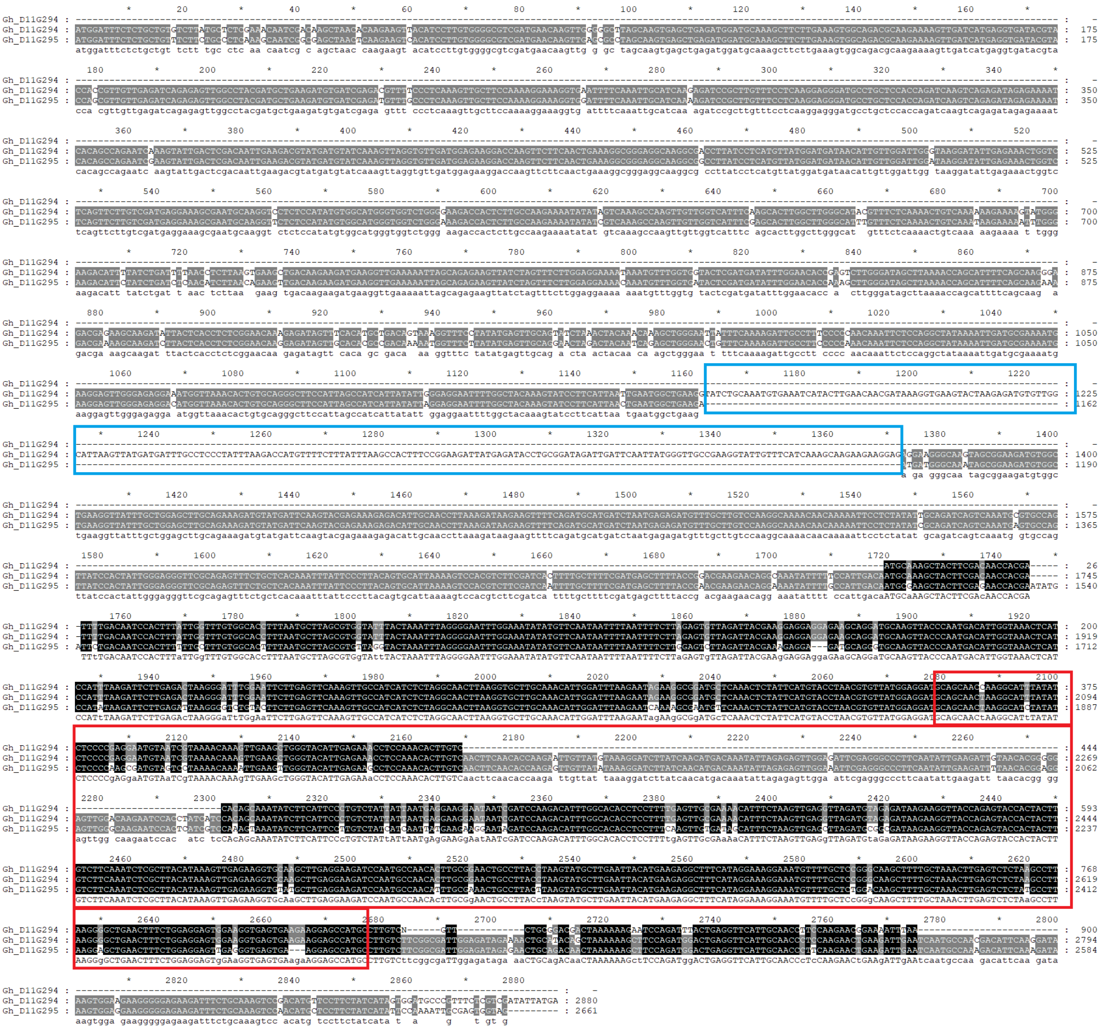


Figure S1. Alignment of the sequences of *Gh_D11G2948, Gh_D11G2949* and *Gh_D11G2950.* Three candidate genes alignment showed that *Gh_D11G2949* had a 216-bp sequence that *Gh_D11G2948* and *Gh_D11G2950* didn’t have (blue grids). A co-owned 599-bp fragment (599-bp in *Gh_D11G2949,* 587-bp in *Gh_D11G2950* and 378-bp in *Gh_D11G2948*) (red grids) been cloned into pTRV2, to silencing all three candidate genes.

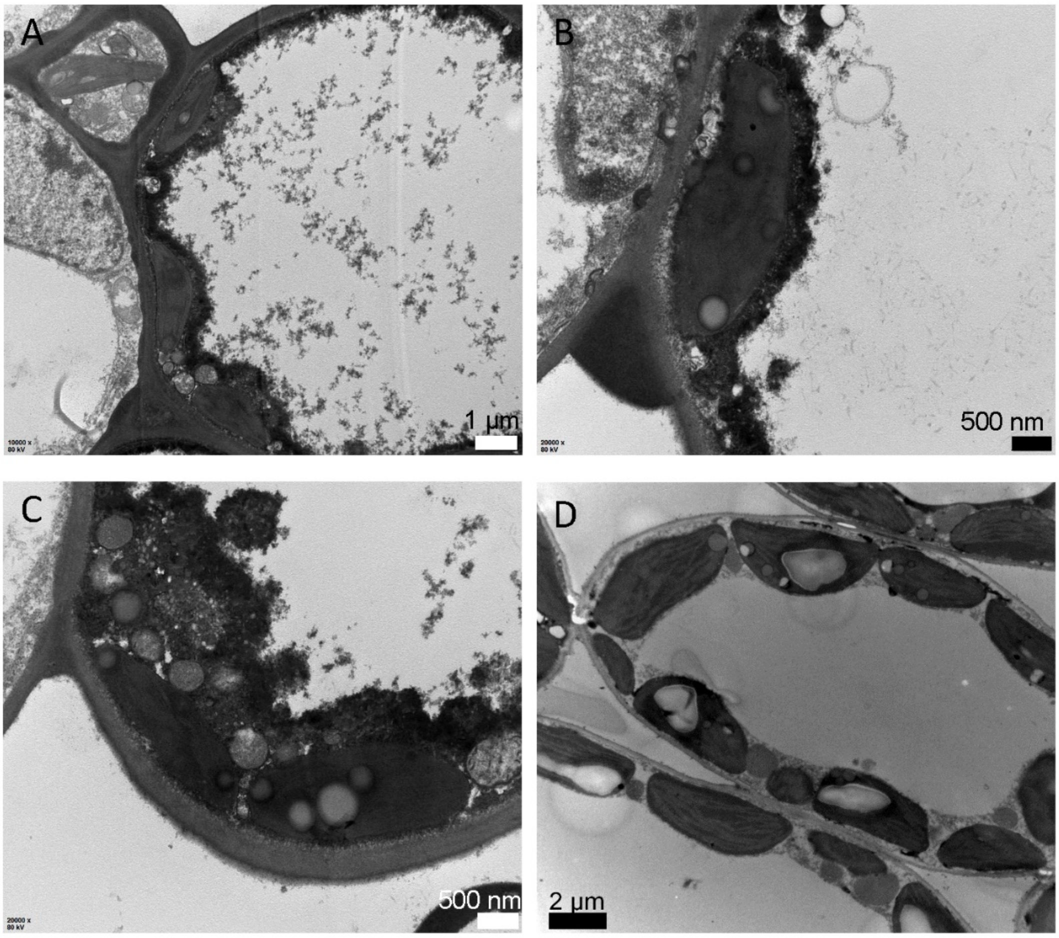


Figure S2. Images of transmission electron microscope of lethal and normal F_1 leaves in S4. Figure A, B and C were the images of cells of lethal F_1 leaves in S4; figure D was the image of cells of normal leaves in S4.

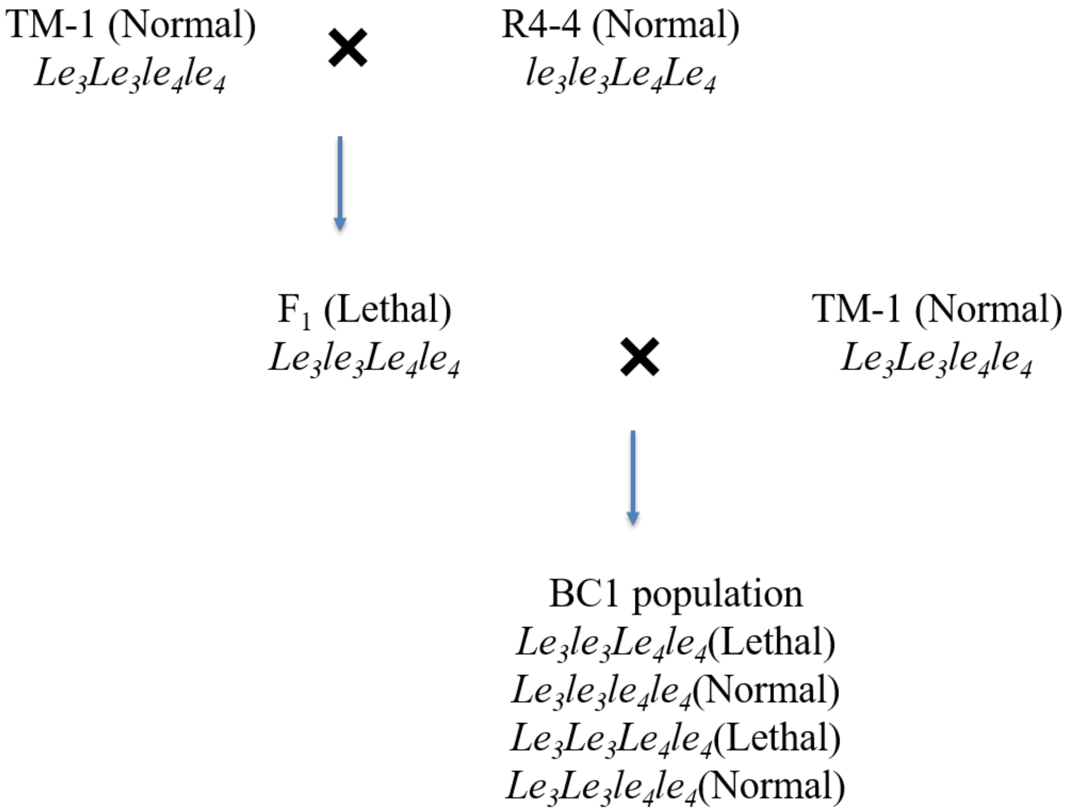
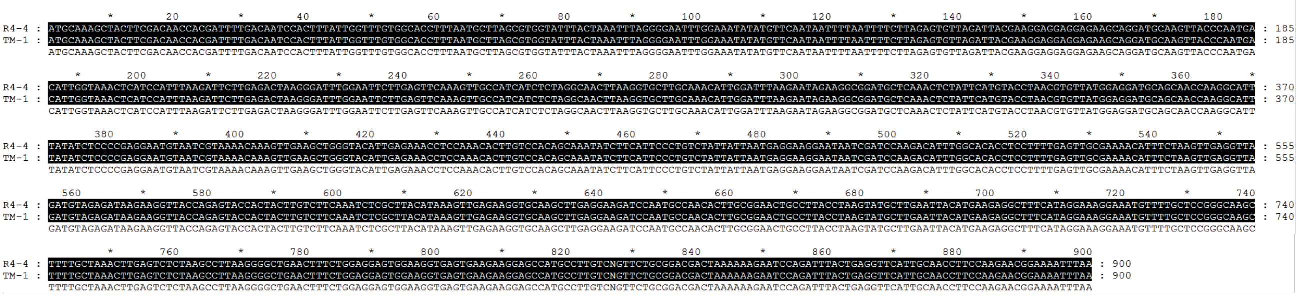


Figure S3. Genotype of BC_1 population. Parent line TM-1 conteined $Le_3Le_3le_4le_4$ genotype and normal phenotype. Parent line R4-4 conteined $le_3le_3Le_4Le_4$ genotype and normal type. When the cross genrated F_1 progeny, the F_1 plants conteined Le_3Le_4 at same time, the phenotype were lethal. Use TM-1 as recurrent parent producted BC_1 population. There were four genotype and two phenotype in BC_1 population, Le_4 locus from *G.barbadense* R4-4 were separated. Thus, we can use the BC_1 population to fine mapping the lethal gene Le_4.



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                 *        20         *        40         *        60         *        80         *       100         *       120         *       140         *       160         *       180
R4-4 : ATGCAAAGCTACTTCGACAACCACGATTTTGACAATCCACTTTATTGGTTTGTGGCACCTTTAATGCTTAGCGTGGTATTTACTAAATTTAGGGGAATTTGGAAATATATGTTCAATAATTTTAATTTTCTTAGAGTGTTAGATTACGAAGGAGGAGGAGAAGCAGGATGCAAGTTACCCAATGA : 185
TM-1 : ATGCAAAGCTACTTCGACAACCACGATTTTGACAATCCACTTTATTGGTTTGTGGCACCTTTAATGCTTAGCGTGGTATTTACTAAATTTAGGGGAATTTGGAAATATATGTTCAATAATTTTAATTTTCTTAGAGTGTTAGATTACGAAGGAGGAGGAGAAGCAGGATGCAAGTTACCCAATGA : 185
       ATGCAAAGCTACTTCGACAACCACGATTTTGACAATCCACTTTATTGGTTTGTGGCACCTTTAATGCTTAGCGTGGTATTTACTAAATTTAGGGGAATTTGGAAATATATGTTCAATAATTTTAATTTTCTTAGAGTGTTAGATTACGAAGGAGGAGGAGAAGCAGGATGCAAGTTACCCAATGA

           *      200         *       220         *       240         *       260         *       280         *       300         *       320         *       340         *       360         *
R4-4 : CATTGGTAAACTCATCCATTTAAGATTCTTGAGACTAAGGGATTTGGAATTCTTGAGTTCAAAGTTGCCATCATCTCTAGGCAACTTAAGGTGCTTGCAAACATTGGATTTAAGAATAGAAGGCGGATGCTCAAACTCTATTCATGTACCTAACGTGTTATGGAGGATGCAGCAACCAAGGCATT : 370
TM-1 : CATTGGTAAACTCATCCATTTAAGATTCTTGAGACTAAGGGATTTGGAATTCTTGAGTTCAAAGTTGCCATCATCTCTAGGCAACTTAAGGTGCTTGCAAACATTGGATTTAAGAATAGAAGGCGGATGCTCAAACTCTATTCATGTACCTAACGTGTTATGGAGGATGCAGCAACCAAGGCATT : 370
       CATTGGTAAACTCATCCATTTAAGATTCTTGAGACTAAGGGATTTGGAATTCTTGAGTTCAAAGTTGCCATCATCTCTAGGCAACTTAAGGTGCTTGCAAACATTGGATTTAAGAATAGAAGGCGGATGCTCAAACTCTATTCATGTACCTAACGTGTTATGGAGGATGCAGCAACCAAGGCATT

              380         *       400         *       420         *       440         *       460         *       480         *       500         *       520         *       540         *
R4-4 : TATATCTCCCCGAGGAATGTAATCGTAAAACAAAGTTGAAGCTGGGTACATTGAGAAACCTCCAAACACTTGTCCACAGCAAATATCTTCATTCCCTGTCTATTATTAATGAGGAAGGAATAATCGATCCAAGACATTTGGCACACCTCCTTTTGAGTTGCGAAAACATTTCTAAGTTGAGGTTA : 555
TM-1 : TATATCTCCCCGAGGAATGTAATCGTAAAACAAAGTTGAAGCTGGGTACATTGAGAAACCTCCAAACACTTGTCCACAGCAAATATCTTCATTCCCTGTCTATTATTAATGAGGAAGGAATAATCGATCCAAGACATTTGGCACACCTCCTTTTGAGTTGCGAAAACATTTCTAAGTTGAGGTTA : 555
       TATATCTCCCCGAGGAATGTAATCGTAAAACAAAGTTGAAGCTGGGTACATTGAGAAACCTCCAAACACTTGTCCACAGCAAATATCTTCATTCCCTGTCTATTATTAATGAGGAAGGAATAATCGATCCAAGACATTTGGCACACCTCCTTTTGAGTTGCGAAAACATTTCTAAGTTGAGGTTA

         560         *       580         *       600         *       620         *       640         *       660         *       680         *       700         *       720         *       740
R4-4 : GATGTAGAGATAAGAAGGTTACCAGAGTACCACTACTTGTCTTCAAATCTCGCTTACATAAAGTTGAGAAGGTGCAAGCTTGAGGAAGATCCAATGCCAACACTTGCGGAACTGCCTTACCTAAGTATGCTTGAATTACATGAAGAGGCTTTCATAGGAAAGGAAATGTTTTGCTCCGGGCAAGC : 740
TM-1 : GATGTAGAGATAAGAAGGTTACCAGAGTACCACTACTTGTCTTCAAATCTCGCTTACATAAAGTTGAGAAGGTGCAAGCTTGAGGAAGATCCAATGCCAACACTTGCGGAACTGCCTTACCTAAGTATGCTTGAATTACATGAAGAGGCTTTCATAGGAAAGGAAATGTTTTGCTCCGGGCAAGC : 740
       GATGTAGAGATAAGAAGGTTACCAGAGTACCACTACTTGTCTTCAAATCTCGCTTACATAAAGTTGAGAAGGTGCAAGCTTGAGGAAGATCCAATGCCAACACTTGCGGAACTGCCTTACCTAAGTATGCTTGAATTACATGAAGAGGCTTTCATAGGAAAGGAAATGTTTTGCTCCGGGCAAGC

                 *       760         *       780         *       800         *       820         *       840         *       860         *       880         *       900
R4-4 : TTTTGCTAAACTTGAGTCTCTAAGCCTTAAGGGGCTGAACTTTCTGGAGGAGTGGAAGGTGAGTGAAGAAGGAGCCATGCCTTGTCNGTTCTGCGGACGACTAAAAAAGAATCCAGATTTACTGAGGTTCATTGCAACCTTCCAAGAACGGAAAATTTAA : 900
TM-1 : TTTTGCTAAACTTGAGTCTCTAAGCCTTAAGGGGCTGAACTTTCTGGAGGAGTGGAAGGTGAGTGAAGAAGGAGCCATGCCTTGTCNGTTCTGCGGACGACTAAAAAAGAATCCAGATTTACTGAGGTTCATTGCAACCTTCCAAGAACGGAAAATTTAA : 900
       TTTTGCTAAACTTGAGTCTCTAAGCCTTAAGGGGCTGAACTTTCTGGAGGAGTGGAAGGTGAGTGAAGAAGGAGCCATGCCTTGTCNGTTCTGCGGACGACTAAAAAAGAATCCAGATTTACTGAGGTTCATTGCAACCTTCCAAGAACGGAAAATTTAA
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Figure S4. Alignment of the sequences of *Gh_D11G2948*. No difference in gene *Gh_D11G2948* between *G. barbadense* R4-4 and *G. hirsutum* TM-1

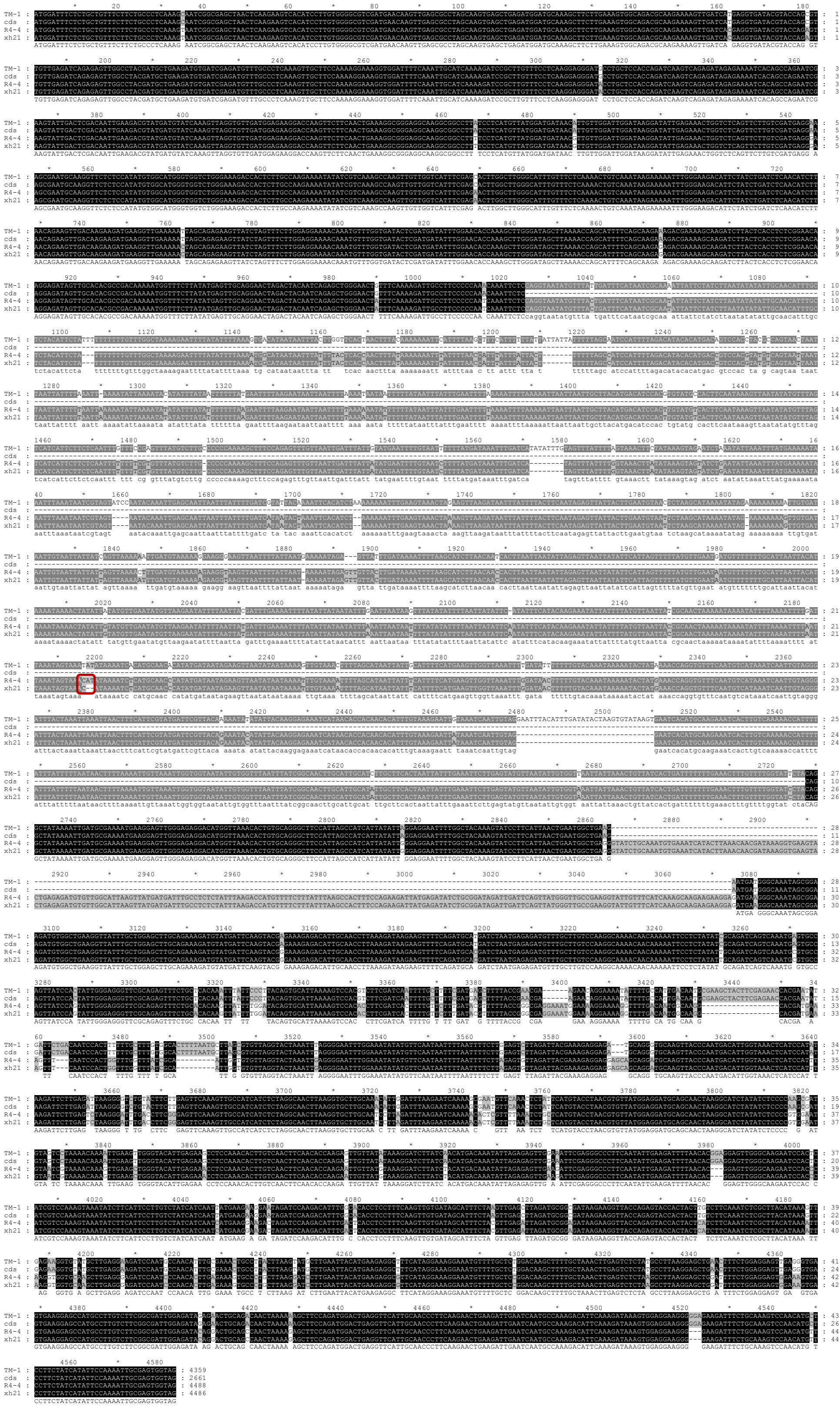


Figure S5. Alignment of the sequences of *Gh_D11G2950*. Hybrid lethality *G. barbadense* parent line R4-4 only has two bases inserted in introns compare with hybrid normal *G. barbadense* parent line Xinhai21. No difference in amino acid sequence between two *G. barbadense* varieties.

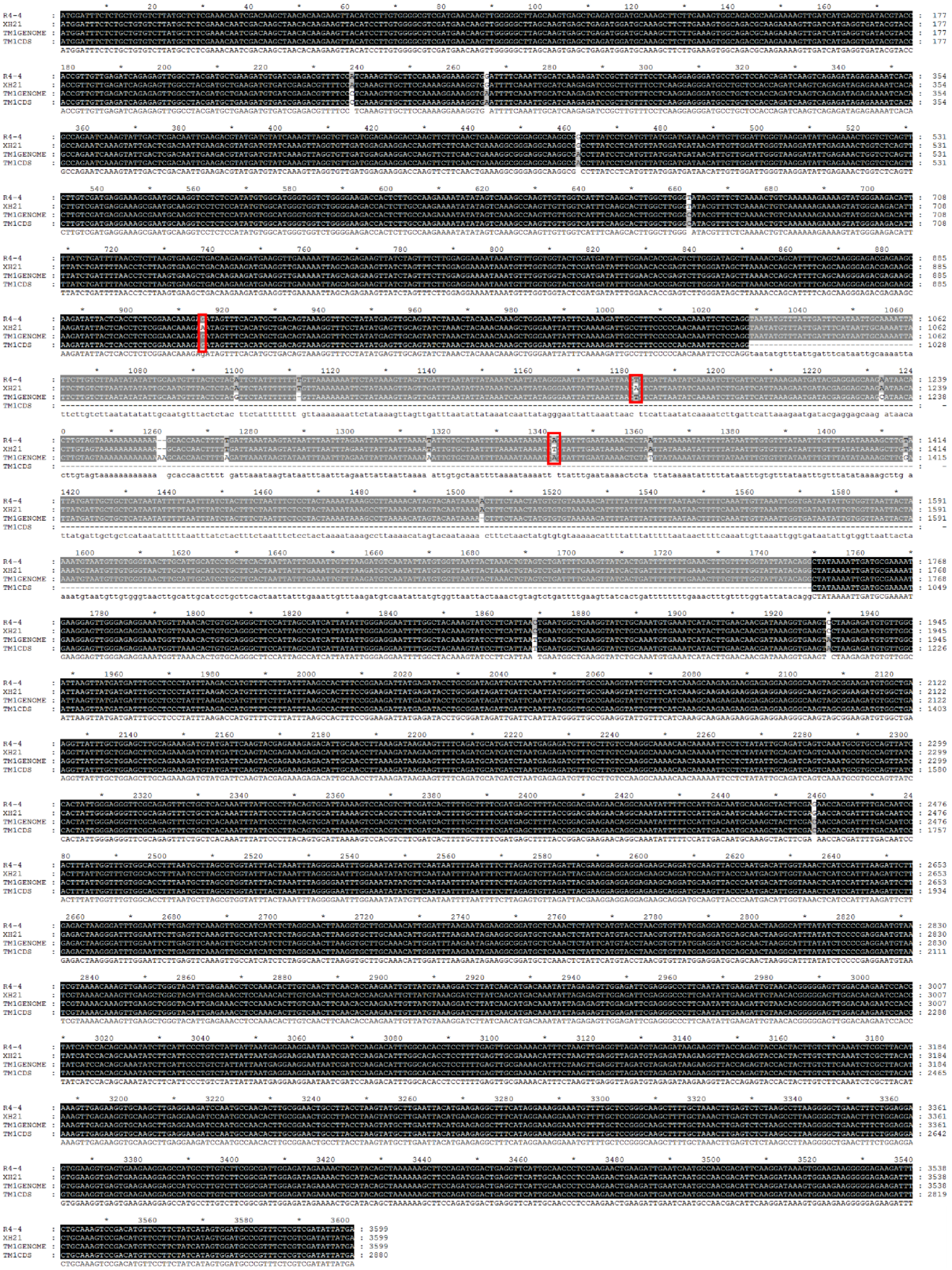
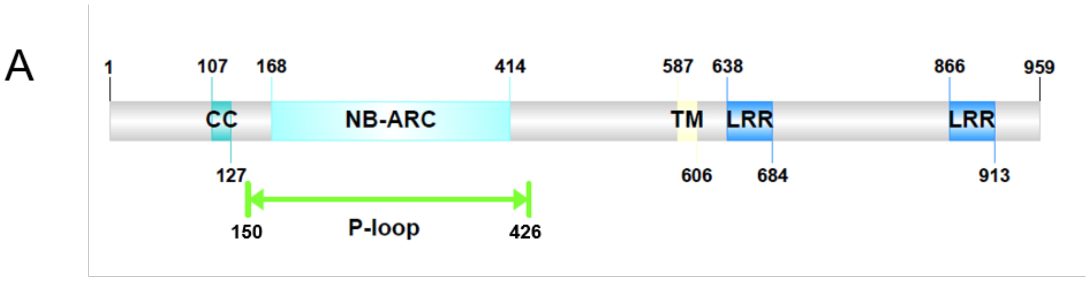


Figure S6. Alignment of the sequences of *Gh_D11G2949*. Three SNPs in genome sequence between *G. barbadense* R4-4 and *G. barbadense* Xinhai21. One SNP was in exon region; two SNPs were in intron. No difference in amino acid sequence between two *G. barbadense* varieties.



B

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MDFSAVSYALETIDKLTQEVTSLWGVDEQVGGLASELRWMQSFLKVADARKVDHEVIRTTVVEIRELAYDAED
VIETFSLKVASKRKGEFSNCIKRSACFLKEGCLLHQIKSEIEKITARIKVLTRQLKTYDVSKLGVDGEGPSSSTER
REARRPYPHVMDDNIVGLGKDIEKLVSVLVDEESECKVLSICGMGGLGKTTLAKKIYSQSQVVGHFKHLAWAY
VSQNCQKRKVWEDILSDFNLLSEADKKMKVEKLAEKLSSFLEENKCLVVLDDIWNTESWDSLKPAFSARETRS
KILLTSRNKEIVSHADSKGFLYELQYLNYKQSWELFQKIAFPPTNSPGYKIDAKMKELGEEMVKHCAGLPLAIIIL
GGILATKYPSLIEWLKVSANVKSYLNNDKGEVLRDVLALSYDDLPPYLRPCFLYLSHFPEDYEIPADRLIQLWVA
EGIVSSKQEEGEEGQVAEDVAEGYLLELAERCMIQVRERDIATLKIRSFQMHDLMRDVCLSKAKQQKFLYIAD
QSNACQLSTIGRVRRVSAHKFIPLQCIKSPRLRSLLLFDELLPDEEQANIFPLTMQSYFDNHDFDNPLYWFVAP
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LEIRGPFNIEDCNTGELDKNPPIIHSKYLHSLSIINEEGIIDPRHLAHLLLSCENISKLRLDVEIRRLPEYHYLSSNLA
YIKLRRCKLEEDPMPTLAELPYLSMLELHEEAFIGKEMFCSGQAFAKLESLSLKGLNFLEEWKVSEEGAMPCL
RRLEIENCIQLKKLPDGLRFIATLQELKIESMPTTFKDKVEEGGEDFCKVRHVPSIIVDARFSSIL
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Figure S7. Protein coding sequence and protein domain structure of Gh_D11G2949. (A) Protein domain structure of Gh_D11G2949. CC: Colied-coil domain, TM: Transmembrane. (B) Protein coding sequence of Gh_D11G2949.

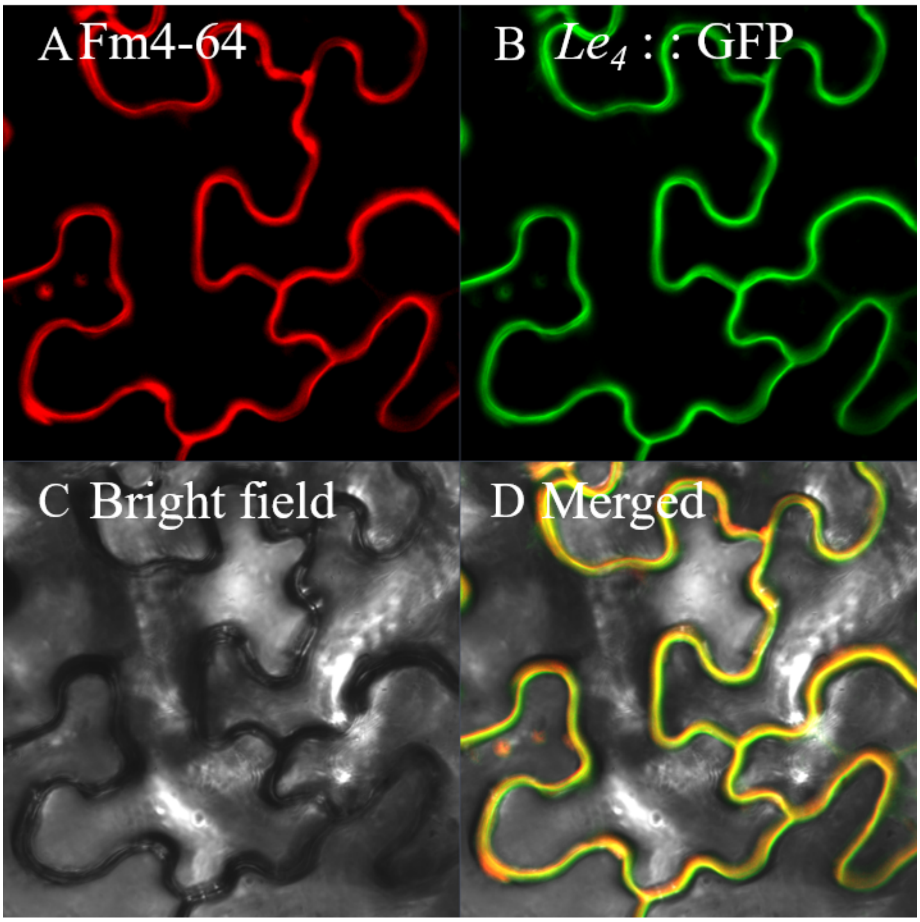

Figure S8. Subcellular localization of *Le₄*(Gh_D11G2949). Subcellular localization of *Le₄::*GFP of *Nicotiana benthamiana* leaf epidermal cell. FM4-64 dye the cell member (red), overlap with *Le₄::*GFP.

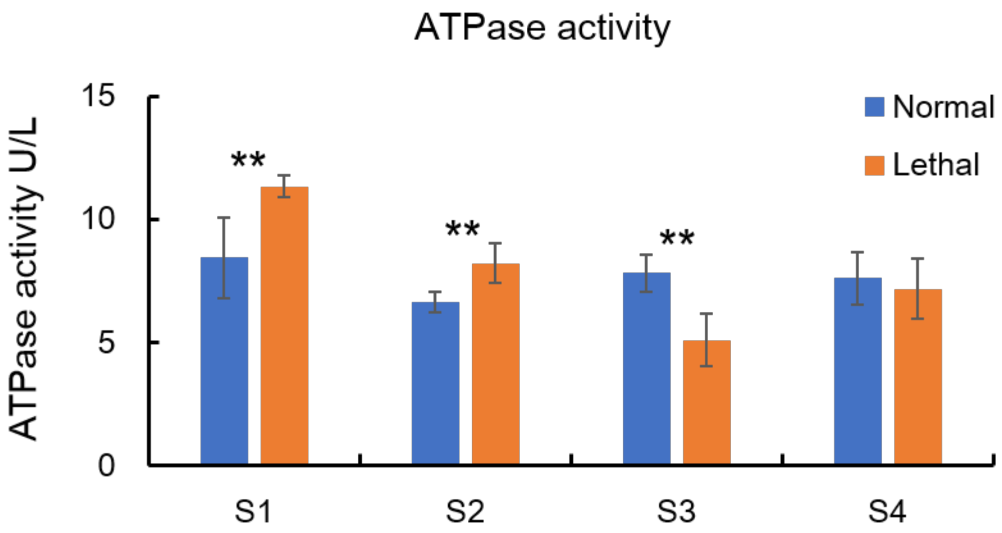

Figure S9. ATPase activity between normal and lethal F_1s in leaves from four different developmental stages. ATPase concentration was significant different higher in lethal F_1 than in normal F_1 in S1 and S2.

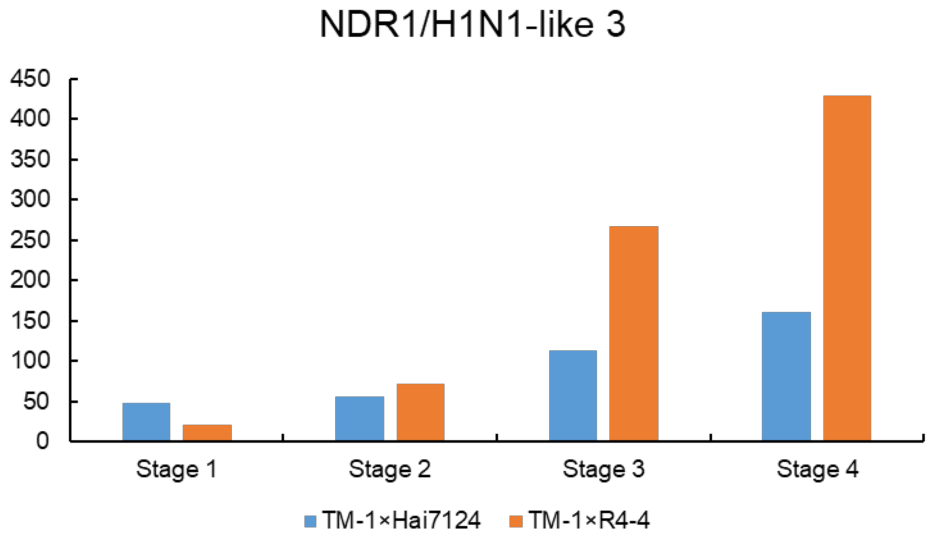

Figure S10. FPKM value of *NDR1/H1N1-like 3* gene. *NDR1/H1N1-like 3* gene up-regulated after stage 2 in lethal F_1.

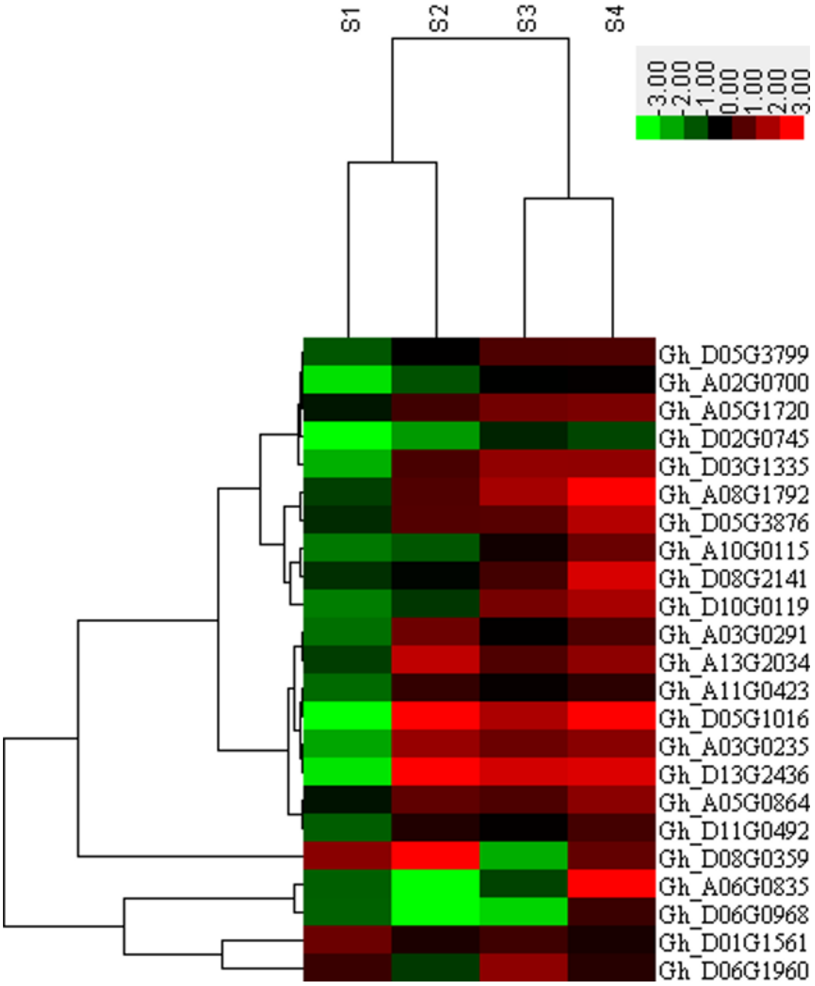

Figure S11 Heatmap of differential expressed *MAPK/MAP2K/MAP3K* genes in the four stages. The quantitative changes in expression are shown in red (indicating up-regulation) and green (indicating down-regulation) in lethal F_1.

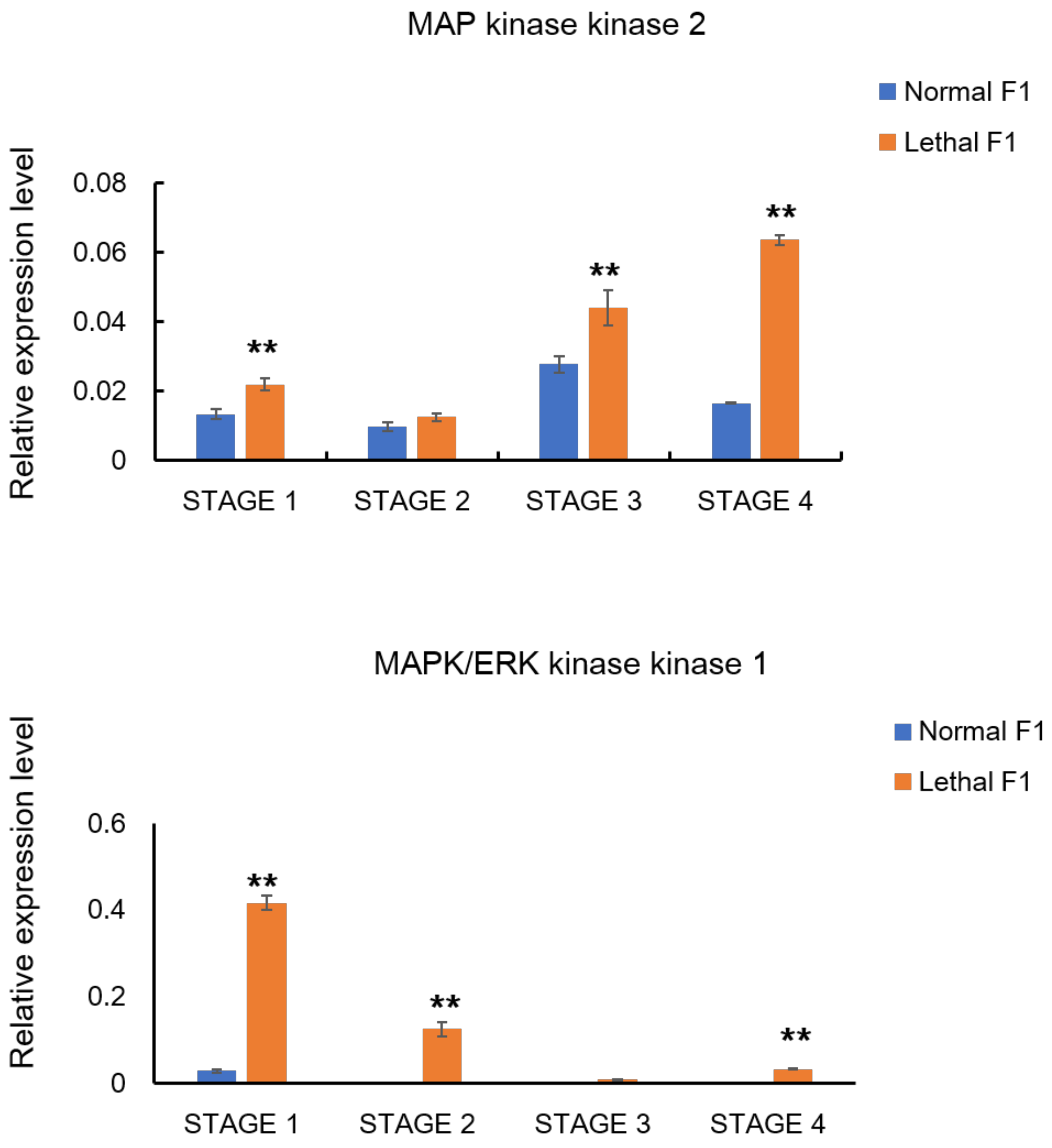

Figure S12. qPCR of *MAPKs* genes. qPCR analysis conformed the *MAPK/MAPKK/MAPKKK* genes transcriptome analysis results. *MAPKs* genes up-regulated in lethal F_1 plants.

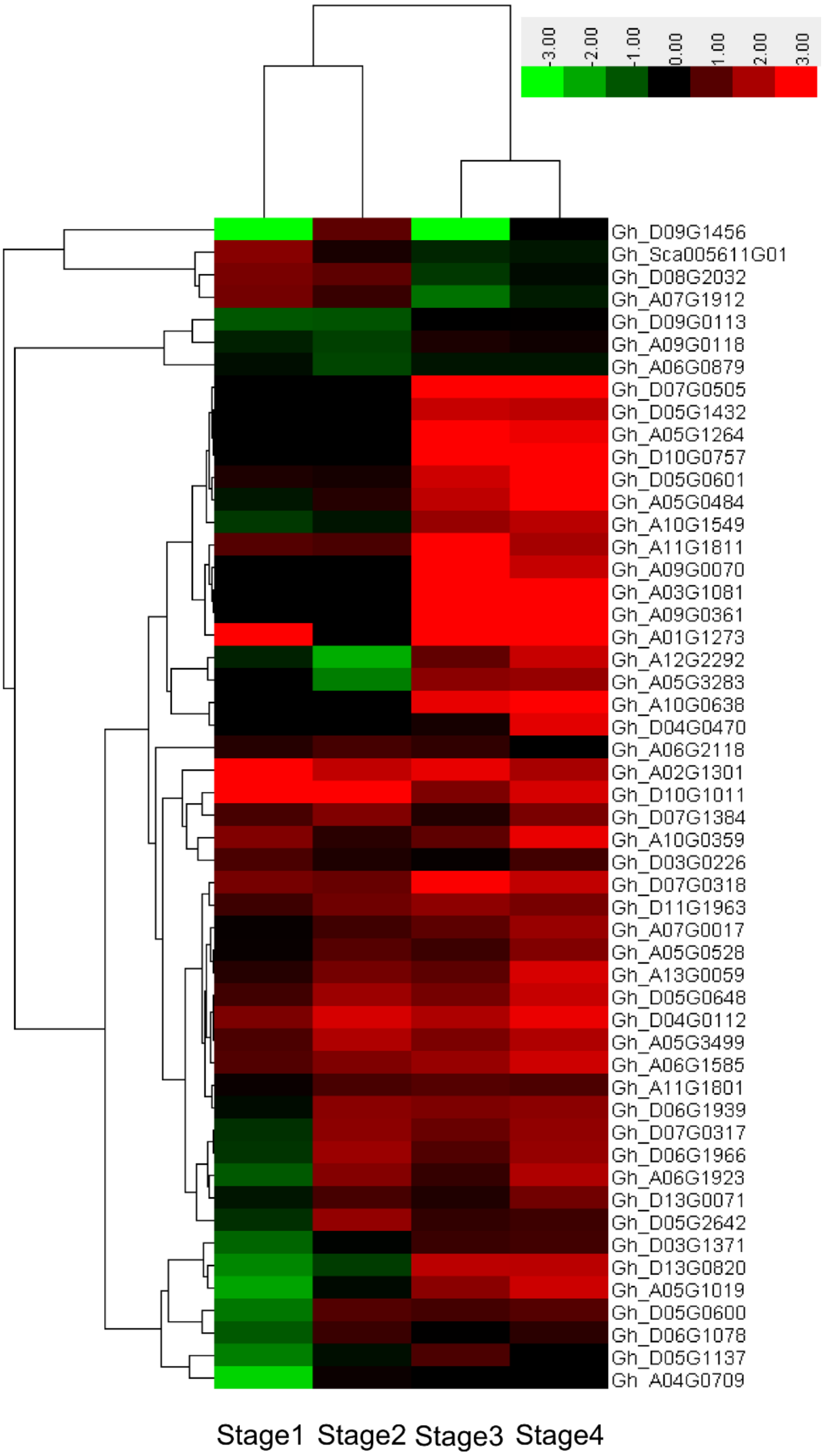

Figure S13. Heatmap of differentially expressed *WRKY* TFs in the four stages. The quantitative changes in expression are shown in red (indicating up-regulation) and green (indicating down-regulation) in lethal F_1.

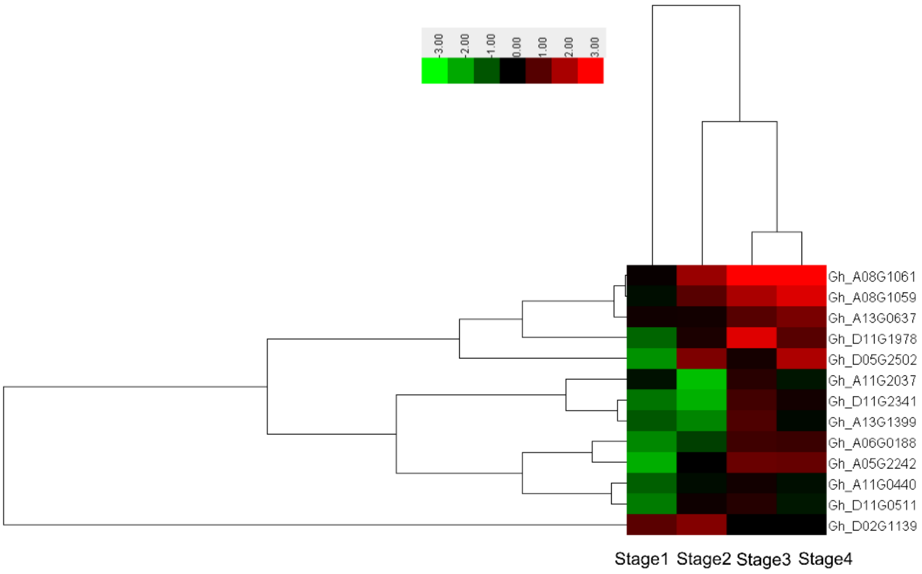

Figure S14. Heatmap of differentially expressed *MYB* TFs in the four stages. The quantitative changes in expression are shown in red (indicating up-regulation) and green (indicating down-regulation) in lethal F_1.

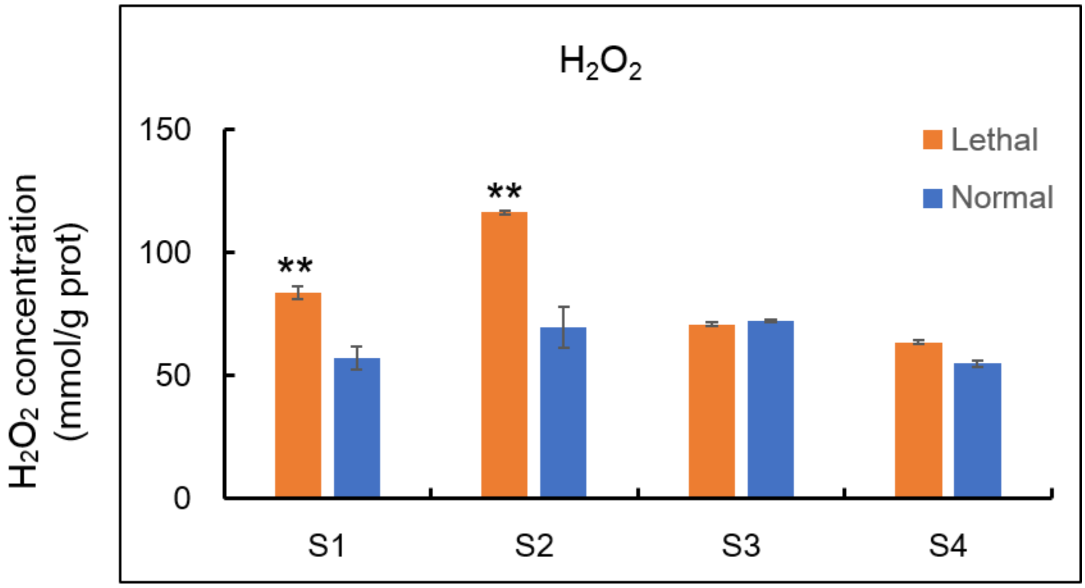

Figure S15. H_2O_2 concentration in lethal and normal F_1s in the four stages. Lethal F_1 had higher H_2O_2 concentration than normal F1 in early stages.

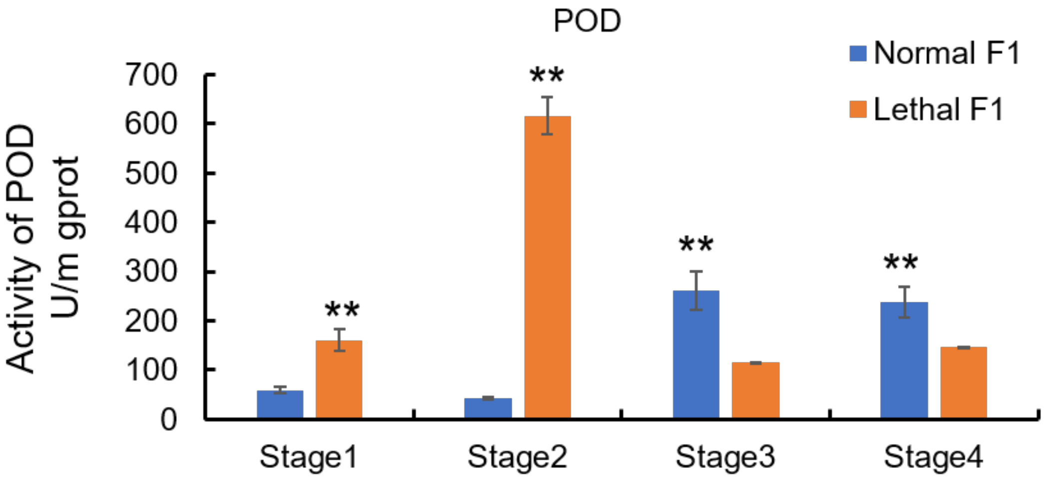

Figure S16. Activity of POD in lethal and normal F_1s in the four stages. Lethal F_1 plants had higher POD activity in early stages, especially in S2.

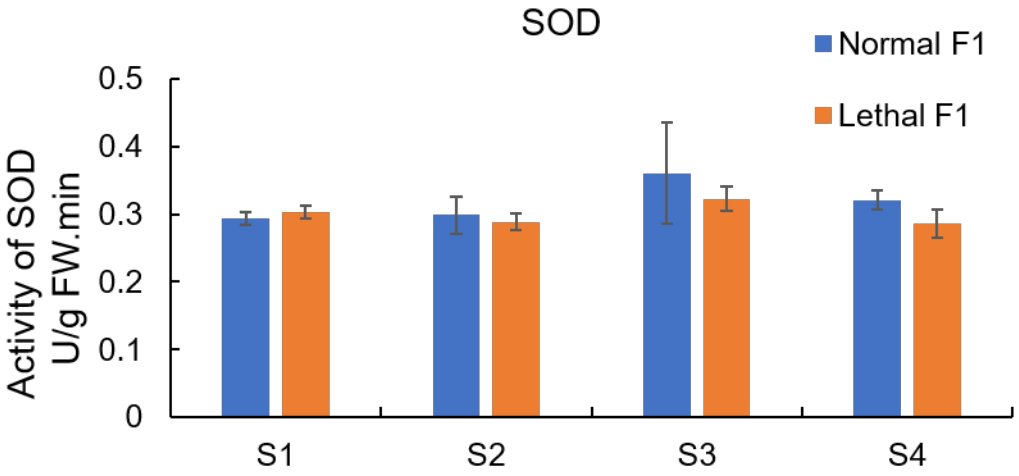

Figure S17. SOD activity in normal and lethal F_1s in the four stages. There was no difference between normal and lethal F_1s in any stage.