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## RESEARCH ARTICLE



# A computational analysis revealed BES1 transcription factor and $\beta$ -amylase as crosstalk elements in Upland cotton species (*Gossypium* sp.)

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#### Abstract

Cotton is a resilient and multipurpose crop, meeting major of the world's textile needs while also yielding byproducts like edible oil and animal feed. Starch plays a crucial role in cotton fabric production. It enhances fabric strength by forming a protective film around cotton fibers, making them more resistant to wear and tear. BES1 (brassinosteroid insensitive 1) is a key regulator in brassinosteroid signaling. It controls thousands of target genes involved in development processes. Interestingly, two  $\beta$ -amylase proteins (BAM7 and BAM8) are part of the BES1 family, despite their primary function as  $\beta$ -amylases.  $\beta$ -Amylase (BAM) and BES1 are two gene families with functional and regulatory roles in controlling shoot growth and development by mediating brassinosteroid effects. They share similar domains and participate in various biological processes, tolerance and responses to stresses like salt and drought. In a computational analysis comparing *Arabidopsis* and *Gossypium* species, BAM and BES1 were characterized. BES1 genes were grouped into four clusters based on the comparison of the two species. Two clusters corresponded to BAM7 and BAM8, while the other two clusters were associated with BES1. The conserved nucleotide domain sequence is GCTGGATGG. Short tandem repeats include TG and TTG, which can serve as molecular markers. BES1 is specifically linked to cellulose and fiber production and holds promise as a candidate for plant selection and breeding in *Gosspipum* (cotton).

Keywords: BAM; bioinformatics; BZR1; crosstalk; gene expression; transcription factor.

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#### 1. Introduction

Brassinosteroids (BRs) are a group of polyhydroxylated steroidal phytohormones that play crucial roles in plant development, growth, and productivity. These hormones regulate processes such as cell division, elongation, and differentiation throughout the entire plant life cycle (Manghwar et al., 2022). Interactions and crosstalk occur between BRs and other phytohormones, impacting signaling, transcription, regulation, and function (Yang et al., 2023). Notably, BRs and gibberellins exhibit crosstalk by employing downstream genes like DELLA for gibberellins and BES1 (BRI1-EMS-SUPPRESSOR 1) for BRs (Yasir & Wasaya, 2021). BES1 gain-offunction plants demonstrate involvement in jasmonic acid (JA)-related defense responses, particularly against necrotrophic pathogens. This BR-JA crosstalk has also been observed in tomatoes as a defense mechanism against insect herbivory (Campos et al., 2009).

BES1, also known as BZR1/BES1, belongs to a family of transcription factors (TFs) that play a pivotal role in mediating brassinosteroid (BR) responses in various plant biological processes (**Shi et al., 2022**). BZR1/BES1 undergoes a two-way process involving phosphorylation and dephosphorylation to regulate its activity. When phosphorylated by BIN1, BZR1 becomes a target for the 20S proteasome, leading to its degradation. Conversely, dephosphorylation of BES1 results in its nuclear accumulation, where it exerts its transcriptional functions. BES1 participates in the BES1/TPL/HDA19 repressor complex, which modulates the influence of abscisic acid (ABA) on seed germination and counteracts the inhibitory effect of BR on ABA (Ryu et al., 2014). Through BR signaling, BES1 mediates the CrRLK (receptor-like kinase protein) family, enhancing cell elongation. BES1 cooperates with other TFs to fine-tune BRregulated mechanisms. A group of WRKY TFs (including WRKY46, WRKY54, and WRKY70) interacts with BES1 in response to drought stress, growth, and development (Shi et al., 2022). Responsive to Desiccation 26 (RD26), an NAC family TF, interacts with BES1 and antagonizes BR activity, resulting in altered drought responses in plants. While BES1 is implicated in various defense and growth mechanisms, its precise roles and functions remain partially undisclosed. In summary, BES1/BZR1 stands at the crossroads of BR signaling, orchestrating intricate processes in plant development and adaptation BES1 is found in the plant kingdom with some copies in each species (https://planttfdb.gaolab.org/) but not necessarily well-distributed among the species. Among plants containing BES1, Gossypium raimondii has the highest abundance, followed by Salix purpurea and Populus trichocarpa, with 42, 38, and 32 BES1 members, respectively. However, non-fibrous plants, such as algae, do not contain BES1. Wu et al. (2016) showed that there are 14 orthologous gene pairs shared by Chinese cabbage and Arabidopsis. It functions in connecting plant signaling to BRs and controlling gene expression by a transcriptional network by which plants respond and tolerate stresses and direct growth and development (Nolan et al., 2017). Its binding motif is a G box (CCACGTGG) that interacts with BIM1 a basic helix-loop-helix protein to synergistically bind to E box (CANNTG) sequences. These sequences are seen in many BR-induced promoters (Yin et al., 2005).

β-amylase proteins (BAM) are the enzymes responsible to starch breakdown. They belong to plant kingdom and usually are found in the nucleus rather than the chloroplast. They catalyse the hydrolysis reaction that results in maltose. They have BRASSINAZOLE RESISTANT1 (BZR1)-type/BES1 DNA binding motifs. There are two β-amylase known as BAM7 and BAM8 that resemble BZR1/BES1. They possess a G box that bind a cisregulatory element in BES1/BZR1 by which they activate gene expression. Mutants of BAM7 and BAM8 (bam7 and bam8) cause leaf growth and development changes (**Reinhold et al., 2011**). However, the other BAM members also show similarity to BZR1/BES1.

BES1-BAM complex plays a role in plant response to stress and growth and development. The double-

faced complex regulates many genes that respond to BR through crosstalk signaling. There may exist metabolic signals via G box by binding a ligand in their BAM domain or regulatory cascades by their BES1 motif. However, it seems that the function of BES1-BAM can be independent and depending on plant requirement, the pertinent element starts its act. Some studies are available explaining βamylase alteration in response to plant stresses (Kaplan & Guy, 2004; Todaka et al., 2000). For example, water stress enhances activity of  $\beta$ -amylase and this can activate sugar signaling or regulation by interactions with other TFs and phytohormones starting from BES1 as a master key. The altered activity of  $\beta$ -amylase occurs in different plant life steps and organs from seeds and germination to fruit sweetening. On the other hand, similar changes in BES1 have been reported as a plant response to stresses to enhance plant tolerance (Ahammed et al., 2020; Cao et al., 2024; Gruszka et al., 2020).

Cotton plant belonging to *Gossypium* genus is one of the most important providers of cellulosic fiber and of great economic importance. Cellulosic fiber is a differentiated epidermal cell of seeds produced by cotton plant (John & Crow, 1992). This importance makes it mandatory to advance the application of biotechnological tools for cotton improvement (Rathore et al., 2015). The genome of three species including *G. raimondii*, (Wang et al., 2012), *G. hirsutum* (Li et al., 2015) and *G. arboreum* (Li et al., 2014) have been published. However, the genes those are involved in fiber and cellulose production are under more consideration.

In a previous study, researchers compared TFs and molecular markers among different Gossypium species. They found asymmetric contributions among TFs in these species (Jazayeri et al., 2020). Specifically, they assigned 11, 14, and 16 BES/BZR genes to G. arboreum, G. hirsutum, and G. raimondii, respectively. Interestingly, when comparing protein clusters, a cluster of BES1/BZR TFs revealed two copies for G. arboreum, while G. hirsutum and G. raimondii had only one copy each. Notably, G. hirsutum, a tetraploid resulting from the hybridization of diploid species G. arboreum and G. raimondii, did not double the copy numbers of BES1 family genes. These findings suggest that evolutionary events may balance the gene numbers within the BES1 family due to its multifunctionality. This article is aimed to briefly study the BES1/BZR1 family in comparatively three species of Gossypium and three species of Arabidopsis. The similarities, short sequence repeats and domains of the BES1 TF genes of these six species were disclosed.

## 2. Methodology

**Figure 1** shows the flowchart that was used to analyze the proteins sequence data. The protein sequences of BES1 TFs were downloaded from PlantTFDB (https://planttfdb.gao-lab.org/) website for three species of Arabidopsis including *A. halleri*, A. *lyrata* and *A. thaliana*, and three species of *Gossypium* including *G. arboreum*, *G. hirsutum* and *G. raimondii*. The number of BES1 genes was 8 for *A. halleri*, 9 for *A. lyrata*, 14 for *A. thaliana*, 12 for *G. arboreum*, 24 for *G. hirsutum* and 42 for *G. raimondii*.



Figure 1. The protein sequence analysis workflow for analyzing BES1/BAM genes involved several steps. First, data were retrieved from public databases. Next, the retrieved sequences were subjected to protein clustering using the OrthoVenn tool. The resulting clusters were then used for further comparative analyses. To visualize the clustered BES1/BAM protein sequences, the ETETOOLKIT tool was employed. Additionally, the MEME tool revealed motifs within the BES1/BAM sequences. Finally, the iTOL tool was used to create a BES1/BAM tree, and sequence alignment and visualization of common sequence domains and repeats were performed using MEGA. The used tools in the study are written in parentheses below corresponding analysis. The flowchart was created by ERASER tool (https://app.eraser.io/).

The OrthoVenn program (Wang et al., 2015) was used to cluster the protein sequences and perform comparative analyses. It is a web-based platform that compares protein sequence datasets among different species, analyzes their annotation and generates Venn diagram and GO enrichment for the clustered proteins (http://www.bioinfogenome.net/OrthoVenn/).

The protein clusters generated by OrthoVenn were visualized by TreeView of Environment for Tree Exploration (ETE) toolkit (Huerta-Cepas et al., 2016) (http://etetoolkit.org/treeview/). It was also used to construct the gene tree for all reported plants in PlantTFDB based on BES1 TF family. The MEME Suite (Multiple Em for Motif Elicitation) was used for finding motifs for each cluster (Bailey & Elkan, 1994). The Universal Protein Resource (UniProt, https://www.uniprot.org/) was employed to reveal pertinent function of each protein cluster. UniProt serves as a comprehensive resource for protein seguence and annotation data. The UniProt databases include the UniProt Knowledgebase (UniProtKB), UniProt Reference Clusters (UniRef), and UniProt Archive (UniParc). The UniProt consortium, along with host institutions EMBL-EBI, SIB, and PIR, is dedicated to the long-term preservation of these valuable databases. The Interactive Tree Of Life (iTOL, https://itol.embl.de/) (Letunic & Bork, **2016**) was used to construct the BES1 phylogenetic tree of six studied species. iTOL is a web-based tool that allows users to display, manipulate, and annotate phylogenetic trees. The Molecular Evolutionary Genetics Analysis (MEGA7.0.21) (Kumar et al., 2016) was employed to align, find and visualize the common sequence domain and repeats among all 109 BES1 input sequences. MEGA is an integrated tool for conducting automatic and manual sequence alignment, inferring phylogenetic trees, mining web-based databases.

## 3. Results and discussion

The phylogenetic tree of BES1 comprises 71 leaves, representing the evolutionary relationships among these genes. Notably, there are two genus-specific branches that exclusively belong to *Gossypium* species (blue oval in **Figure 2**). Additionally, two other genera exhibit synteny pairs in different branches. As anticipated, the genes of *Arabidopsis* form clusters on branches where no *Gossypium* gene is present. However, intriguingly, certain branches within the *Gossypium* lineage feature sole *Gossypium* genes, distinct from *Arabidopsis* (red rectangles in **Figure 2**). These findings suggest that *Gossypium's* BES1 genes

evolved independently over time, separate from their Arabidopsis counterparts. *Gossypium* species possess a greater number of BES1 genes compared to *Arabidopsis* (**Table 1**). This observation aligns with the fact that BES1 is intricately involved in processes related to cellulose production and is associated with activating brassinosteroids (BRs)—key regulators of cell elongation and size. Notably, BES1 interacts with CESA (cellulose synthase) to modulate cellulose synthesis (**Xie et al., 2011**). The abundance of BES1 members in *Gossypium* underscores their potential impact on advancing cellulose production and downstream fiber-related processes.

In the context of protein clustering, here are the key findings: Proteins from each species tend to cluster together. However, there are singletons (individual proteins not grouped with others) for all species except A. thaliana (Table 1). The proteins form seven clusters in total: four intergenic clusters and three interspecies clusters. Among these, two clusters are specific to the pair of A. thaliana and A. lyrata, while one cluster is unique to G. hirsutum and G. raimondii. Interestingly, no clustered species-specific BES1 was identified. A notable observation is that A. thaliana shares all its BES1 with other studied species. In contrast, G. hirsutum possesses the most speciesspecific BES1. This suggests the potential for G. hirsutum to contribute more significantly to fiber production. By comparing different species of Arabidopsis and Gossypium, researchers can uncover valuable insights into these proteins' roles and functions (Jazayeri et al., 2019; Jazayeri et al., 2018).

#### Table 1

The proteins and clusters formed based on proteins. Singletons are the genes, which did not form any cluster within the species and are species-specific

Species	Proteins	Clusters	Singletons
A. halleri	8	6	1
A. lyrata	9	8	1
A. thaliana	14	8	0
G. arboreum	12	9	2
G. hirsutum	24	10	6
G. raimondii	42	10	5

All coding sequences share a common protein domain with the following sequence motif [S/G]WM\*C[\*/S][\*/\*][\*/R]W[N/I]Y (**Figure 3**).

First position: Most genes have a serine (S) at this position, except for the Cotton\_A\_07763\_BGI-A2\_v1.0 gene which has a glycine (G).

Last position: Most genes have an asparagine (N) at this position, while *A. lyrata* 485421 and *G. hirs*utum Gh\_D06G2134 genes have an isoleucine (I).

Downstream repeats: Following the conserved domain, there is a recurring pattern rich in arginine (R) and lysine (K) residues, although not perfectly consistent. Studies on  $\alpha$ -amylase suggest that the ratio of lysine to arginine can influence protein stability in cold-adapted environments in

Pseudoalteromonas haloplanktis. The conserved nucleotide sequence domain in accordance with the conserved protein domain is as follow while the red nucleotides are conserved in

all 109 genes. AATGA[A/T][G/C]TT[A/C]TT[G/A][C/A][T/G]GCT[C/G]TT[G/T][C/G ]T[A/T/C/G][G/T/C/A][A/T][G/A]A[A/G]GCTGGATGG[G/T/A][T/C] TGTT[C/G][T/A][T/A][C/G][C/A][T/A]GATGGA[A/T]CTA[C/T]TT[T/ A]T. However, a box as GCTGGATGG and short sequence repeats of TG in coding sequence those might bind to downstream binding sites for BES1 family was retrieved. These repeats can be used as molecular markers for screening *Gossypium* species based on BES1 to evaluate their fiber size and production. The TG repeats are shown in green. **Figure 4** shows the alignment of all 109 genes around the conserved region demonstrating TG repeats in vellow.

AATGA[A/T][G/C]TT[A/C]TT[G/A][C/A][G/T]GCT[G/C]TT[G/T][G/C] ]T[A/T/C/G][G/T/C/A][A/T][G/A]A[A/G]GCTGGATGG[G/T/A][T/C] TGTT[G/C][T/A][T/A][C/G][C/A][T/A]GATGGA[A/T]CTA[C/T]TT[T/ A]T.



Figure 2. BES1 family tree of protein sequences for three Arabidopsis and three Gossypium. The blue circles show genus-specific gene groups of *Gossypium*, and the red rectangles show the genes of Gossypium that have separated from other genes as the unique gene of the groups they belong to.

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Species/Abbry A Gro					*		* * * *
1. 346398	ARSSWN ??WRRR? ? * ? ?	I * T S * R K G K N *	A*RKT*KSYYC*N	ISWRT*KTWKL*	s · s · s · <sub>Y</sub> · ·	CYCCSC*R	SWMGCSS*WNYFSC*V
2. 356484	WRRRR IFFWRRRRRK G??	ERKRKGKGKN*	A * R K T * K S Y Y F * N	IACWT*TIWKFS	SSC*S* <mark>YE</mark> *	C	SWMVC*C*WNYL*??1
3. 472158	SYFYFCCCCC? - ? YGY * ? ?	KAFLERKRK**	KKRKKKKSCCC * D	LYWTSSRKL	SS <sup>A</sup> L	SS*GSLF*	SWMGC*RRWNYL*K?-
4. 4/664/	REPRINCE2	NATLERKRK**	EKRKKKKSCCC*D	EYWT * ISRKI *	A S A A L A A A A	SSAGSLCA	SWMDCS BWLYL K 2
6. 492971	? <b>D</b> F W N *	NSYLEGKRK**	EKRKKKKSYCC*D	FCWT*NSWKF*	A S * A L * * * *	SS*GSL**	SWMDC*R*WNYL*K?-
7.856241	A R S S W N ? ? W R R R ? ? * ? ?	I*TS*RKGKN*	A * R K T * K S Y Y C * N	SW	<mark>S</mark> * <mark>Y</mark> * *	CYCCSC*R	SWMGCSS*WN <mark>Y</mark> FSC*V
8.895772	DDDDD2252	NAYLERKRK**	EKRKKKKSYCC*D	FYWT*NVWKL*	T S * A L * * * *	SS*GSL**	SWMDC*T*WNYL*KG?
10. Araha.12428s0016.1.p	ARSSWN ??WRRR? ?*??	I * T S * R K G K N *	A*RKT*KSYYC*N	SWRT*KTWKL*	S · S · S · Y · ·	CYCCSC*R	SWMGCSS*WNYFSC*V
11. Araha.1486s0003.1.p	SYFYFCCCC? ? YGY * ? ?	K A F L E R K R K * *	KKRKKKKSCCC*D	<mark>                                     </mark>	S	S	SWMGC* <mark>RRWNYL</mark> *K? -
12. Araha.15647s0004.1.p	RRRIYCC? ?W??	NAYLEGKRK**	EKRKKKKSYCC	FYWT ISRKL		SS'GSLS'	SWMGCS RWNYL N? -
14. Araha. 17172s0006.1.p	WRRRR IFFWRRRR ??G??	ERKRKGKGKN*	A * R KT * KSYYF * N	ACWT*TIWKFS	SSC · S · YE ·	CYCCSC*R	SWMVC*C*WNYL*??I
15. Araha.17406s0004.1.p	RRRRR??F??W??	NSYLEGKRK**	EERKKKKSYYC*D	LFWT*SSRKL*	A S * A L * * * *	SS*GSLS*	SWMDC*R*WNYL*K?-
16. Araha.4300s0012.1.p	? <b>D</b> F W N *	NAYLERKRK**	EKRKKKKSYCC*D	FYWT * NVWKL *	T S * A L * * * *	SS*GSL**	SWMDC*T*WNYL*KG?
17. Araha.49879s0016.1.p	SYFYFCCCCCCCCCCCCCCCC*??	KAFLERKRK**	KKRKKKKSCCC*D		SSAL · · · ·	SS*GSLC*	SWMGC*RRWNYL*K?-
19. AT1G19350.3	SYFYFCCCCCC??YGY*??	KAFLERKRK**	KKRKKKKSCCC*D	LYWT*SSRKL*	SS*AL****	SS*GSLF*	SWMGC*RRWNYL*K?-
20. AT1G19350.4	<mark>S</mark> YFYFCCCCCC?? <mark>YG</mark> Y*??	K A F L <mark>E</mark> R K R K * *	KKRKKKK <mark>S</mark> CCC*D	<mark>L Y</mark> W T * S S <mark>R K</mark> L *	S	S S * G S L F *	SWMGC*RRWN <mark>YL</mark> *K? -
21. AT1G19350.5	SYFYFCCCCCC??YGY*??	KAFLERKRK**	KKRKKKKSCCC*D		S S * A L * * * *	SS*GSLF*	SWMGC*RRWNYL*K?-
23. AT1G75080.1	SYFYFCCCCCCCCCCCC * ? ?	KAFLERKRK	KKRKKKKSCCC D	LYWT'SSRRL'	SS'AL	SSIGSLCI	SWMGC*RRWNYL*K?-
24. AT1G75080.2	SYFYFCCCCCCCCCCC	K A F L E R K R K * *	KKRKKKK <mark>S</mark> CCC*D		S	S	SWMGC*RRWN <mark>YL</mark> *K?-
25. AT1G78700.1	? <b>D</b> F WN *	NAYLERKRK	EKRKKKKSYCC	FYWT * NVWKL *	TS <sup>*</sup> AL <sup>****</sup>	SS*GSL**	SWMDC*T*WNYL*KG?
26. A12G45880.1 27. AT3G50750.1	R 2 2 Y C C 2 2 W 2 2		FERKKKKSYCC*D	FYWT*ISRKI*	A S * A L * * * *	SS*GSIS*	SWMGCSS*WNYFSF*V SWMDCS*RWNYI*K?-
28. AT4G18890.1	? <mark>D</mark> F W N *	N S Y L E G K R K * *	E K R K K K K <mark>S y</mark> C C * D	F C W T * N S W K F *	A <mark>S</mark> * <mark>A L</mark> * * * *	S S * G S L * *	SWMDC*R*WNYL*K?-
29. AT4G36780.1	RRRRRIF? ? W??	NSYLEGKRK**	EERKKKKSYYC*D	LFWT*SSRKL*	A S * A L * * * *	SS*GSLS*	SWMDC*R*WNYL*K?-
30. A15G45300.1		ERKRKGKGKN*	A RKI KSYYF N	ACWITTUWKFS	SSC'S'YE'		
32. Cotton_A 03434_BGI-A2			AKRKKEKSCCS	FCWT GTWKI	SS*ES*F**	SS*GCI*R	SWMAC*S*WNYL*??!
33. Cotton_A_05312_BGI-A2_	? <b>D</b> FWN*	NAYLEGKRK**	EKRKKKKSYCC*D	FCWT * N VWKL *	A S * A L * * * *	SS*GSL**	SWMDC*RRWNYL*E?
34. Cotton_A_07763_BGI-A2_	? G R Y * C * Y * D * G R	SYC*RRKRK**	KKRKGKKSYCC*D	L*WT*SSRKL*	SS*AL***	SS*GSLC*	GWMGC*R*WNYL*K*G
35. Cotton_A_13575_BGI-A2_ 36. Cotton_A_13729_BGI-A2_	SYFYSCSC2	KAFLERKRK**	KRKKKKSYCC*	LYWTSSRKI	SSAL ****	SS*GSLC*	SWMCC*T*WNYL*??F
37. Cotton_A_14161_BGI-A2_		TSYLEGKRK**	EKRKKKKSYCC	FCWTINVWKLI	A S * A L * * * *	SSYGSLYY	SWMDC*T*WNYL*K?-
38. Cotton_A_18778_BGI-A2_	S Y F Y A C C ? ? ? * ? ?	KAFS*GKRK**	KKRKKKK <mark>Sy</mark> cc * D	LYWT*SSWKL*	S	SS*GSLC*	SWMGC*RRWNYL*K?-
39. Cotton_A_21459_BGI-A2_	SRTSFY??WRR??*??		A * R K T * K S S Y C * N	ECWT * KAWKL *	S*S*S*Y**	CYCCSC*R	SWMCCSS*WNYFSF*I
41. Cotton_A_34134_BGI-A2_	SRTSF*SRRRR? ? * ? ?	I * T S * R K G K N *	A*RKT*KSYYC*N	SCWT*KTWKL*	S*S*S*Y**	CYCCSC*R	SWMGCSS*WNYFSF*I
42. Cotton_A_34170_BGI-A2_	? <b>D</b> F W N *	NAYLEGKRK**	EKRKKKK <mark>S</mark> YCC*D	FCWT・NVWKL・	A <mark>S</mark> * A L * * * *	5 5 * G 5 L * *	SWMDC*RRWNYL*K?-
43. Cotton_A_35761_BGI-A2_	SYFYSCS? ???	KAFLERKRK**	KKRKKKKSYCC*D		SS*AL****	SS*GSLF*	SWMGC*R*WNYL*K?-
45. Gh A03G1024	SYFYSCS? ? ? ?	KAFLERKRK**	KKRKKKKSYCC*D	LYWT'SSRKL'	SS*AL****	SS*GSLF*	SWMGC*R*WNYL*K?-
46. Gh_A04G0814	TLGCS*NY*GR??KSY*I*	I SY * R * K T * E *	A K R K K E K S C C S * D	F C W T * G T W K L * I	S S * <mark>E</mark> S * F * *	SS*GCL*R	SWMAC*S*WNYL*E? -
47. Gh_A05G1683	SYFYSCSC? ? ? * ? ?	KAFLERKRK**	KKRKKKKSYCC * D	LYWT * SSRKL *	SS <sup>·</sup> AL <sup>····</sup>	SS*GSLC*	SWMGC*R*WNYL*K?-
48. Gn_A05G2217 49. Gh_A06G1990	STFTESCY 2DEWN*	NAYLEGKRK**	EKRKKKKSYCC*D		AS ALASA	SSAGSLCA	SWMDC*RRWNYL*R?-
50. Gh_A08G1175	SRTSF*SIWRRR? ? * ? ?	I * T S * R K G K N *	A * R K T * K S Y Y C * N	SCWT*KTWKL*	S * S * S * Y * *	C	SWMGCSS*WNYFSF*I
51. Gh_A10G0312	? <b>D</b> F W N *	NAYLEGKRK**	EKRKKKKSYCC*D	FCWT*NVWKL*	ASTAL	SS*GSL**	SWMDC*RRWNYL*K? -
52. Gh_A11G0794	SRISFY ? ? WRR ? ? * ? ?		A TRKITKSSYCTN	ECWT * NYWKL *	<b>S · S · S · Y</b> · ·	SS*GSL * *	SWMGCSS*WNYFSF*I SWMDC*T*WNYI*K2
54. Gh_D01G0390	? <b>D</b> F W N *	TSYLEGKRK					
			ENRINANDICC U	FGWISNVWKLS	ASTAL	SS*GSL**	SWMDC*T*WNYL*K?-
55. Gh_D02G0939	S Y F Y S C S ? ? ? ? ?	K A F L E R K R K * *	KKRKKKKSYCC * D		A S * A L * * * * S S * A L * * * *	S S * G S L * * S S * G S <mark>L F</mark> *	SWMDC*T*WNYL*K?- SWMGC*R*WNYL*K?-
55. Gh_D02G0939 56. Gh_D04G1312	SYFYSCS? ? ?? TLGCS*NY*GR??KSY*I			FCWT SSRKL*	A S * A L * * * * S S * A L * * * * S S * E S * F * *	S S * G S L * * S S * G S L F * S S * G C L * R	SWMDC*T*WNYL*K?- SWMGC*R*WNYL*K?- SWMAC*S*WNYL*K?-
55. Gh_D02G0939 56. Gh_D04G1312 57. Gh_D05G1874 58. Gh_D05G2477	S         Y         F         Y         S         C         S         ?         -         -         -         ? <td?< td="">         ?         ?         ?</td?<>	K A F L E R K R K * * I S Y * R * K T * E * K A F L E R K R K * * K A F S * G K R K * *	KKRKKKKSYCC*D KKRKKKKSYCC*D KKRKKKKSYCC*D	F C W T * S S R K L * F C W T * S S R K L * L Y W T * S S R K L * L Y W T * S S R K L *	AS*AL***** SS*AL**** SS*ES*F*** SS*AL***** SS*AL	S S * G S L * * S S * G S L F * S S * G C L * R S S * G S L C * S S * G S L C *	SWMDC*T*WNYL*K?- SWMCC*R*WNYL*K?- SWMCC*R*WNYL*K?- SWMCC*R*WNYL*K?- SWMCC*R*WNYL*K?-
55. Gh_D02G0939 56. Gh_D04G1312 57. Gh_D05G1874 58. Gh_D05G2477 59. Gh_D06G2134	S         Y         F         Y         C         S         ?	K A F L E R K R K * * I S Y * R * K T * E * K A F L E R K R K * * K A F S * G K R K * * N A Y L E G K R K *	K K K K K K K K K K K K K K K K K K K	F C W T * S S R K L * F C W T * G T W K L * L Y W T * S S R K L * L Y W T * S S W K L * F C W T * N Y W K L *	A S * A L * * * * * * * * * * * * * * * * *	S     S <td>SWMDC*ERWNYL*K?- SWMCC*R*WNYL*K?- SWMCC*R*WNYL*K?- SWMCC*R*WNYL*K?- SWMCC*RRWNYL*K?- SWMCC*RRWNYL*K?- SWMDC*RRWNYL*K?-</td>	SWMDC*ERWNYL*K?- SWMCC*R*WNYL*K?- SWMCC*R*WNYL*K?- SWMCC*R*WNYL*K?- SWMCC*RRWNYL*K?- SWMCC*RRWNYL*K?- SWMDC*RRWNYL*K?-
55. Gh_D02G0939 56. Gh_D04G1312 57. Gh_D05G1874 58. Gh_D05G2477 59. Gh_D05G2134 60. Gh_D08G1459	S Y F Y S C S ?       ?	K A F L E R K R K * * * I S Y * R * K T * E * K A F L E R K R K * * K A F S * S K R K * * N A Y L E G K R K * * I * T S * R K G K N *	K K K K K K K K K K K K K K K K K K K	I V V T S S K L L Y V T S S K L K V V K L S C V T K V K L S C V T K V K L S C V T K V K L	A S • A L • • • • • • • • • • • • • • • • •	S     S     S     S     L     *       S     S     *     G     S     L     *       S     S     *     G     C     L     *       S     S     *     G     S     L     *       S     S     *     G     S     L     *       S     S     *     G     S     L     *       S     S     *     G     S     L     *       C     Y     C     C     C     *     R	SW M G C * R * W N Y L * K ? - SW M G C * S * W N Y L * K ? - SW M G C * R * W N Y L * K ? - SW M G C * R * W N Y L * K ? - SW M G C * R W N Y L * K ? - SW M D C * R W N Y L * K ? - SW M D C * R W N Y L * K ? -
55. Gh_D02C0039 56. Gh_D04G1312 57. Gh_D05G1874 58. Gh_D05G2477 59. Gh_D06G2134 60. Gh_D08G1459 61. Gh_D10G0318 62. Gh_D10G0318	S         F         Y         S         S         7         5         Y         Y         S         C         7         7         7         7         7         7         5         Y         Y         S         C         7	K       A       F       L       R       K	KKRKKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKSYCC KKRKKKKSYCC KKRKKKKSYCC KKRKKKKSYCC KKRKKKKSYCC KKRKKKKSYCC KKRKKKKSYCC KKRKK KKRKK KKRKK KKRKK KKRKK KKRK KKRK KKRK KKRK KKR	L Y W T * S S R K L * F C W T * S S R K L * L Y W T * S S R K L * L Y W T * S S R K L * F C W T * N V W K L * S C W T * K T W K L *	A         S         A         L         A	S S * G S L * * S S * G S L F * S S * G S L C * S S * G S L C * S S * G S L C * S S * G S L * C Y C C S C * R S S * G S L *	N M M C         *         N M N L         *         ?           S M M C         *         N M N L         *         ?           S M M C         *         N M N L         *         ?           S M M C         *         N M N L         *         ?           S M M C         *         N M N L         *         ?           S M M C         *         N M N L         *         ?           S M M C         *         N M N L         *         ?           S M M C         *         R M M Y L         *         ?           S M M C         *         R M M Y L         *         ?           S M M C         *         N M Y L         *         ?           S M M C         *         N M Y L         *         ?
55. Gh_D02C0939 56. Gh_D04C1312 57. Gh_D05G1874 58. Gh_D05G2477 59. Gh_D05G2134 60. Gh_D06G1459 61. Gh_D010G0318 62. Gh_D01G0932 63. Gh_D11G2965	S         F         Y         S         S         7	K       A       F       R       K	N         N	Image: Construction         Image: Construction           Image: Constretin         Image: Constructin  <	A         S         A         L         A	S S * G S L * * S S * G S L C * C Y C C S C * R S S * G S L * * C Y C C S C * R S S * G S L * *	N         N
55. Gh_D02C0939 56. Gh_D04G1312 57. Gh_D05C1874 59. Gh_D05C2477 59. Gh_D06C2134 60. Gh_D06C2134 60. Gh_D08C1459 61. Gh_D10C0318 62. Gh_D11G2955 63. Gh_D11G2995 64. Gh_D215C1995	S         F         Y         S         2         7	K       A       F       L       R       K       K       K       I	K         K	Image: Construction of the construction of	A         A	S         S         G         S         L         F           S         S         G         S         L         F           S         S         G         S         L         F           S         S         G         S         L         F           S         S         G         S         L         C           S         S         G         S         L         C           S         S         G         S         L         C           S         S         G         S         L         C           C         Y         C         S         L         C           Y         C         C         S         L         C           Q         Y         C         S         L         C           Q         S         S         S         L         C           Q         S         S         S         L         C           Q         S         S         S         L         C           Q         S         C         S         C         R	N         N
55. Gh.,D0200939 56. Gh.,D0401312 57. Gh.,D0601874 58. Gh.,D0602477 59. Gh.,D0602474 50. Gh.,D0602474 60. Gh.,D0602189 61. Gh.,D1160932 63. Gh.,D1160932 63. Gh.,D1162965 64. Gh.,D1221989 65. Gh.,Sac033106001 65. Gh. Sac033106001	S         F         Y         S         0         -         -         0	K A F L E R K R K K I S Y R K K K K K A F L E R K R K K A F L E R K R K N A Y L E G K R K K K N A Y L E G K R K K K N A Y L E G K R K K K K N A Y L E G K R K K K K N A Y L E G K R K K K K K K K K K K K K K K K K K	K K R K K K K S Y C C Y K K R K K K K S Y C C Y K K R K K K K S Y C C Y K K R K K K K S Y C C Y K K R K K K K S Y C C Y K K R K K K K S Y C C Y K K R K K K S Y C C Y K K R K K K S Y C C Y K K R K K K S Y C Y K K R K K K S Y C Y K K R K K K S Y C Y K K R K K K S Y C Y K K R K K K S Y C Y K K R K K K S Y Y F Y K K R K K K S Y Y F Y K R K K K K S Y Y F Y K R K K K K S Y Y F Y K R K K K K S Y Y F Y K R K K K K S Y Y F Y K R K K K K S Y Y F Y K R K K K K S Y Y F Y K R K K K K S Y Y F Y K R K K K K S Y Y F Y K R K K K K S Y K S Y Y F Y K R K K K K S Y K S Y Y F Y K R K K K K S Y K S Y Y F Y K R K K K K S Y K S Y Y F Y K R K K K K S Y K S Y Y F Y K R K K K S Y K S Y K S Y Y F Y K R K K K K S Y K S Y Y F Y K R K K K K S Y K S Y K S Y Y F Y K R K K K K S Y K S Y K S Y Y F Y K R K K K K S Y K S Y K S Y K S Y K S Y K S Y K S Y F Y S Y K R K K K K S Y K S	L         W	A         A         L         A         L         A         A         A         L         A	S         S	M         M         C         I         M         M         K
55. Gh_D0200939 55. Gh_D040112 57. Gh_D05G1874 58. Gh_D04G2477 59. Gh_D05G2477 59. Gh_D05G2473 60. Gh_D05G2134 60. Gh_D08G1459 61. Gh_D102032 63. Gh_D1102956 64. Gh_D212G1996 66. Gh_Sca033106G01 66. Gh_Sca033106G01 66. Gh_Sca0132277G01 67. Gh_Sca113434001	S         F         Y         S         S         F         S	K A F L E R K K K I S Y - R K K K K A F L E R K K K A F L E R K K I S S G K R K I S S G K R K I S S G K R K K A F L E K K K K I S S S S S S S S S S S S S S S S S S S	NKKKKKKK         YC           NKKKKKKKK         YC           NKKKKKKK         YC           NKKKKKKK         YC           NKKKKKKK         YC           NKKKKKKK         YC           NKKKKKK         YC           NKKKKKK         YC           NKKKKKK         YC           NKKKKK         YC           NKKKKK         YC           NKKKK         YC           NKKKK         YC           NKKKK         YC           NKKK         YC           NKK         YC           NK         YC			S         S	N         N
55. Gh.,D0200939 55. Gh.,D041312 57. Gh.,D05G1874 58. Gh.,D05G2477 59. Gh.,D09G2134 60. Gh.,D09G2134 60. Gh.,D09G1459 61. Gh.,D1000318 62. Gh.,D1100932 63. Gh.,D1102965 64. Gh.,D12C1996 65. Gh.,Sca3104601 66. Gh.,Sca122277601 67. Gh.,Sca151434601 68. Garail02022400.1	S         F         Y         S         S         Y         S         Y	K         A         F         E         C         K         F         K         F         K         F         L         C         K         F         L         C         K         F         L         C         K         K         F         L         C         K				S         S	N         M         C         N
55. Gh.,D0200939 55. Gh.,D0401312 57. Gh.,D05G1874 58. Gh.,D04G2477 59. Gh.,D06G2477 59. Gh.,D06G2474 59. Gh.,D06G2477 59. Gh.,D06G2479 62. Gh.,D11G0932 63. Gh.,D11G0932 63. Gh.,D112G2955 65. Gh.,Sca0333105G01 65. Gh.,Sca023277G01 67. Gh.,Sca0152277G01 67. Gh.,Sca0152277G01 69. Geral,002G52400.2 20. Geral,002G5400.2 20. Geral	S         F         Y         S         F		N         N	Image: Constraint of the second sec		S         G         S         C         S         C         S         R	N         N
55. Gh.,D0200939 55. Gh.,D0401312 57. Gh.,D05G1874 58. Gh.,D05G2137 59. Gh.,D05G2134 60. Gh.,D05G2134 60. Gh.,D08G1459 61. Gh.,D1000318 62. Gh.,D11G2985 63. Gh.,D11G2985 65. Gh.,Sca033106001 66. Gh.,Sca12277001 67. Gh.,Sca151434001 68. Goral.0026052400.1 69. Goral.0026052400.2 70. Goral.0026052400.3 71. Goral.0026052400.3	S         F         Y         S         S         P				A         B         A	S         S	M         M         C         M         M         M         K
55. Gh.,D0200939 56. Gh.,D0401312 57. Gh.,D05G1874 58. Gh.,D05G2477 59. Gh.,D05G2134 60. Gh.,D05G2134 60. Gh.,D08G1459 61. Gh.,D102095 63. Gh.,D1102995 65. Gh.,Sca033106G01 66. Gh.,Sca122277G01 66. Gh.,Sca122277G01 68. Grant,002G52400.1 69. Grant,002G52400.2 70. Grant,002G52400.3 71. Grant,002G52400.3 71. Grant,002G52400.4 72. Grant,004G158800.1	S         F         Y         S         Q         -         -         2         2         Q         1         Q         S         Q         -         Q         2         Q	X         A         L         E         K					N         N
55. Gh.,D0200939 55. Gh.,D0401312 57. Gh.,D05G1874 58. Gh.,D05G2174 58. Gh.,D05G2174 59. Gh.,D09G2134 60. Gh.,D09G2134 60. Gh.,D09G2134 60. Gh.,D0100318 62. Gh.,D1100932 63. Gh.,D1102965 64. Gh.,D12C1996 65. Gh.,Sca31306001 66. Gh.,Sca122277G01 67. Gh.,Sca151434G01 68. Grafi 002G62400 1 69. Grafi 002G62400 1 71. Grafi 002G62400 2 71. Grafi 002G62400 3 73. Grafi 002G62400 3 74. Grafi 002G62400 3 75. Grafi 002G6762400 3 75. Grafi 002G6762400 3 75. Grafi 002G67676 75. Grafi 002G67676767 75. Grafi 002G676776776777677777777777777777777777	S         F         Y         S         C         P	X         A         F         E         R         K         F         I         X         I         S         X         I         S         X         I         X					N         N
55. Gh.,D0200939 55. Gh.,D0401312 57. Gh.,D05C1874 58. Gh.,D04C1474 59. Gh.,D06C1474 59. Gh.,D06C1459 60. Gh.,D04C1459 60. Gh.,D04C1459 60. Gh.,D0120932 63. Gh.,D1120932 63. Gh.,D1120955 64. Gh.,Dsca1277G01 67. Gh.,Sca151434G01 68. Grani,0020652400 1 69. Grani,0020652400 3 71. Grani,004G158600 1 73. Grani,004G158600 1 74. Grani,004G158600 1 75. Grani,004G158600 1 7	S         F         Y         S         C         9         9         9         1         9         9         1         1         0         S         7         9         1         1         0         S         7         9         1         1         0         1         7         1         1         0         1         7         1						N         N
55. Gn.,D0200939 55. Gn.,D0401312 57. Gn.,D05G1874 58. Gh.,D0462477 59. Gh.,D06G2477 59. Gh.,D06G2477 59. Gh.,D06G2477 59. Gh.,D0160232 63. Gh.,D1160932 63. Gh.,D1160932 63. Gh.,D1162985 64. Gh.,D21621986 65. Gh.,Sca12277G01 67. Gh.,Sca13134601 68. Gran 0026052400 71. Goral 0026052400 27. Goral 004158800 17. Goral 0026052400 37. Gh. Gana 004158800 17. Goral 004158800 17. Goral 004158800 13. Goral 004158	S F I S C S 7       <						M         M         C         M         M         M         K
55. Gh.,D0200939 55. Gh.,D0401312 57. Gh.,D05G1874 58. Gh.,D04G1874 59. Gh.,D05G2137 60. Gh.,D05G2137 60. Gh.,D06G1459 61. Gh.,D100318 62. Gh.,D11G2955 63. Gh.,D11G2955 65. Gh.,Sca033106G01 66. Gh.,Sca12277C01 67. Gh.,Sca151434G01 88. Goral 002G52400.3 71. Goral 002G52400.3 71. Goral 002G52400.3 71. Goral 002G52400.1 73. Goral 004G158600.11 74. Goral 004G158600.11 75. Goral 004G158600.12 76. Goral 004G158600.12 77. Goral 004G158600.12 77. Goral 004G158600.12 76. Goral 004G158600.12 77. Goral 004G158600.14	S       F       Y       S       S       P						N         N
55. Gh.,D0200939 55. Gh.,D0401312 57. Gh.,D05G1874 58. Gh.,D05G2174 58. Gh.,D05G2174 58. Gh.,D05G2174 59. Gh.,D09G2134 60. Gh.,D09G2134 60. Gh.,D09G2134 60. Gh.,D0120199 62. Gh.,D11201998 65. Gh.,Sca122277G01 67. Gh.,Sca131434G01 68. Grafi 002G052400 1 69. Grafi 002G052401 71. Grafi 002G052401 72. Grafi 004158800 112 73. Grafi 004158800 112 76. Grafi 004158800 112 76. Grafi 004158800 112 76. Grafi 004158800 112 76. Grafi 004158800 12 76. Grafi 004158800 1	S       F       Y       S       S       P						N         N
55. Gh.,D0200939 55. Gh.,D0401312 57. Gh.,D05C1874 58. Gh.,D04C1474 59. Gh.,D06C1474 59. Gh.,D06C1459 60. Gh.,D04C1459 62. Gh.,D1100932 63. Gh.,D1102955 64. Gh.,D1201926 65. Gh.,Sca12277G01 67. Gh.,Sca151434G01 68. Grafl,002G052400,1 67. Gh.,Sca151434G01 68. Grafl,002G052400,2 70. Grafl,002G052400,3 71. Grafl,004G158600,1 73. Grafl,004G158600,1 74. Grafl,004G158600,1 75. Grafl,004G158600,1 75. Grafl,004G158600,1 75. Grafl,004G158600,1 76. Grafl,004G158600,1 77. Grafl,004G158600,1 77. Grafl,004G158600,1 77. Grafl,004G158600,1 77. Grafl,004G158600,1 78. Grafl,004G158600,1 79. Grafl,004G158600,2 79. Grafl,004G158600,2 79. Grafl,004G158600,3 80. Grafl,004G158600	S F F S C S       7       7       7       7         S F S S S S S S S S S S S S S S S S S S						M         M
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55         Gh., D0200939           55         Gh., D0401312           57         Gh., D0501874           58         Gh., D0501874           58         Gh., D0401312           57         Gh., D0902134           60         Gh., D0902134           61         Gh., D1000318           62         Gh., D1100932           63         Gh., D1201996           65         Gh., Sca122277001           67         Gh., Sca122277001           67         Gh., Sca122277001           68         Grafi 0026052400 2           70         Grafi 0026052400 3           71         Grafi 0026052400 3           72         Grafi 004515800 11           73         Grafi 004515800 11           76         Grafi 004515800 12           76         Grafi 004515800 12           78         Grafi 004515800 12           79         Grafi 004515800 2           79         Grafi 004515800 2           79         Grafi 004515800 2           70         Grafi 004515800 2           71         Grafi 004515800 2           73         Grafi 004515800 2           74         Grafi 004515800 2	S       F       Y       S       S       P				A     B     A     B     A     B     A     B     A     B     A     B     A     B     A     B     A     B <td></td> <td>N         N</td>		N         N
55. Gh., D0200939           55. Gh., D0401312           57. Gh., D0501312           57. Gh., D0501314           58. Gh., D0501374           58. Gh., D0501374           58. Gh., D0501374           58. Gh., D0501374           59. Gh., D0601378           62. Gh., D1000318           62. Gh., D1100932           63. Gh., D1201996           65. Gh., Scat122277001           67. Gh., Scat15134G01           68. Grafi 002062400 1           70. Grafi 002052400 2           70. Grafi 002052400 3           71. Grafi 004158600 11           73. Grafi 0044158600 13           74. Grafi 0044158600 13           76. Grafi 0045158600 13           77. Grafi 0044158600 13           78. Grafi 0044158600 2           79. Grafi 0044158600 13           79. Grafi 0044158600 14           78. Grafi 0044158600 2           79. Grafi 0044158600 2           79. Grafi 0044158600 3           70. Grafi 0044158600 4           80. Grafi 0044158600 4           81. Grafi 0044158600 5           82. Grafi 0044158600 6           83. Grafi 0044158600 7           84. Grafi 0044158600 7           84. Grafi 0044158600 7           84. Grafi 004415	S       F       Y       S       S       F       S       Y       S       Y       Y       S       Y       Y       S       Y       Y       S       Y       Y       Y       S       Y						N         N
55. Gh., D0200939           55. Gh., D0401312           57. Gh., D05C1874           58. Gh., D04C1474           59. Gh., D06C1474           59. Gh., D06C1474           59. Gh., D06C1474           59. Gh., D06C1474           59. Gh., D06C1459           61. Gh., D1000318           62. Gh., D1100932           63. Gh., D1120955           64. Gh., D12C1998           65. Gh., Sca033306501           66. Gh., Sca12277051           67. Gh., Sca151434601           68. Grafi, 002052400.1           70. Grafi, 002652400.2           70. Grafi, 002652400.3           71. Goral, 004C158600.11           75. Grafi, 004C158600.13           76. Grafi, 004C158600.13           77. Grafi, 004C158600.3           0. Grafi, 004G158600.3           0. Grafi, 004G158600.4           82. Grafi, 004G158600.5           82. Grafi, 004G158600.7           84. Grafi, 004G158600.7           84. Grafi, 004G15	S F I S C S 7       -       -       7       7       7         S F I S C S C 7       -       7       7       7       7       7         S F I S C S C 7       -       7 <td< td=""><td></td><td></td><td></td><td></td><td></td><td>M         M         C         M         M         M         K</td></td<>						M         M         C         M         M         M         K
55. Gn., D0200939           55. Gn., D0201312           57. Gn., D0501312           57. Gn., D0501312           57. Gn., D0501314           60. Gn., D0402477           58. Gn., D0402477           59. Gn., D040247           50. Gn., Scat21227           61. Gn., Scat2277031           67. Gn., Scat31434601           58. Grani, 002052400, 2           70. Grani, 002052400, 3           71. Grani, 002052400, 3           71. Grani, 002052400, 3           71. Grani, 00245158000, 13           77. Grani, 004158800, 13           77. Grani, 004158800, 13           77. Grani, 004158800, 14           81. Grani, 004158800, 13           77. Grani, 004158800, 14           81. Grani, 004158800, 5           82. Grani, 004158800, 7           84. Grani, 004158800, 7           84. Grani, 004158800, 7           85. Grani, 004158800, 7           86. Grani, 004158800, 7	S       F       Y       S       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y       S       Y						M         M
55         Gh., D0200939           55         Gh., D0401312           57         Gh., D0501874           58         Gh., D0402134           60.         Gh., D0402134           60.         Gh., D0402134           60.         Gh., D0402134           62.         Gh., D1000318           62.         Gh., D1100932           63.         Gh., D1201996           65         Gh., Sa3104001           66.         Gh., Sa3104001           66.         Gh., Sa3104001           68.         Grafi 002052400.1           69.         Grafi 002052400.2           70.         Grafi 002052400.2           70.         Grafi 002052400.3           71.         Grafi 002052400.1           73.         Grafi 00405158000.11           74.         Grafi 004158800.13           77.         Grafi 004158800.13           76.         Grafi 004158800.13           76.         Grafi 004158800.13           76.         Grafi 004158800.13           76.         Grafi 004158800.13           77.         Grafi 004158800.13           77.         Grafi 004158800.13           77.         Grafi 004158800.13 </td <td>S       F       Y       S       S       P</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>M         M         C         M         M         N</td>	S       F       Y       S       S       P						M         M         C         M         M         N
55. Gh., D0200939           55. Gh., D0201312           57. Gh., D0501312           57. Gh., D0501312           57. Gh., D0501312           58. Gh., D0501312           59. Gh., D0501314           58. Gh., D0501314           58. Gh., D0501318           62. Gh., D100932           63. Gh., D1100932           63. Gh., D1201996           65. Gh., Scat1512277G01           67. Gh., Scat151243601           68. Grail 002062400 1           70. Grail 002052400 2           70. Grail 002052400 3           71. Grail 004158800 110           74. Grail 004158800 113           75. Grail 0044158800 12           76. Grail 0044158800 13           77. Grail 004158800 12           78. Grail 0044158800 13           79. Grail 0044158800 13           79. Grail 0044158800 18           81. Grail 0044158800 5           82. Grail 0044158800 5           83. Grail 0044158800 7           84. Grail 0044158800 8           85. Grail 0044158800 7           86. Grail 0046158800 8           86. Grail 0046158800 8           86. Grail 0046158800 8           86. Grail 0046158800 7           86. Grail 0046158800 7           86. Grail 0046	S       F       Y       S       S       F       S       Y       S       Y						M         M         C         M         M         N
55. Gh., D0200939           55. Gh., D0401312           57. Gh., D05C1874           58. Gh., D04C1474           59. Gh., D06C1474           59. Gh., D06C1474           59. Gh., D06C1474           59. Gh., D06C1474           59. Gh., D06C1459           60. Gh., D01G0318           62. Gh., D11G0932           63. Gh., D11G0932           63. Gh., D11G2955           64. Gh., Sca033306501           66. Gh., Sca12277G01           67. Gh., Sca151434G01           68. Grafi, 002062400.1           70. Grafi, 002652400.2           70. Grafi, 002652400.3           71. Gorali, 002652400.3           73. Grafi, 004G158600.1           74. Grafi, 004G158600.1           75. Grafi, 004G158600.1           76. Grafi, 004G158600.1           77. Grafi, 004G158600.1           78. Grafi, 004G158600.3           80. Grafi, 004G158600.3           81. Grafi, 004G158600.3           82. Grafi, 004G158600.4           83. Grafi, 004G158600.5           82. Grafi, 004G158600.8           83. Grafi, 004G158600.9           86. Grafi, 007G099100.1           87. Grafi, 007G099100.1           88. Grafi, 007G099100.1           80. Grafi,	S       F       Y       S       S       F       S       Y       S       Y						M         M         N         M         N
65         Gh., D0200939           65         Gh., D0401312           57         Gh., D0501874           58         Gh., D0401312           57         Gh., D0501874           58         Gh., D0402134           60.         Gh., D0402134           60.         Gh., D0402134           62.         Gh., D1100932           63.         Gh., D1102955           64.         Gh., D1201996           65.         Gh., Sca122277001           67.         Gh., Sca122277001           68.         Gh., Sca122277001           69.         Gh., Sca122277001           60.         Gh., Sca122277001           61.         Gh., Sca122277001           62.         Gh., Sca122277001           70.         Gh., Sca122277001           71.         Gh., Sca122277001           72.         Gh., Sca12434001           74.         Gh., Sca122277001           70.         Gh., Sca122277001           71.         Gh., Sca122277001           72.         Gh., Sca122277001           73.         Gh., Sca102262400.3           71.         Gh., Sca102262400.3           71.         Gh., Sca102262400.3	S       F       Y       S       S       F       S       Y       S       S       Y       S       S       Y       Y						M         M         C         M         M         M         K
55         Gn., D0200939           55         Gn., D0401312           57         Gn., D0501312           57         Gn., D0501312           57         Gn., D0501312           58         Gn., D0501314           58         Gn., D0501314           50         Gn., D0402134           60         Gn., D0100318           62         Gn., D1100932           63         Gn., D1120198           65         Gn., Sca151434601           68         Grafi 002052400           70         Grafi 002052400           71         Grafi 002052400           72         Grafi 002052400           73         Grafi 002052400           74         Grafi 002052400           73         Grafi 002052400           74         Grafi 002052400           72         Grafi 004158800           74         Grafi 004158800           75         Grafi 004158800           76         Grafi 004158800           76         Grafi 004158800           76         Grafi 004158800           76         Grafi 004158800           78         Grafi 004158800           79         Grafi 0	S       F       Y       S       S       F       P						N         N
55. Gh., D0200939           55. Gh., D0201312           57. Gh., D0501312           57. Gh., D0501312           57. Gh., D0501314           58. Gh., D0501314           58. Gh., D0501314           58. Gh., D0501314           58. Gh., D0501318           62. Gh., D100032           63. Gh., D1100932           63. Gh., D1102955           64. Gh., D1201996           65. Gh., Sca03106011           67. Gh., Sca03104052401           68. Grail 0020052400 2           70. Grail 002052400 3           71. Grail 0040158600 11           75. Grail 0044158600 11           76. Grail 0044158600 13           77. Grail 0044158600 13           77. Grail 0044158600 13           78. Grail 0044158600 13           79. Grail 0044158600 13           79. Grail 0044158600 14           78. Grail 0044158600 1           79. Grail 00441586	S       F       Y       S       S       P						M         M
55. Gh., D0200939           55. Gh., D0401312           57. Gh., D05C1874           58. Gh., D04C1474           58. Gh., D04C2477           58. Gh., D04C1459           61. Gh., D1000318           62. Gh., D04C1459           63. Gh., D102032           63. Gh., D1102032           63. Gh., D1102055           64. Ch., D12C1998           65. Gh., Sca0133106G01           66. Gh., Sca12277G01           67. Gh., Sca013277G01           67. Gh., Sca115434G01           68. Grafi, 0020052400.1           70. Grafi, 002052400.2           70. Grafi, 002052400.3           71. Gorafi, 002052400.3           72. Grafi, 004158600.1           73. Grafi, 004158600.1           74. Grafi, 004158600.1           75. Grafi, 004158600.2           79. Grafi, 004158600.2           79. Grafi, 004158600.2           70. Grafi, 004158600.5           82. Grafi, 004158600.8           83. Grafi, 004158600.8           84. Grafi, 004158600.9           86. Grafi, 004158600.9           86. Grafi, 007G099100.1           87. Grafi, 007G099100.1           88. Grafi, 007G099100.1           89. Grafi, 007G099100.5           80. Grafi, 007G09	S       F       S       S       F       S						M         M         N         M         N
55         Gn., D0200939           55         Gn., D0401312           57         Gn., D0501874           58         Gn., D0501874           58         Gn., D0501874           58         Gn., D0501874           58         Gn., D0501874           59         Gn., D0402134           60.         Gn., D0100318           62.         Gn., D1100932           63.         Gn., D1120955           64         Gn., D1221996           65         Gn., Sa31080011           66.         Gn., Sa3102277001           67.         Gn., Sa3102277001           68.         Gna1.0026052400.2           70.         Grafi.0026052400.3           71.         Grafi.0026052400.3           71.         Grafi.002615800.11           73.         Grafi.004515800.12           74.         Grafi.004515800.12           76.         Grafi.004515800.13           78.         Grafi.004515800.14           78.         Grafi.004515800.14           78.         Grafi.004515800.8           82.         Grafi.004515800.1           83.         Grafi.004515800.8           85.         Grafi.004515800.8 </td <td>S       F       Y       S       Y</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>N         N</td>	S       F       Y       S       Y						N         N
55. Gh., D0200939           55. Gh., D0201312           57. Gh., D0501312           57. Gh., D0501312           57. Gh., D0501314           58. Gh., D0501314           58. Gh., D0501314           58. Gh., D0501314           58. Gh., D0501318           62. Gh., D1100932           53. Gh., D1120956           64. Gh., D1201996           65. Gh., Scat122277001           67. Gh., Scat151434G01           68. Grafi 002062400 2           70. Grafi 002052400 2           71. Grafi 004158600 110           74. Grafi 0046158600 112           76. Grafi 0046158600 112           76. Grafi 0046158600 12           76. Grafi 0046158600 13           77. Grafi 0046158600 13           77. Grafi 0046158600 2           79. Grafi 0046158600 3           70. Grafi 0046158600 3           80. Grafi 0046158600 3           81. Grafi 0046158600 3           82. Grafi 0046158600 3           83. Grafi 0046158600 7           84. Grafi 0046158600 7           84. Grafi 0046158600 7           85. Grafi 0046158600 7           86. Grafi 00561900 7           87. Grafi 0046158600 7           88. Grafi 0076199100 5           80. Grafi 0076	S       F       Y       S       S       P						N         N
55. Gh., D0200939           55. Gh., D0201312           57. Gh., D0501312           57. Gh., D0501312           57. Gh., D0501314           58. Gh., D0501312           58. Gh., D0501314           58. Gh., D0501314           58. Gh., D0501318           52. Gh., D100032           53. Gh., D1100932           53. Gh., D1102955           64. Gh., D1201996           65. Gh., Sca03106011           67. Gh., Sca031040601           68. Gml, D202052400           69. Granil 0020052400           70. Granil 002052400           71. Granil 0040158600           73. Granil 0040158600           74. Granil 0040158600           75. Granil 004158600           76. Granil 004158600           77. Gra							M         M
55         Gn., D0200939           55         Gn., D0401312           57         Gn., D0501874           58         Gn., D0501874           59         Gn., D0501874           59         Gn., D0501874           59         Gn., D0602134           60.         Gn., D0100318           62         Gn., D1100932           63.         Gn., D1120985           54         Gn., D1201998           55         Gn., Sa3108001           66         Gn., Sa3108001           70         Gn., Sa142277001           67         Gn., Sa31020012400.1           68         Grani 002052400.1           68         Grani 002052400.2           70         Grani 002052400.3           71         Grani 002052400.3           72         Grani 004158800.1           74         Grani 004158800.1           74         Grani 004158800.1           76         Grani 004158800.2           76         Grani 004158800.2           76         Grani 004158800.2           76         Grani 004158800.2           76         Grani 004158800.3           80         Grani 004158800.8           <	S       F       S       S       F       S						N         N
55         Gh., D0200939           55         Gh., D0401312           57         Gh., D0501874           58         Gh., D0501874           50         Gh., D050188           62         Gh., D1100932           53         Gh., D1201996           55         Gh., Scall 3016011           56         Gh., Scall 3016011           56         Gh., Scall 3016011           56         Gh., Scall 3016011           57         Gerail 002052400 2           70         Gerail 002052400 2           70         Gerail 002052400 2           70         Gerail 004158800 11           73         Gerail 004158800 12           76         Gerail 004158800 13           77         Gerail 004158800 13           76         Gerail 004158800 13           76         Gerail 004158800 13           77         Gerail 004158800 2           79         Gerail 004158800 2           79         Gerail 004158800 3           80         Gerail 004158800 4	S       F       Y       S       S       P						
55         Gn., D0200939           55         Gn., D0201312           57         Gn., D0501312           57         Gn., D0501312           57         Gn., D0501312           58         Gn., D0501314           58         Gn., D0501314           58         Gn., D0501318           62         Gn., D1100932           53         Gn., D1201995           55         Gn., Scal 312277001           67         Gn., Scal 312277001           67         Gn., Scal 312277001           68         Grafi 002052400 2           70         Grafi 002052400 2           70         Grafi 002052400 3           71         Grafi 0040158800 11           73         Grafi 0040158800 11           74         Grafi 0040158800 11           75         Grafi 0040158800 13           77         Grafi 0040158800 13           76         Grafi 0040158800 13           77         Grafi 004158800 13           76         Grafi 004158800 13           77         Grafi 004158800 2           80         Grafi 004158800 3           80         Grafi 004158800 3           81         Grafi 004158800 3	S       F       Y       S       S       P						M         M         N         M         N
55. Gh., D0200939           55. Gh., D0201312           57. Gh., D0501312           57. Gh., D0501312           57. Gh., D0501312           58. Gh., D0501312           59. Gh., D0501314           58. Gh., D0501312           59. Gh., D0501314           58. Gh., D0501318           52. Gh., D100032           53. Gh., D1100932           53. Gh., D112095           64. Gh., D1201996           65. Gh., Sca13122277G01           67. Gh., Sca151434G01           68. Grail 0020052400 2           70. Grail 002052400 3           71. Grail 0040158600 10           73. Grail 0046158600 11           75. Grail 004158600 13           77. Grail 004158600 12           76. Grail 004158600 13           77. Grail 004158600 2           78. Grail 004158600 13           78. Grail 004158600 14           81. Grail 004158600 18           82. Grail 004158600 18           83. Grail 004158600 18           84. Grail 004158600 19           86. Grail 005017000 1           88. Grail 007G099100 18	S       F       Y       S       S       P						
55         Gn., D0200939           55         Gn., D0401312           57         Gn., D0501874           58         Gn., D0501874           58         Gn., D0501874           58         Gn., D0501874           58         Gn., D0501874           59         Gn., D0602134           60         Gn., D0100318           62         Gn., D1100932           63         Gn., D1120955           64         Gn., D1201996           65         Gn., Sca122277001           67         Gn., Sca122277001           70         Grad. 1002652400.2           71         Grad. 1002652400.2           72         Grad. 1004515800.1           73         Grad. 1004515800.1           74         Grad. 1004515800.1           75         Grad. 1004515800.2           76         Grad. 1004515800.2           78         Grad. 1004515800.2	S       F       Y       S       S       P						
65         Gh., D0200939           65         Gh., D0401312           57         Gh., D0501874           58         Gh., D0501874           50         Gh., D050181           62         Gh., D100318           62         Gh., D1100932           53         Gh., D1201996           55         Gh., Sca3104001           66         Gh., Sca151434001           68         Garail 002052400.1           69         Garail 002052400.2           70         Garail 002052400.3           71         Garail 0046158600.10           74         Garail 0046158600.11           75         Garail 0046158600.12           76         Garail 0046158600.13           77         Garail 0046158600.13           78         Garail 0046158600.2           79         Garail 0046158600.2           79         Garail 0046158600.2           80         Garail 0046158600.3           81         Garail 0046158600.4           81         Garail 0046158600.5	S       F       Y       S       S       P						

Figure 3. The conserved protein sequence among 109 genes. The stars on the top mean the conserved amino acid in all proteins.

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Pageige/Abbr/	
1 2/6209	
0.056404	
2.300404	
3.472138	
4. 470047 E. 496494	
6 402071	
7 856241	
8 895772	CTARGCATTGTGATAATAATGARGTTCTTAAGGCTCTTTGTAATGAAGCTGGATGGATGTTGAACCTGATGGAACTACTTATAGAA
9 912686	CTA A GC A T I G I G A T A A T G A G T T C T T A A G G C T C T T G T C T I G A A G C T G G A T G
10 Araba 12428s0016.1 p	CACTER AGA C C GA TA TEA T CATGET G C T G
11 Araba 1486s0003 1 p	CTA AGCATTG GATA ATA ATGA AGTTC TTA AGGCTC TTTG TTC TG AAGCTG GGTTG TTG AAGAAGATG GAACTACTTA TA GAA
12. Araha 15647s0004.1.p	CTA AGC ATT GT GA TA ATT GA AGT T CTT TA AGG CT CTT T GT CTT GA AGC T GG A T GG GT T GT T
13. Araha 17172s0006.1.p	C T S C T A G A G C T S A T A T G A T S A T S A T S C T S C T S C T S C T A G A G A A G C T S G A T S G T S A T S C T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S C T S A T S C T S A T S C T S C T S A T S C T S C T S A T S C T S C T S C T S A T S C T S
14. Araha.17172s0006.2.p	C T S C T A S A S C T S A T S A T S A T S A T S A T S T T S C T S C T S C T S A S A S C T S S A S C T S T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S A T S C T S
15. Araha.17406s0004.1.p	C TA A C C A T T G T G A T A A T G A G T T C T T A A G G C T C T T G T G A A G C T G G A T G T G T G A A G A T G A T G A C T A C T T A T A G A A G
16. Araha.4300s0012.1.p	C T A A C C A T T G T G A A T A A T G A A G T T C T T A A G G C T C T T T G T A A G G A G C T G G A T G G A A C C T G A T G G A A C T A C T T A T A G A A
17. Araha.49879s0016.1.p	C T A A C C A T T G T G A A T A A T G A G T T C T T A A G G C T C T T G T G T G A A G C T G G A T G G A A G A T G G A A C T A C T T A T A G A A
18. AT1G19350.1	CTARGCATIGEGATAATAATAATGAAGTECTTAAGGCTCTTTGTTCTGAAGCTGGATGGGTTGTCAAGAAGATGGAACTACTTATAGAA
19. AT1G19350.3	C T A A C C A T T G T G A A T A A T G A G T T C T T A A G G C T C T T G T T C T G A A G C T G G A T G G A A C A A G A T G G A A C T A C T T A T A G A A
20. AT1G19350.4	CTARGCATIGE GATAATAATAATGAAGTECTTAAGGCTCTTEGTECTGAAGCTGGGTEGTEGAAGAAGATGGAACTACTTATAGAA
21. AT1G19350.5	C T A A G C A T T G T G A T A A T A A T G A G T T C T T A A G G C T C T T G T T C T G A A G C T G G A T G G A C A G A T G G A A C T A C T T A T A G A A
22. AT1G19350.6	CTAAGCAT TGTGATAATAATGAAGTTCTTAAGGCTCTTTGTTCTGAAGCTGGATGTTGTTGAAGAAGATGGAACTACTTATAGAA
23. AT1G75080.1	C TAAGCATTGTGATAATAATGAAGTTCTTAAGGCTCTTTGTGTTGAAGCTGGATGGGTTGTTGAAGAAGATGGAACTACTTATAGAA
24. AT1G75080.2	CTAAGCATIGIGATAATAATGAAGTICITAAGGCICITIGIGAGCIGGAIGGGIIGIIGAAGAAGAIGGAACAIGAACAITGIAAGAAGAIGAAGAIGAACIACIIAAAAA
25. AT1G78700.1	C TAAGCATTGTGATAATAATGAAGTTCTTAAGGCTCTTTGTAATGAAGCTGGATTGTTGAACCTGATAGAACTACTTATAGAA
26. AT2G45880.1	GAGTTAGASCTGATATTAATGATGTTATTGCTGCTCTTGCTAGAGAASCTGGATGGGTTGTTCTTCCTGATGGAACTACTTTTCCTT
27. AT3G50750.1	C TAAGCATTGTGATAATAATGAAGTTCTTAAGGCTCTTTGTCTTGAAGCTGGATGGA
28. AT4G18890.1	C TAAGCAT I G I G ATAATAATGAAGTI C I TAAGGCI CITII G TAATGAAGCI GGATGGACTGI I GAAGAI GAI GAI GGAACTACTI ATAGAA
29. AT4G36780.1	C TA A G C A I T G T G A T A A T G A A G I I C I T A A G G C I C I I T G A A G C I G G A T G G I T G A A G A T G A T G A T G A A C A C T A C T A A A G A A
30. A15G45300.1	CIGUING NOTION A LIGNATION IS THE TOUR STATES IS A LIGNAGE A CITACITATAGAC
31. AT5G45300.2	CIGCIAGAGCIGANAIGAIGHIAIIGCIGCICIGCIGCIGGAGCIGGIGCIGAIGGAGCIGGIGAIGGAGCIGAIGGAGCIGAIGGAGCIGAIGGAAC
32. Cotton_A_03434_BGI-A2_	C TA A G A G A G C T G A T I C T A A T G A T C T T C A T G A G G A G G A G G A T G G C A G G A G C T A T T T A T A G A A
33. Cotton_A_05312_BGI-A2_	CTANSCATTG TGATAATAA GAAGATCTTATAAGA
34. Cotton_A_07763_BGI-A2_	U HAN YA HAN MAN MAN HAN I YANGHUC HIMAN SUC TU TU TU TU TU CU ANG A TU GANGATU A TU GANGATU A TU ATA CAA
35. Gotton_A_135/5_BGI-A2_	CIGO INCOLO I GALA IGANI GALGE I CII GOLGO I I GOLGO I GOLGO I GOLGO I GALGO I GALGO I GALGO I GALGO I GALGO I G
30. Cotton_A_13/29_BGI-A2_	STANDART GIVEN ANA TANDART CANAGA CONTRACTOR AND TACK AND
38 Cotton & 18779 POLA2	
39 Cotton & 21/59 DCL42	
40 Cotton A 28046 BGL42	
41 Cotton A 34134 BGLA2	
42 Cotton A 34170 BGI-A2	C TA A G C A T T G T G A A T A A T G A A T C T T A A G G C T C T T T G T A A T G A A G C T G G A C T G C T G A A G A T G G A A C T A C T T A T A G A A
43 Cotton A 35761 BGI-A2	CTA A C C A T I G I G A T A A T G A A C T C T T A A C C C C T T I G T C G A A C C I G G A T G A
44. Gh A01G0360	CTAAGCATTGTGATAATAATGAAGTTCTTAAGGCTCTTTGTAATGAAGCTGGACTGTTGAACCTGATGGAACTACTTATAGAA
45. Gh_A03G1024	C TA A C C A T I G T A A T A A T G A A G T T C T T A A G C C T C T T G T T C T G A A C C T G G A T G G T T G T A A G A T G A A C T A C T T A T A G A A
46. Gh_A04G0814	C T A A G A G A G C T G A T C T A A T C A T C T T A A G G C T G T T G T G A G A A G C T G G A T G G A G C T G A T G A G A G C T G A T G A G A G C T G A T G A G A G C T G A T G A G A G C T G A T G A G A G C T G A T G A G A G C T G A T G A G A G C T G A T G A G A G C T G A T G A G A G C T G A T G A G A G C T G A T G A G C T G A T G A G C T G A T G A G A G C T G A T G A G C T G A T G A G C T G A T G A G C T G A T G A G C T G A T G A G C T G C T G C T G C T G C C T G A G C T G C T G C C T G C C T G C C T G C C T G C C T G C C C C
47. Gh_A05G1683	C T A A C C A T T G T G A A T A A T G A A G T T C T T A A G C C T C T T G T G C T G A A G C T G G A T G G A T G A T G A A G A T G A A G A T G A A G A T G A A G A T G A A G A A G A G
48. Gh_A05G2217	C T A A C C A T T G T G A A T A A T G A A G T T C T T A A G C C T C T T G T G C T G A A G C T G G T T G T G A A G A T G G A A C T A C T T A T A G A A
49. Gh_A06G1990	C T A A C C A T T G T G A T A A T A A T G A G T T C T T A A G G C T C T T T G T G A G G C T G G A T G G A C T A C T A C T A A G A G A T G G A A C T A C T A A G A G A T G G A A C T A C T A A G A G A T G G A A C T A C T A A G A G A T G G A A C T A C T A A G A G A T G G A A C T A C T A A G A G A T G G A C T A C T A A G A G A T G G A C T A C T A A G A G A T G G A C T A C T A A G A G A T G G A A C T A C T A A G A G A T G G A C T A C T A A G A G A T G G A C T A C T A A G A G A T G G A A C T A C T A A G A G A T G G A C T A C T A A G A G A T G G A C T A C T A A G A G A T G G A C T A C T A A G A G A T G G A C T A C T A A G A G A T G G A C T A C T A G A G A T G G A C T A C T A C T A A G A G A T G G A C T A C T A C T A C T A A G A G A T G G A C T A C
50. Gh_A08G1175	GAGTTAGAGCTGATATTAATGATGTTATTGCTGCTCTTCCTAGAAGCTGGATGGGTTGTTCTTCCTGATGGATG
51. Gh_A10G0312	CTAAGCATTGTGATAATAATAATGAAGTTCTTAAGGCTCTTTGTAATGAAGCTGGACTGTTGAAGAAGATGGAACTACTTATAGAA
52. Gh_A11G0794	GAGTTAGAGCTGATATTAATGATGTTATTGCTGCTCCTTGCTAGAGAAGCTGGGTTGTTCTTCCTGATGGAACTACTTTTCCTT
53. Gh_A11G2598	CTAAGCATTGTGATAATAATGAAGTTCTTAAGGCTCTTTGTAATGAAGCTGGATGGA
54. Gh_D01G0390	CTAAGCATTGTGATAATAATGAAGTTCTTAAGGCTCTTTGTAATGAAGCTGGATGGA
55. Gh_D02G0939	C TA AGC A TT G T G A TA A T G A AGT T C TT A AGG C TC TT T G TT C TG A AGC T G G A T G G G G T G T T G A AG A T G A T G A AC T A C T TA TA G A A
56. Gn_D04G1312	CTANDADADCIGATCIAACGACCIGITCIGAAGAAGCIGGATGGCATGIGAAGCIGATGGAACCIATTAAAGAA
57. Gh_D05G1874	
58. Ch_D05C2124	
60 Ch D08C1459	
61 Ch D1000318	
62 Ch D11C0932	
63 Gh D11G2965	CTA A C C A T G C G A T A A T C A A C T C T T A A C C T C T
64. Gh D12G1996	C T G C T A G A G C T G A T A T G A A T G A T G T T C T T G C T C C T A G A G A A G C T G G A T G G A C C T G A T G G A A C T A C T T A T A G A C
65. Gh Sca033106G01	A T G A A T G A T G T T C T T G C T G C T C T T G C T A G A G A A G C T G G A T G G A C T G A T G G A C T A C T T A T A G A C
66. Gh_Sca122277G01	GAGTTAGAGCTGATATTAATGATGTTATTGCTGCTCCTTGCTAGAGAAGCTGGATGGGTTGTTCTTCCTGATGGAACTACTTTTCCTT
67. Gh_Sca151434G01	C TAACCAT TG TG ATAATAATGAAGTTCT TAAGGCTCTTTG TAATGAAGCTGGATGGACTG TG AACCTGATGGAACTACTTATAGAA
68. Gorai.002G052400.1	C T A A G C A T T G T G A A T A A T G A A G T T C T T A A G G C T C T T G T A A T G A A G C T G G A T G G A C C T G A T G G A A C T A C T T A T A G A A
69. Gorai.002G052400.2	C T A A C C A T T G T G A A T A A T G A A G T T C T T A A G G C T C T T G T A A T G A A C C T G G A C T G T G A A C C T G A T G G A A C T A C T T A T A G A A
70. Gorai.002G052400.3	CTANGCATIGIGATAATAATGAAGTICTIAAGGCTCTIIGTAATGAAGCIGGAIGGAACCIGAIGGAACCIGAIGGAACTACTAAAAA
71. Gorai.002G052400.4	C T A A G C A T T G T A A T A A T G A A G T T C T T A A G G C T C T T T G T A A T G A A G C T G C A C T G A C C T G A A C C T G A A C T A C T A C T A C A A C T A C A A C T A C A A C T A C A A C T A C A A C A C
72. Gorai.004G158600.1	CASTINGASCIGATATIAN TONICATORICITIC TOCICITIC TAGAGAAGCIGGATGGGTTCTTCCIGATGGAACTACTATICCIT
/3. Gorai.004G158600.10	GAGINAGAGCIGA MANTAATGATGITATTCCTGCTCCTGCTGCAGAAGCTGGGTTGTTCCTGATGGAACTACTACTTTCCTT
74. Goral.004G158600.11	SASTINASASCISA TATINA ISA ISTINIISCISCICIISCIASASAKACISGATGGGITGITCIICCIGATGGAACTACTACTITICCIT
75. G0ral.004G158600.12	
77. Goral 004C 158600 14	
78 Gorai 004G158600 2	
79. Gorai.004G158600.3	GAGTTAGAGCTGATATTAATGATGTTATTGCTGCTCTTGCTAGAAAAGCTGGATGGGTTGTTCTTCCTCATAGAAGTGCTTG
80. Goral.004G158600.4	GNGTTAGAGC TCATATTAATCATCATCATCATCCTCCTCCTCCTAGAGAAGCTGGATGGGTTCTTCCTCATGGAACTACTTTCCTT
81. Gorai.004G158600.5	GAGTTAGAGCTGATATTAATGATGTTATTGCTGCTGTTGTAGAGAAGCTGGATGGGTTGTTCTTCCTGATGGAACTACTTTTCCTT
82. Gorai.004G158600.6	CAGTINGAGCIGATATTAATCATCTITATIGCIGCICTICTIGCIAGAGAAGCIGGGIIGIICIICCICATCGAACIACIIIICCII
83. Gorai.004G158600.7	GAGTTAGAGCTCATATTAATCATGTTATTCCTCCTCCTCCTAGAGCAAGCTGGGTTCTTCCTGATGGAACTACTACTTCCTT
84. Gorai.004G158600.8	CACTINGAGOTGATATINATCATCATGCTGCTCTTGCTAGAGAAGCTGGATGGGTTGTTCTTCCTGATGGAACTACTATTCCTT
85. Gorai.004G158600.9	GAGETAGAGCTGATATTAATGATGTTGTTGCTGCTGCTGCTGGTGGAGCTGGATGGGTTGTTCCTGATGGAACTACTACTTTCCTT
86. Gorai.005G107000.1	CHARGCANIGIGATAATAATGAAGTICITAAAGGCICITITGIICIGAAGCTGGATGGGTTGIIGAAGATGGTGGAAGATGGAAGA
87. Goral.007G099100.1	SASTINASASCISATATINA ISA ISTATISCISCISCISCIASASASASCISGATGGGITGTICCIGATGGAACTACTACTITICCIT
00. G01al.00/G099100.2	NON- THE REAL PARTY AND A TO THE THE TO THE TO THE TO THE AND AND THE AND A TO THE TO THE ATO A TO ATO ATO ATO ATO ATO ATO ATO AT
05. G01al.007G099100.3	
91. Gorai.007G099100.5	GAGTTAGAGCTGATATTAATGATGTTATTGCTGCTCTTGCTAGAAAACTGGGTTGGGTTGTCCTCCTCATGCAACTACTTTTGCTAGAACTGCTGGATGGGGTTGTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTC
92. Goral.007G099100.6	GAGTTAGASCTGATATTAATGATGTTATTGCTGCTCTTGCTAGAGAAGCTGGATGGGTTGTTCTTCCTGATGCAACTACTTTTCCT
93. Gorai.007G099100.7	GAGETTAGAGE TGATATTAATGATGTTATTGETGETGETGETAGAGAAGETGGGTGGG
94. Gorai.007G099100.8	GAGETTAGAGO TGATATTAATGATGTTATTGCTGCTGCTGCTAGAGAAGO TGGGTGGGTGCTTCCTGATGGAACTACTTTTCCTT
95. Goral.007G327000.1	CTAAGCATTGTGATAATAATGAAGTTCTTAAGGCTCTTTGTAATGAAGCTGGACGGCTGTGGACCTGATGGAACCTGATGGAACTACTTATAGAA
96. Gorai.008G218300.1	CTGCTAGAGCTGATATGAATGTATGTTCTTGCTGCTCTTGCTAGAGAGCTGGACTGTTGAACCTGATGGAACCTGAAGCTACTACTATAGAC
97. Gorai.008G218300.2	CTGCTAGAGCTCATATGATGTTCTTCCTCCTCCTCCTAGAGCAGCTGGACTGGACTGATGGACCTGATGGAACTGATGGAACTACTAATAGAC
98. Gorai.008G218300.3	CTGCTAGAGCTGATATGATGTTCTTCCTCCTCCTGCAGGAGCTGCAGCTGCACCTGATGGAACCTGATGGAACCTGATGGAACTACTAAAGAC
99. Gorai.008G218300.4	CTGCTAGAGCTGATATGATGTTCTTCCTGCTGCTGCTAGAAGCTGCAGGAAGCTGGACTGATGAACCTGATGAACTAGAACTACTAAAGAC
100. Gorai.009G204600.1	C TARGE A TEGER TARTA TEA TEA STICTIAN GEORETICITE TEA A GETEGETEGETEGETEGETEGETEGETEGETEGETEGET
101. Gorai.009G204600.2	CTARGCATTGTGATAATAATGAAGTTCTTAAGGCTCTTTGTGTTGAAGCTGGATGGTTGTTGAAGATGATGATGAAGA
102. Gorai.009G274000.1	CINANGCALIGIGA MANTANTGANGTICITANGGCTCTTTGTGCTGANGCTGGGTTGGTGGAGAAGATGAAGAT
103. Goral.010G240700.1	CTARSCALLS IGA TAATAA TGAAGATCTTTAASSCTCTTTTGTGATGAAGCTGGATGGACTACTACTTATAAGA
104. G0rai.010G240700.2	STANSYA I GI'SA NAA TAA I GAAGHIC HAAG SC ICCHI I GI GAAG I GGAAC TOR TO AAGAAGA TGGAAC TA CTTATAAGA
105. G0rai.011G036300.1	C THE COLOR TO THE
107. Gorai 0116036300.2	
108 Gorai 011G036300.4	
109. Gorai 012G118900.1	C TA A G A G A G C T G A T C T A A T C A T C T T C A T A A G C T G T A T G A A G C T G G A T G C T C A T G C T A T T T T A T A G A A G C T G A T G C T C A T G C T A T T T T A T A G A A G C T G A T G C T C A T G C T A T T T T A T A G A A G C T G A T G C T G A T G C T C A T G C T A T T T T A T A G A A G C T G A T G C T G C A T G C T G

Figure 4. The alignment of 109 BES1 genes among the 3 Arabidopsis and 3 Gossypium species. The stars on the top of columns means conserved nucleotide. TG motif is highlighted in yellow.

In an intriguing discovery, the four clusters of common BES1 genes shared between Arabidopsis and Gossypium were categorized into two distinct groups: β-amylase and BES1. Specifically, there were two clusters associated with each of these gene families (as indicated in Table 2). Notably, BMY7 and BMY8 exhibit attributes of BES1 TFs, consistent with previous reports by (Reinhold et al., 2011). When overexpressed in plants, BMY7 and BMY8 enhance tolerance to drought, heat, and Conversely, osmotic stress. plants with overexpressed BES1 genes also demonstrate resilience to these environmental challenges. The fascinating aspect lies in the polyvalent nature of BES1 and BMY genes, driven by their shared functional and regulatory motifs. This dual functionality allows them to respond differentially in various contexts, depending on the plant's requirements. BMY genes, acting as β-amylases, help balance starch content and produce maltose as a signaling sugar. Meanwhile, BES1 TFs regulate

gene expression and facilitate crosstalk among phytohormones and other TFs (**Song et al., 2018**).

In Figure 5, G. arboreum genes exhibit greater proximity to the Arabidopsis clade compared to the two other Gossypium species. Meanwhile, G. hirsutum genes are partially situated within their own clade. Notably, G. raimondii genes form a cohesive cluster, except for the outlier Gorai.008G218300.2. The Arabidopsis species follow a similar pattern: A. halleri and A. lyrata share a branch, while A. thaliana occupies a separate branch. Interestingly, A. halleri and A. lyrata are more closely related to each other than to A. thaliana, suggesting distinct evolutionary patterns in A. thaliana compared to the other two species. Furthermore, among the 28 BAM7 genes, three G. hirsutum genes exhibit varying relationships: one closely resembles G. arboreum, another is similar to G. raimondii, and a third stands alone in a distant clade. Additionally, Cluster 2 encompasses 11 BAM8 genes (Figure 6).



Figure 5. The Venn diagram for the BES1 TF / BAM7/8 comparing three species of Arabidopsis with three species of Gossypium.

#### Table 2

The four common clusters among all 6 species. GO terms are presented for each cluster. In total 39 genes clustered as BAM and 18 genes as BES1 while the inputs were known as BES1

ID	No. of proteins	Swiss-Prot Hit	GO Annotation
Cluster 1	28	B-amylase 7 O80831	GO:0005737; C:cytoplasm; GO:0005634; C:nucleus; GO:0016161; F: $\beta$ -amylase activity; GO:0003700; F:sequence-specific DNA binding transcription factor activity; GO:0000272; P:polysaccharide catabolic process; GO:0048831; P:regulation of shoot system development; GO:0006355; P:regulation of transcription, DNA-templated
Cluster 2	11	B-amylase 8 Q9FH80	GO:0005737; C:cytoplasm; GO:0005634; C:nucleus; GO:0016161; F:β-amylase activity; GO:0003700; F:sequence-specific DNA binding transcription factor activity; GO:0000272; P:polysaccharide catabolic process; GO:0048831; P:regulation of shoot system development; GO:0006355; P:regulation of transcription, DNA-templated;
Cluster 3	9	Protein BRASSINAZOLE- RESISTANT 1 Q8S307	GO:0005829; C:cytosol; GO:0005634; C:nucleus; GO:0003677; F:DNA binding; GO:0003700; F:sequence-specific DNA binding transcription factor activity; GO:0009742; P:brassinosteroid mediated signaling pathway; GO:0045892; P:negative regulation of transcription, DNA-templated; GO:0048481; P:ovule development; GO:0040008; P:regulation of growth; GO:0006355; P:regulation of transcription, DNA-templated; GO:0006351; P:transcription, DNA-templated; GO:0048481; P:ovule development; GO:0048481; GO:0048316; P:seed development; GO:0006351; P:transcription, DNA-templated;
Cluster 4	9	BES1/BZR1 homolog protein 4 Q9ZV88	GO:0003677; F:DNA binding; GO:0006355; P:regulation of transcription, DNA-templated; TAS:TAIR; GO:0006351; P:transcription, DNA-templated



Figure 6. Gene tree for cluster 1 including 28 genes of BAM7 (above) and cluster2 with 11 genes of BAM8 (below).

In the context of BES1 clusters, as depicted in **Figure** 7, a consistent pattern emerges among *Arabidopsis* species: *A. lyrata* and *A. halleri* share a branch, while *A. thaliana* occupies a distinct branch. Notably, all *G. raimondii* genes cluster together within a single clade. In contrast, *G. hirsutum* genes form a separate clade, except for Gh\_A05G1683, which aligns with the *G. arboreum* clade. These findings align with those observed for BAM genes, suggesting that these specific genes may serve as representative markers for evolutionary events within the studied species.

Among the motif within Cluster 1, which comprises 28 genes (as illustrated in **Figure 8**), recurring occurrences of the amino acid glycine (G) in the peptide sequences was observed. Glycine stands out due to its unique properties: it lacks a chiral carbon and possesses a hydrogen side chain (unlike

other amino acids, which feature carbon-based side chains). Consequently, glycine exhibits greater conformational flexibility. This intriguing observation leads us to hypothesize that glycine may play a role in the functionality of amylases. Notably, glycine is highly abundant among the 39 genes of both BAM7 and BAM8. Additionally, another recurring amino acid residue: glutamic acid (E) was identified. Glutamic acid is essential for  $\alpha$ -amylase activity, as previously reported by (MacGregor et al., 2001). It is speculated that a similar pattern may exist for βamylase. Furthermore, both L-glycine and Lglutamic acid are known to be effective chelating agents in proteins, aligning with existing literature. These findings highlight the importance of further investigation to ascertain whether these amino acid residues are involved in the dual functionalities of BES1 and BAM.







Figure 8. The most conserved motif of cluster1. It is rich of E and G.

## 4. Conclusions

The BES1 and BAM7/8 gene families share common domains, which makes them crosstalk elements capable of regulating gene expression and the function of downstream genes. Initially, their similar stress response patterns in plants suggested that they belonged to the same group due to their common binding domain and conserved motifs. However, recent findings indicate that they belong to two distinct gene groups. Despite this separation, their shared domains allow them to collaborate with each other. This study disclosed that these genes are known as BES1 but they share highly similar domains with BAM7 and BAM8. Therefore, motifs and BES1/BAM-based family are the same suggesting these genes are multifunctional.

Interestingly, BES1 and BAM7/8 directly generate maltose, a sugar that may function as a regulatory signal and play a role in plant responses to stress, growth, and development processes. In the context of Arabidopsis and Gossypium (cotton), a specific domain—G box GCTGGATGG—and repeats of TG have been identified in all 109 BES1 genes. This domain can be used for marker-assisted selection in Gossypium species. Notably, G. hirsutum exhibits less kinship, while G. arboreum and G. raimondii are closer species. This research proposes a similar structure and function for the RK repeats in plant βamylase, potentially impacting enzyme activity. However, this connection needs further investigation by functional genomics and molecular biology to disclose their functionality.

Given their regulatory functionality in cellulose and fiber production, BES1/BAM members are promising candidate genes for further analysis in *Gossypium*. Additionally, in *Arabidopsis* species, BES1 and BAM7/8 relationships reveal that *A. halleri* and *A. lyrata* are more closely related to each other than to *A. thaliana*. The precise bifunctionality of the BES1/BAM complex remains to be fully uncovered.

#### Declaration of conflict of interest

The authors have no conflicts of interest to declare.

#### Author Statement

R. O. Villamar-Torres: Conceptualization, Data curation, Methodology, Visualization, Writing – original draft, Writing – review & editing, C. A. Mestanza Uquillas: , H. D. Chévez-Vera: Investigation, Validation, Visualization, M. R. Heredia-Pinosi: Investigation, Validation, Visualization, C. R. Viot. Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, S. M. Jazyeri: Conceptualization, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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