# The large repertoire of conifer NLR resistance genes includes drought responsive and highly diversified RNLs

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## Supplementary files

**Supplementary Methods:** Detailed methods for the plant material, RNA extraction, RNA-Seq library synthesis, sequencing, assembly and additional data sampling.

**Supplementary Data S1:** NLR peptide sequences from the seven conifer transcriptomes.

**Supplementary Data S2:** Functional annotation of the NLR peptide sequences from the seven conifer transcriptomes (excel file).

## Supplementary Figures:

**Supplementary Figure S1:** Alignment of Post-LRR domains
**Supplementary Figure S2:** Alignment of representative RNL sequences
**Supplementary Figure S3:** progressive clustering of the RPW8 domains in seven conifer species.
**Supplementary Figure S4:** Unrooted ML phylogenetic tree of the RPW8 domain in conifers after using 60% sequence identity cut-off and clustering using the WAG model
**Supplementary Figure S5:** Library size of the transcriptomes and Principal component analysis of the data from the study of Stival Sena et al. (2018)
**Supplementary Figure S6:** Detailed ML phylogenetic trees corresponding to the Figure 3
**Supplementary Figure S7:** Detailed ML phylogenetic trees corresponding to the Figure 4
**Supplementary Figure S8:** Unrooted ML phylogenetic tree of the RPW8 domain in land plants using the JTT substitution model
**Supplementary Figure S9:** Count number average of the five most differentially expressed RNL sequences

## Supplementary Tables:

**Supplementary Table S1:** Ratio of NLR to the total number of transcripts in our study and other plants
**Supplementary Table S2:** Details of the NLR subfamilies numbers in 49 land plant species used to build the figure 2
**Supplementary Table S3:** Details of the RPW8 motifs identified in Figure 3

**Supplementary Table S4:** List of the 119 differentially expressed NLR genes

**Supplementary Table S5:** Position of the RNL genes on the *P. glauca* composite linkage map of Pavy *et al.* (2017)

**Supplementary Table S6:** RNL and TNL genes present on the chromosome 7 of the peach genome.

**Supplementary Figure S1.** Alignment of Post-LRR motifs in *Amborella trichopoda, Ginkgo biloba* (Gb) and five reference TNLs in dicots.

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                                                   Motif 1                                          Motif 2
                              10        20        30        40        50        60        70        80        90       100
                      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
motifs                --------------------------P-GSEIPEWFSHQSVGD------------------------~~~~~~KWMGLALCAVFE---------------
Gb_20740              SGSSVSVCNLNKWIREV-PSLQELNIY-GSSVPLWFRHQMHGQLDGKE--------HARDLCLDVPIRVKPNVKIEAVIFCFVSHD--QGWSDYEWDHGR
Gb_06500              SGCGVLKVDFLKWIKGM-PLLRELSFY-ASEIPEWFDHRMKTKFFVK---------DFPRLCMDRVSMIFDEGRYAGTDVGYM-----------------
A.trichopoda00001.586 -GCSPHTLRGQSLAKETFTSLDKLSIP-GSKVPDWFMFQTP--------------------SFTVPRLSDVGLHIREVLLCFSYS-----TYDTSP----
A.trichopoda00109.130 -GCSPHVWRGQNLAKEMFTSLVQLSVP-GSKVPNWFACKCTPRCSLIQDGE-----MLQQVSCALPRPSHAGRRIKEIMVCLVIY-----VHHSSYTR--
A.trichopoda00056.119 -GCKPHVLRGQSLLEEMFTGLYSLSVP-GSKVPNWFMCKCTSRYIRIQDIENPDWFMYQTLSCTVSRISEEGLHIREMMVWFVIY-----VNELSCTH--
A.trichopoda00085.83  -GCGSYVSNSPGLGKETFIQLRHFGVS-GSKVPSWFMFQTL--------------------SCVIPRVSDEGLKVRGVILCLVLS-----VDSEMPIDDG
A.trichopoda00085.87  -GCSPRVSRS--IAKETFNHLWCFGIS-GSKVPDWFMFQKL--------------------SCTVPRLSDQDLKIRGVILCFVIS-----HNSEIPIDFV
A.trichopoda00085.80  -GCT-HPSRS--IAKETFEHLRCFGVS-GSNVPDWFMCQSL------------------~~SYMISGMSDHGVEIRGVILCLVIS-----LDNEIPTEID
N(N.g.)Q40392.1       --QNISSMRHDISASDSLSLTVFTGQPYPEKIPSWFHHQGW----------------DSSVSVNLPENWYIPDKFLGFAVCYS----------RSLIDTT
Bs4(S.l.)AAR21295.1   -FLNISSFQHNISASDSLSLRVFTSL--GSSIPIWFHHQG----------------TDTSVSVNLPENWYVSDNFLGFAVCYY----------------G
RPS4(A.t.)CAB50708.1  --QLLSYARKRYNGGLVSESLFSTCFP-GCEVPSWFCHET----------------VGSELEVKLLPHWHDK-KLAGIALCAVVS-------CLDPQDQV
Gro14(S.t.)AAP44390.1 -TSMVDSLLKQMLEALYMNVRFCLYVP-GMEIPEWFTYKSW---------------GTQSMSVALPTNWFTP-TFRGFTVCVILDKKMLFILGRFNTHKV
Ma(P.c.)CAR94514.1    -----DAIRR--------DERFEYGYR-SNEIPAWLSRRS----------------TESTITIPLPHDVDGKSKWIKLALCFI---------------CE

                             110       120       130       140       150       160       170       180       190       200
                      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
motifs                ----------------------------------------------------------------------------------------------------
Gb_20740              YNSNTWMEATIIRGE--EEVHNTRILR----NRHADRRDQLHLSVYREG----HPFVMN--LQNGDRIRV--------------------------WARS
Gb_06500              -------KATIVRGG--QEVFETVFIRI----REANRRDQLYLSMYLEN----DSFVKR--LHSGDRIRI--------------------------RVCS
A.trichopoda00001.586 --GSPRSMVSIKRED--RGIRIEEQVDIQYFPLS--HQNQVFFRRLRHGHMP-RLWFKW--FKDENQIEL--------------------------LTKF
A.trichopoda00109.130 --NRHVVGLWIKRED---ETL--PQIPLQIYPVTNLNQNEIYFYRFREGQYY-GNRKFW--LEDGDEIEVEYDPIQCRCQAADVDEPTECEDQVEDIEEL
A.trichopoda00056.119 --QKHVVNLLIKRKG-----REKDRLPLGIYPGT--HRNQIYIYHFRDGIGF-EFQMNW--LKDGDQIEF-------------VSESTYYEDQIEVINES
A.trichopoda00085.83  L-DIPHVQFKVMNNG---MQRMSPTLKLGN-PKA--NQDQVYLYHFQED----DALVLN--LEDGDEVKI--------------------------INNC
A.trichopoda00085.87  P-DIPDVLFMIIRNG---TRRFSTMLRLGV-PKA--HQDQVYLFHFQEG----GHILPM--LEGGDQIEV--------------------------AKRS
A.trichopoda00085.80  DWGIPDVELTITRDG---IERFSSTLRLEMLPKA--RQDQVYLFHFQEG----DDVLLM--LGDGDQIEV--------------------------LNRS
N(N.g.)Q40392.1       AHLIPVCDDKMSRMT--QKLALSECDT----ESSNYSEWDIHFFFVPFA----GLWDTS--KANGKTPND--------------------------YGII
Bs4(S.l.)AAR21295.1   NLTENTAELIMSSAG-----MPCITWKLLLSNHSECTYIRIHFFLVPFA----GLWDTS--NANGKTPND--------------------------YKHI
RPS4(A.t.)CAB50708.1  SRLSVTCTFKVKDED---KSWVAYTCPVGSWTRHGGGKDKIELDHVFIGYTSCPHTIKC--HEEGNSDEC----------------------NPTEASLK
Gro14(S.t.)AAP44390.1 YGLENMIWLNLKRYDGLRQKISTSFGPIG--SEKPGGLGDTLITHIAFE----RSWK----LENDLDYYR--------------------------NNAF
Ma(P.c.)CAR94514.1    AAQKHDSLEDVPEFDEELGLKFTRNHRIEL-CTTEDPHERLLALDYRDCNFA-GPFIHWCFIPQSDLAES--------------------------SNKR

                                           Motif 3
                             210       220       230       240       250       260       270       280       290       300
                      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
motifs                ---------~~~~~VKKCGVRLVYEQDVEELNQT------------------------------------------------------------------
Gb_20740              IYA------AWRIWVKEGGMHILYREGEEKHEQDSLCTNIILYNHPSYTIHSNSAHKYAISL------------------------
Gb_06500              QSI------SHQICVKQGGMHLLYTKEETTTSNNKKDLNDTVFERLSKDLTWLHWWNVVWDKHVPIHQMDIKSFIAYDLQSCFIHRQTATLKPSVMHASR
A.trichopoda00001.586 ---------MVCL---KGAMHLVYKPADTDESIQERLANFFNPLLSE-----------------------------------------------------
A.trichopoda00109.130 RAS---SLRLPCVRLKKVGIHFIYESEDTEDSTEEKLANFFNSL--------------------------------------------------------
A.trichopoda00056.119 GPSLTPSLGFPCYHLKQAGIHLVYESEDTEDSIEEKLADFFNSLHVQWQLTASLYVRYPFWFRIATPYFLCGIWILK---------
A.trichopoda00085.83  PPL------TPSIHIKKVAIHLVYKPEDTKDSIQEKLAEFFNSMDAHEDALEIVQFHSLQAHNLQTYHPERRMRSGILLLVVILCCFYFWMM--------
A.trichopoda00085.87  PPM------FPSIQLKKGGIHLVYKPEQTQDYIQEKLAEFCNSMDCHENLPPHYLRRKVQAGILLWVFVLLYFVWMRSNSENYSPFLPSLMTYSALYALK
A.trichopoda00085.80  PPE------FPCVQMKKGGIHLVYKPEDTHESIQEKLADFFNSLNCHDSS--------------------------------------------------
N(N.g.)Q40392.1       RLS------FSG-EEKMYGLRLLYKEGPEVNALL-QMRENSNEPTEHSTGIRRTQYNNRTSFYELING--------------------
Bs4(S.l.)AAR21295.1   MLS------FPQ-ELKECGVRLFYEDESVLETTNDELTIGVRRIRYDDDDSEHYEEAGCSSSKKQRS---------------------------------
RPS4(A.t.)CAB50708.1  FTVTGGTSENGKYKVLKCGLSLVYAKDKDKNSALETKYDMLIGKSFQETSEGVDGRVKKTKGKYVMPVEKNFQETTEGVDGRVNKKKKTRMDNGRPKKKQ
Gro14(S.t.)AAP44390.1 QLEFSACDHYQKDVVKGLGVRLVYEN--------------------------------------------------------------------------
Ma(P.c.)CAR94514.1    LIQATITPDSPGTRVTGCGVSLIYLEDVPKFVRKLNKHYSYCYHGYQPEQEEDGMRSILSTSGIQTEQEEVQEQETTTSTRIVGQLRRNVESLLEKLFE-
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**Supplementary Figure S2:** Alignment of representative RNL sequences from basal land plants, gymnosperm, angiosperm, monocots, dicots and the reference RNLs (ADR1, NRG1, RPW8.1 and RPW8.2)

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                                        10        20        30        40        50        60        70        80        90       100
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P.patens_Pp3c10_50V3.1          -MGDPLVAPALVGFGVNL--LLTEVIKAGRMAYRCKDECNELVAKLESVRPLVEQV-NDNSSKGNEAC-----TLWLSG-FQSLLERAKVVTNECKLE--
M.polymorpha_Mapoly3272s0001    ---MAELVSVVIGFGVEL--LLQGVIDEVKQAVDRNKNCKELLVLLEGLAPLVQFL-CDSSKKGQGQNKSTPVTEWLEK-LRSRLEEADKVVRECISQGS
S.fallax_Sphfalx0171s0003.1.p   ---MLIEPGLVFGILGQLQPLLNRVIDAVEITLKYDKSCTKLSSLLLRLQPLLQHI-ANQAPNLSPNV--SLVHTWLTE-LEKRLKAAETTLNVSNEQHP
G.biloba_Gb_38648               --MGDLLSQTLAGVVATE--LLSVVKDVAKKCYGCKSYCKRLEKTLEGIIPILDEI-FQTGAEVSQHR-----QRELKE-LYEKLQKGIELVENCSKV--
A.trichopoda_scaffold00033.264  ---MDLVSGAIIGAIAGE--ILKALLDATNIVYECKSQCEKLKSTLQHIVPKLEDI-EQLRLQLTDCE-----PADFKE-LKSRLESGKVLVYKCMKL--
A.coerulea_Aqcoe5G206900.1      ----MAVTDFFAGEIATE--LLKQLFQITRKCSLCRASAEQLMISINELLPIITEI-KYSGVELPQGR-----QNHLDR-LSETLTAGRELAVKVLSS--
P.dactylifera_XP_008792596.1    ------MDTFFAGEIATE--LVKELVKVIRRTVLCRPTAEQLKRNVDALLPIIQEI-RHSGVELPQAR-----QSQLSD-LAEHLHLALELARKAAAS--
A.comosus_Aco004926.1           ------MDTLFPGEVATE--LLRELAKVVRRAYGCRATAEQLKRGVEALLPIVQEI-RYAGVELPQPR-----QRQLSE-LAERLARGLELARAAAAA--
M.acuminata_GSMUA_Achr7T08250_  ------METFFAGEIATE--LVKELLKVVRRTYLCRPAAEQLKRSVDALLPIVQEI-RHSGVELPQHR-----QSQLSE-LADQLRLALDLARKAAAS--
N.benthamiana_NRG1_AAY54606.1   -MAATLLGGAALGPVFDI--LLKAVLDVGIKIATFRSKFQSLIKTLNDIKPVFDDI-ERLNKALDGRD------YEIEM-FKKQLFAGEELVRKCSKT--
A.thaliana_ADR1_BAF00531.1      ---MASFIDLFAGDITTQ--LLKLLALVANTVYSCKGIAERLITMIRDVQPTIREI-QYSGAELSNHH-----QTQLGV-FYEILEKARKLCEKVLRC--
A.thaliana_RPW8.1_ACJ05907.1    MPIGELAIGAVLGVGAQA--IYDRFRKARDISV-----VNRLCATIISIRPLLVQIDKRSKVEGSPLR-------EVNERLTCFLELAYVFVEAYPKL--
A.thaliana_RPW8.2_ACJ72031.1    -MIAEVAAGGALGLALSV--LHEAVKRAKDRSVTTRFILHRLEATIDSITPLVVQI-DKFSEEMEDSS-----SRKVNKRLKLLLENAVSLVEENAEL--
                                                                         RPW8 DOMAIN

                                       110       120       130       140       150       160       170       180       190       200
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P.patens_Pp3c10_50V3.1          SRWSRRKRMVKFWRWKKLPSLVMKI---------------SSDIEEARSNTSLVTLGIVLSMHIPKQVEAFSFVKQ------------------------
M.polymorpha_Mapoly3272s0001    RPWNIVKNRSTSKKLVEVTRGITDLVGQAPLVQLGYTKETTDEQNKILADLQESMKNANQLHSDLIHEVHCA--DLSQITRVTVNTPTEIFGRSYYGTIT
S.fallax_Sphfalx0171s0003.1.p   RLTAPVSRYRAASEINKLTESVEELLKQADLVTVDLALSISAKVENATEDTKHALQSVENQLSIVTRQLPNVRRQLTDVTRQCLKPSIAKSWNIEEATVA
G.biloba_Gb_38648               SCLNMYQRYKFAKRILALEKYISSF------SDKQGWAHVMSDLHHLRVDTGRRLEYIEKQVEGNHECILFEQMMKDQMPLFGIGSEASYPNLQIP----
A.trichopoda_scaffold00033.264  RFWKVHKKYSYGKKLIELDEYLVRF------FDLNLAADTNLDTKQLRIE----FKEMRRLLDGRGIASESSYALP------------------------
A.coerulea_Aqcoe5G206900.1      NRWNVYKNLQYARKMEKLEKNVSRF------INGPLQFHVLADVHHLRVG----LDRLEEKIGIDKLEERIGGLRI-----------EGNGWVEESMRRK
P.dactylifera_XP_008792596.1    SRWNVYRSMQLAHRMEKLDRWIARW------LERQMPAHVLADVHHLRVESAVRLDRIERHLQNGAPATAAAAAAMTMMKPAAVGVAQMMEGLELGGGG-
A.comosus_Aco004926.1           PRWNVYRSVKLAREMEKVERWIARW------LQRQMPAHVLADVHHLRVDAEVRMERIERRIDHVEAAAAAAAAAAAPGPVVGVRAPSSAAKGAGEMMMM
M.acuminata_GSMUA_Achr7T08250_  PRWNVYRSMQLAHRMERLDRWISRW------VERHMPAHVLADVHHLRVDYSARLDRIERTLDMTAASAALAAARV----PVAVGSVPFSGSPLTEMMDG
N.benthamiana_NRG1_AAY54606.1   KCYDALKKWNYSRKLTKLENSLVRF------CQVHGFIQVCRDSKIILVNVIEHGKKLDQITSMLRGISLRNGSSI-----GFTNSNGSSGWMNGNSFGS
A.thaliana_ADR1_BAF00531.1      NRWNLKHVY-HANKMKDLEKQISRF------LNSQILLFVLAEVCHLRVNGDRIERNMDRLLTERNDSLSFPETMMEIE---------------------
A.thaliana_RPW8.1_ACJ05907.1    RRRQVLRKYRYIKAIETIELALRSIIVVDFQVDQWDDIKEIKAKISEMDTKLAEVISACSKIRT
A.thaliana_RPW8.2_ACJ72031.1    RRRNVRKKFRYMRDIKEFEAKLRWV-VGVDVQVNQLADIKELKAKMSEIRTKFDSTS
                                                                         RPW8 DOMAIN

                                       210       220       230       240       250       260       270       280       290       300
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P.patens_Pp3c10_50V3.1          -----------------------------------------GTPSLIVGVDEHFKKLKMSILQSHGGDKASHFGLQGRGGAGKTLLVQMLNNDDDIQMKY
M.polymorpha_Mapoly3272s0001    LERAFEDIRAQADEHFLTQTQGSATGSSADVFPSPQHQFERQISARVFGGDELLDSARSILL---EEDGGQWVGLWSMGGAGKTLLASRLSNDLPLKQHF
S.fallax_Sphfalx0171s0003.1.p   IITAFQNLNASDDHDNAMEAVPLTLATIDPQQPSPKL----HVPQFVFGLDDSIGRLQQLLLSRHSDPDPHSIGVWGKGGVGKTLLARKVHNSMEVRQHF
G.biloba_Gb_38648               -----------------------------------------EMPEHPVGLKISVEELKKRLF----PQDVSLISVKGMGGSGKTTLAVALCSDQQVQEFF
A.trichopoda_scaffold00033.264  -----------------------------------------GLPDCPVGLSDSLRELKAKFF----ADDVSVLGICAQGGYGKTTLASMLCREEDVKGRF
A.coerulea_Aqcoe5G206900.1      VEEE-------------------------------------EKVVNLIGVGMVLAKKKMMEV-VVGRDDLKVIGICGIGGSGKTTLATEISKDPQVRSHF
P.dactylifera_XP_008792596.1    -----------------------------------------GEAAAPVGAAVRVGKERVKEMLMATGGSAEVVGICGIGGSGKTTLAKEICRDPRIQNYF
A.comosus_Aco004926.1           MQGLEMGVVVGGGE---------------------------AVGPPMIGAGARVGNERVKEV-LMGGAGWGAVGICGIGGSGKTTLAREICRDPQIRHYF
M.acuminata_GSMUA_Achr7T08250_  -----------------------------------------GEGEKPVGVGIRVGKERVKEMLMAGGDRAAVVGISGIGGSGKTTLAKEICRDPQIRSYF
N.benthamiana_NRG1_AAY54606.1   TNGSGFSGWSDVP----------------------------QFSDSVVGFDLPLQELKVKLL----EEKEKVVVLSAPAGCGKTTLAAMLCQEDDIKDKY
A.thaliana_ADR1_BAF00531.1      -----------------------------------------TVSDPEIQTVLELGKKKVKEM-MFKFTDTHLFGISGMSGSGKTTLAIELSKDDDVRGLF
A.thaliana_RPW8.1_ACJ05907.1
A.thaliana_RPW8.2_ACJ72031.1
                                                                         NBS DOMAIN
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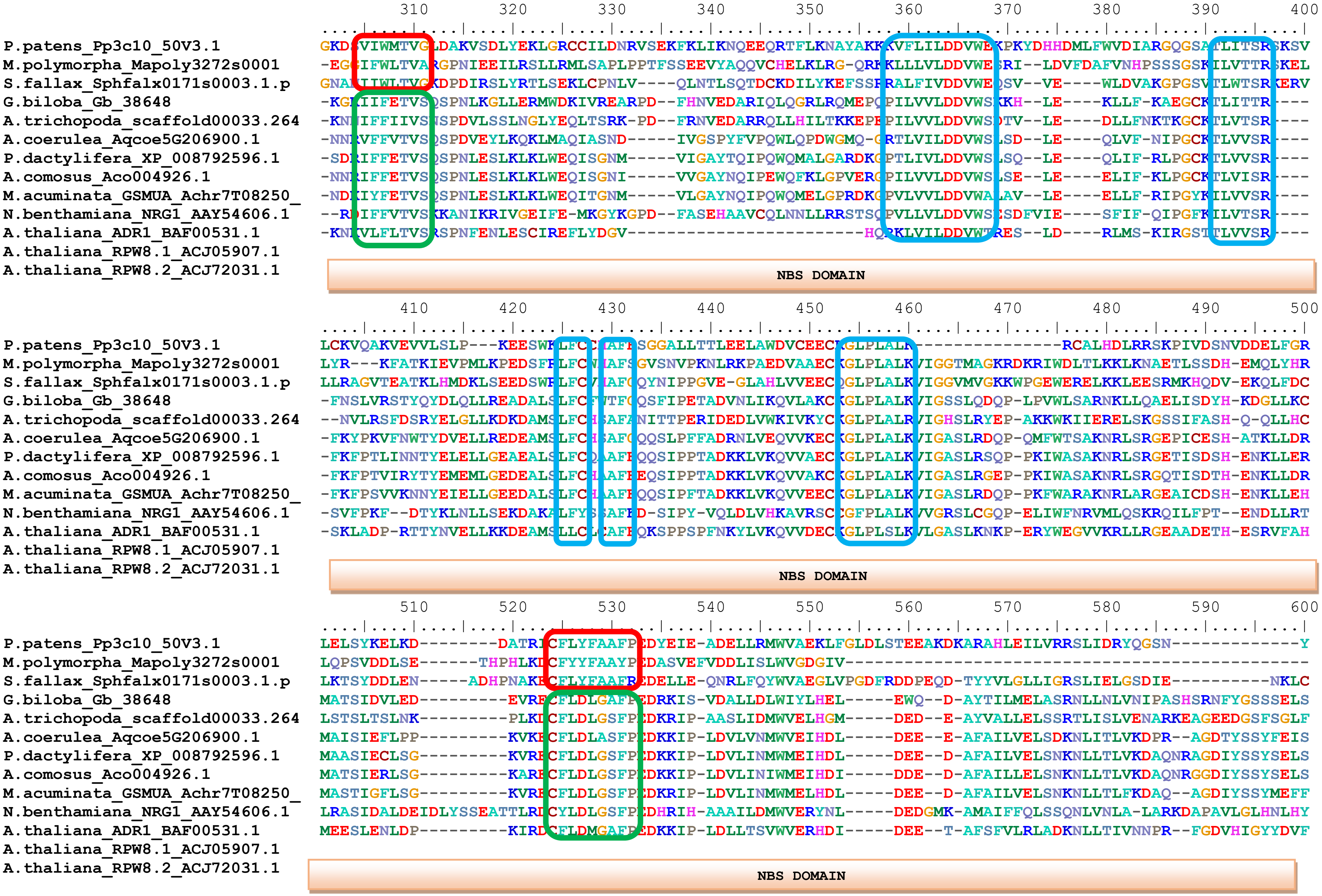
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A.thaliana_RPW8.2_ACJ72031.1
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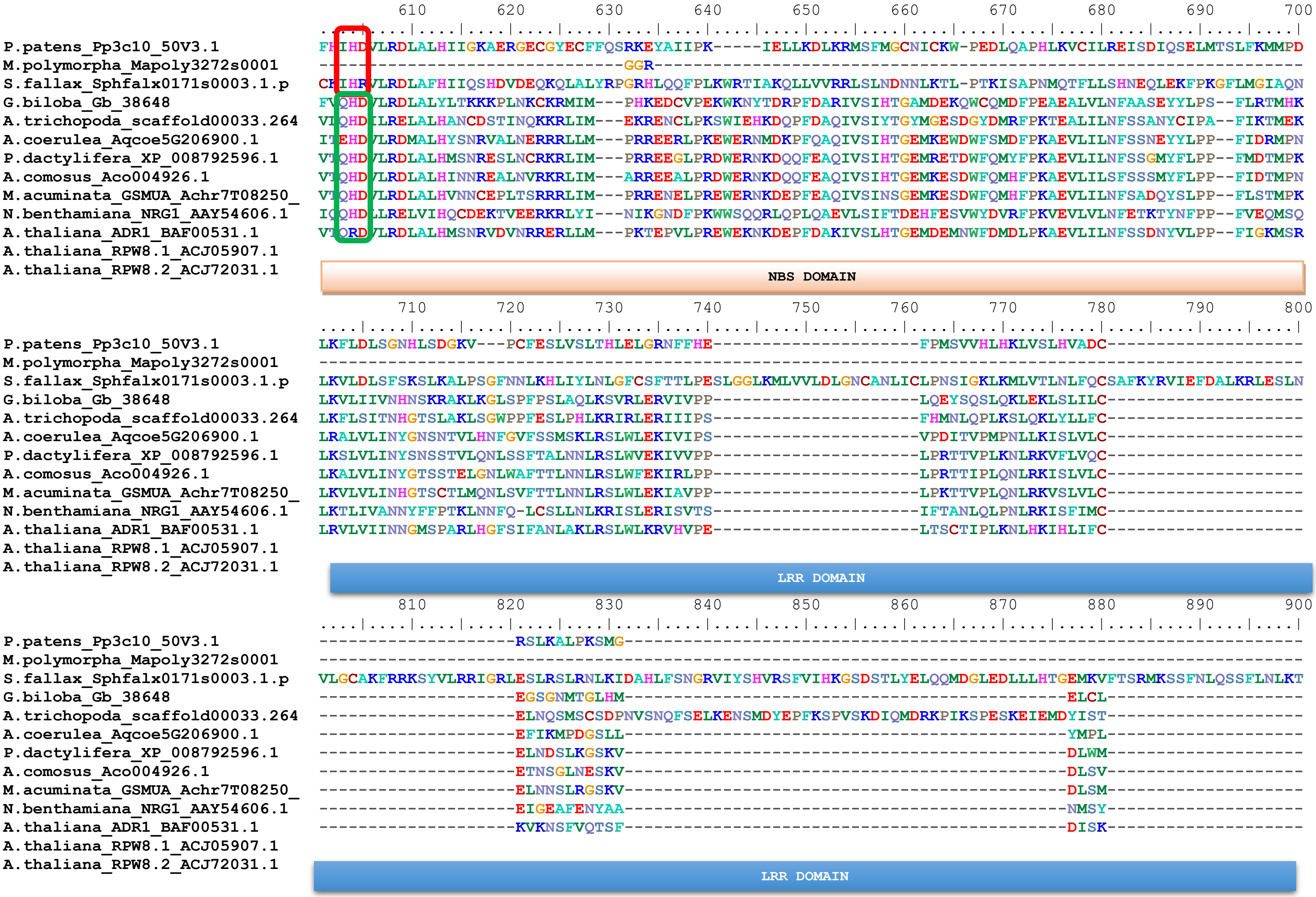
NBS DOMAIN



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                                       610       620       630       640       650       660       670       680       690       700
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P.patens_Pp3c10_50V3.1         FHIHDVLRDLALHIIGKAERGECGYECFFQSRKEYAIIPK-----IELLKDLKRMSFMGCNICKW-PEDLQAPHLKVCILREISDIQSELMTSLFKMMPD
M.polymorpha_Mapoly3272s0001   -------------------------------GGR------------------------------------------------------------------
S.fallax_Sphfalx0171s0003.1.p  CKIHRVLRDLAFHIIQSHDVDEQKQLALYRPGRHLQQFPLKWRTIAKQLLVVRRLSLNDNNLKTL-PTKISAPNMQTFLLSHNEQLEKFPKGFLMGIAQN
G.biloba_Gb_38648              FVQHDVLRDLALYLTKKKPLNKCKRMIM---PHKEDCVPEKWKNYTDRPFDARIVSIHTGAMDEKQWCQMDFPEAEALVLNFAASEYYLPS--FLRTMHK
A.trichopoda_scaffold00033.264 VIQHDILRELALHANCDSTINQKKRLIM---EKRENCLPKSWIEHKDQPFDAQIVSIYTGYMGESDGYDMRFPKTEALILNFSSANYCIPA--FIKTMEK
A.coerulea_Aqcoe5G206900.1     ITEHDVLRDMALHYSNRVALNERRRLLM---PRREERLPKEWERNMDKPFQAQIVSIHTGEMKEWDWFSMDFPKAEVLILNFSSNEYYLPP--FIDRMPN
P.dactylifera_XP_008792596.1   VTQHDVLRDLALHMSNRESLNCRKRLIM---PRREEGLPRDWERNKDQQFEAQIVSIHTGEMRETDWFQMYFPKAEVLILNFSSGMYFLPP--FMDTMPK
A.comosus_Aco004926.1          VTQHDVLRDLALHINNREALNVRKRLIM---ARREEALPRDWERNKDQQFEAQIVSIHTGEMKESDWFQMHFPKAEVLILSFSSSMYFLPP--FIDTMPN
M.acuminata_GSMUA_Achr7T08250_ VTQHDVLRDLALHVNNCEPLTSRRRLIM---PRRENELPREWERNKDEPFEAQIVSINSGEMKESDWFQMHFPKAEVLILNFSADQYSLPP--FLSTMPK
N.benthamiana_NRG1_AAY54606.1  IQQHDLLRELVIHQCDEKTVEERKRLYI---NIKGNDFPKWWSQQRLQPLQAEVLSIFTDEHFESVWYDVRFPKVEVLVLNFETKTYNFPP--FVEQMSQ
A.thaliana_ADR1_BAF00531.1     VTQRDVLRDLALHMSNRVDVNRRERLLM---PKTEPVLPREWEKNKDEPFDAKIVSLHTGEMDEMNWFDMDLPKAEVLILNFSSDNYVLPP--FIGKMSR
A.thaliana_RPW8.1_ACJ05907.1
A.thaliana_RPW8.2_ACJ72031.1

                                                                              NBS DOMAIN

                                       710       720       730       740       750       760       770       780       790       800
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P.patens_Pp3c10_50V3.1         LKFLDLSGNHLSDGKV---PCFESLVSLTHLELGRNFFHE---------------------FPMSVVHLHKLVSLHVADC--------------------
M.polymorpha_Mapoly3272s0001   ----------------------------------------------------------------------------------------------------
S.fallax_Sphfalx0171s0003.1.p  LKVLDLSFSKSLKALPSGFNNLKHLIYLNLGFCSFTTLPESLGGLKMLVVLDLGNCANLICLPNSIGKLKMLVTLNLFQCSAFKYRVIEFDALKRLESLN
G.biloba_Gb_38648              LKVLIIVNHNSKRAKLKGLSPFPSLAQLKSVRLERVIVPP---------------------LQEYSQSLQKLEKLSLILC--------------------
A.trichopoda_scaffold00033.264 LKFLSITNHGTSLAKLSGWPPFESLPHLKRIRLERIIIPS---------------------FHMNLQPLKSLQKLYLLFC--------------------
A.coerulea_Aqcoe5G206900.1     LRALVLINYGNSNTVLHNFGVFSSMSKLRSLWLEKIVIPS---------------------VPDITVPMPNLLKISLVLC--------------------
P.dactylifera_XP_008792596.1   LKSLVLINYSNSSTVLQNLSSFTALNNLRSLWVEKIVVPP---------------------LPRTTVPLKNLRKVFLVQC--------------------
A.comosus_Aco004926.1          LKALVLINYGTSSTELGNLWAFTTLNNLRSLWFEKIRLPP---------------------LPRTTIPLQNLRKISLVLC--------------------
M.acuminata_GSMUA_Achr7T08250_ LKVLVLINHGTSCTLMQNLSVFTTLNNLRSLWLEKIAVPP---------------------LPKTTVPLQNLRKVSLVLC--------------------
N.benthamiana_NRG1_AAY54606.1  LKTLIVANNYFFPTKLNNFQ-LCSLLNLKRISLERISVTS---------------------IFTANLQLPNLRKISFIMC--------------------
A.thaliana_ADR1_BAF00531.1     LRVLVIINNGMSPARLHGFSIFANLAKLRSLWLKRVHVPE---------------------LTSCTIPLKNLHKIHLIFC--------------------
A.thaliana_RPW8.1_ACJ05907.1
A.thaliana_RPW8.2_ACJ72031.1

                                                                              LRR DOMAIN

                                       810       820       830       840       850       860       870       880       890       900
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P.patens_Pp3c10_50V3.1         --------------------RSLKALPKSMG---------------------------------------------------------------------
M.polymorpha_Mapoly3272s0001   ----------------------------------------------------------------------------------------------------
S.fallax_Sphfalx0171s0003.1.p  VLGCAKFRRKSYVLRRIGRLESLRSLRNLKIDAHLFSNGRVIYSHVRSFVIHKGSDSTLYELQQMDGLEDLLLHTGEMKVFTSRMKSSFNLQSSFLNLKT
G.biloba_Gb_38648              --------------------EGSGNMTGLHM---------------------------------------------ELCL--------------------
A.trichopoda_scaffold00033.264 --------------------ELNQSMSCSDPNVSNQFSELKENSMDYEPFKSPVSKDIQMDRKPIKSPESKEIEMDYIST--------------------
A.coerulea_Aqcoe5G206900.1     --------------------EFIKMPDGSLL---------------------------------------------YMPL--------------------
P.dactylifera_XP_008792596.1   --------------------ELNDSLKGSKV---------------------------------------------DLWM--------------------
A.comosus_Aco004926.1          --------------------ETNSGLNESKV---------------------------------------------DLSV--------------------
M.acuminata_GSMUA_Achr7T08250_ --------------------ELNNSLRGSKV---------------------------------------------DLSM--------------------
N.benthamiana_NRG1_AAY54606.1  --------------------EIGEAFENYAA---------------------------------------------NMSY--------------------
A.thaliana_ADR1_BAF00531.1     --------------------KVKNSFVQTSF---------------------------------------------DISK--------------------
A.thaliana_RPW8.1_ACJ05907.1
A.thaliana_RPW8.2_ACJ72031.1

                                                                              LRR DOMAIN
```

```
                                        910       920       930       940       950       960       970       980       990      1000
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P.patens_Pp3c10_50V3.1          ----NLNSLVKLDLYGCRSLKALPESMGNLNSLVELDLGGCESLEAFP--KSMGKLNSLVKLNLGGCESLEALPESMGK---------------------
M.polymorpha_Mapoly3272s0001    ----------------------------------------------------------------------------------------------------
S.fallax_Sphfalx0171s0003.1.p   LRLSSLPNLQSLQLTDCPNLVNLTLNDC--PNLENGALTNCPNLVSLPTLDTLPRLHSL-ILRL----SIKKLPQSFTHRGAFPALNLFNLGQSKIVEFP
G.biloba_Gb_38648               ----NLPRLHEINFDHCSNLEELAASICNMTSLQRLSVRNCHDLYKLS--DDLGNLSSLQMLRLYACPSLEELPHSICK---------------------
A.trichopoda_scaffold00033.264  ----IFPKLKEIEMDYCGDLRDFPSWVCRLTHLKKFSITNCHDLCNLP--EEVGGLRYLEVLSLHACTRLSELPRSITR---------------------
A.coerulea_Aqcoe5G206900.1      ----ILPRLLELTMDHCTNLTELPSSVCSFSSLRSLSITNCHDLQNLP--IELGQLKSLQILRFCSCPALEKLPQSLCG---------------------
P.dactylifera_XP_008792596.1    ----TFPRLSSLTIDHCIDLNELPSSICEVSSLESISISNCHDLSELP--HEFGKLGSLQILRIYACPALKRLPQSICQ---------------------
A.comosus_Aco004926.1           ----TFPKLSDLTIDHCIDLKELPSSICEVRSLNSISISNCHDLYELP--SELGKLESLEILRLYACPSLKRLPQSICR---------------------
M.acuminata_GSMUA_Achr7T08250_  ----TLPRLSHLTIDHCIDLTKLPSSICNIGSLQCISISNCHDLSELP--GEFGKLSSLEILRVYACPSIKRLPQSICR---------------------
N.benthamiana_NRG1_AAY54606.1   ----MWPKLVEMNIEYCSDLVEVPAETCDLVGLKKLSICYCHELVALP--EELGKLSNLEVLRLHSCTNVSKLPESVVK---------------------
A.thaliana_ADR1_BAF00531.1      ----IFPSLSDLTIDHCDDLLEL-KSIFGITSLNSLSITNCPRILELP--KNLSNVQSLERLRLYACPELISLPVEVCE---------------------
A.thaliana_RPW8.1_ACJ05907.1
A.thaliana_RPW8.2_ACJ72031.1                                              LRR DOMAIN

                                       1010      1020      1030      1040      1050      1060      1070      1080      1090      1100
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
P.patens_Pp3c10_50V3.1          ------LNSLVQLNLYGCGSLEALPESMGNLNSLVELNLGSCESL-------------------------------------------------------
M.polymorpha_Mapoly3272s0001    ----------------------------------------------------------------------------------------------------
S.fallax_Sphfalx0171s0003.1.p   EVEEDAMPKLQWLDFNDCISLHTLPASISLLTSIQTINLGSKNEKLMTSCQTNFSNSPIRKSFIMDGKPLIPEEEVFELVIPMQEGTTRVQGNDKRPFQK
G.biloba_Gb_38648               ------LRQLEFLDISLCECMKQLPEALGQLSSLREIDMRECSRV-------------------------------------------------------
A.trichopoda_scaffold00033.264  ------LSSLKLLDISECLNIVGLPNDLHGLSGLEKIDMSRCSQI-------------------------------------------------------
A.coerulea_Aqcoe5G206900.1      ------LENLKYLDISQCVNLTSLPERIGGLIMLEKIDMRECSQI-------------------------------------------------------
P.dactylifera_XP_008792596.1    ------LKRLKYLDISQSLNLRDLPEELGNLKNLEKIDMRECS-L-------------------------------------------------------
A.comosus_Aco004926.1           ------LRRLKYLDISQCINLGDLPEELGHLTSLEKIDMRECSQL-------------------------------------------------------
M.acuminata_GSMUA_Achr7T08250_  ------LKRLKYLDISQSFNLRELPEELGHLTSLEKIDMRECSQL-------------------------------------------------------
N.benthamiana_NRG1_AAY54606.1   ------LNRLGFLDVYDCVELDFLPREMDQLCSLRTICMGSRLGF-------------------------------------------------------
A.thaliana_ADR1_BAF00531.1      ------LPCLKYVDISQCVSLVSLPEKFGKLGSLEKIDMRECS-L-------------------------------------------------------
A.thaliana_RPW8.1_ACJ05907.1
A.thaliana_RPW8.2_ACJ72031.1                                                LRR DOMAIN

                                       1110      1120      1130      1140      1150      1160
                                ....|....|....|....|....|....|....|....|....|....|....|....|...
P.patens_Pp3c10_50V3.1          ------EAFPESMGNLKNLKVFK----------------------------------------
M.polymorpha_Mapoly3272s0001    ---------------------------------------------------------------
S.fallax_Sphfalx0171s0003.1.p   VHGDDEERMFKRGGSIMGSDLFIPSSPTRFVFRGSSSLAEPTKSEKEHGLCEAL---------
G.biloba_Gb_38648               ------KQLPQSASDLRSLVHVICDEKIGHHWSAIKASTIPQLCVEVAEKHFHLDWLED----
A.trichopoda_scaffold00033.264  ------RRLPGSVAELRGLKNVICDEEIANQWEEIKLQSLSGLHVRVVEEEATLDWLER----
A.coerulea_Aqcoe5G206900.1      ------SSVPASAVSLRRLSRVICDEEASWLWKEVE-RVVPDLRVEVAEECFNLDWLAE----
P.dactylifera_XP_008792596.1    ------RNIPRSLSSLKSLGHVICDEEMALLWKEAE-RHIPDLRIQIAEQCYNLDWLVE----
A.comosus_Aco004926.1           ------RNLPRSSSSLKSLGHVVCDEEMALFWKEAE-RNIPDLRVQVAEVCYNLDWLVE----
M.acuminata_GSMUA_Achr7T08250_  ------RTIPRSSSSLKSLGHVICDEEVALLWKEAE-RCIPDLRVQVAEECFNLDWLVE----
N.benthamiana_NRG1_AAY54606.1   ------TELPDSVLRLVKLEDVVCDEETASLWEYYK-EHLRNLRITVIKEDINLNLLHKSLFI
A.thaliana_ADR1_BAF00531.1      ------LGLPSSVAALVSLRHVICDEETSSMWEMVK-KVVPELCIEVAKKCFTVDWLDD----
A.thaliana_RPW8.1_ACJ05907.1
A.thaliana_RPW8.2_ACJ72031.1                                               LRR DOMAIN
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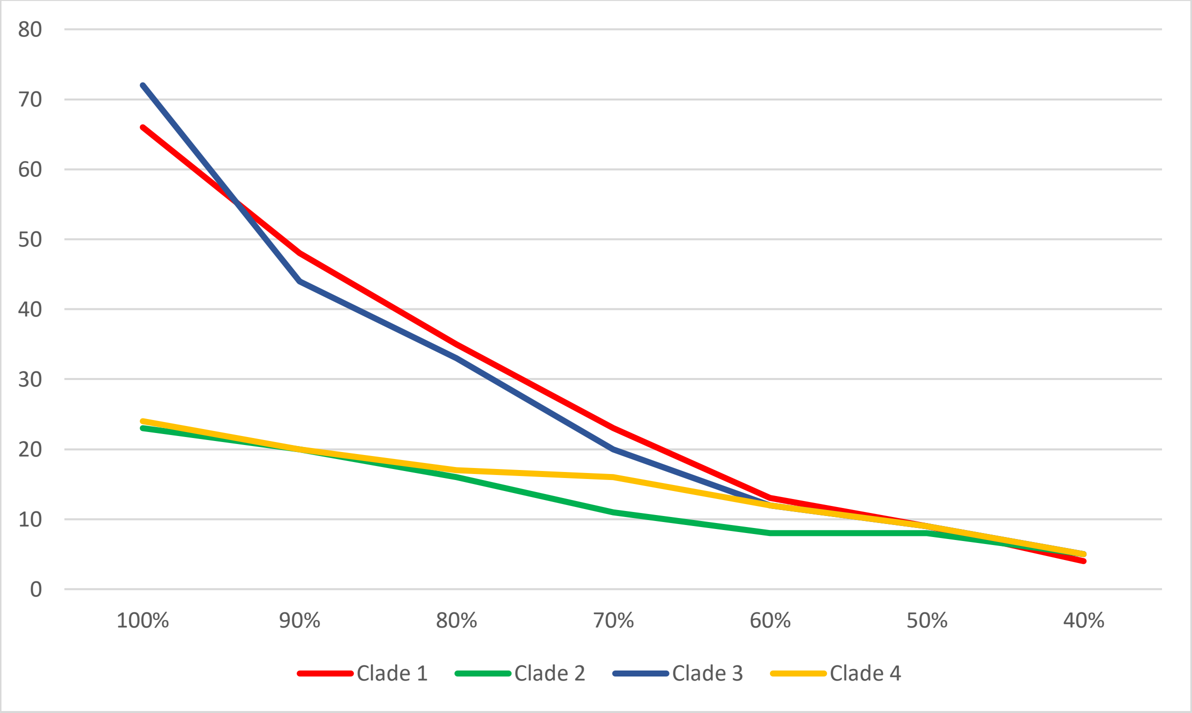
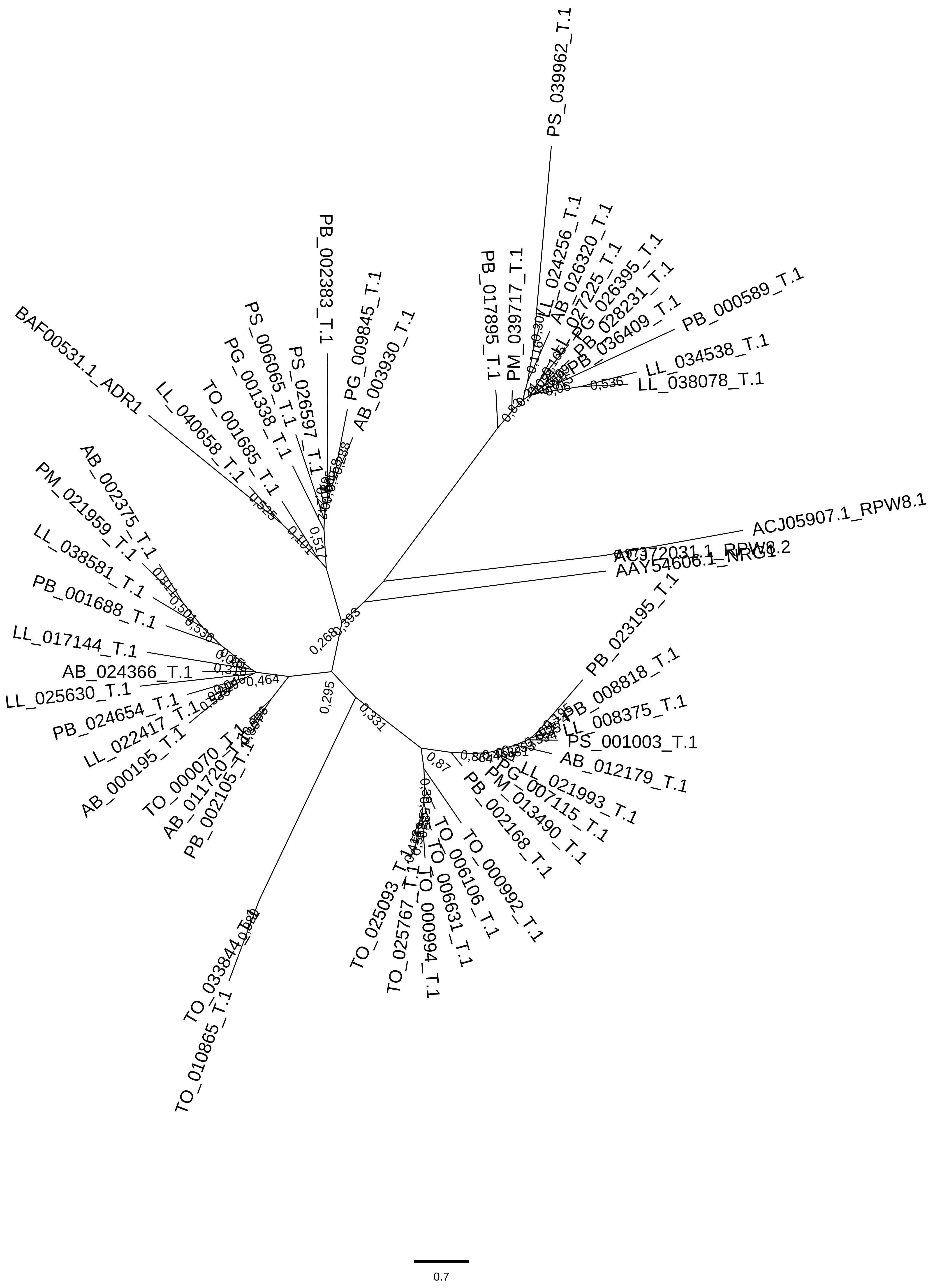
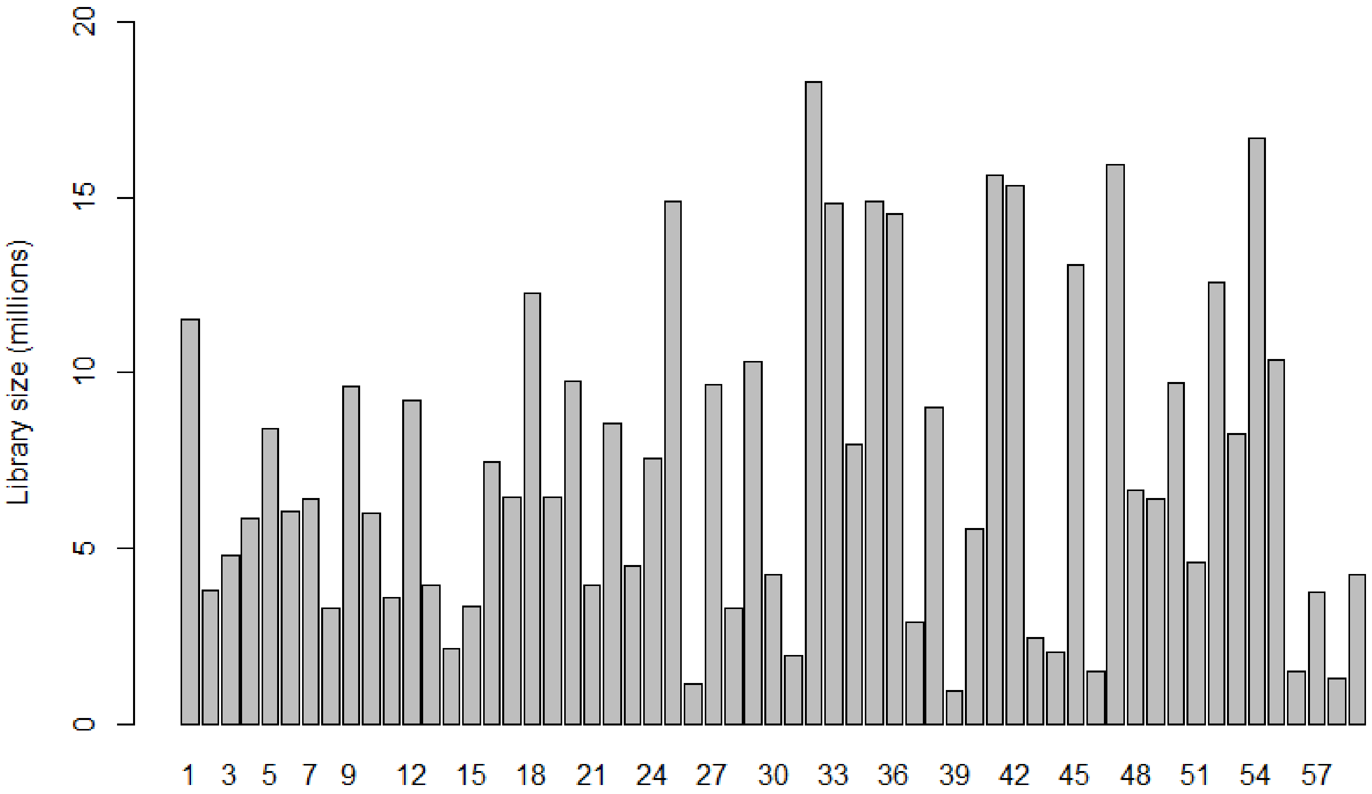
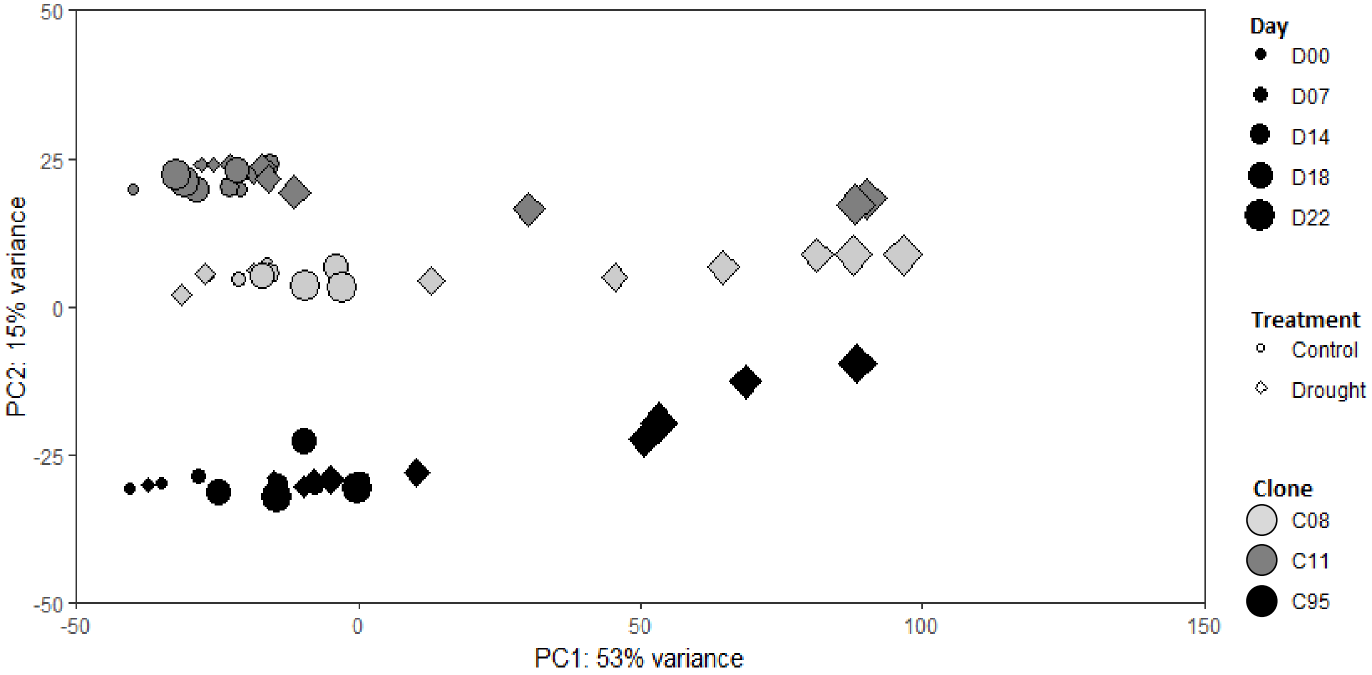
**Supplementary Figure 3 :** Progressive clustering of the RPW8 domain in seven conifer species. Y-axis shows the respective number of sequences of each clade and the X-axis represents sequence identity cut-off.



**Supplementary Figure S4:** Unrooted ML phylogenetic tree using the WAG model of the RPW8 domain in conifers after a 60% sequence identity cut-off





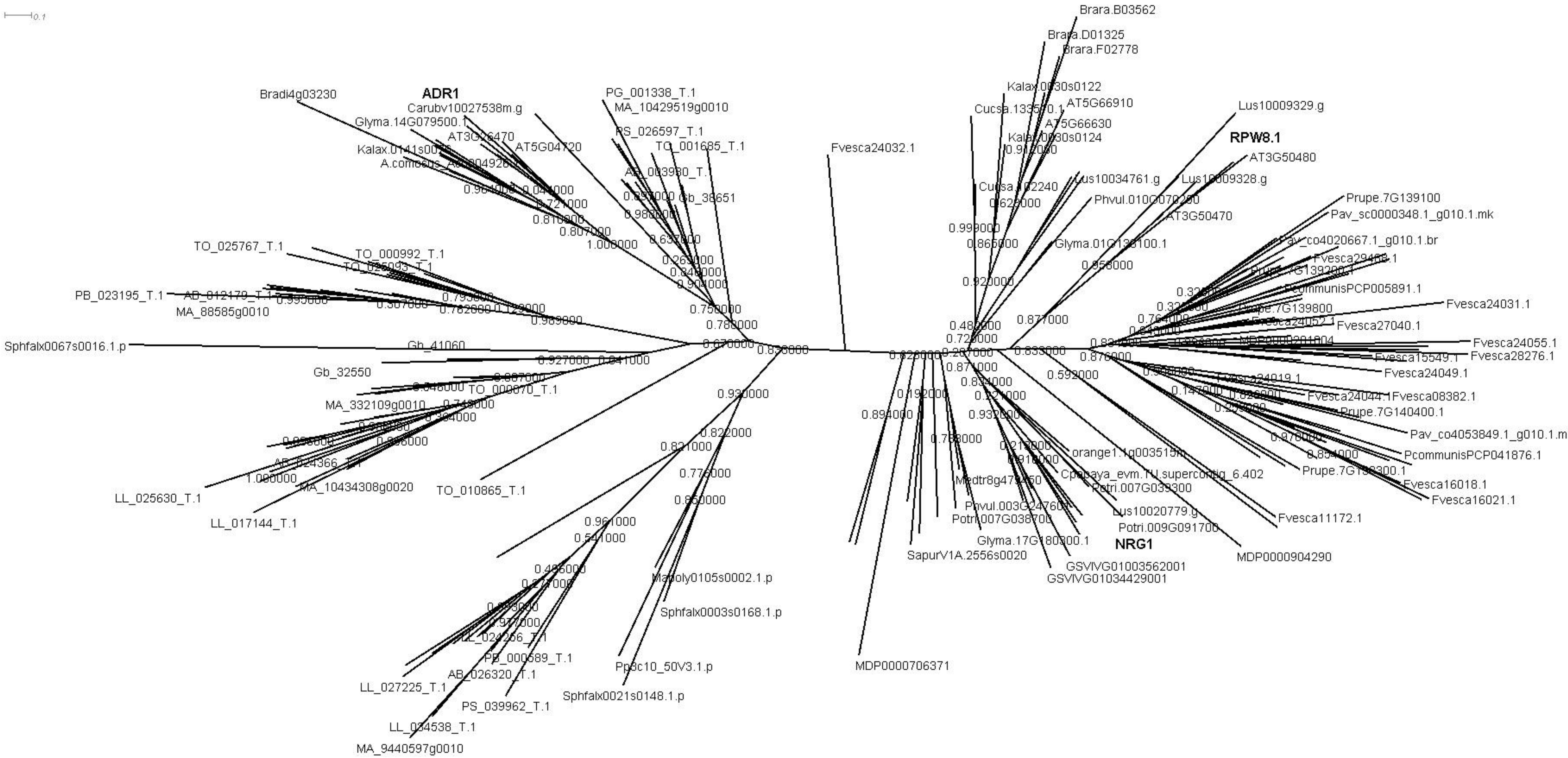


**Supplemental Figure S5:** Analysis of the RNA sequencing data from the study of Stival-Sena et al. (2018). Library size of the transcriptomes for the 59 samples (top). Principal component analysis based on the expression level of all genes in each library (below). The three *P. glauca* clones C08, C11 and C95 are depicted in light grey, dark grey and black, respectively. Control and drought stressed plants are shown in circle and diamond-shaped. The different time points are illustrated by increasing shape-size from 0-day post treatment (J64) with small-size shape to 22-days post treatment (J86) with large-size shape.

**Supplementary Figure S6:** Detailed ML phylogenetic tree of the RPW8 domain of conifer sequences corresponding to the Figure 3

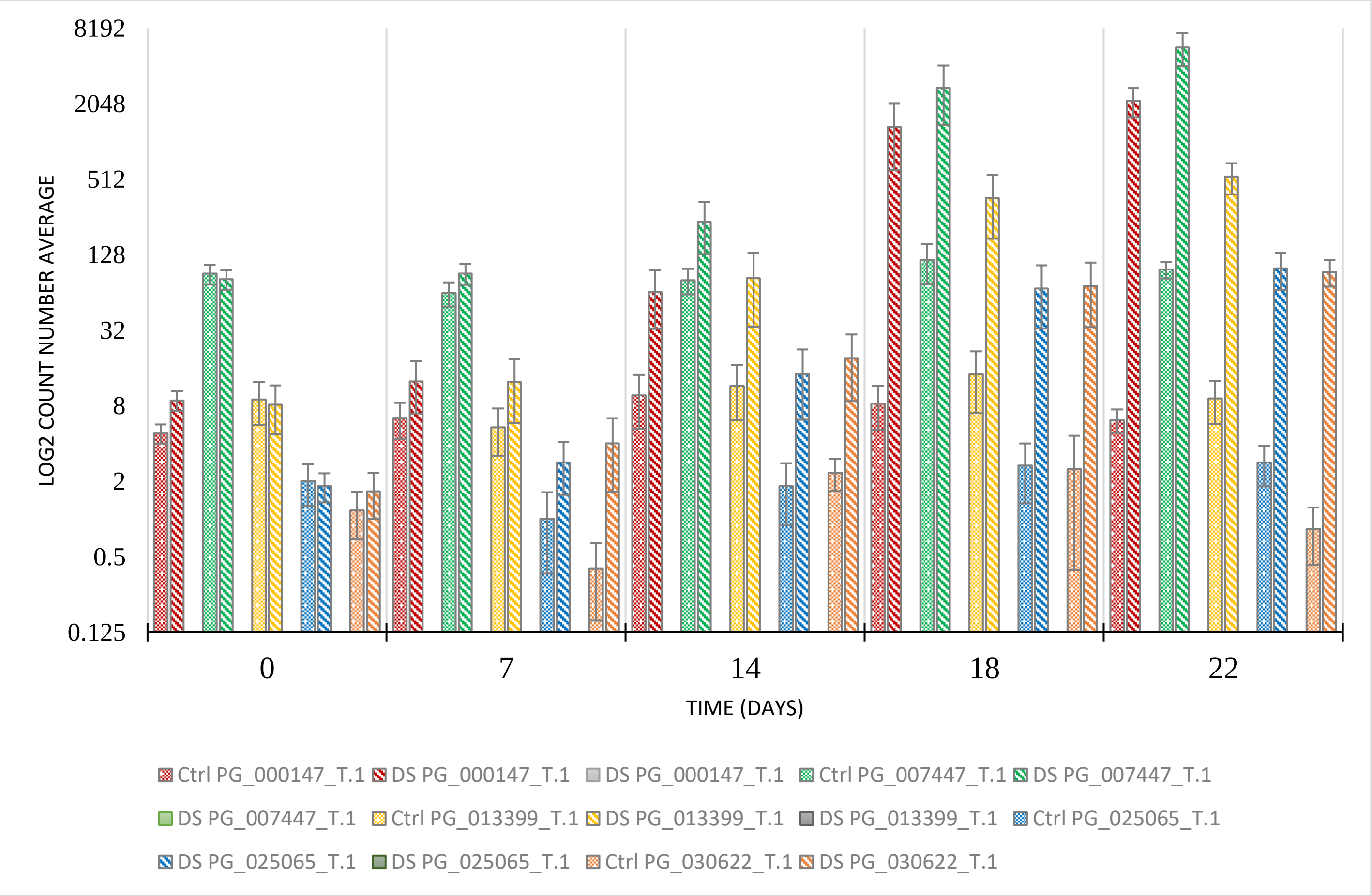
**Supplementary Figure S7:** Detailed ML phylogenetic tree of the RPW8 domain of clustered land plant sequences corresponding to the Figure 4.

**Supplementary Figure S8:** unrooted ML phylogenetic tree of the RPW8 domain in land plants using the JTT substitution model



**Supplementary Figure 9:**

Count number average, obtained from six biological replicates (two replicates for the three genotypes C08, C11, C95; with SEM), of the five most differentially expressed RNL sequences (Adjusted P-value <0.05) over the five time points of sampling.



**Supplementary Table S1 :** Ratio of NLR-related sequences to the total number of transcripts in this study and in other plants.

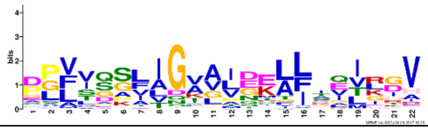
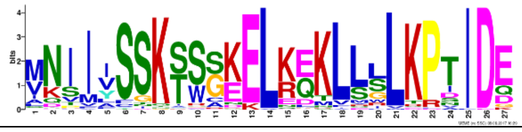
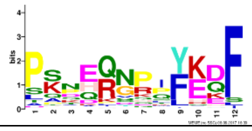
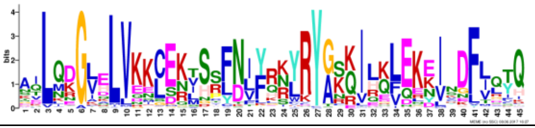
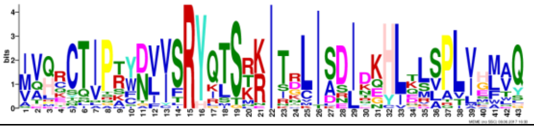
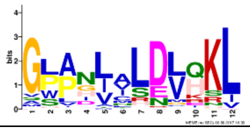
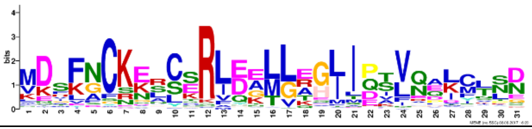
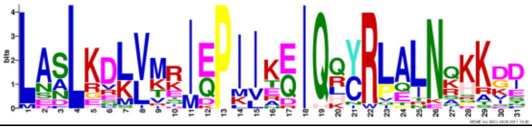
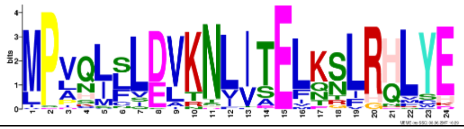
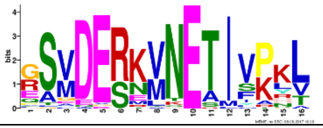
| Species | Coding genes | Gene transcripts | TOTAL NLR | NLR /coding genes ratio | NLR /gene transcripts ratio | Annotation Genebuild by EnsemblPlants | References |
|---|---|---|---|---|---|---|---|
| Abies balsamea | - | 46178 | 338 | - | 0,73% | | |
| Larix laricina | - | 50712 | 633 | - | 1,25% | | |
| Picea glauca | - | 37491 | 506 | - | 1,35% | | |
| Picea mariana | - | 58751 | 725 | - | 1,23% | | |
| Pinus banksiana | - | 47473 | 486 | - | 1,02% | | |
| Pinus strobus | - | 45447 | 560 | - | 1,23% | | |
| Thuja occidentalis | - | 38767 | 486 | - | 1,25% | | |
| Arabidopsis thaliana | 27655 | 55442 | 205 | 0,74% | 0,37% | TAIR10, INSDC Assembly GCA_000001735.1, Sep 2010 | Meyers et al. 2002 |
| Glycine max | 56679 | 90023 | 319 | 0,56% | 0,35% | Glycine_max_v2.0, INSDC Assembly GCA_000004515.3, Nov 2015 | Kang et al. 2012 |
| Medicago truncatula | 50444 | 60059 | 571 | 1,13% | 0,95% | MedtrA17_4.0, INSDC Assembly GCA_000219495.2, Jun 2014 | Shao et al. 2014 |
| Oryza sativa | 40745 | 89723 | 458 | 1,12% | 0,51% | ASM465v1, INSDC Assembly GCA_000004655.2, Jan 2005 | Li et al. 2010 |
| Populus trichocarpa | 41377 | 46745 | 317 | 0,77% | 0,68% | JGI2.0, INSDC Assembly GCA_000002775.2, Jan 2010 | Kohler et al. 2008 |
| Prunus persica | 26873 | 47323 | 354 | 1,32% | 0,75% | Prunus_persica_NCBIv2, INSDC Assembly GCA_000346465.2, Sep 2017 | Zhong et al. 2015 |
| Solanum Lycopersicum | 33810 | 35216 | 326 | 0,96% | 0,93% | SL2.50, INSDC Assembly GCA_000188115.2, Oct 2014 | Andolfo et al. 2014 |
| Vitis vinifera | 29971 | 30609 | 459 | 1,53% | 1,50% | IGGP_12x, INSDC Assembly GCA_000003745.2, Jun 2011 | Yang et al. 2008 |
| Zea mays | 39498 | 138270 | 95 | 0,24% | 0,07% | B73 RefGen_v4 (Zm-B73-REFERENCE-GRAMENE-4.0), INSDC Assembly GCA_000005005.6, Mar 2016 | Li et al. 2010 |

**Supplementary Table 2 :**Details of the NLR subfamilies numbers in 49 land plant species. Numbers in blue are retrieved from the mentionned surveys and numbers in green are extracted from the related peptide dataset (using BlastP analysis and verified with HMM models).

| **common name** | **name** | **NLR** | **TNL** | **CNL** | **RNL** | **NLR survey** | **Source for RNL search** | **Peptide dataset** |
|---|---|---|---|---|---|---|---|---|
| common liverwort | *Marchantia polymorpha* | 39 | 0 | 7 | 2 | Xue et al. 2012 | Phytozome v12.1 | Marchantia polymorpha v3.1 |
| flat-topped bogmoss | *Sphagnum fallax* | 252 | 38 | NA | 10 | - | Phytozome v12.1 | Sphagnum fallax v0.5 |
| spreading earthmoss | *Physcomitrella patens* | 115 | 9 | 11 | 4 | Xue et al. 2012 | Phytozome v12.1 | Physcomitrella patens v3.3 |
| spike moss | *Selaginella moellendorffii* | 2 | 0 | 0 | 0 | Yue et al. 2012 | Phytozome v12.1 | Selaginella moellendorffii v1.0 |
| Gingko biloba | *Gingko biloba* | NA | 150 | NA | 13 | - | Guan et al. 2016 - Gb.pep.fa | http://gigadb.org/dataset/100209 |
| Northern white-cedar | *Thuya occidentalis* | NA | 317 | 85 | 31 | - | Transcriptome | - |
| Eastern larch | *Larix laricina* | NA | 343 | 166 | 46 | - | Transcriptome | - |
| white spruce | *Picea glauca* | NA | 279 | 134 | 31 | - | Transcriptome | - |
| Black spruce | *Picea mariana* | NA | 379 | 184 | 43 | - | Transcriptome | - |
| White pine | *Pinus strobus* | NA | 339 | 124 | 40 | - | Transcriptome | - |
| Jack pine | *Pinus banksiana* | NA | 250 | 119 | 55 | - | Transcriptome | - |
| balsam Fir | *Abies balsamea* | NA | 150 | 112 | 32 | - | Transcriptome | - |
| Amborella | *Amborella trichopoda* | NA | 11 | NA | 1 | - | Phytozome v12.1 | Amborella trichopoda v1.0 |
| Date palm | *Phoenix dactylifera* | NA | 0 | NA | 1 | - | NCBI genomes assembly DPV01 | Phoenix dactylifera DPV01 |
| Banana | *Musa acuminata* | NA | 0 | NA | 1 | - | Phytozome v12.1 | *Musa acuminata v1* |
| Pineapple | *Ananas comosus* | NA | 0 | NA | 1 | - | Phytozome v12.1 | *Ananas comosus v3* |
| Maize | *Zea mays* | 95 | 0 | 71 | 1 | Li et al. 2010 | Phytozome v12.1 | Zea mays Ensembl-18 |
| Sorghum | *Sorghum bicolor* | 184 | 0 | 130 | 1 | Li et al. 2010 | Phytozome v12.1 | Sorghum bicolor v3.1.1 |
| Brachypodium | *Brachypodium distachyon* | 212 | 0 | 205 | 1 | Li et al. 2010 | Phytozome v12.1 | Brachypodium distachyon v3.1 |
| Rice | *Oryza sativa* | 458 | 0 | 274 | 1 | Li et al. 2010 | Phytozome v12.1 | Oryza sativa v7_JGI |
| Aquilegia coerulea | *Aquilegia coerulea* | NA | 0 | NA | 1 | - | Phytozome v12.1 | *Aquilegia coerulea v3.1* |
| Milky Widow's Thrill | *Kalanchoe laxiflora* | NA | 80 | NA | 6 | - | Phytozome v12.1 | *Kalanchoe laxiflora v1.1* |
| Prince-of-Wales feather | *Amaranthus hypochondriacus* | NA | 1 | NA | 1 | - | Phytozome v12.1 | *Amaranthus hypochondriacus v1.0* |
| robusta coffee | *coffea canephora* | NA | 14 | NA | 3 | - | sol genomics network | Coffea canephora protein sequences v1.0 |
| Nicotiana benthamiana | *Nicotiana benthamiana* | NA | 14 | NA | 5 | - | sol genomics network | N. benthamiana Genome v1.0.1 predicted proteins |
| Potato | *Solanum tuberosum* | 438 | 77 | 361 | 5 | Jupe et al.2012 | sol genomics network | Potato PGSC DM v3.4 protein sequences |
| currant tomato | *Solanum pimpinellifolium* | 355 | 45 | 300 | 3 | Andolfo et al. 2014 | sol genomics network | S. pimpinellifolium protein sequences LA1589 |
| Tomato | *Solanum lycopersicum* | 326 | 40 | 280 | 3 | Andolfo et al. 2014 | sol genomics network | Tomato Genome proteins (ITAG release 3.20) |
| Pepper | *Capsicum annuum* | 306 | 16 | 288 | 2 | Qian et al. 2017 | - | Cpsicum annuum cv CM334 Genome protein sequences (release 1.55) |
| Wine grape | *Vitis vinifera* | 535 | 111 | 215 | 16 | Yang et al. 2008 | Phytozome v12.1 | Vitis vinifera Genoscope.12X |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Barrelclover | *Medicago truncatula* | 571 | 275 | 279 | 17 | Shao et al. 2014 | - | Medicago truncatula Mt4.0v1 |
| common bean | *Phaseolus vulgaris* | 337 | 103 | 224 | 10 | Shao et al. 2014 | - | - |
| soybean | *Glycine max* | 465 | 178 | 269 | 18 | Shao et al. 2014 | - | Glycine max Wm82.a2.v1 |
| pigeon pea | *Cajanus cajan* | 289 | 112 | 167 | 10 | Shao et al. 2014 | - | - |
| strawberry | *Fragaria vesca* | NA | 153 | 193 | 52 | Jia et al. 2015 | Genome Database for Rosaceae | Stawberry Genome v1.0 ab initio gene proteins |
| sweet cherry | *Prunus avium* | NA | 156 | NA | 14 | - | Genome Database for Rosaceae | Sweet cherry genome v1.0 proteins |
| Peach | *Prunus persica* | 443 | 194 | 249 | 19 | Van Ghelder et al. 2016;<br>Jia et al.2015 | Genome Database for Rosaceae | Peach Genome v2.0.a1 all transcript peptide |
| pear | *Pyrus bretschneideri Rehd* | 617 | 342 | 275 | 42 | Jia et al. 2015 | Genome Database for Rosaceae | Pear draft genome v1.0 hybrid proteins |
| apple | *Malus domestica* | 1303 | 664 | 639 | 49 | Jia et al. 2015 | Genome Database for Rosaceae | Apple Genome V1.0 predicted peptides |
| Flax | *Linum usitatissimum* | 147 | 98 | 49 | 10 | Kale et al. 2013 | Phytozome v12.1 | *Linum usitatissimum v1.0* |
| Purple willow | *Salix purpurea* | NA | 164 | NA | 9 | - | Phytozome v12.1 | *Salix purpurea v1.0* |
| Poplar | *Populus trichocarpa* | 402 | 123 | 277 | 9 | Kohler et al. 2008 | Phytozome v12.1 | Populus trichocarpa v3.0 |
| Cucumber | *Cucumis sativus* | 53 | 11 | 17 | 4 | Wan et al. 2013 | Phytozome v12.1 | Cucumis sativus v1.0 |
| Mustard | *Brassica rapa* | 80 | 52 | 28 | 11 | Mun et al. 2009 | Phytozome v12.1 | Brassica rapa FPsc v1.3 |
| Pink shepherd's-purse | *Capsella rubella* | NA | 91 | NA | 8 | - | Phytozome v12.1 | Capsella rubella v1.0 |
| Lyre-leaved rock-cress | *Arabidopsis lyrata* | 138 | 103 | 21 | 11 | Guo et al. 2011 | Phytozome v12.1 | Arabidopsis lyrata v2.1 |
| Thale cress | *Arabidopsis thaliana* | 151 | 94 | 55 | 11 | Meyers et al. 2003 | TAIR | TAIR10 proteins |
| Papaya | *carica papaya* | 54 | 6 | 6 | 3 | Porter et al. 2009 | Phytozome v12.1 | Carica papaya ASGPBv0.4 |
| sweet orange | *Citrus sinensis* | NA | 152 | NA | 11 | - | Phytozome v12.1 | Citrus sinensis v1.1 |

**Supplementary Table S3:** Details of the RPW8 motifs identified in figure 3.

| motif | MEME | Nb of sites | e-values | Width |
|---|---|---|---|---|
| a |  | 84 | 2.8e-332 | 22 |
| b |  | 71 | 5.0e-1038 | 27 |
| c |  | 132 | 5.4e-418 | 12 |
| d |  | 157 | 1.2e-3475 | 45 |
| d* |  | 24 | 1.5e-402 | 43 |
| e |  | 82 | 1.1e-257 | 12 |
| f |  | 122 | 7.5e-1295 | 31 |
| g |  | 19 | 1.1e-178 | 31 |
| h |  | 49 | 4.2e-598 | 24 |
| i |  | 48 | 2.2e-347 | 16 |

**Supplementary Table S4:** List of the 119 differentially expressed NLR genes (Adjusted Pvalue <0.05) with their subfamily and expression group assignations.

| Differentially Expressed NLR | baseMean | log2FoldChange | lfcSE | stat | Pvalue | Adjusted Pvalue | NBS-LRR Subfamily | Expression group |
|---|---|---|---|---|---|---|---|---|
| PG_001471_T.1 | 13.66855083 | 2.450547425 | 0.865418942 | 18.94433341 | 0.000805985 | 0.002115279 | CNL | 1 |
| PG_011798_T.1 | 1154.483568 | 0.962059964 | 0.168405879 | 53.39583691 | 7.04E-11 | 9.52E-10 | RNL | 1 |
| PG_018623_T.1 | 68.07760019 | 0.589946431 | 0.317997279 | 13.13450767 | 0.010636928 | 0.020782289 | TNL | 1 |
| PG_020032_T.1 | 119.6129448 | 3.158357801 | 0.574544738 | 53.9227415 | 5.46E-11 | 7.60E-10 | RNL | 1 |
| PG_021812_T.1 | 196.7851559 | 2.257576457 | 0.311751807 | 80.21065113 | 1.57E-16 | 7.77E-15 | TNL | 1 |
| PG_026838_T.1 | 62.81941299 | 0.010673059 | 0.847345107 | 12.44157594 | 0.014352604 | 0.026941669 | RNL | 1 |
| PG_000147_T.1 | 454.6327066 | 8.617440953 | 0.820836302 | 166.7146012 | 5.30E-35 | 2.60E-31 | RNL | 2 |
| PG_007447_T.1 | 1203.423106 | 7.066495126 | 0.688865522 | 135.8840669 | 2.15E-28 | 2.11E-25 | RNL | 2 |
| PG_013399_T.1 | 157.8220042 | 7.364781125 | 0.733023068 | 136.6303383 | 1.49E-28 | 1.54E-25 | RNL | 2 |
| PG_019724_T.1 | 44.00186757 | 7.965987357 | 1.11145701 | 75.09382209 | 1.90E-15 | 7.41E-14 | U | 2 |
| PG_025065_T.1 | 34.07984528 | 6.466260061 | 1.059575018 | 49.7327906 | 4.11E-10 | 4.61E-09 | RNL | 2 |
| PG_030622_T.1 | 26.17277113 | 7.590556448 | 1.21711405 | 53.82779567 | 5.72E-11 | 7.92E-10 | RNL | 2 |
| PG_001975_T.1 | 126.5367603 | -4.642758124 | 0.651369846 | 62.22673798 | 9.87E-13 | 2.10E-11 | CNL2 | 3 |
| PG_003813_T.1 | 303.1528528 | -1.160856559 | 0.503404947 | 23.71939465 | 9.09E-05 | 0.000301984 | TNL | 3 |
| PG_016168_T.1 | 69.93504192 | -3.78489389 | 0.651036855 | 41.65805645 | 1.96E-08 | 1.52E-07 | CNL | 3 |
| PG_030758_T.1 | 65.90984909 | -4.537298352 | 0.707877044 | 54.53546445 | 4.07E-11 | 5.90E-10 | U | 3 |
| PG_030759_T.1 | 66.36499118 | -4.47384608 | 0.696530501 | 51.72759587 | 1.57E-10 | 1.95E-09 | CNL2 | 3 |
| PG_032601_T.1 | 23.12206662 | -4.201030728 | 0.761142056 | 34.47644297 | 5.95E-07 | 3.24E-06 | CNL | 3 |
| PG_023129_T.1 | 104.6630694 | -2.531400929 | 0.415916275 | 52.86661926 | 9.09E-11 | 1.20E-09 | U | 4 |
| PG_023520_T.1 | 42.27907251 | -2.144389575 | 0.595158543 | 24.60194623 | 6.05E-05 | 0.000209127 | TNL | 4 |
| PG_023535_T.1 | 94.93489561 | -2.538803176 | 0.479327493 | 35.70120308 | 3.33E-07 | 1.92E-06 | CNL2 | 4 |
| PG_023965_T.1 | 21.24298972 | -1.816117862 | 0.531362906 | 13.5470234 | 0.008890343 | 0.017761685 | TNL | 4 |
| PG_025567_T.1 | 60.22093264 | -3.019162588 | 0.510433424 | 55.44525513 | 2.62E-11 | 3.98E-10 | CNL | 4 |
| PG_026514_T.1 | 55.30180247 | -1.303832876 | 0.331295901 | 17.14696092 | 0.0018099 | 0.004364347 | TNL | 4 |
| PG_027609_T.1 | 42.23665206 | -2.194485926 | 0.490044659 | 28.78175933 | 8.66E-06 | 3.62E-05 | CNL2 | 4 |
| PG_030965_T.1 | 19.98854145 | -1.602304019 | 0.4825323 | 14.01007491 | 0.00726297 | 0.0149126 | TNL | 4 |
| PG_032035_T.1 | 36.32098043 | -2.543761889 | 0.479207083 | 49.29935164 | 5.06E-10 | 5.58E-09 | CNL | 4 |
| PG_000915_T.1 | 63.38803716 | -2.759108663 | 0.679969234 | 22.29136321 | 0.000175353 | 0.000544333 | TNL | 5 |
| PG_018964_T.1 | 26.76858277 | -2.628668648 | 0.576219602 | 31.52195183 | 2.40E-06 | 1.14E-05 | TNL | 5 |
| PG_030046_T.1 | 100.9200444 | -1.730344318 | 0.675588051 | 10.79058881 | 0.029021106 | 0.049747933 | TNL | 5 |
| PG_031163_T.1 | 49.7212265 | -2.686052791 | 0.76808363 | 14.24888412 | 0.006541656 | 0.013592214 | TNL | 5 |
| PG_022851_T.1 | 112.1294323 | -3.868486953 | 0.637975857 | 37.77128262 | 1.25E-07 | 8.00E-07 | CNL2 | 6 |
| PG_025525_T.1 | 108.4130107 | -4.069264073 | 0.789541005 | 26.84974793 | 2.13E-05 | 8.18E-05 | CNL2 | 6 |
| PG_036715_T.1 | 20.5698958 | -4.28233979 | 0.753577594 | 35.47274244 | 3.71E-07 | 2.12E-06 | CNL2 | 6 |
| PG_002086_T.1 | 64.85685646 | -4.147860455 | 0.70437581 | 42.80282853 | 1.14E-08 | 9.27E-08 | CNL | 7 |
| PG_005099_T.1 | 63.33578162 | -4.554452585 | 0.802123073 | 35.21336138 | 4.20E-07 | 2.36E-06 | CNL | 7 |
| PG_005233_T.1 | 53.39038078 | -4.064269404 | 0.727301141 | 34.38237824 | 6.22E-07 | 3.37E-06 | CNL | 7 |
| PG_013121_T.1 | 96.62762652 | -4.471619134 | 0.808072645 | 34.49306931 | 5.90E-07 | 3.21E-06 | CNL | 7 |
| PG_033031_T.1 | 25.55387424 | -3.91731213 | 0.856219097 | 22.5020579 | 0.000159189 | 0.000499048 | CNL | 7 |
| PG_001161_T.1 | 54.10061107 | -2.130084277 | 0.577936715 | 17.5661263 | 0.00149982 | 0.003689131 | TNL | 8 |
| PG_002418_T.1 | 252.8121285 | -2.618591326 | 0.458933465 | 48.21657648 | 8.51E-10 | 8.96E-09 | TNL | 8 |
| PG_013663_T.1 | 38.19068774 | -3.456289223 | 0.527170406 | 60.43620572 | 2.35E-12 | 4.55E-11 | CNL | 8 |
| PG_020429_T.1 | 101.0200953 | -4.94932683 | 0.683020599 | 80.05027739 | 1.70E-16 | 8.32E-15 | CNL | 8 |
| PG_022220_T.1 | 94.96966871 | -5.050136833 | 0.800594648 | 60.90102294 | 1.88E-12 | 3.73E-11 | CNL | 8 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| PG_022823_T.1 | 44.71730791 | -3.568524874 | 0.635262345 | 34.12287305 | 7.03E-07 | 3.75E-06 | CNL2 | 8 |
| PG_023416_T.1 | 89.60857445 | -2.006389623 | 0.587944315 | 21.48811531 | 0.000253357 | 0.000755875 | TNL | 8 |
| PG_024393_T.1 | 81.6559838 | -4.331841513 | 0.647269824 | 62.05616348 | 1.07E-12 | 2.25E-11 | U | 8 |
| PG_027550_T.1 | 32.04023514 | -2.975991853 | 0.63282373 | 31.05784588 | 2.98E-06 | 1.38E-05 | CNL | 8 |
| PG_030072_T.1 | 57.63111363 | -4.180792975 | 0.613595477 | 69.6134584 | 2.74E-14 | 8.59E-13 | CNL2 | 8 |
| PG_032528_T.1 | 132.5588063 | -1.175167371 | 0.436738839 | 23.0530549 | 0.000123573 | 0.000397487 | TNL | 8 |
| PG_024369_T.1 | 10.34221701 | -1.726291057 | 0.707336991 | 11.8502798 | 0.018500054 | 0.033692787 | TNL | 9 |
| PG_030268_T.1 | 27.16756693 | -2.580465791 | 0.785817968 | 18.45491226 | 0.00100539 | 0.002578626 | TNL | 9 |
| PG_010062_T.1 | 18.82308515 | -2.435621672 | 0.686564156 | 30.18465372 | 4.49E-06 | 2.00E-05 | CNL | 10 |
| PG_019615_T.1 | 32.96847495 | -1.686565957 | 0.485751922 | 24.66200819 | 5.88E-05 | 0.000204041 | CNL2 | 10 |
| PG_021165_T.1 | 62.44948818 | -0.919657288 | 0.575615773 | 13.00444501 | 0.011254095 | 0.02182623 | TNL | 10 |
| PG_024997_T.1 | 40.69575945 | -2.124083976 | 0.440362373 | 32.60236133 | 1.44E-06 | 7.14E-06 | CNL | 10 |
| PG_027305_T.1 | 17.06346923 | -3.314147182 | 0.695703658 | 25.05561518 | 4.90E-05 | 0.000172961 | CNL | 10 |
| PG_027423_T.1 | 31.24823132 | -4.447745951 | 0.657873666 | 64.51863955 | 3.25E-13 | 7.95E-12 | TNL | 10 |
| PG_028579_T.1 | 40.29778808 | -3.822941478 | 0.661760161 | 32.70628245 | 1.37E-06 | 6.84E-06 | CNL2 | 10 |
| PG_029570_T.1 | 122.4037371 | -1.612713909 | 0.537969686 | 20.05200386 | 0.000487731 | 0.001357722 | TNL | 10 |
| PG_031236_T.1 | 12.75522078 | -2.21678313 | 0.64747364 | 23.24363588 | 0.000113196 | 0.000366752 | TNL | 10 |
| PG_032108_T.1 | 17.46866298 | -1.865769178 | 0.563457152 | 24.95263731 | 5.14E-05 | 0.00018066 | TNL | 10 |
| PG_001027_T.1 | 161.5715712 | -1.031062234 | 0.363706762 | 11.44447221 | 0.021997747 | 0.039168958 | TNL | 11 |
| PG_002977_T.1 | 65.00590861 | -0.67777785 | 0.252211026 | 18.10512926 | 0.001177058 | 0.002966979 | CNL | 11 |
| PG_003080_T.1 | 41.14477415 | -1.954068217 | 0.349801845 | 51.61383177 | 1.66E-10 | 2.05E-09 | CNL | 11 |
| PG_005613_T.1 | 41.72700027 | -1.880404521 | 0.412871922 | 36.7457954 | 2.03E-07 | 1.24E-06 | CNL | 11 |
| PG_009545_T.1 | 34.78267968 | -1.481033871 | 0.391503444 | 17.58808829 | 0.001485105 | 0.003657062 | TNL | 11 |
| PG_011665_T.1 | 34.68241541 | -1.253654738 | 0.346787633 | 25.15549246 | 4.68E-05 | 0.000166068 | TNL | 11 |
| PG_013569_T.1 | 47.66285744 | -0.916186698 | 0.371909318 | 11.57333464 | 0.020822788 | 0.037337609 | TNL | 11 |
| PG_014034_T.1 | 90.69627304 | -1.591659617 | 0.387987418 | 26.61009712 | 2.38E-05 | 9.03E-05 | TNL | 11 |
| PG_014262_T.1 | 38.177421 | -1.36031812 | 0.377384386 | 15.14860842 | 0.004402713 | 0.009602228 | CNL | 11 |
| PG_024247_T.1 | 51.1213908 | -0.959916607 | 0.40026069 | 10.9908364 | 0.026667194 | 0.046188784 | TNL | 11 |
| PG_026136_T.1 | 47.89023568 | -1.497486026 | 0.475179719 | 10.98336543 | 0.026751602 | 0.046318635 | TNL | 11 |
| PG_026526_T.1 | 31.32844764 | -1.614285564 | 0.386154877 | 21.57084417 | 0.000243947 | 0.000730799 | CNL | 11 |
| PG_028266_T.1 | 84.82777644 | -1.541650801 | 0.418689027 | 18.31051559 | 0.001073033 | 0.00272965 | TNL | 11 |
| PG_028823_T.1 | 21.27612319 | -2.630351034 | 0.483195303 | 47.36376561 | 1.28E-09 | 1.29E-08 | CNL | 11 |
| PG_029130_T.1 | 35.03753941 | -1.635264917 | 0.493262809 | 14.29919818 | 0.006398892 | 0.013352111 | CNL | 11 |
| PG_030244_T.1 | 42.72735024 | -1.777911641 | 0.380729803 | 23.97659572 | 8.07E-05 | 0.000271041 | U | 11 |
| PG_031683_T.1 | 96.35560312 | -1.776782864 | 0.384168178 | 25.52315451 | 3.95E-05 | 0.000142998 | TNL | 11 |
| PG_003292_T.1 | 94.89065323 | 0.565879191 | 0.351994239 | 15.04870974 | 0.004601249 | 0.009977589 | TNL | 12 |
| PG_010112_T.1 | 31.04581951 | 1.484548949 | 0.49360684 | 14.73020848 | 0.005294772 | 0.011290643 | TNL | 12 |
| PG_026810_T.1 | 20.95937916 | -1.934545381 | 0.592122454 | 13.52907765 | 0.008960119 | 0.017879254 | TNL | 12 |
| PG_033929_T.1 | 30.20519774 | 1.346815978 | 0.471286512 | 15.82813764 | 0.003258664 | 0.00735466 | TNL | 12 |
| PG_000173_T.1 | 60.68427734 | -1.329337119 | 0.349102487 | 21.63603528 | 0.000236777 | 0.000712089 | RNL | 13 |
| PG_000389_T.1 | 75.69006458 | -2.715578045 | 0.465288902 | 38.56151786 | 8.58E-08 | 5.73E-07 | CNL | 13 |
| PG_003633_T.1 | 58.58000727 | -2.074512023 | 0.648566474 | 12.54688614 | 0.013715711 | 0.025914642 | CNL | 13 |
| PG_003976_T.1 | 31.05365071 | -1.602981113 | 0.432981079 | 18.93394418 | 0.000809781 | 0.002123821 | TNL | 13 |
| PG_006607_T.1 | 43.07682985 | -2.652887188 | 0.556816468 | 22.11757739 | 0.000189903 | 0.000584417 | U | 13 |
| PG_007137_T.1 | 24.19273955 | -4.415076688 | 0.653452545 | 56.79017683 | 1.37E-11 | 2.23E-10 | CNL | 13 |
| PG_010581_T.1 | 1247.26345 | -2.347340021 | 0.278682408 | 85.22064331 | 1.36E-17 | 8.62E-16 | RNL | 13 |
| PG_019409_T.1 | 39.61601954 | -1.966184761 | 0.52100778 | 19.3782101 | 0.000662242 | 0.001778693 | TNL | 13 |
| PG_022962_T.1 | 43.29620255 | -2.884348498 | 0.550496513 | 29.90588836 | 5.12E-06 | 2.25E-05 | CNL | 13 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| PG_027346_T.1 | 114.4947097 | -2.682939906 | 0.602321885 | 21.14510885 | 0.000296365 | 0.00086847 | TNL | 13 |
| PG_028426_T.1 | 27.22517091 | -1.661573711 | 0.496468878 | 21.96234787 | 0.000203909 | 0.000622831 | TNL | 13 |
| PG_029059_T.1 | 37.14968146 | -1.409700204 | 0.372162057 | 26.02668853 | 3.13E-05 | 0.000115636 | CNL | 13 |
| PG_029408_T.1 | 35.7229738 | -4.078132414 | 0.753797523 | 30.7849868 | 3.39E-06 | 1.56E-05 | CNL | 13 |
| PG_029409_T.1 | 83.51748434 | -2.443438316 | 0.767259599 | 14.99019329 | 0.0047216 | 0.01019689 | TNL | 13 |
| PG_030995_T.1 | 14.84798199 | -3.366374962 | 0.79233794 | 19.78984998 | 0.000549431 | 0.001508092 | CNL | 13 |
| PG_032342_T.1 | 38.56739212 | -3.036606397 | 0.567025025 | 32.68832488 | 1.38E-06 | 6.90E-06 | U | 13 |
| PG_002263_T.1 | 89.78943946 | -2.329554751 | 0.762927717 | 11.83514554 | 0.018620208 | 0.033880185 | RNL | 14 |
| PG_002809_T.1 | 41.19800658 | -1.83551034 | 0.516296252 | 17.30213343 | 0.001688359 | 0.004100987 | CNL | 14 |
| PG_010841_T.1 | 42.71141734 | -1.034107428 | 0.362064416 | 11.40745909 | 0.022346948 | 0.039707905 | U | 14 |
| PG_011683_T.1 | 54.07302573 | -1.104980905 | 0.33933562 | 17.17473289 | 0.001787531 | 0.004319425 | CNL | 14 |
| PG_012075_T.1 | 171.9369843 | -1.344107138 | 0.239120268 | 54.36591039 | 4.41E-11 | 6.32E-10 | TNL | 14 |
| PG_012802_T.1 | 376.1679886 | -0.808815432 | 0.247471407 | 14.39266465 | 0.00614175 | 0.012870271 | RNL | 14 |
| PG_013996_T.1 | 182.7643113 | -1.195285507 | 0.378602913 | 23.12230687 | 0.000119697 | 0.000386223 | TNL | 14 |
| PG_016503_T.1 | 90.50226088 | -1.015731267 | 0.283381251 | 15.46284356 | 0.00383152 | 0.00848754 | RNL | 14 |
| PG_019732_T.1 | 72.16617877 | -1.559483228 | 0.44358853 | 15.38932295 | 0.003958254 | 0.008741645 | TNL | 14 |
| PG_019785_T.1 | 44.98527101 | -0.249587365 | 0.35077238 | 12.65842411 | 0.013071199 | 0.024864409 | TNL | 14 |
| PG_020200_T.1 | 159.7914378 | -1.168589846 | 0.319886811 | 14.88062609 | 0.004955319 | 0.010655918 | TNL | 14 |
| PG_020291_T.1 | 62.67867129 | -1.637604927 | 0.410999878 | 22.78754465 | 0.000139621 | 0.000442724 | CNL | 14 |
| PG_021434_T.1 | 38.13073435 | -1.793628192 | 0.455378934 | 18.49638759 | 0.000986752 | 0.002536124 | TNL | 14 |
| PG_023840_T.1 | 57.64926664 | -1.563447034 | 0.440550379 | 14.2394534 | 0.00656876 | 0.013638781 | TNL | 14 |
| PG_024538_T.1 | 78.90344311 | -1.652324331 | 0.415549383 | 18.88019809 | 0.000829701 | 0.002171136 | TNL | 14 |
| PG_026017_T.1 | 27.25549826 | -2.489154522 | 0.421522743 | 39.50007944 | 5.49E-08 | 3.83E-07 | CNL | 14 |
| PG_026413_T.1 | 50.51917933 | -0.668992338 | 0.308376356 | 13.71326107 | 0.008268744 | 0.016703407 | TNL | 14 |
| PG_027685_T.1 | 38.47900144 | -2.347668898 | 0.613558749 | 19.98185702 | 0.000503535 | 0.001397157 | TNL | 14 |
| PG_028025_T.1 | 30.89383893 | -1.427341915 | 0.40181666 | 21.70792093 | 0.000229112 | 0.000691316 | U | 14 |
| PG_035021_T.1 | 17.37134006 | -1.74716938 | 0.811981421 | 14.42315996 | 0.006060066 | 0.012712668 | TNL | 14 |

**Supplementary Table S5**: List of RNL sequences with, when available, their corresponding *P. glauca* gene catalogue accession number and genetic position (Rigault et al. 2011, Pavy et al. 2013).

| RNL (reference transcriptome accession number) | RNL sequence group assignation | DEG | Expression group | Cluseq accession (Rigault et al. 2011) | | Composite map linkage group (Pavy et al. 2018) | Composite map position (Pavy et al. 2018) |
|---|---|---|---|---|---|---|---|
| PG_001338_T.1 | 2 | - | - | GQ0168_L19 | GQ02747_D08 | LG12 | 2,09 |
| PG_004977_T.1 | 3 | - | - | GQ02905_L23 | GQ03418_F13 | LG12 | 43,32 |
| PG_004976_T.1 | 3 | - | - | GQ02905_L23 | - | LG12 | 43,32 |
| PG_020277_T.1 | 3 | - | - | WS00725_G10 | GQ03715_H16 | LG12 | 46,54 |
| PG_016503_T.1 | 3 | downregulated | 14 | GQ04109_A14 | - | LG08 | 129,52 |
| PG_010581_T.1 | 1 | downregulated | 13 | GQ03702_K04 | GQ03321_G12 | LG06 | 3,86 |
| PG_012538_T.1 | - | - | - | GQ03612_J11 | GQ0133_H03 | LG06 | 4,13 |
| PG_008655_T.1 | 1 | - | - | GQ03222_P15 | - | LG06 | 4,16 |
| PG_011798_T.1 | 1 | upregulated | 1 | GQ03512_J05 | - | LG06 | 4,18 |
| PG_003625_T.1 | 2 | - | - | GQ02813_L10 | - | LG02 | 75 |
| PG_002263_T.1 | 1 | downregulated | 14 | - | - | - | - |
| PG_007115_T.1 | 1 | - | - | - | - | - | - |
| PG_020032_T.1 | 1 | upregulated | 1 | WS0011_L03 | - | - | - |
| PG_026838_T.1 | 1 | upregulated | 1 | - | - | - | - |
| PG_009845_T.1 | 2 | - | - | GQ03308_B18 | WS00747_F12 | - | - |
| PG_032324_T.1 | 2 | - | - | - | - | - | - |
| PG_000147_T.1 | 3 | upregulated | 2 | GQ0025_J06 | GQ03619_N18 | - | - |
| PG_000172_T.1 | 3 | - | - | - | - | - | - |
| PG_000173_T.1 | 3 | downregulated | 13 | GQ0031_B13 | GQ03316_N22 | - | - |
| PG_002106_T.1 | 3 | - | - | GQ0205_A13 | - | - | - |
| PG_007447_T.1 | 3 | upregulated | 2 | - | - | - | - |
| PG_012802_T.1 | 3 | downregulated | 14 | GQ03701_F14 | - | - | - |
| PG_013399_T.1 | 3 | upregulated | 2 | GQ03714_K21 | - | - | - |
| PG_025065_T.1 | 3 | upregulated | 2 | - | - | - | - |
| PG_030622_T.1 | 3 | upregulated | 2 | - | - | - | - |
| PG_026395_T.1 | 4 | - | - | - | - | - | - |
| PG_000071_T.1 | - | - | - | - | - | - | - |
| PG_000073_T.1 | - | - | - | - | - | - | - |
| PG_002264_T.1 | - | - | - | - | - | - | - |
| PG_005390_T.1 | - | - | - | GQ03007_O23 | - | - | - |
| PG_017209_T.1 | - | - | - | - | - | - | - |
| PG_018001_T.1 | - | - | - | - | - | - | - |
| PG_024644_T.1 | - | - | - | - | - | - | - |
| PG_029492_T.1 | - | - | - | - | - | - | - |
| PG_029972_T.1 | - | - | - | - | - | - | - |
| PG_035029_T.1 | - | - | - | - | - | - | - |

**Supplementary Table S6:** RNL and TNL genes present on the chromosome 7 of the peach genome.

| | **RNLs** | **TNLs** |
|---|---|---|
| Sequence accession numbers | Prupe.7G138300 | Prupe.7G012000 |
| | Prupe.7G138400 | Prupe.7G017000 |
| | Prupe.7G138500 | Prupe.7G026100 |
| | Prupe.7G138600 | Prupe.7G037000 |
| | Prupe.7G138700 | Prupe.7G037600 |
| | Prupe.7G138800 | Prupe.7G065300 |
| | Prupe.7G138900 | Prupe.7G065400 |
| | Prupe.7G139100 | Prupe.7G065500 |
| | Prupe.7G139200 | Prupe.7G065600 |
| | Prupe.7G139500 | |
| | Prupe.7G139600 | |
| | Prupe.7G139700 | |
| | Prupe.7G139800 | |
| | Prupe.7G140100 | |
| | Prupe.7G140400 | |
| number on the Chromosome 7 / total number | 15/19 | 9/192 |
| percentage | 79% | 5% |

## Supplementary Methods

## Reference transcriptome development

### Plant Materials

RNA-sequencing was carried in seven Pinaceae species including white spruce (*Picea glauca*), black spruce (*Picea mariana*), estern white pine (*Pinus strobus*), jack pine (*Pinus banksiana*), balsam fir (*Abies balsamea*), tamarack (*Larix laricina*), eastern white cedar (*Thuya occidentalis*). We isolated several different tissue samples including four to six vegetative tissues (for details, see Table S1) from 4 year old plants grown under greenhouse conditions with natural light; we sampled the tissues from two well-watered plants and two plants that had not been watered for 14 days. The tissues also included several megagametophytes and whole embryos isolated from germinating seeds.

### RNA extraction

Total RNA was extracted by grinding tissues in liquid nitrogen to a fine powder and by utilizing either 1) the cetyltrimethyl ammonium bromide (CTAB) extraction method as described by Chang et al. (1993) with modifications (Pavy et al. 2008) for the tissue preferential analysis and the drought experiment or 2) MasterPure™ Plant RNA Purification kit (Epicenter, Madison, WI, USA) for the RNA-Squencing. The total RNA concentration was determined using a NanoDrop 1000 (Thermo cientific, http://www.thermoscientific.com/) and assessed for quality with an Agilent 2100 Bioanalyzer and Agilent RNA 6000 Nano Kit LabChips (Agilent Technologies Inc.,http://www.agilent.com/), and stored at −80°C. Only RNA samples that display RIN score >7 and 1.9<A260/A280<2.2 were used for subsequent experiments. The minimum RIN score for RNA extracted from foliage was set to 6.5 due to chloroplast content.

### RNA-Seq library synthesis and sequencing

Total RNAs from all of the tissues were combined to form a single RNA pool for each as shown (Table 1). We used 500 ng of total RNA to synthetize mRNA libraries with TruSeq® Stranded mRNA kit (Illumina Canada Inc, Victoria, BC), following the manufacturer's protocol with a few modifications. First, Illumina technology suitable custom adapters were synthetized (IDT, Coralville, Iowa, USA) and their concentration was reduced by half at 25nM per reaction to avoid adapter dimer molecules and tris-NaCl (10mM, 50mM) at 25nM was used to complete the volume. Second strand synthesis and marking clean-up and library amplification clean-up were performed using Axygen® AxyPrep™ Mag PCR Clean-Up Kit (Axygen Biosciences, Union City, CA, USA) whereas post ligation clean-up was done using a ratio of 0.85 of PEG/NaCl SPRI® Solution over Beads with adapter-ligated DNA. Quantifications of each library were determined by a Nanodrop ND-1000 (Thermo Scientific, Wilmington, DE, USA) and library fragment size distribution and the absence of dimer molecules were verified with an Agilent Bioanalyzer 2100 using High Sensitivity DNA chips (Agilent Technologies Inc., Santa Clara, CA, USA). Four equimolar pools of 17 to 18 libraries each were prepared and purified following the Library Amplification Cleanup protocol as described in the library construction protocol. The concentration and the fragment size distribution of each purified equimolar pool was evaluated with a Nanodrop ND-1000 and an Agilent Bioanalyzer 2100 respectively. Each of the pools were sequenced by using the rapid run procedure (2×250 bp) at the Genome Quebec Innovation Centre at McGill University (Montreal, Quebec, Canada) with an Illumina HiSeq 2500 sequencing system.

### Transcriptome assemblies

The transcriptome assemblies were performed with Gydle software NUCLEAR version 3.2.16, RESOLVE verison 2.6.12 and VISION version 2.6.12, and guided using the white spruce gene catalog obtained by the sequencing of full-length insert cDNAs (FLICs) from P. glauca cDNA libraries as described in Rigault et al. (2011). Raw sequence reads were filtered by trimming adapters and retaining segments with 50 or more consecutive Q20+ bases to produce high-quality (HQ) sequences. HQ sequences were assembled using reference-initiated read-mapping, followed by iterative rounds of consensus resolution, extension, remapping and de-novo assembly of unmapped reads, until the assembly captured 95% of HQ sequences. The assembly process represented each gene by a single transcript sequence defined by its maximal set of exons, clustering isoforms showing a nucleotide similarity of 97% or above in order to capture splicing and allelic variation. Representative sequences were oriented using RNA-Seq strand information and coding sequence annotation. Quality control and curation of each transcriptome assembly was performed by coverage analysis, visualisation and interactive edition.

## Transcriptomic of drought stress modified from Stival Sena et al. (2018)

### Plant material

We used young trees from three genetically unrelated *Picea glauca* genotypes (clones 8, 11 and 95) that were propagated in vitro by somatic embryogenesis and grown in containers for 2 years at the Vegetative Propagation Centre of the Saint-Modeste tree nursery of the Ministère des Forêt, de la Faune et des Parcs (Saint-Modeste, Québec, Canada). The plants were 40 cm on average, were potted in pots of 5 l containing a mix of peat, perlite and vermiculite (3:1:1, by weight) and grown in a greenhouse with day temperature of 23 °C, night temperature of 20 °C, 16/8 h (day/night) photoperiod and watered three times per week, for 2 months prior to the experiment. For the experiment, one-half of the plants were watered and for the other half, water was withheld; the plants were arranged in a completely randomized design. Plants were destructively sampled and the newly formed foliage (needles) was collected at 0 (Julian day, JD 64), 7 (JD71), 14 (JD78), 18 (JD82) and 22 (JD86) days from the beginning of the watering treatments. Two plants per genotype (replicates) in both watering treatments were sampled at each sampling point (a total of 150 plants). The watered plants were sampled 2 h after the last watering. The sampling time was at midday. At each sampling day, the midday water potential (branch) of four plants per genotype in both watering regimes was measured using a Scholander pressure chamber (Model 610, PMS Instruments, Albany, OR, USA). Foliage samples were frozen in liquid nitrogen immediately after removal from the trees and stored at −80 °C. The needles were ground to powder using a MixerMill 300 (Retsch, http://www.retsch.com/) and steel grinding balls cooled in nitrogen. Powdered foliage tissue was stored at −80 °C until RNA extraction.

### RNA extraction and cDNA synthesis

RNA was extracted by grinding tissues in liquid nitrogen to a fine powder and by utilizing either (i) the cetyltrimethyl ammonium bromide (CTAB) extraction method as described by Chang et al. (1993) with modifications (Pavy et al. 2008). The total RNA concentration was determined using a NanoDrop 1000 (Thermo Scientific, http://www.thermoscientific.com/) and assessed for quality with an Agilent 2100 Bioanalyzer and Agilent RNA 6000 Nano Kit LabChips (Agilent Technologies Inc., http://www.agilent.com/), and stored at −80 °C. Complementary DNAs were prepared from 500 ng of total RNA using Quantitect Reverse Transcription Kit (Qiagen, Germantown, MD, USA) and then diluted 1:4 in RNase-free water.

### RNA-seq library synthesis

We used 500 ng of RNA per sample to synthetize mRNA libraries with TruSeq® Stranded mRNA kit (Illumina Canada Inc., Victoria, BC, Canada), following the manufacturer's protocol with a few modifications. First, Illumina technology-suitable custom adapters were synthetized (IDT, Coralville, IA, USA) and their concentration was reduced by half at 25 nM per reaction to avoid adapter dimer molecules and Tris-NaCl (10 mM, 50 mM) at 25 nM was used to complete the volume. Second strand synthesis and marking clean-up and library amplification clean-up were performed using Axygen® AxyPrep™ Mag PCR Clean-Up Kit (Axygen Biosciences, Union City, CA, USA) whereas post ligation clean-up was done using a ratio of 0.85 of PEG/NaCl SPRI® Solution over Beads with adapter-ligated DNA. Distribution of library fragment size and the absence of dimer molecules were verified with an Agilent Bioanalyzer 2100 using High Sensitivity DNA chips (Agilent Technologies Inc., Santa Clara, CA, USA). DNA quantification was performed using Quant-iT™ PicoGreen ® dsDNA Reagent and Kits (Molecular Probes Inc., Eugene, OR, USA).Three equimolar pools of 20 libraries each were prepared and purified following the Library Amplification Cleanup protocol as described in the library construction protocol. The concentration and the fragment size distribution of each purified equimolar pool was evaluated with a Nanodrop ND1000 and an Agilent Bioanalyzer 2100, respectively. Each of the pools were sequenced by using the rapid run procedure (2 × 250 bp) at the Genome Quebec Innovation Centre at McGill University (Montreal, Quebec, Canada) with an Illumina HiSeq 2500 sequencing system.

### Read assembly and annotation

Raw RNA-Seq sequence (FASTQ) files were filtered into High-Quality (HQ) sequences, trimming adapters using the NUCLEAR version 3.2.4 software (Gydle Inc.) and retaining only segments of 50 consecutive Q20+ bases. The HQ sequences were mapped to ribosomal and gene sequences using the *Picea mariana* Gene catalogue (Rigault et al. 2011) using NUCLEAR. A total of 22532 gene sequences (i.e. cluster representative clone sequences) were annotated by performing protein-level similarity searches with BLASTX (E-value # 1e-10) against proteins of Arabidopsis (Arabidopsis thaliana; The Arabidopsis Information Resource [TAIR] version 10) (see Rigault et al. 2011 for more details).

**Table 1: Tissues and RNA used to prepare the pools used for the preparation of RNA-Sequencing libraries.**

| Plant | Tissue type | Amount of RNA used | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Picea glauca* | *Picea mariana* | *Pinus strobus* | *Pinus banksiana* | *Abies balsamea* | *Larix laricina* | *Thuya occidentalis* |
| Well-watered, 4 year-old tree, 2 trees | Young foliage | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | Stem Apex (1 cm) | 0 | 0 | 0 | 100 | 0 | 0 | 100 |
| | Stem secondary phloem and bark | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | Stem secondary xylem | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | Root apex (1 cm) | 0 | 0 | 100 | 100 | 100 | 100 | 100 |
| | Root secondary xylem | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Not watered (14 days), 4 year-old tree, 2 trees | Young foliage | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | Stem Apex (1 cm) | 0 | 0 | 100 | 100 | 0 | 0 | 100 |
| | Stem secondary phloem and bark | 100 | 0 | 100 | 100 | 100 | 100 | 100 |
| | Stem secondary xylem | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | Root apex (1 cm) | 0 | 100 | 100 | 0 | 100 | 100 | 100 |
| | Root secondary xylem | 100 | 100 | 100 | 0 | 100 | 100 | 100 |
| Seedlings at 1 cm root stage | Megagametophyte | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| | Embryo | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| Total amount of RNA in species pool | | 1.2 | 1.2 | 1.5 | 1.4 | 1.4 | 1.4 | 1.6 |

**Table 2: Accession numbers related to the seven transcriptomes**

| study | bioproject_accession | biosample_accession | sra_accession | tsa_accession | taxid | organism |
|---|---|---|---|---|---|---|
| SRP134160 | PRJNA437248 | SAMN08646834 | SRR6816980 | GGJG00000000 | 90345 | Abies balsamea |
| SRP134160 | PRJNA437248 | SAMN08646875 | SRR6816981 | GGJA00000000 | 3326 | Larix laricina |
| SRP134160 | PRJNA437248 | SAMN08646877 | SRR6816983 | GGJI00000000 | 3330 | Picea glauca |
| SRP134160 | PRJNA437248 | SAMN08646878 | SRR6816977 | GGJJ00000000 | 3335 | Picea mariana |
| SRP134160 | PRJNA437248 | SAMN08646879 | SRR6816982 | GGJH00000000 | 3353 | Pinus banksiana |
| SRP134160 | PRJNA437248 | SAMN08646880 | SRR6816978 | GGJK00000000 | 3348 | Pinus strobus |
| SRP134160 | PRJNA437248 | SAMN08646881 | SRR6816979 | GGJL00000000 | 3317 | Thuja occidentalis |

**Table 3: Additional data sampling:**

| name | Resource | Peptide dataset |
|---|---|---|
| *Amaranthus hypochondriacus* | Phytozome v12.1 | Amaranthus hypochondriacus v1.0 |
| *Amborella trichopoda* | Phytozome v12.1 | Amborella trichopoda v1.0 |
| *Ananas comosus* | Phytozome v12.1 | Ananas comosus v3 |
| *Aquilegia coerulea* | Phytozome v12.1 | Aquilegia coerulea v3.1 |
| *Arabidopsis lyrata* | Phytozome v12.1 | Arabidopsis lyrata v2.1 |
| *Arabidopsis thaliana* | TAIR | TAIR10 proteins |
| *Brachypodium distachyon* | Phytozome v12.1 | Brachypodium distachyon v3.1 |
| *Brassica rapa* | Phytozome v12.1 | Brassica rapa FPsc v1.3 |
| *Cajanus cajan* | Shao et al. 2014 | Shao et al. 2014 |
| *Capsella rubella* | Phytozome v12.1 | Capsella rubella v1.0 |
| *Capsicum annuum* | sol genomics network | Cpsicum annuum cv CM334 Genome protein sequences (release 1.55) |
| *carica papaya* | Phytozome v12.1 | Carica papaya ASGPBv0.4 |
| *Citrus sinensis* | Phytozome v12.1 | Citrus sinensis v1.1 |
| *coffea canephora* | sol genomics network | Coffea canephora protein sequences v1.0 |
| *Cucumis sativus* | Phytozome v12.1 | Cucumis sativus v1.0 |
| *Fragaria vesca* | Genome Database for Rosaceae | Stawberry Genome v1.0 ab initio gene proteins |
| *Ginkgo biloba* | Guan et al. 2016 | http://gigadb.org/dataset/100209 |

| | | |
|---|---|---|
| *Glycine max* | Phytozome v12.1 | Glycine max Wm82.a2.v1 |
| *Kalanchoe laxiflora* | Phytozome v12.1 | Kalanchoe laxiflora v1.1 |
| *Linum usitatissimum* | Phytozome v12.1 | Linum usitatissimum v1.0 |
| *Malus domestica* | Genome Database for Rosaceae | Apple Genome V1.0 predicted peptides |
| *Marchantia polymorpha* | Phytozome v12.1 | Marchantia polymorpha v3.1 |
| *Medicago truncatula* | Phytozome v12.1 | Medicago truncatula Mt4.0v1 |
| *Musa acuminata* | Phytozome v12.1 | Musa acuminata v1 |
| *Nicotiana benthamiana* | sol genomics network | N. benthamiana Genome v1.0.1 predicted proteins |
| *Oryza sativa* | Phytozome v12.1 | Oryza sativa v7_JGI |
| *Phaseolus vulgaris* | Phytozome v12.1 | Phaseolus vulgaris v2.1 |
| *Phoenix dactylifera* | NCBI genomes | Phoenix dactylifera assembly DPV01 |
| *Physcomitrella patens* | Phytozome v12.1 | Physcomitrella patens v3.3 |
| *Picea abies* | Congenie.org | Picea abies v1.0 |
| *Populus trichocarpa* | Phytozome v12.1 | Populus trichocarpa v3.0 |
| *Prunus avium* | Genome Database for Rosaceae | Sweet cherry genome v1.0 proteins |
| *Prunus persica* | Genome Database for Rosaceae | Peach Genome v2.0.a1 all transcript peptide |
| *Pyrus bretschneideri Rehd* | Genome Database for Rosaceae | Pear draft genome v1.0 hybrid proteins |
| *Salix purpurea* | Phytozome v12.1 | Salix purpurea v1.0 |
| *Selaginella moellendorffii* | Phytozome v12.1 | Selaginella moellendorffii v1.0 |
| *Solanum lycopersicum* | sol genomics network | Tomato Genome proteins (ITAG release 3.20) |
| *Solanum pimpinellifolium* | sol genomics network | S. pimpinellifolium protein sequences LA1589 |
| *Solanum tuberosum* | sol genomics network | Potato PGSC DM v3.4 protein sequences |
| *Sorghum bicolor* | Phytozome v12.1 | Sorghum bicolor v3.1.1 |
| *Sphagnum fallax* | Phytozome v12.1 | Sphagnum fallax v0.5 |
| *Vitis vinifera* | Phytozome v12.1 | Vitis vinifera Genoscope.12X |
| *Zea mays* | Phytozome v12.1 | Zea mays Ensembl-18 |