

**Online Resource 4:** Multiple sequence alignment of closely related proteins to TaGW2 using ClustalOmega. In yellow is the conserved RING domain and in grey are the three amino acid residues (EEQ) which are absent from the -9 bp mutant *gw2-A1* transcript. Protein names include GenBank ID followed by the species genus name. The wheat TaGW2-A1 protein is based on the GenBank nucleotide accession number KP749899.

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TaGW2-A1_Triticum      MGNRIGGRRKAGVEERYTRPQGLYEHRDIDQKKLRKLILEAKLAPCYPGADDA--GGDLEECPICFLFYPSLNRSKCCSKGICTECLFQMKPHTHTARPTQCPFC KTPNYAVEYRGVKT
ABY51682.1_Hordeum    MGNRIGGRRKAGVEERYTRPQGLYEHRDIDQKKLRKLILETKLAPCYPGADDA--GADLEECPICFLFYPSLNRSKCCSKGICTECLFQMKPHTHTARPTQCPFC KTPNYAVEYRGVKT
XP_010234090.1_Brachypodium MGNRIGGRRKAGVEERYTRPQGLYEHRDIDQKKLRKLILEAKLAPCYPGADDA--GGDLEECPICFLFYPSLNRSKCCSKGICTECLFQMKPHTHTARPTQCPFC KTPNYAVEYRGVKT
ABO31101.1_Oryza      MGNRIGGRRKAGVEERYTRPQGLYEHRDIDQKKLRKLILEAKLAPCYMGADDA--AADLEECPICFLFYPSLNRSKCCSKGICTECLFQMKPHTHTAQTQCPFC KTPSYAVEYRGVKT
XP_004951330.1_Setaria MGNRIGGRRKAGVEERFTRPQGLYEHRDIDQKKLRKLILEAKLAPCYPGADAPAGGGDLEECPICFLFYPSLNRSKCCSKGICTECLFQMKPHTHTARPTQCPFC KTPNYAVEYRGVKT
XP_002453598.1_Sorghum MGNRKGGRRPKSGGEKRFTPPQGLYEHKIDIDQKKLRKLILEAKLAPCYPGADDAAGGGDLEECPICFLFYPSLNRSKCCSKGICTECLFQMKPHTHTARPTQCPFC KTPNYAVEYRGVKT
XP_008681635.1_Zea    MGNRIGGRRKSGVEERFTRPQGLYDHKIDIDQKKLRKLILEAKLAPCYPGADDAAPGGGGDLEECPICFLFYPSLNRSKCCSKGICTECLFQMKPHTHTARPTQCPFC KTPNYAVEYRGVKT
XP_009387884.1_Musa   MGNNIRRRR--QMVDEKYTRPQGLYHRIDYKLRKLILESKLAPCYPGLEEY--AYDLEECPICFLFYPSLNRSRCCMKGICTECLFQMKPPHSTRPSQCPFC KTSNYAVEYHGAKTK
XP_010925650.1_Elaeis MGNKIGRRR--QVDEKYTRPQGLYQHRDIDHKKLRKLI LIDSKLAPCYPGNEEC---GLDLEECPICFLFYPSLNRSRCCVKGICTECLFQMKPPHSTRPTQCPFC KTPNYAVEYRGMKTK
XP_008801976.1_Phoenix MGNKIGRRR--QVDEKYTRPQGLYQHRDIDHKKLRKLI LIDSKLAPCYPGDDEC---AMDLEECPICFLFYPSLNRSRCCMKGICTECLFQMKAPHSIRPTQCPFC KTSNYAVEYRGMKTK
XP_006302337.1_Capsella MGNKLGRKR--QVVEERYTKPQGLYVNDVDIKKLRKLI LIVESKLAPCYPGDDESC---HDLEECPICFLFYPSLNRSRCCMKS ICTECLFQMKPNPN SARPTQCPFC KTPNYAVEYRGVKS
AEE36104.1_Arabidopsis MGNKLGRKR--QVVEERYTKPQGLYVNDVDVKKLRKLI LIVESKLAPCYPGDDESC---HDLEECPICFLFYPSLNRSRCCMKS ICTECLFQMKPNPN SARPTQCPFC KTPNYAVEYRGVKS
XP_011653276.1_Cucumis MGNKLGRRR--QVDEKYTRPQGLYNHKEVDHKKLRKLI LILES KLAPCYPGDEESA---SDLEECPICFLFYPSLNRSRCCMKS ICTECLFQMKVPNSTRPTQCPYCKTSNYAVEYRGVKS
XP_004243185.1_Solanum MGNKLGRRK--QVVDKYTRPQGLYQHKDVIDVKKLRKLI LIDSKLAPCYPGDDDCP--NANLEECPICFLFYPSLNRSRCCMKGICTECLFQMKTPNSTRPTQCPFC KTSNYAVEYRGVKT
XP_003543150.1_Glycine MGNKLGRRR--QVDEKYTRPQGLYNHKEVDHKKLRKLI LILES KLAPCYPGDEETA---YDREECPICFLFYPSLNRSRCCTKS ICTECLFQMKVPNSTRPTQCPFC KTSNYAVEYRGVKS
XP_010062730.1_Eucalyptus MGNKLGRRR--QVDEKYTRPQGLYHHKDV DDKLRKLI LILES KLAPCYPGDEEST---CDLEECPICFLFYPSLNRSRCCTKGICTECLFQMKIPNSTRPTCPFC KTSNYAVEYRGVKT
XP_008226243.1_Prunus  MGNKLGRRR--QVDEKYTRPQGLYQHKDVIDHKKLRKLI LIDSKLAPCYPGDEEAT---NDFEECPICFLFYPSLNRSRCCTKGICTECLFQMKPNPNSTRP -TCPYCKTANYAVEYRGVKT
CAN66658.1_Vitis      MGNKLGRRR--QVDEKYTRPQGLYQHKDVIDHKKLRKLI LIDSKLAPCYPGDEEAT---NDFEECPICFLFYPSLNRSRCCTKGICTECLFQMKPNPNSTRP -TCPYCKTANYAVEYRGVKT
XP_007022166.1_Theobroma MGNKLVRRK--QVVDERYTRPQGLYTHNDVDIKKLRKLI LILES KLAPCYPGDEECC---CDLEECPICFLFYPSLNRSRCCTKR ICTECLFQMKPNPNSTRPTQCPFC KTSNYAVEYRGVKT
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TaGW2-A1_Triticum      EERSIEQFEEQKVIEAQMRVRQALQDEEDKMKRKQSRCSSTIAPTTEVEYRDI CSTSFSVPSY-QCTQETECSSSEPSCSAQANMRFSH----SRHTRDDNIDMNIEDMMVMEAI
ABY51682.1_Hordeum    EERSIEQFEEQKVIEAQMRMRQALQDEEDKMKRKQSRCSSTIAPTTEVEYRDI CSTSFSAPPY-RCTEQETECSSSEPSCSAQANMRFSH----SRHTRDGNIDMNIEDMMVMEAI
XP_010234090.1_Brachypodium EERSIEQLEEQKVIEAQMRMRQALQDEEDKMKRKQSRCSSTIAPTTEVEYRDI CSTSFSVPSY-QCTEQEAECSSSEPSCSAQSNMRPVH----SRHNRDDNIGMNI EEMMVMEAI
ABO31101.1_Oryza      EERSIEQFEEQKVIEAQMRMRQALQDEEDKMKRKQNRCSSTITPTKEVEYRDI CSTSFSVPSY-RCAEQETECSSSEPSCSAQTSMRPFH----SRHNRDDNIDMNI EEDMMVMEAI
XP_004951330.1_Setaria EERSIEQFEEQKVIEAQLRIRQKELQDEEAKLKRKQSRCSSTVTPTTEVEYRDI CSTSFSVPSY-QCAEQETECSSSEPSCSQASMRPFH----SRHNRDDNVD MNL EEDMMVMEAI
XP_002453598.1_Sorghum EERSIEQFEEQKVIEAQLRMRQKELQDEEAKMKRKQSRCSSTVTPTTEVEYRDI CSTSFSVPSY-QCTEQNECCSSSEPSCSQANMRPFH----SRHNRDDNVD MNL EEDMMVMEAI
XP_008681635.1_Zea    EERSIEQFEEQKVIEAQLRMRQKELQDEEAKMRKQGRCSSTVTPTTEVEYRDI CSTSFSVPSY-QCTEQNECCSSSEPSCSQANRRPFH----SRHNRDDNVD MNL ENMMVMEAI
XP_009387884.1_Musa   EEKGMEQVEEQVIEAQIRIRQELQDEAERMKQRNLSSSSTMTPT-DVGHHDISNTSIVSPSM-KCSIQSSDLGSRQASCASAPASTRPSQ----LRQNRDNNLDLLEDV MMEAI
XP_010925650.1_Elaeis EEKGMEQVEEQRVIEAQIRMRQELQDEAERMKQRQDVSMPSRRMTS-AEVEHRDMCSTLSVPSF-TCTALGNE SVTSQASCASAPASTRPSH----SRQNRDGNFDL DLEEDIMVMEAI
XP_008801976.1_Phoenix EEKGMEQLEEQRVIEAQIRMRQEI QDEAERMKQRKDVSSSSGIMTP-AEVEYQDICTSLS-----GNE SVSSQASCSSPDSTRPSH----SRQNRDNDNF DLEEDIMVMEAI
XP_006302337.1_Capsella EEKGIEQVEEQRVIEAKIRIRQKEMQDDEEKMQKRLESCSSSTAMT-GEMEYG----SASAVSY-NSPMDEGETASSQNA---SAVRQHSR----PRGNR DDEVDV DLEELMVMEAI
AEE36104.1_Arabidopsis EEKGIEQVEEQRVIEAKIRMRQKEMQDDEEKMQKRLESCSSSTAMT-GEMEYG----STSAISY-NSLMDGDIAPSQNA---SVVRQHSR----PRGNR DDEVDV DLEELMVMEAI
XP_011653276.1_Cucumis EEKSLEQIEEQRVIEAKIRIRQELQDDEERMQKRHELSTSNADTTV-E-----NVPSS-QSPAEDDEIVSLQDPCMTQIRPPPIPIRSNRRNFRDDEFDLDLEDIMVMEAI
XP_004243185.1_Solanum EEKGIEQIEEQRVIEAKIRMRQELQDDEEKMHKRRLESTSSSIAGP-SEIEYC----STAAPSF-ASAVEGGEVTVTQETCTAPTTRQPQR----TRQNRDEDFDLDLEDIMVMEAI
XP_003543150.1_Glycine EEKGLEQIEEQRVIEAKIRMRQELQDEEEMHKRLEMSSSNVNAV-ADVEYS----SNAVSSSVVENEIVSSQDSCATSVVRANAT----TRTNRDDEFDLDLEDIMVMEAI
XP_010062730.1_Eucalyptus EEKGIEQIEEQRVIEAKIRMRQKELQDEEERMQKRQLSSSSTMAP-VEIDNG----SVGQSR-RSPVDEEIVSADQSYSTISRPPH----LRTNRDDEFDLDLEDIMVMEAI
XP_008226243.1_Prunus EEKGLEQIEEQRVIEAKIRMRQEI QDEEERMQIRQLSSSSTRNMAP-VGDEYS----V-TVPS-ASPAGEEIVSSQDSCAASMRQPPP----PRVYREDEFDLDLEDIMVMEAI
CAN66658.1_Vitis      EEKGMEQIEEQRVIEAKIRMRQKELQDEEERMQKRQEISSSSSILAQ-GEVEYS----TTAVPSF-RSPVEGDEIDSSQDPRASMI IQTLP----PRQNRDDEFDLDLEDIMVMEAI
XP_007022166.1_Theobroma EEKGIEQIEEQRVIEAQIRMRQELQDEEERMQKRQELSSSSTAVAP-GEVQYS----SVAARS----SGEEEIVSSQDSCAASMRQPSH----PRANRNEEFDLDLEDIMVMEAI
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Simmonds et al. *A splice acceptor site mutation in TaGW2-A1 increases thousand grain weight in tetraploid and hexaploid wheat through wider and longer grains*

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TaGW2-A1_Triticum      WRSIQEQGSIGNPSCGSMFMPF-----EQPT-RERQAFVAAPP-----LEMPHPGGFSCAVAAMAEHQPSSMDFSYM-TGSSAFPVDFMFRRCNIIAGGSMGAAES-SPDSW----
ABY51682.1_Hordeum    WRSIQEQGSIGNPACGSFMPF-----EQPT-RERQAFVAASP-----LEIPHPGGFSCAVAAMTEHQPSMDFSYM-TGSSAFPVDFMFRRCNIIAGGSLRAVES-SLDSW----
XP_010234090.1_Brachypodium WRSIQEQGSMGNPVCGNFMPV-----IEPPSRERQAF-VPAP-----LEIPHPGGFSCAVASMAEHQPPSMDFSYM-AGNSAFPVDFMFRRCNIIAGGSMCAVDS-SPDSW----
ABO31101.1_Oryza      WRSIQEQGSIGNPVCGNFMPV-----TEPPSRERQAFVPAAS-----LEIPHPGGFSCAVAAMAEHQPPSMDFSYM-AGSSAFPVDFMFRRCNIIAGGSMCNLES-SPESW----
XP_004951330.1_Setaria WRSIQEQGHLVNPVCGSYFPV-----IEPQTRERQAFVPAAP-----MEMPHPGGYSCAVAAMAELHQPPSMDFSYM-AGSSTFPVDFMIRRPCNIIAGGSMCAVENSLDWTW----
XP_002453598.1_Sorghum WRSIQEQGHLVNPVCGSYFPV-----IEPPSRERQAFVPAAP-----LEMPHPGGYSCAVAALAELHQPPASMDFSYM-AGSSTYPVDFMIRRPCNIIAGGSLCGVENSLDWTW----
XP_008681635.1_Zea    WRSIQEQGHLVNPVCGSYFPV-----IEPPSRERQAFVPAAP-----LEMPHPGGYSCV-AALAELHQPPSMDFSYM-AGSSTYPVDFMIRRPCNMSSGSLCVENSLDWTW----
XP_009387884.1_Musa   WLSIQEQGSQGNPSCVGSYLP-----RPSTSGEWHSSHGTP-----MRPSSSGGLACAAAALAERQHMHGNSAVH-MAIDNAAACDMLQRSGSSSPGITRFVHDNPSGRW----
XP_010925650.1_Elaeis WLSIQEQGSPGNPCCGGDVLV-----EPSFSD--NSVA--VA-----PVEASPGGLACAVAALAERQHINGDSAAG-MAGSDASSFDMLRQPSLLPVGMTATAAENDPPGSW----
XP_008801976.1_Phoenix WLSIQEQGAPGNPTCGSNVLP-----EPSVEECYNLSA--VP-----PIEVSPGGLACAVASLAELHQHMNGDSAAS-MAGSGALAFDMVRQSSSLPVGMTRVIGKNPPGFW----
XP_006302337.1_Capsella WLSVQETGTQRNSASGEMTS-----SRQYETE-DHSYVSQPPMASTVEPAMPSSSSSSGGITCAIAALAERQMVGESSN--HNHNVNVSSYSMLPGNCDSYDIIEQEVDDIGNHHHHHQ
AEE36104.1_Arabidopsis WLSVQETGTQRNSASGEITS-----SRQYVTD-NHSYVSSPPRVTPIVEPATPSS-SSGGLSCAISALAERQMVGESSSNHNHNVNVSSYSMLPGNCDSYDIIEQEVVDIGNHHHRH-
XP_011653276.1_Cucumis WLSIQEKGRNKDPVYAEAAS-----SDQYATE-GRYVSPATIP-----LAGTSSSPSGGLACIAALAELHQIIGRGPSS-CTNGDSPVFTMLPGATEFYNRMNTNVENYPPQTQGSISA
XP_004243185.1_Solanum WLSIQENGRNRMSDYSDVGP-----SEQYTEE-DHRCVPAVNP-----TAGSSSSPSGGLACIAALAERQQMGESSSY--SRNMSSSYNEHPSCDRFNSNGEELENDDYPHAEG----
XP_003543150.1_Glycine WLSIQENGRRRNLSFVDATSGHYVADGRYVSS-VSSVSSVMGP-----PTGSSSSPSGGLACIAALAERQQMAGESSMS-LTNENMPSFNTLPGRSRRFYNRGRDMANYPGDN----
XP_010062730.1_Eucalyptus WLSIQEKGFNSLAQSEAAP-----SEQYVSE-DHYVVSQATAP-----LAGSGSSPSGGLACIAALAERQQMGESSFS--HNTTMSTYVNPVPCGQFYPTAVQDSDGYAAPES----
XP_008226243.1_Prunus WLSIQENGRHKSPSYGDVTP-----SEQFVTR-QSYVSPAMVS-----VSG-SSSPSGGLACIAALAERQQTSGESSTN--PGGNVPGFSMVPPTSRFYNRVDRESENYSAAVS----
CAN66658.1_Vitis      WLSIQDNGRHRNPLYGDTT-----AEQYVTE-EHYVLPAMAP-----QVSSSSPSGGLACIAALAERQQMGESSSTN--YNGNMPAFNMPGSSRFSNRVEQYYPENYPPIES----
XP_007022166.1_Theobroma WSIQENNRQRNSNYGDAAS-----SVQYVSG-DRYISPAMTT-----VAGSSTSPSGGLACIAALAERQQMGESSLN--HNGDIPSFNMLCSGSRLYNRVDRVVENYPPAES----
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TaGW2-A1_Triticum      -----SGIAPSCSRREVVEEGECSTDHLSEGAEGT--SYAGSDIVDAGTMLPLPFAD---NYSMVASHFRPESIEEQMMYSMAVSLAEAHGRTH--TQGLAWL-
ABY51682.1_Hordeum    -----SGIAPSGTRREVMVEEGECSDHWSEGAEGT--SYAGSDIMADAGTMPPLPFAD---NYSMAASHFRPESIEEQMMYSMAVSLAEAHGRTH--TQGLTWL-
XP_010234090.1_Brachypodium -----SGIAPPSCSR-EMIREEGECSTDHWSEGAEGT--SYAGSDIVADAGTMQQLPFAE---NYNMAPSHFRPESIEEQMMYSMTVSLAEAHGRTH--SQGLAWL-
ABO31101.1_Oryza      -----SGIAPSCSR-EVVREEGECSDHWSEGAEGT--SYAGSDIVADAGTMPQLPFAE---NFAMAPSHFRPESIEEQMMFMAVSLADG--HGRTH--SQGLAWL-
XP_004951330.1_Setaria -----SGIAPSCSR-EVLREEGECSTDHWSEGAEGT--SYAGSDIMADAGSMQPLPFAE---NFAMAPSHFRPESIEEQMMFMAVSLTDAHGHRAH--AQGMAWL-
XP_002453598.1_Sorghum -----SGIAPSCSR-EVVREEGECSTDHWSEGAEGT--SYAGSDIMADTGTMQPLPFAE---NFTMAPSHFRPESIEEQMMFMAVSLAEAHHGRTQ--AQGLAWL-
XP_008681635.1_Zea    -----SGMAPNSCR-GVVREEGECSTDHWSEGAEGT--SYAGSDIMVDAGAMQPLPFAE---NFTMAPSHFRPESIEEQMMFMAVSLAEHGHGRTQ--AQGLAWL-
XP_009387884.1_Musa   -----TEISPDNGREVHGQEFGECLADHQSEVAEAGT--SYNVE-----TGAMT-GPLSE---GVSIPPGHFVPEFEEQMMMLAMAVSLAEARARMS--NHGVSLL-
XP_010925650.1_Elaeis -----NEVLPSDGRGVTR-EEGECSTDHWSEVTEGGT--NYAGSDVRVHARSAA-VSLPE---AGSMAVSHLLPESFEEQMMMLAMAVSLADAGARTN--AQGLTWL-
XP_008801976.1_Phoenix -----NEVSPDSGREVPR-EEGECSTDHWSEVTEAGT--SYAGSDVMVDAGAAA-ASFPG---V-NMAANHLLPESFEEQMMMLATAVSLAEARARTN--AQGLTWL-
XP_006302337.1_Capsella QHY-----HNNTMGTGSSNNYVSSYM-----TGESFHNFPPLVIVPEFEEQMMMLAMAVSLAEV-HATTTASTEVTWQ-
AEE36104.1_Arabidopsis -----HYEMGETGSSNSYVSSYM-----TGEFHNFPPLVIVPEFEEQMMMLAMAVSMAEV-HATTTCAPTEVTWQ-
XP_011653276.1_Cucumis ENYAPAQVSTVDTPDCRM-ILTRNDGEWNLDHQSEAEAGT--SYPTSDLNEDNSTECALPTVNAMDGGNQ--ATIPIIPQNFEEQMMMLAMAVSLAEAREVST--GPGHSWQE
XP_004243185.1_Solanum -----T-MHVSPESHL-EIPREDGEWA-DHGSMAEAVGT--SYAVSDEMEDDA---SFPLQGEVMSDLQNTSSIVPENFEEQMMMLAMAVSLAEASARSS--PPGVAWH-
XP_003543150.1_Glycine -----L-NEEPLDEAV-TMTRSHGEWDMDGTQLTETAT--SYTNSVAADRGLSSLPRSDNDGSLQSATEPIVPEFEEQMMMLAMAVSLAEARAMSS--QGSASWQ-
XP_010062730.1_Eucalyptus -----GTTELVHGGGI-PLARDDGEWNMDRGSEVAEAGT--SYASSDSPEDAGGISALPQNEVDVGFQNI PAPIVPEFEEQMMMLAMAVSLAEARAVTS--GPGITWQ-
XP_008226243.1_Prunus -----S-SEMSINHGM-PLTQDDREWNADT-----RT--SYASSDTTEDAGSTAPPTGNEIEGDLQNVDPPIVPEFEEQMMMLAMAVSLAEARAVAS--GPGVPWQ-
CAN66658.1_Vitis      -----S-MDALPDGGL-AVTKDDGEWGVDRGSEVAEAGT--SYASSDATDEAGGVAALPPTDEAEGSFQNVGGPIVPEFEEQMMMLAMAVSLAEARARTS--TQGV-WQ-
XP_007022166.1_Theobroma -----P-VDMPADGGM-TPARDEGEWGDHGESEVAEAGT--SYASSDVTEDEAGGICTISQDDIRGSHFNVPGPIVPEFEEQMMMLAMAVSLAEARAMTS--GPGVSWQ-
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