Functional characterization of *Cs*BGlu12, a β-glucosidase from *Crocus sativus* provides insights into its role in abiotic stress through accumulation of antioxidant flavonols

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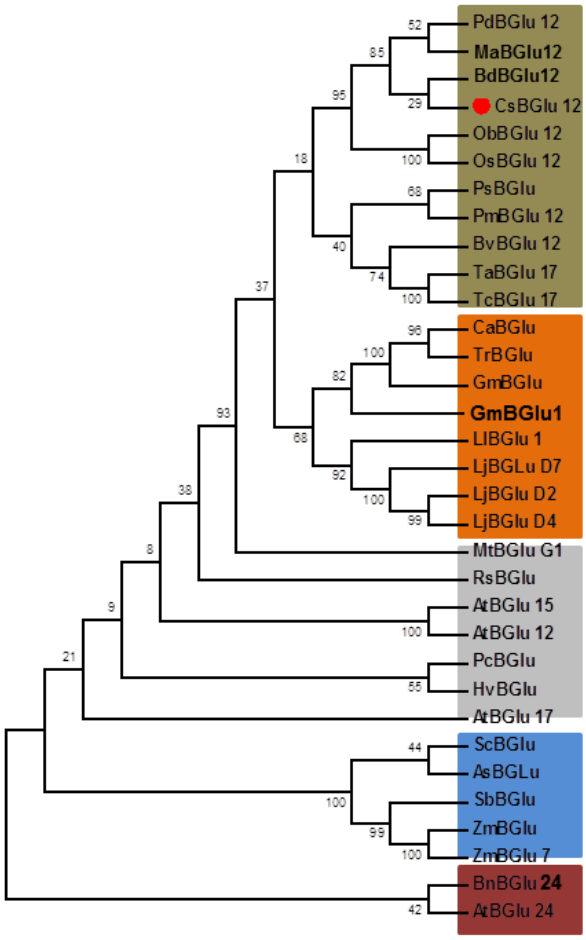
Supplemental Information

Supplemental Table S1: List of substrates used for docking analysis, free energy change (ΔG) for best pose of the enzyme-ligand complex

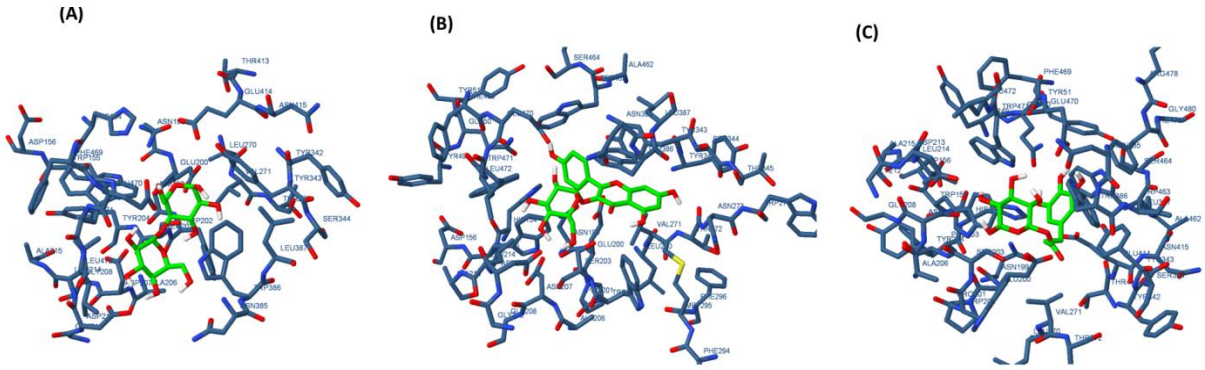
Substrates	Free energy change (ΔG) kcal/mol
Cellobiose	-8.71
Cellotriose	-7.65
Cellotetraose	-6.34
Cellopentose	-6.54
Kaempherol 3-*O*-β-glucoside	-8.71
Quercetin 3-*O*-β-glucoside	-7.01
Naringenin 7-*O* -β-glucoside	-5.8
Iridin	-4.7
1-*O*-Sinopyl-β-D-glucoside	-3.11
Esculin	-2.1
Leucoseptoside	-1.98
Acteoside	-1.76
Martynoside	-1.82
Picrocrocin	-1.32

Supplemental Table S2. List of primer sequences used in the study.

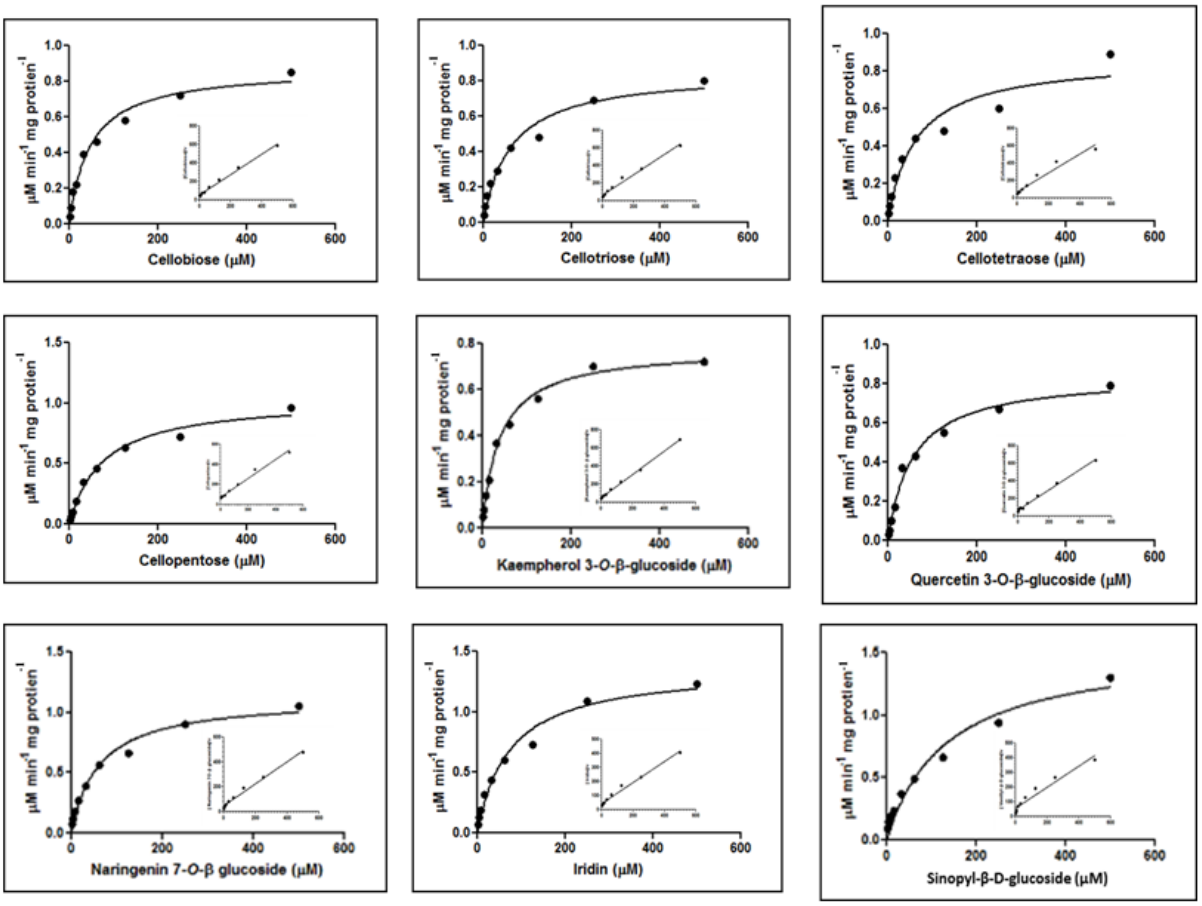
Name of Primer	Sequence of Primer (5′ to 3′)
Full Length primers	
CsBGlu-F	ATGGGGTTCGCTACATCTCTC
CsBGlu-R	TTCTATTTCTTCAGAAATTTC
Primers for semi-quantitative PCR	
CsSBG-F1	TCACCTTGAACGAACCGTGGA
CsSBG-R1	CCTTGGTGTAGAGCAAG
Primers for qRT-PCR	
QRT-F	TGAACGAACCGTGGAG
QRT-R	CAGTGTGATGCCTATT
Primers for bacterial expression	
CsBGlu-PGex-F	GTCGACTTATGGGGTTCGCTACATCTCTC
CsBGlu-PGex-R	GCGGCCGCTTCTATTTCTTCAGAAATTTC
Primers for site directed mutagenesis	
M1-F	ACCTTGAACGCCCCGTGGAG
M1-R	CTCCACGGGGCGTTCAAGGT
M2-F	TACATCACAGCCAATGGTGTC
M2-R	GACACCATTGGCTGTGATGTA
Primers for PBI121 cloning	
CsBGluPBI-F	AATCTAGAATGGGGTTCGCTACATCTCTC
CsBGluPB1-R	AAGGAGCTCTATTTCTTCAGAAATTTC
Primers for PAM-PAT-35S-YFP	
YFP-F	GAGCTCATGGGGTTCGCTACATCTCTC
YFP-R	AAGCTTGTCTATTTCTTCAGAAATTTC
18S primers	
18S-F	GTAACCCGTTGAACCCCATT
18S-R	CCATCCAATCGGTAGTAGCG
GAPDH primers	
GAPDH-F	ATAGCTTATTATTAGACGGA
GAPDH-R	CTCAAGGGAATCATGGGCT



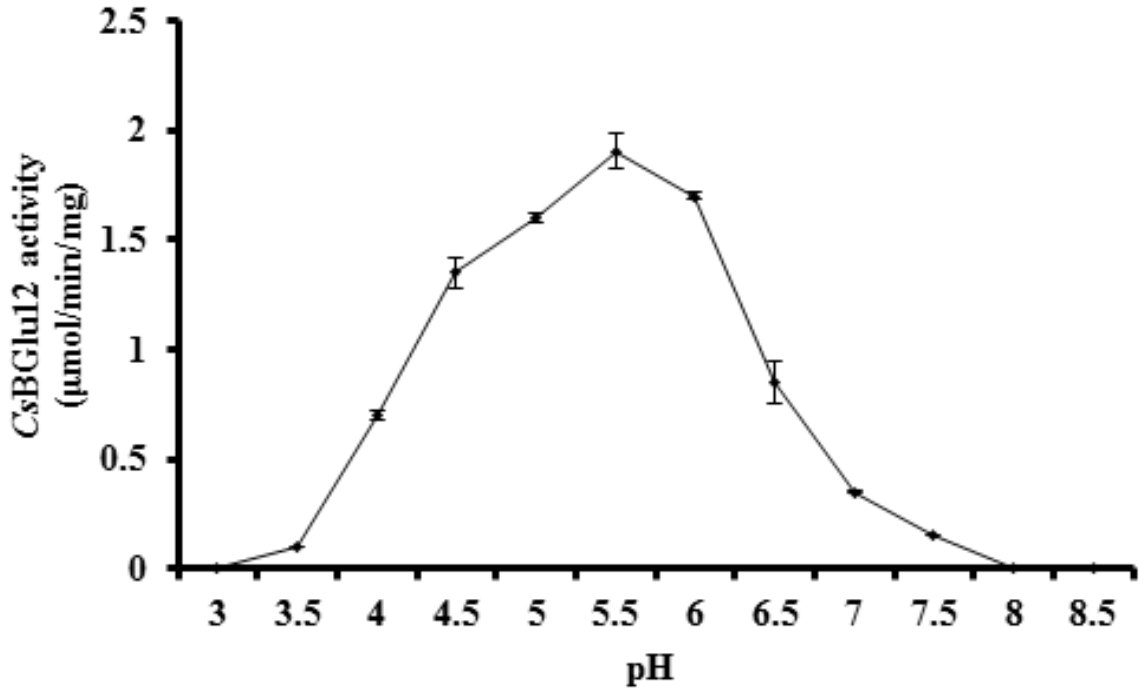
Supplemental Figure S1: Phylogenetic tree of *Cs*BGlu12. The phylogenetic tree was developed using the Neighbor-Joining method by MEGA software (Tamura et al., 2013). The protien sequences used for the analysis include *Crocus sativus Cs*BGlu12 (KX790358), *Medicago tranculata Mt*BGluG1 (CM001222.2), *Arabidopsis thaliana At*BGlu15 (O64879.1), *At*BGlu17 (O64882.1), *At*BGlu12 (Q9FH03.1), *At*BGlu24 (Q9LKR7.2), *Lotus Japonica Lj*BGluD2 (ACD65510.1), *Lj*BGluD4 (ACD65509.2), *Lj*BGluD7 (ACD65511.1) , *Glycine max Gm*BGlu (XP_006590951.1), *Gm*BGlu1 (BAF34333.1) *Leucaena leucocephala Ll*BGlu1 (EU328158.1), *Phoenix dactylifera Pd*BGlu12 (XP_008775422), *Brachypodium distachyon Bd*BGlu12 (XP_003563902), *Oryza brachyantha Ob*BGlu12 (XP_006656039), *Musa acuminata Ma*BGlu12 (XP_009410330), *Theobroma cacao* Tc*BGlu17 (XP_007014814), *Prunus mume Pm*BGlu12 (XP_008230315), *Beta vulgaris Bv*BGlu12 (XP_010673565), *Brassica napus Bn*Glu24 (CAA42775.1), *Zea mays Zm*BGlu (CAA52293.1), *Cicer arietinum Ca*BGlu (CAG14979.1), *Trifolium repens Tr*BGlu (CAA40058.1), *Prunus serotina Ps*BGlu (AAF34650.1), Rauvolfia *serpentina Rs*BGlu (CAC83098.1), *Pinus contorta Pc*BGlu (AAC69619.1), *Sorghum bicolor Sb*BGlu (AAC49177.1), *Secale cereale Sc*BGlu (AAG00614.1), *Avena sativa As*BGlu (AAD02839.1), *Hordeum vulgare Hv*BGlu (AAA87339.1) and *Oryza sativa Os*4BGlu12 (Q7XKV4.2).



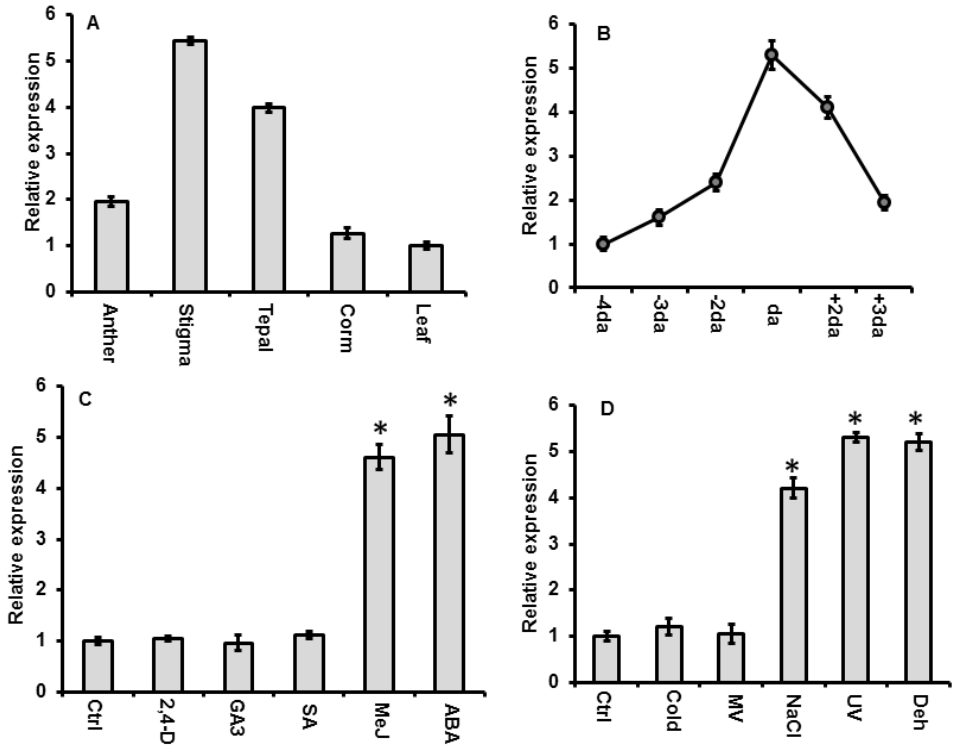
Supplemental Figure S2: Docking analysis of CsBGlu12. The analysis was carried out with all the substrates using DockingServer (http://www.dockingserver.com). However, only representative substrates are shown here which docked in the active site of CsBGlu12 (A) cellobiose (B) Kaempherol 3-O-β glucoside and (C) 1-O-sinopyl-β-D-glucoside.



Supplemental Figure S3: Michaelis-Menten and Hanes-Woolf plot analyses of *Cs*BGlu12 activity. Activity was measured using different substrates at varying substrate concentrations (1.9 to 500 µM). For each substrate, the main graph represents the velocity versus substrate concentration; inside is a Hanes-Woolf plot of the mean values. All values are means of three replicates ± SD



Supplemental Figure S4: Dependence of recombinant *Cs*BGlu12 activity on assay pH. Activity was measured using assay in presence of 500 µM cellobiose and a 15 min incubation period. For the pH profile, assays were buffered in 100 mM universal buffer and pH 3-8.5 with 0.5 pH unit increments. All values represent the mean ± SD of three independent determinations using a typical recombinant *Cs*BGlu 12.



Supplemental Figure S5: Expression pattern of CsBGlu12. Expression of CsBglu12 was determined using quantitative real time PCR (A) different tissues (B) different developmental stages of flower, four days before anthesis (-4da), three days before anthesis (-3da), two days before anthesis (-2da), day of anthesis (da), two days after anthesis (+2da) and three days after anthesis (C) hormonal treatments, Ctrl (control), MeJ (methyl jasmonate), 2,4-D (2, 4-dichlorophenoxyacetic acid), ABA (abscisic acid), SA (salicylic acid), GA3 (gibberellic acid) (D) stress treatments, NaCl (sodium chloride), UV (ultraviolet-B), MV (methylviologen), Dehyd (dehydration) and cold (4◦C). GAPDH was used as endogenous control. Experiment was done in triplicates.

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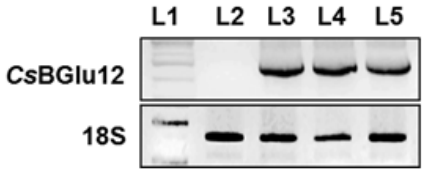
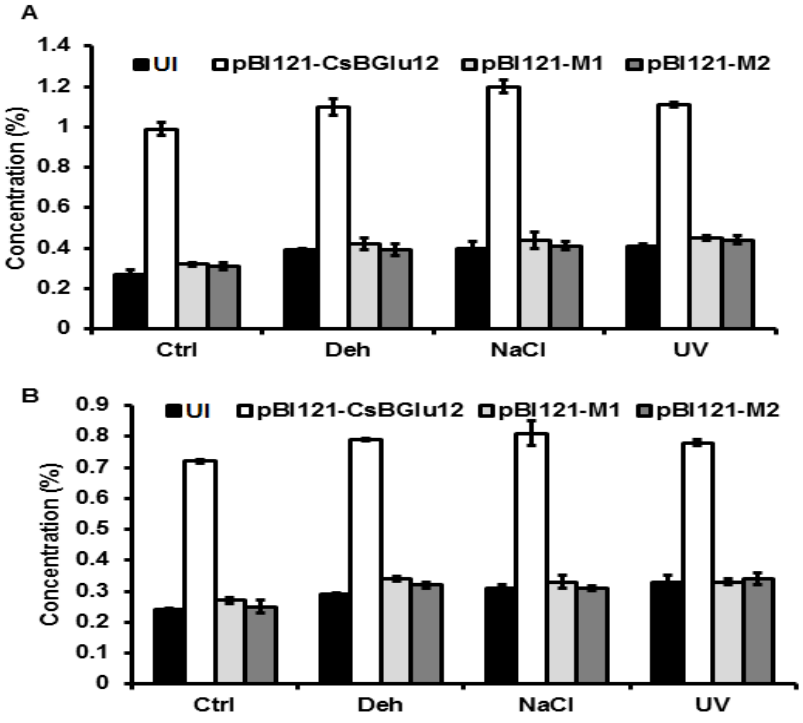


Supplemental Figure S6: Semiquatitative RT- PCR of CsBGlu12 in transient overexpression lines of *N. Benthamiana.*, L1: Marker; L2: uninoculated (UI); L3: CsBGlu12 overexpression line; L4: M1 overexpression line and L5: M2 overexpression line. The reaction mixture for each sample contained 10 mM Tris HCl pH 9.0, 50 mM KCl, 2.5 mM MgCl2, 200 mM dNTP, 1 mM primers (Table S1), 25 ng of cDNA template, and 0.5 U of Taq DNA polymerase (Fermentas). The PCR conditions were as follows: one cycle 95ºC for 1 min, 27 cycles of 94ºC for 30 s, 57ºC for 30 s and 72ºC for 1.5 min. The samples were run on 1.5% agarose gel and visualized under gel doc (Bio-Rad).



Supplemental Figure S7: Quantification of flavonols (a) Kaempferol (B) Quercetin in *N. benthamiana* plants subjected to different stress conditions: NaCl (sodium chloride), UV (ultraviolet-B), MV (methylviologen), Dehyd (dehydration) and cold (4◦C).