experiments in this study.	
<i>ACTIN2</i>	F: 5'-ATGGCAGACGGTGAGGATATTCA-3'
	R: 5'-GCCTTTGCAATCCACATCTGTTG-3'
<i>ASPG1</i>	F: 5'-ATGGCTTCCCGCGATTCTT-3'
	R: 5'-GCATTTATCCCTGACAATCCGAT-3'
<i>KAT1</i>	F: 5'-TTAGCAGCTGTGTAATTGTTTAC-3'
	R: 5'-ACATGTTCTCACTGATGGATGATG-3'
<i>CBP</i>	F: 5'-CTTCAAACCTAAAGACGGAGGC-3'
	R: 5'-TGCTCTTGCTCGCTGACC-3'
<i>β-ACTIN8</i>	F: 5'-AGTGGTCGTACAACCGGTATTGT-3'
	R: 5'-GAGGATAGCATGTGGAAGTGAGAA-3'
<i>ASPG1</i>	F: 5'-TCGACGTCGTTTCATCTCTC-3'
	R: 5'-GTTTGGTGGTGGTGAGTGAG-3'
<i>DREB2A</i>	F: 5'-AACCTGTCAGCAACAACAGC-3'
	R: 5'-AAACACATCGTCGCCATTTA-3'
<i>DREB2B</i>	F: 5'-AAGCTGCTTCCGCTTATGAT-3'
	R: 5'-AGGGAAGTTAAGACGAGCCA-3'
<i>ABF2</i>	F: 5'-TTACAGGCAAGGATCATGGA-3'
	R: 5'-CACGGAAACAACAACCAAG-3'
<i>MYB2</i>	F: 5'-ATGGACCGAGGAAGAAGATG-3'
	R: 5'-TTGATGATACCAGAGGAACGA-3'
<i>MYC2</i>	F: 5'-GGTGAGAACGACCCGTCTAT-3'
	R: 5'-CGTTACCCGGTTCGTTAGAT-3'
<i>KIN1</i>	F: 5'-GCAATGTTCTGCTGGACAAG-3'
	R: 5'-TACACTCTTCCCGCCTGTT-3'
<i>KIN2</i>	F: 5'-CAGAGACCAACAAGAATGCC-3'
	R: 5'-GAAAGAGTACCTCAGCTTTGCC-3'

Table S2 Primer Sequences were used for semi-quantitative and real-time RT-PCR experiments in this study (continued).	
<i>RAB18</i>	F: 5'-AGCTCTAGCTCGGAGGATGA-3'
	R: 5'-CATGATGACCTGGCAACTTC-3'
<i>RD20</i>	F: 5'-TATGGCAGGCTTTCAAACAA-3'
	R: 5'-GAGAATTGGCCCTCTCTTTG-3'
<i>RD22</i>	F: 5'-TTATTGAAGGTAGTGGCGATTG-3'
	R: 5'-ATGGAGAGAGTTGGGAATGG-3'
<i>RD20</i>	F: 5'-TATGGCAGGCTTTCAAACAA-3'
	R: 5'-GAGAATTGGCCCTCTCTTTG-3'
<i>RD26</i>	F:5'-TGTTACAGTTGGATGATTGGG-3'
	R:5'-AGAAGACGATGACGACCCAT-3'
<i>RD29A</i>	F: 5'-AGGAACCACCACTCAACACA-3'
	R: 5'- GCTCATGCTCATTGCTTTGT-3'
<i>RD29B</i>	F: 5'- ACGAGCAAGACCCAGAAGTT-3'
	R: 5'- AGGAACAATCTCCTCCGATG-3'
<i>ABI1</i>	F: 5'- AGATGGTCGGTTTGATCCTC-3'
	R: 5'- AGTTCGCTACCTGAGAACCG-3'
<i>ABI2</i>	F: 5'-TCAAGATCCATTGGCGATAG-3'
	R: 5'-CAAATCGCACACTTCTTCGT-3'
<i>OST1</i>	F: 5'- TGGAATATGCATCTGGAGGA-3'
	R: 5'- AGAACAACCTCGTCTTCGCT-3'
<i>RbohD</i>	F: 5'- AACAACAGGTGGCTGTTTACC-3'
	R: 5'-TGTGATTGAGAAAGGATGCC-3'
<i>RbohF</i>	F: 5'-TTCAGTATCCGTGGGCAATA-3'
	R: 5'-CACTCCTGCGAAAGATCAAA-3'
<i>NCED3</i>	F: 5'-TTGATGCTCCAGATTGCTTC-3'
	R: 5'-GGACCCTATCACGACGACTT-3'
<i>ABA3</i>	F: 5'-AAGTCCATGGATCCACACAA-3'
	R: 5'-TTATCATCTGGCACCGGTTA-3'