Table 1 Suppl. Primers used in this study (Y2H - yeast two-hybrid).

Primer name	Forward sequence	Reverse sequence	Use
MaGα	5’-ATGGGCTTACTATGCAGCAGGA-3’	5’-TCACAACAAGCCAGCCTCGAAG-3’	Gene Cloning
MaGβ	5’-ATGTCTGTTGCCGAGCTGAAAG-3’	5’-TCAAAGTACCCTCCTATGCCCT-3’	
MaGγ1	5’-ATGGAGCCCGAAACGGCATCGT3’	5’-TCAGAGTATGACACACCTACAG-3’	
MaGγ2	5’-ATGCAATCGAGTGGGTCCCAAT-3’	5’-TCACAGGATCCAGCATCTGCAG-3’	
MaRGS	5’-ATGGGAAGCTGTGCAGTGGAAG-3’	5’-TCAGTCGAAAGAATCGCAGCCA-3’	
MaRACK1	5’-ATGGCGAGCGAGGGACTGGTTC-3’	5’-CTAGTAGCGGCCAATACCCCAA-3’	
MaGα	5’-GCAAGCAGCAGAAATTGAAA-3’	5’-ATTGGCATGGATGACTGAGA-3’	RT-qPCR
MaGβ	5’-TGGTGACAAAGATGAACCGT-3’	5’-AACAACCTCGGCCAATAAAG-3’	
MaGγ1	5’-CACCGACGAACATATCGGT3’	5’-TCCAAGCATCTGAGTTCCTG-3’	
MaGγ2	5’-CACCGGATTCCGTCTTTAAT-3’	5’-TGCTGGGAGTAGTGGATCAG-3’	
MaRGS	5’-TATGTGTGGGCAGTCTGGAT-3’	5’-AGACGCCTCTTGACGAAGAT-3’	
MaRACK1	5’-ATGGCGAGCGAGGGACTGGTTC-3’	5’-CTAGTAGCGGCCAATACCCCAA-3’	
MaACTIN3	5’-GCATGAAGATCAAGGTGGTG-3’	5’-CATCTGCTGGAAGGTGCTAA-3’	
MaGα	5’-ATTAACAAGGCCATTACGGCCCGGATGGGCTTACTATGCAGCAGGA-3’	5’-AACTGATTGGCCGAGGCGGCCGTCACAACAAGCCAGCCTCGAAG-3’	Split-ubiquitin-based system
MaGβ	5’-ATTAACAAGGCCATTACGGCCATGTCTGTTGCCGAGCTGAAAG-3’	5’-AACTGATTGGCCGAGGCGGCCCCAAGTACCCTCCTATGCCCT-3’	
MaRGS	5’-ATTAACAAGGCCATTACGGCCATGGGAAGCTGTGCAGTGGAAG-3’	5’-AACTGATTGGCCGAGGCGGCCCCGTCGAAAGAATCGCAGCCA-3’	
MaGβ	5’-GGAATTCCATATGATGGCGAGCGAGGGACTGGTTC-3’	5’-GGAATTCTCAAAGTACCCTCCTATGCCCT-3’	Y2H analysis
MaGγ1	5’-GGAATTCCATATGATGGAGCCCGAAACGGCATCGT-3’	5’-GGAATTCTCAGAGTATGACACACCTACAG-3’	
MaGγ2	5’-GGAATTCCATATGATGCAATCGAGTGGGTCCCAAT-3’	5’-GGAATTCTCACAGGATCCAGCATCTGCAG-3’	

Table 2 Suppl. Accession numbers for the protein encoded by G-protein signalling genes found in the plant kingdom (RGS - regulator of G-protein signaling protein, RACK1 - receptor for activated C kinase 1).

Species	Gα-subunit	Gβ-subunit	Gγ-subunit	RGS	RACK1
Eudicots:					
Arabidopsis thaliana	At2g26300	At4g34460	AGG1:At3g63420 AGG2:At3g22942 AGG3:At5g20635	At3g26090	AtRACK1A: At1g18080 AtRACK1B: At1g48630 AtRACK1C: At3g18130
Malus domestica	XP_008377920.1	XP_008375922.1		XP_008340147.1	XP_008393404.1
Vitis vinifera	XP_010648394.1	XP_003631647.1		XP_002267857.1	XP_002281279.1
Populus euphratica	XP_011019332.1			XP_011029599.1	
Ricinus communis	XP_002516152.1	XP_002509478.1		AHB52760.1	
Solanum lycopersicum	NP_001292984.1	XP_004230907.1			
Medicago truncatula	XP_003588959.1	XP_003603911.1			XP_003631071.1
Glycine max	GmGα2: Glyma14g11140.1 GmGα3: Glyma06g05960.1 GmGα4: Glyma17g34450.1	GmGβ1: Glyma11g12600.1 GmGβ2: Glyma12g04810.1 GmGβ3: Glyma06g01510.1 GmGβ4: Glyma04g01460.1	GmGγ1: Glyma10g03610.1 GmGγ2: Glyma02g16190.1 GmGγ6: Glyma14g17060.1 GmGγ7: Glyma17g29590.1 GmGγ9: Glyma17g05640.1 GmGγ10: Glyma07g04510.1	GmRGS1: Glyma18g01490.1 GmRGS2: Glyma11g37540.1	Glyma05g243800
Lotus japonicus		AFK33707.1			
Phaseolus vulgaris					ACJ24167.1
Brassica napus	ACX43273.1	ACX43274.1		NP_001302894.1	XP_013664597.1
Brassica nigra			AIS73133.1 AIS73134.1 AIS73136.1		
Eucalyptus grandis					XP_010036939.1
Jatropha curcas					XP_012072542.1
Mosses:					
Physcomitrella patens			Pp1s22_182V6.1 Pp1s39_119V6.1		
Monocots:					
Oryza sativa subsp. indica	Os05g26890.1	ADL27745.1	OsGS3: ABC84855.1 RGG1: BAD15277.1		RACK1A: Os01g49290.1 RACK1B: Os05g47890.1
Sorghum bicolor		XP_002466691.1			
Phoenix dactylifera				XP_008796039.1	
Brachypodium distachyon	XP_014755105.1				
Zea mays	DAA43682.1	NP_001105365.1			NP_001146976.1
Gymnosperms:					
Picea abies	AKA58772.1	PAHJ60245.1			
Green Alga:					
Chara braunii	AHB52757.1	AHB52758.1	AHB52759.1		
Volvox carteri f. nagariensis					XP_002945887.1
Chlamydomonas reinhardtii					XP_001698065.1

Table 3 Suppl. Information regarding G-protein signaling genes in *Morus notabilis* (*Mn*) and *Morus atropurpurea* (*Ma*) (CDS - coding sequence).

Gene Name	CDS length [bp]	Proteins size [aa number]	Mr [Da]	PI	GenBank ID
MnGα	1155	384	44635.8	5.95	KX289875
MaGα	1155	384	44652.7	5.81	KX099864
MnGβ	1134	377	41061.1	7.13	KX279361
MaGβ	1134	377	41025.1	7.56	KX099865
MnGγ1	321	106	11518.8	4.70	KX268500
MaGγ1	321	106	11608.9	4.55	KX099866
MnGγ2	318	105	12034.5	5.39	KX268501
MaGγ2	321	106	12024.5	5.39	KX099867
MnRGS	1386	461	52246.8	6.76	KX268502
MaRGS	1386	461	52321.8	6.97	KX099868
MnRACK1	993	330	36007.6	6.45	KX268499
MaRACK1	993	330	36136.8	6.71	KX099869

Table 4 Suppl. Comparison of G-protein signalling genes from different species.

	Morus notabilis	*Arabidopsis thaliana*	*Oryza sativa*	*Chara braunii*	*Glycine max*	*Picea abies*	*Homo sapiens*
Gα	1	1	1	1	4	1	23
Gβ	1	1	1	1	4	1	5
Gγ	2	3	3	1	10	4	12
RGS	1	1	-	1	1	-	>30
RACK1	1	3	1	-	1	-	1

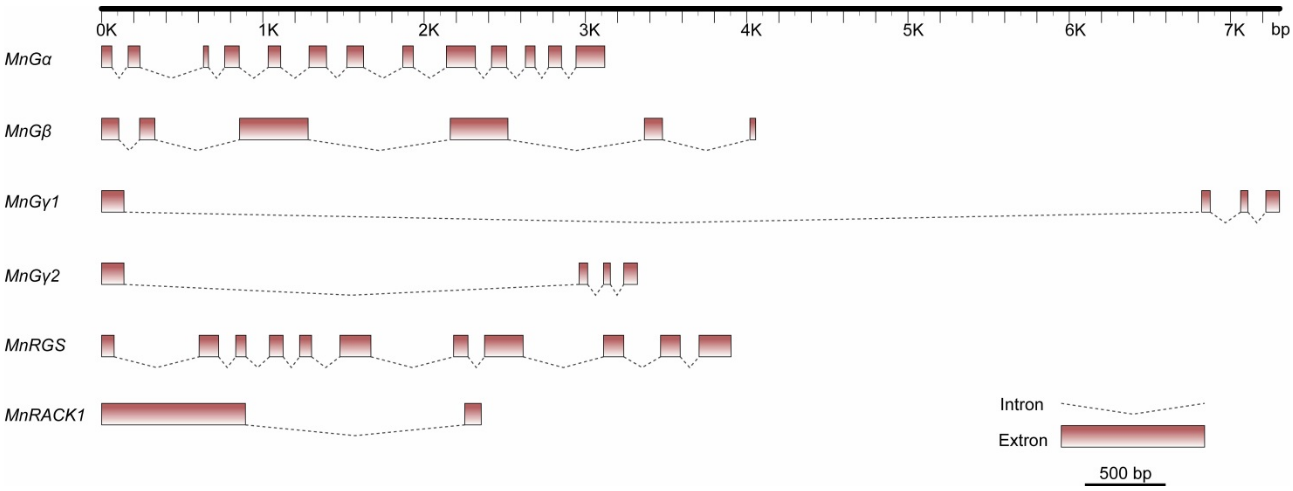

Fig. 1 Suppl. Gene structure and predicted functional domains of G-protein signalling genes in *Morus notabilis*. Gene structures were obtained by aligning the cloned cDNA sequences with the *M. notabilis* genome data and displayed by Fancy Gene (http://bio.ieo.eu/fancygene/).

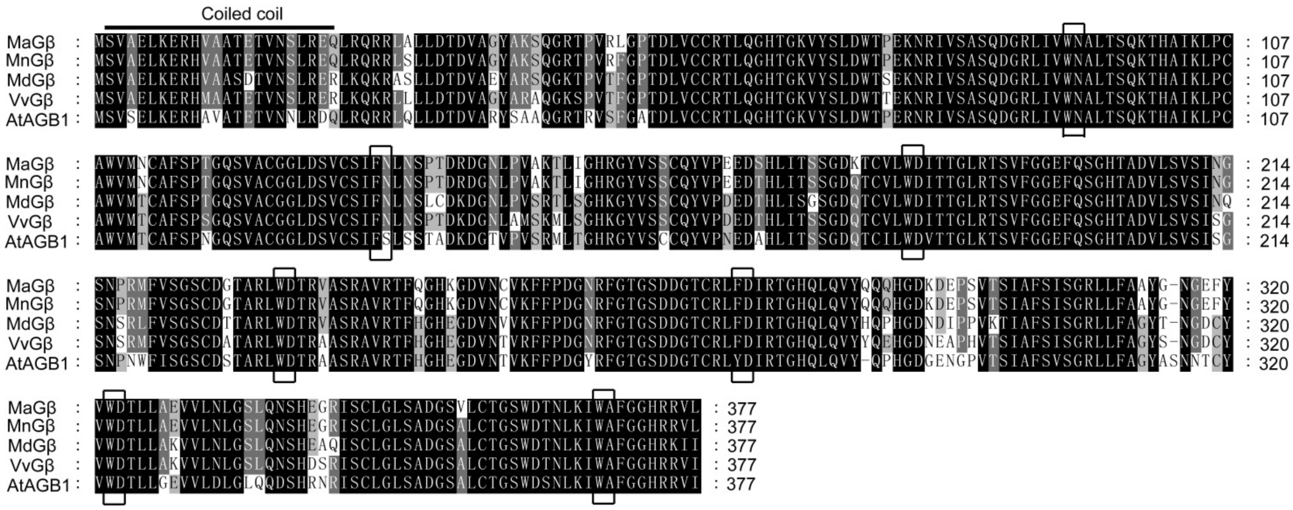

Fig. 2 Suppl. Amino acid sequence alignments of *Morus* Gβ protein sequences with other Gβ proteins from other plant species (Ma - *Morus atropurpurea*, Mn - *Morus notabilis*, Md - *Malus domestica*, Vv - *Vitis vinifera*, At - *Arabidopsis thaliana*). The tryptophan-aspartic acid (WD) repeats are marked within boxes.

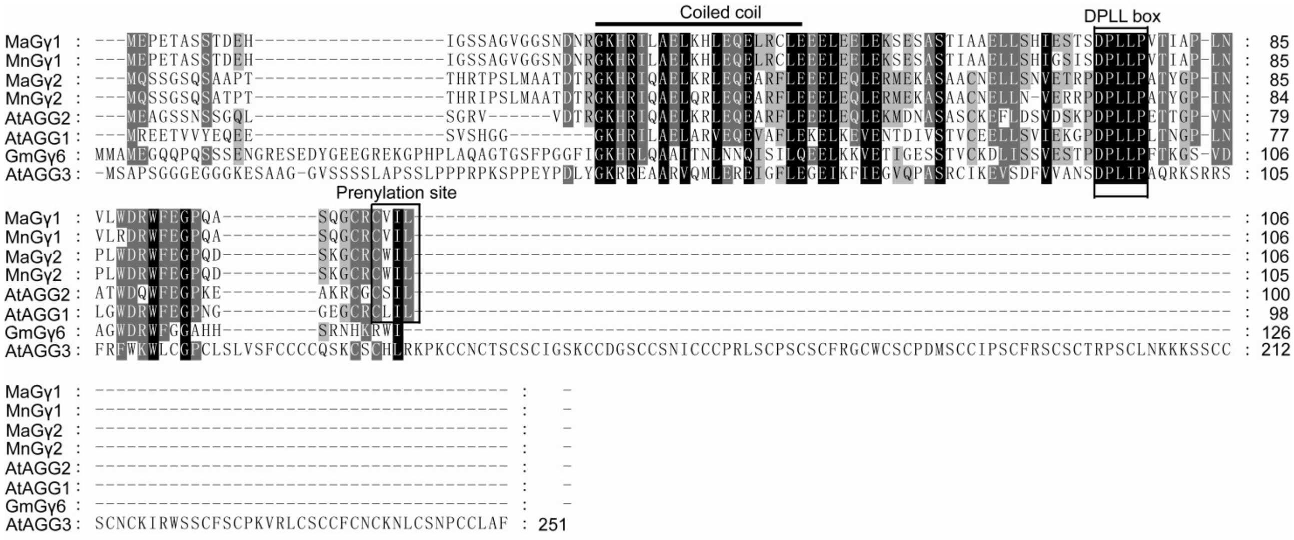

Fig. 3 Suppl. Amino acid sequence alignments of *Morus* Gγ protein sequences with other Gγ protein from other plant species (Ma - *Morus atropurpurea*, Mn - *Morus notabilis*, At - *Arabidopsis thaliana*, Gm - *Glycine max*).

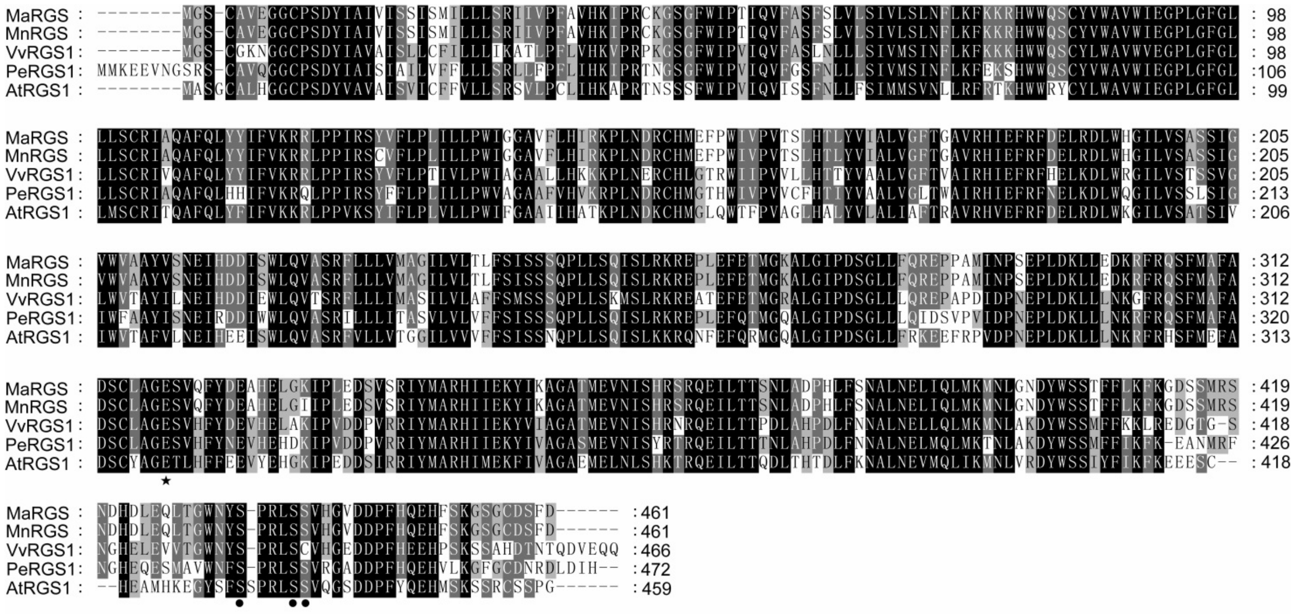

Fig. 4 Suppl. Amino acid sequence alignments of the *Morus* RGS protein sequence with RGS proteins from other plant species (Ma - *Morus atropurpurea*, Mn - *Morus notabilis*, Vv - *Vitis vinifera*, Pe - *Populus euphratica*, At - *Arabidopsis thaliana*) (● indicates the sites required for the endocytosis of RGS, ★ indicates the site for G-proteins activation).

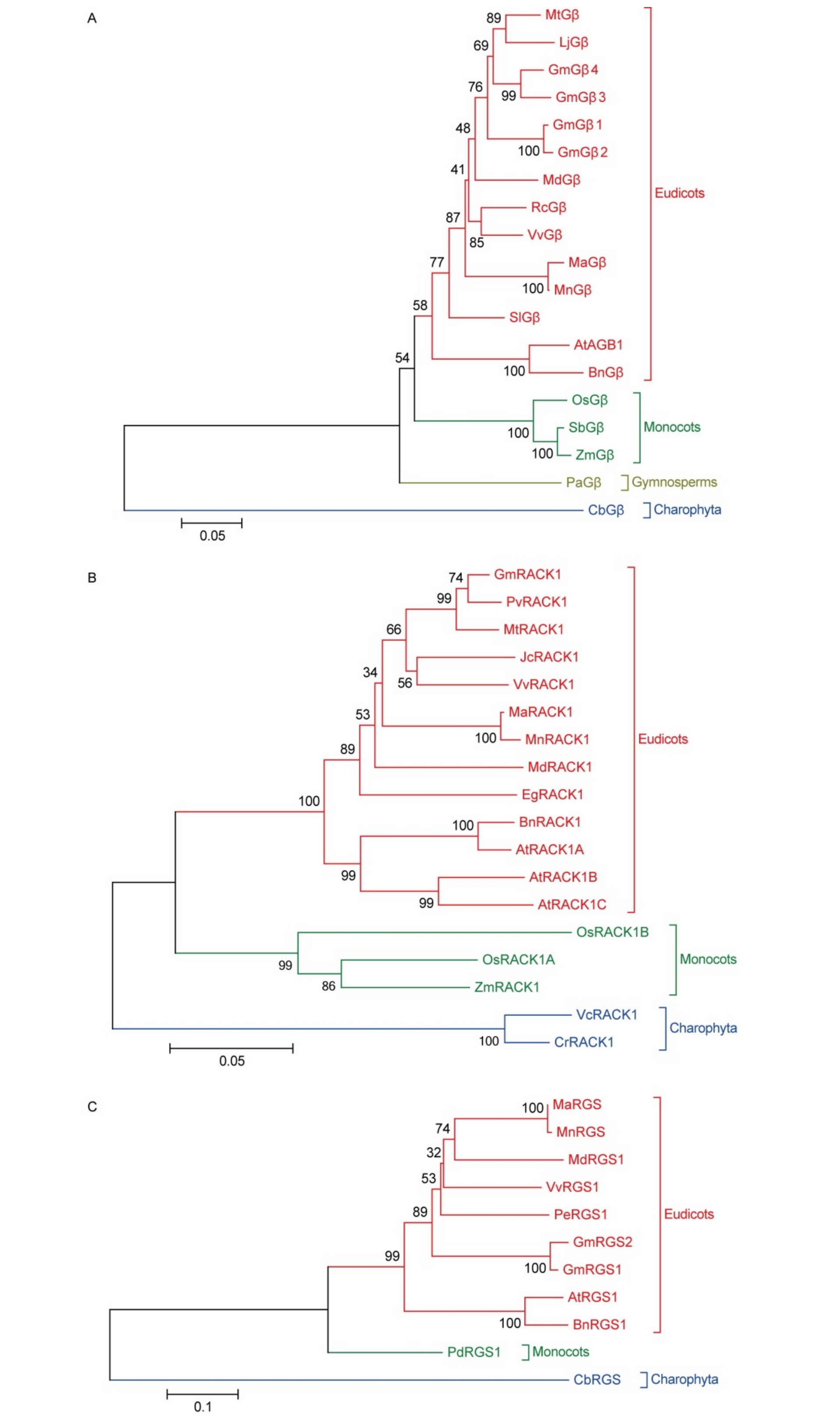

Fig. 5 Suppl. Phylogenetic analysis of G-protein β subunit (Gβ) (*A*), RACK1 (*B*), and RGS (*C*) isolated from *Morus* and their corresponding G-protein signalling proteins from other plant species (Ma - *Morus atropurpurea*, Mn - *Morus notabilis*, Vv - *Vitis vinifera*, Pe - *Populus euphratica*, Gm - *Glycine max*, At - *Arabidopsis thaliana*, Bn - *Brassica napus*, Cb - *Chara braunii*). The amino acid sequences were analyzed with *ClustalW* (http://www.clustal.org) and the phylogenetic tree was constructed with *MEGA 5.0* using a bootstrap test of phylogeny with a minimum evolution test and default parameters.

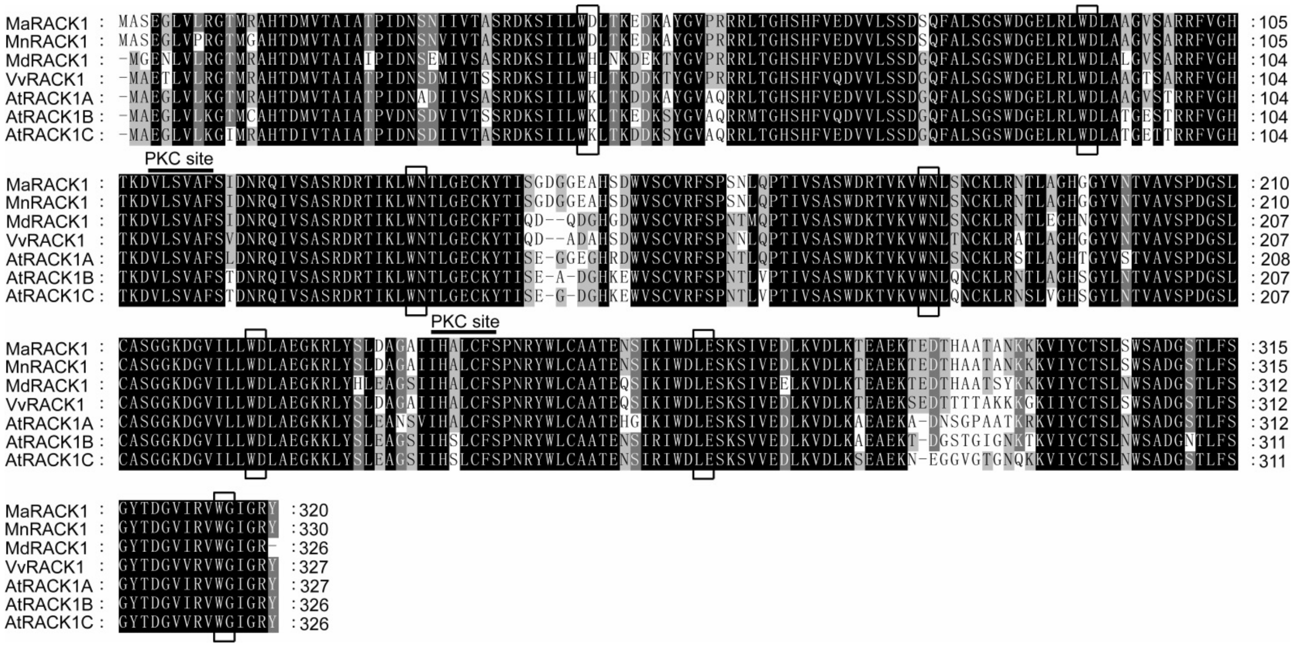

Fig. 6 Suppl. Amino acid sequence alignments of the *Morus* receptor for activated C kinase 1 (RACK1) protein with other RACK1 proteins from other plant species (Ma - *Morus atropurpurea*, Mn - *Morus notabilis*, Vv - *Vitis vinifera*, Md - *Malus domestica*, At - *Arabidopsis thaliana*). The WD repeats are marked within boxes.

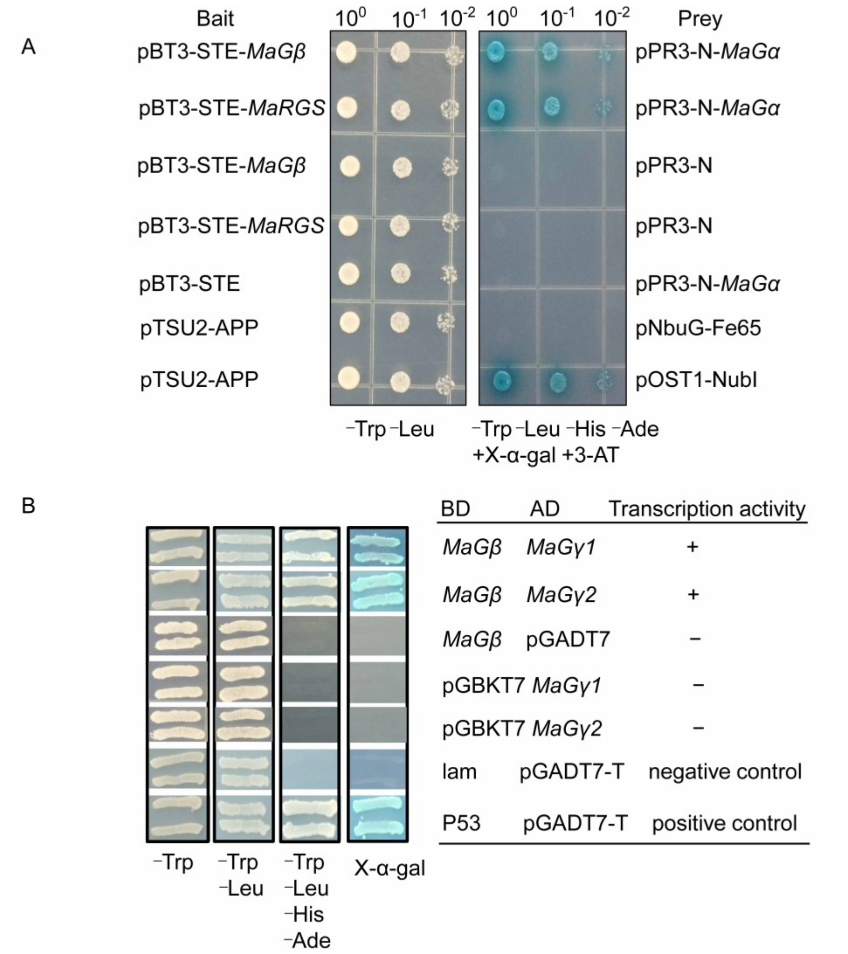

Fig. 7 Suppl. Physical interactions among *Morus* G-protein signalling components. *A* - Interaction between MaGα and MaGβ as well as between MaGα and MaRGS were tested in a split-ubiquitin-based system (RGS - regulator of G-protein signaling protein). Two biological replicates were performed with similar results. *B* - An interaction between MaGβ and MaGγ subunits, as determined using a yeast two-hybrid (Y2H) interaction screen. Two biological replicates were performed with similar results.

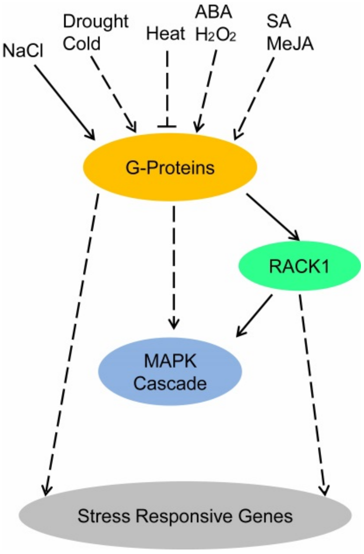

Fig. 8 Suppl. A possible model for *Morus* G-protein signalling pathway in response to abiotic stresses and signal molecules. *Solid lines* indicate signalling pathways that have been published, and *dashed lines* indicate possible new signalling pathways. Positive effects are indicated by *arrows*, and a *bar* indicate repression (ABA - abscisic acid, MeJa - methyljasmonate, SA - salicylic acid, RACK1 - receptor for activated C kinase 1, MAPK - mitogen-activated protein kinase).