*Supplementary Material*

# High-Level Production of Recombinant Snowdrop Lectin in Sugarcane and Energy cane

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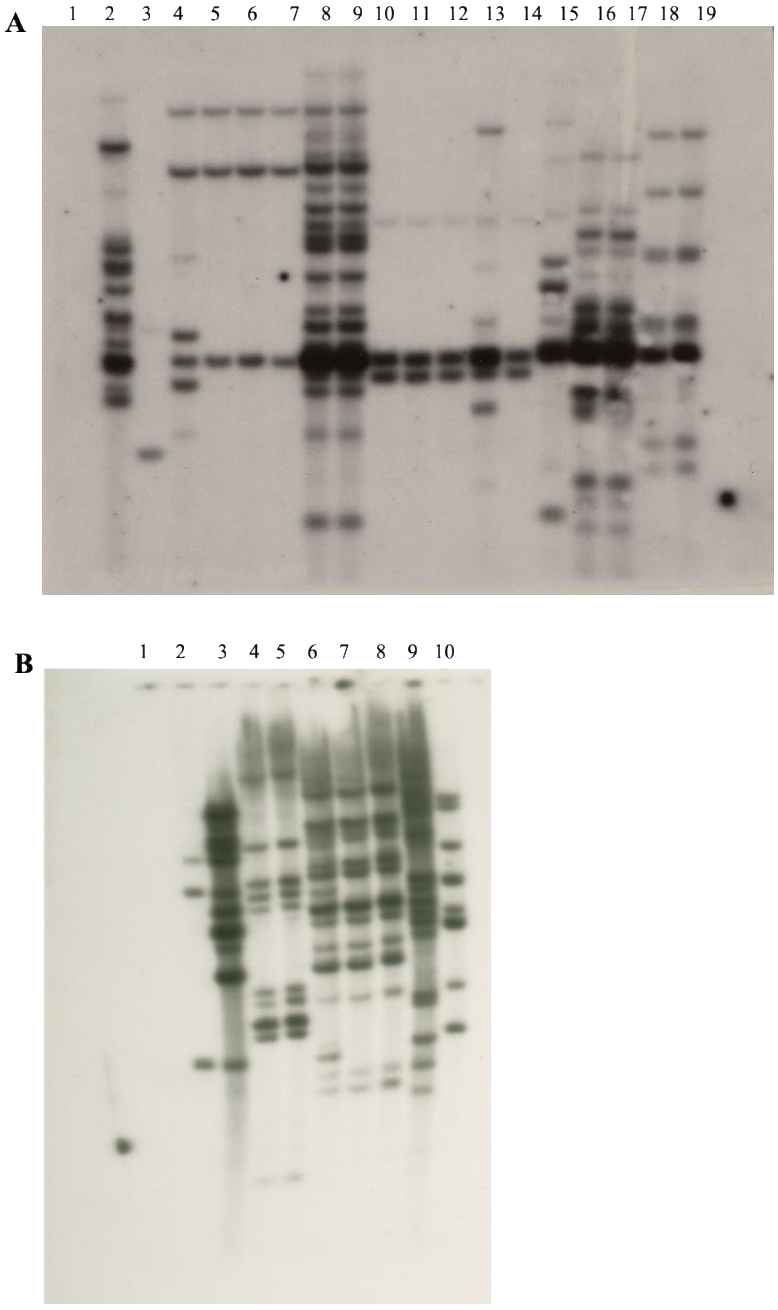
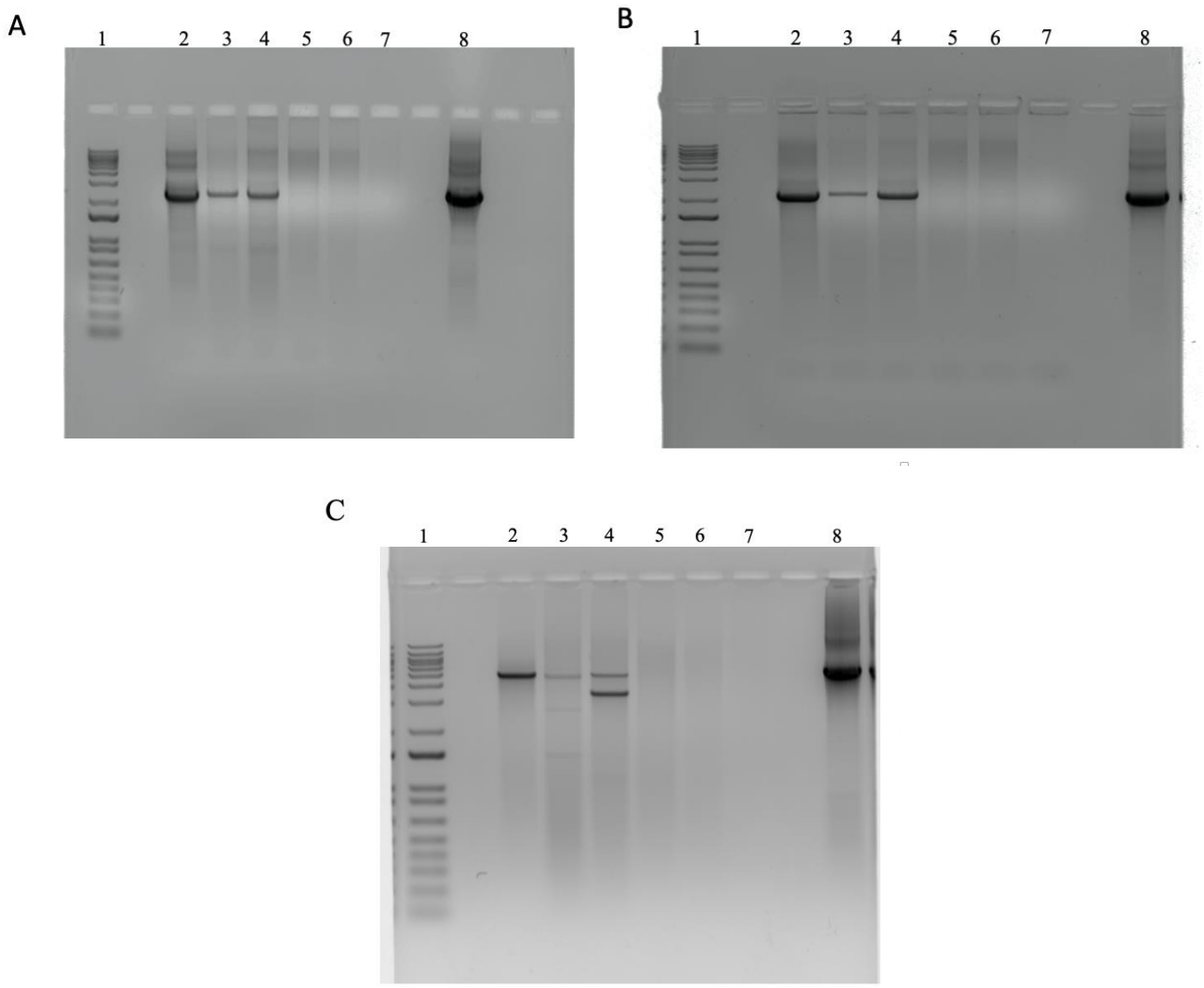
**Running title:** Recombinant therapeutic snowdrop lectin from *Saccharum* spp.

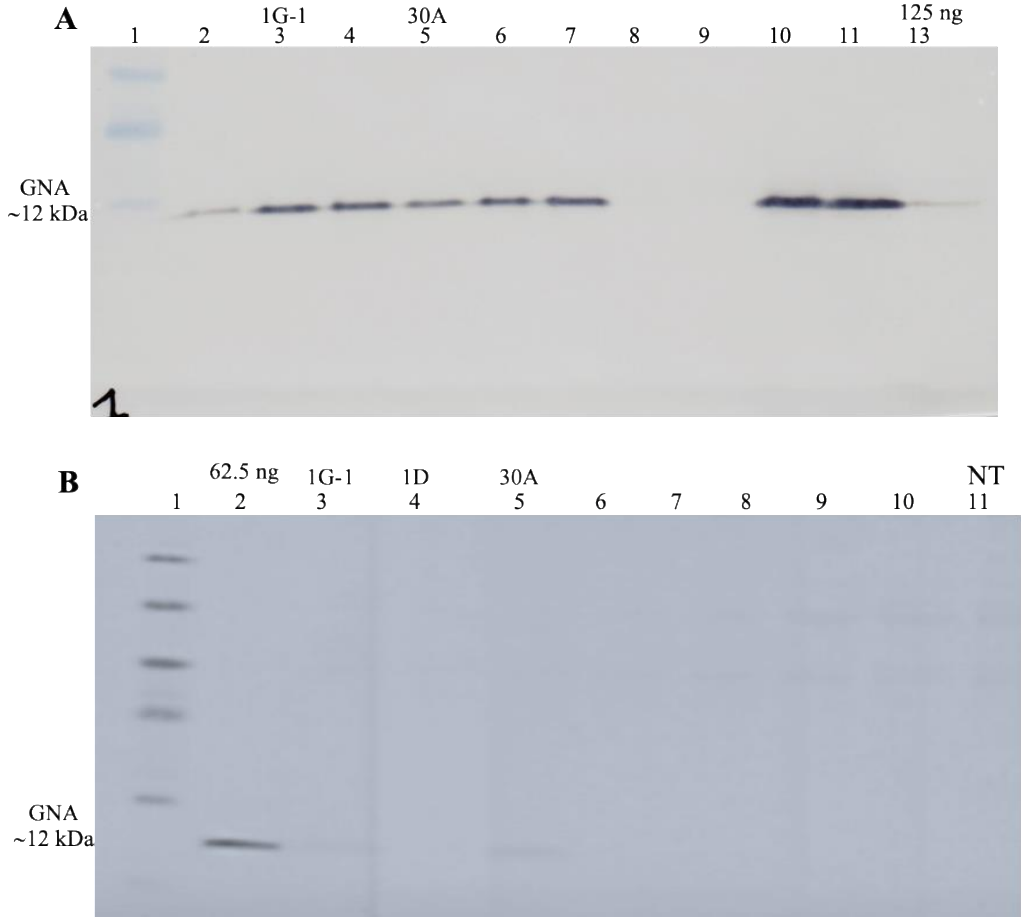



**Supplementary Figure S1. (A)** and (**B**) Full-length uncropped DNA gel blot autoradiograms used to prepare Southern blots in FIGURE 1B and FIGURE 2B, respectively. **(A)** Single-promoter *pUbi:GNA* lines 1G-1: lane 8, 1D: lane 5 and 30A: lane 18. **(B)** Triple-promoter *pUBD5-1:GNA* lines 1-2T: lane 3, 5-1G: lane 7 and 5-3C: lane 4. Non-transformed control plant: lane 1 in both blots. (**A**) and (**B**) Full-length uncropped DNA gel blot autoradiograms used to prepare Southern blots in FIGURE 1B and FIGURE 2B, respectively.

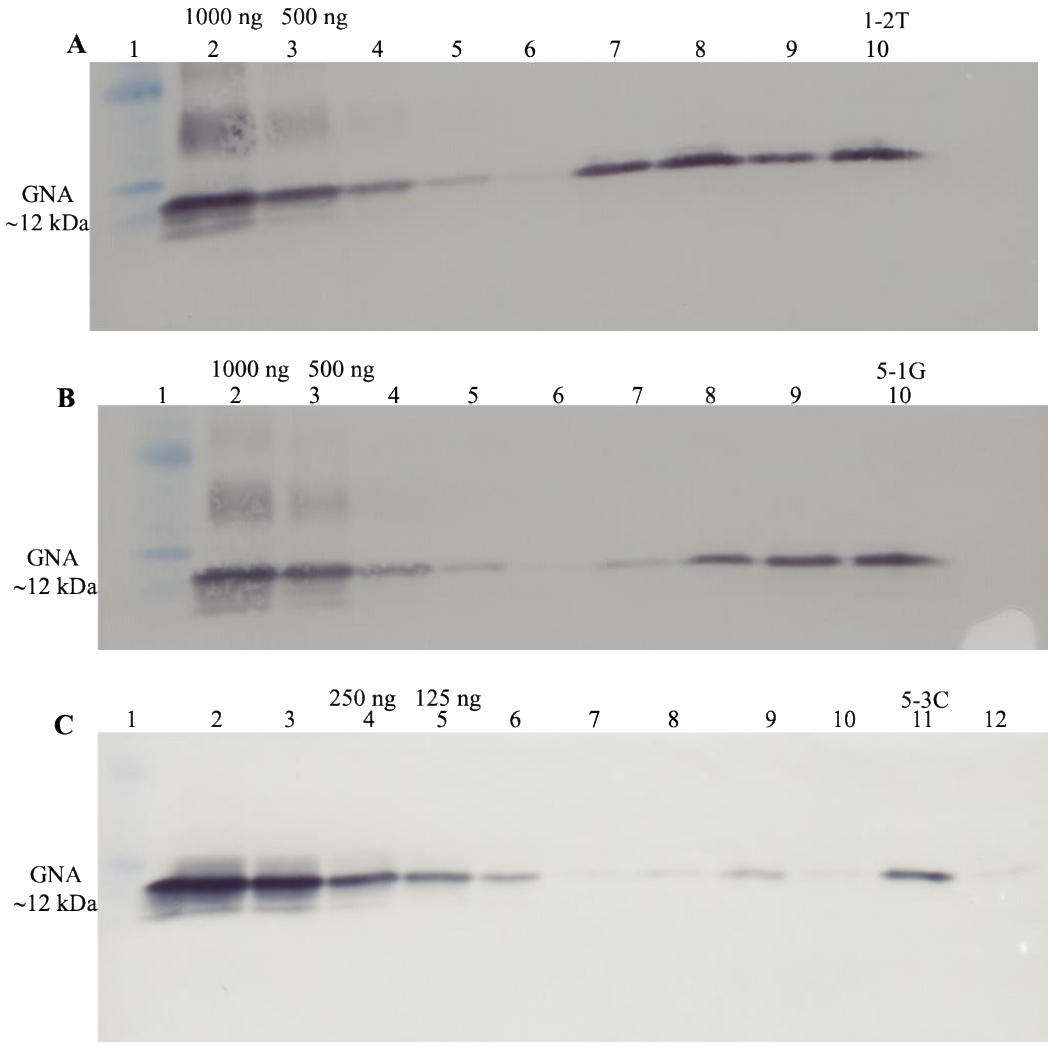

**Supplementary Figure S2.** Qualitative phenotype of the triple-promoter:*GNA*-expressing sugarcane lines. One-year-old mature plants are shown. GNA: *GNA* transgenic plants; WT: wild type plants.



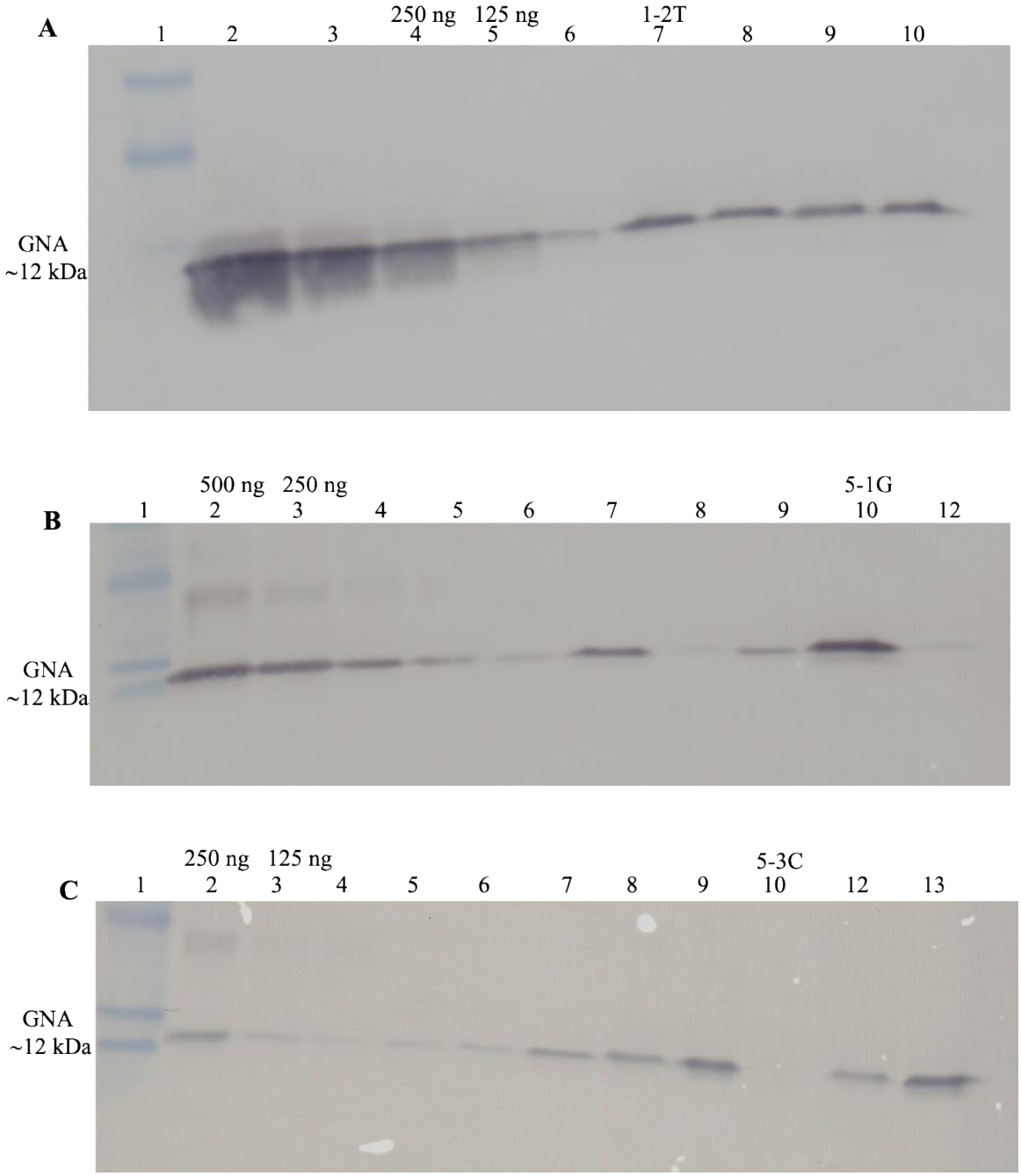

**Supplementary Figure S3. (A), (B) and (C)** Uncropped agarose gel used to prepare FIGURE 2C. **(A)** shows Integration of *pUbi:GNA* expression cassettes: lane 1, 1 kb DNA plus ladder; lane 2, *pUBD5-1:GNA* line 1-2T; lane 3, *pUBD5-1:GNA* line 5-1G; lane 4, *pUBD5-1:GNA* line 5-3C; lanes 5 and 6, non-transformed (NT) CP72-1210 and CP89-2143, respectively; lane 7, NC-no DNA template and lane 8, *pUbi:GNA* plasmid. **(B)** shows Integration of *pSCBV21:GNA* expression cassettes: lane 1, 1 kb DNA plus ladder; lane 2, *pUBD5-1:GNA* line 1-2T; lane 3 *pUBD5-1:GNA* line 5-1G; lane 4, *pUBD5-1:GNA* 5-3C; lanes 5 and 6, NT CP72-1210 and CP89-2143, respectively; lane 7, NC-no DNA template and lane 8, *pSCBV21:GNA* plasmid. **(C)** shows Integration of *pSHDIR5-1:GNA* expression cassettes: lane 1, 1 kb DNA plus ladder; lane 2, *pUBD5-1:GNA* line 1-2T; lane 3 *pUBD5-1:GNA* line 5-1G; lane 4, *pUBD5-1:GNA* line 5-3C (full and truncated amplicons); lanes 5 and 6, NT CP72-1210 and CP89-2143, respectively; lane 7, NC-no DNA template and lane 8, *pSHDIR5-1:GNA* plasmid.



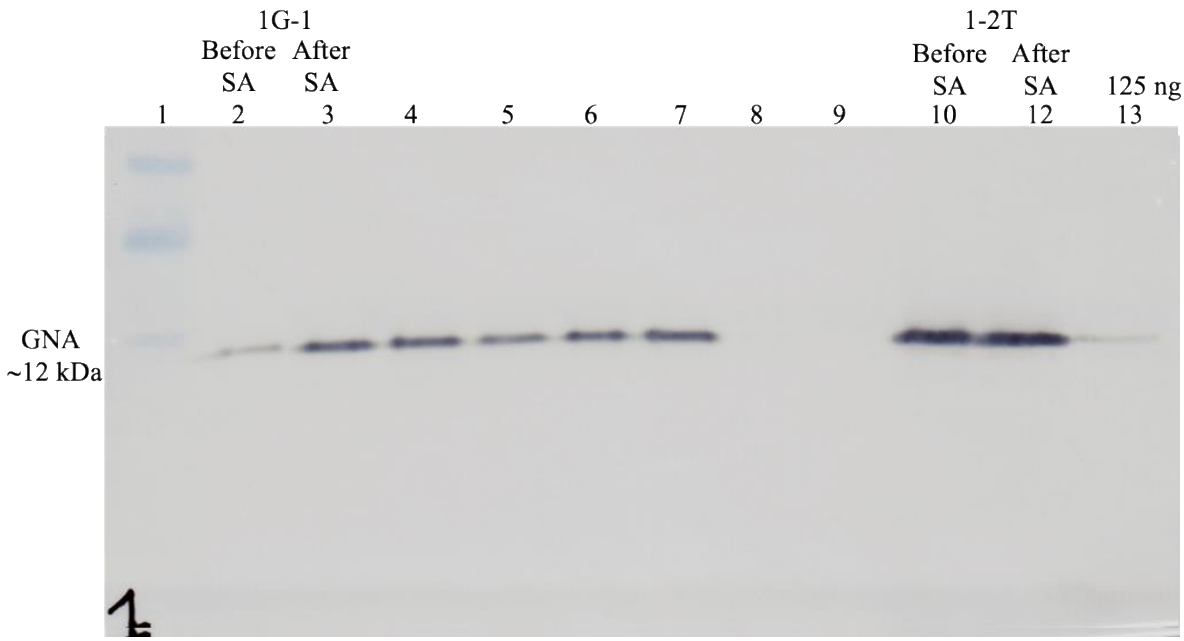

**Supplementary Figure S4. (A)** and (**B**) Full-length uncropped immunoblot membranes used to prepare FIGURE 1C and 1D. **(A)** shows uncropped immunoblot used to prepare FIGURE 1C (leaves) single-promoter *pUbi:GNA* lines 1G-1: lane 3 and 30A: lane 5 and GNA standard 125 ng: lane 13. **(B)** shows uncropped immunoblot used to prepare FIGURE 1D (culms) single-promoter *pUbi:GNA* lines 1G-1: lane 3, 1D: lane 4 and 30A: lane 5 and non-transformed (NT): lane 11.



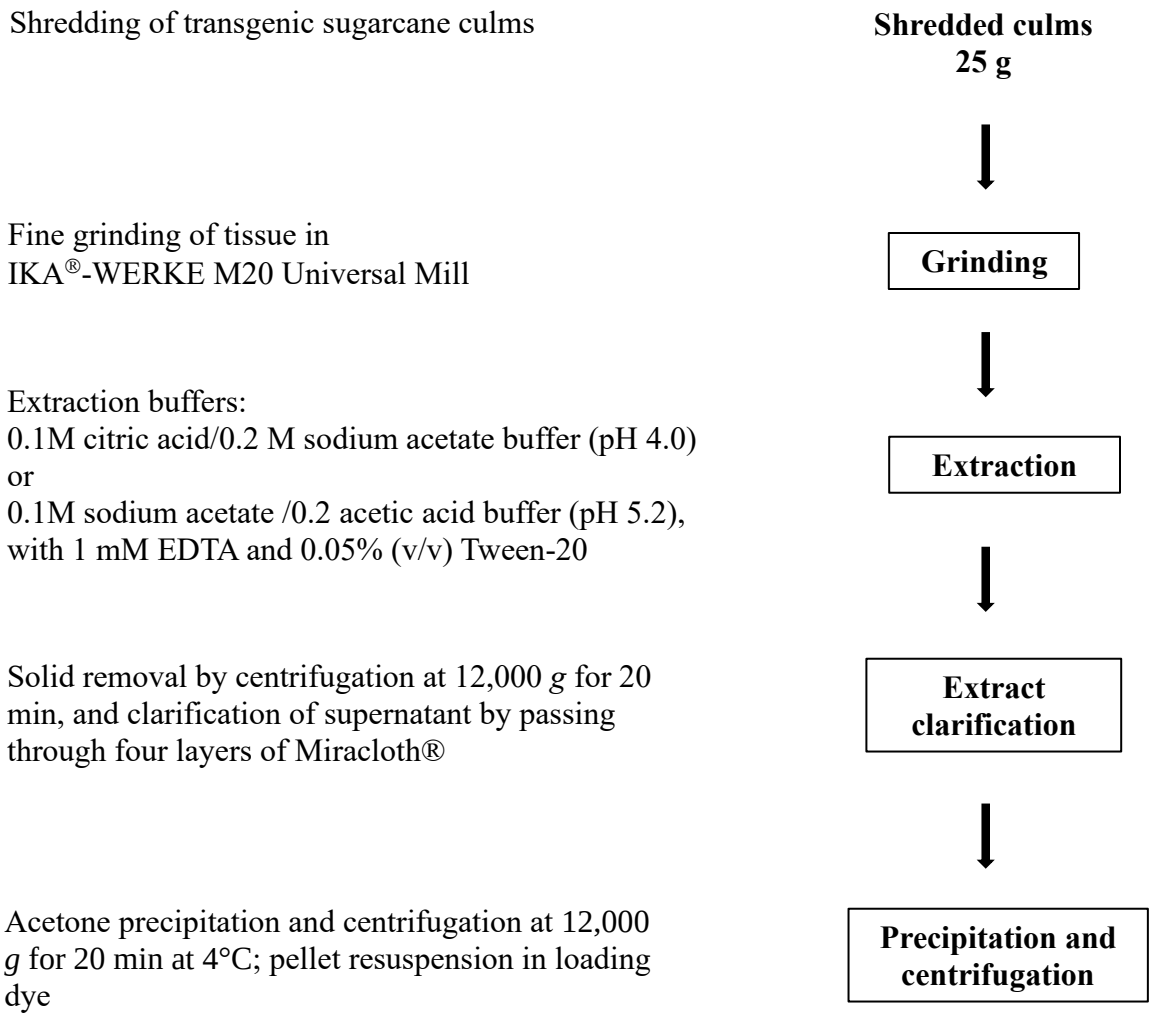

**Supplementary Figure S5. (A), (B)** and (**C**) Full-length uncropped immunoblot membranes used to prepare FIGURE 2D (Immunoblot for leaf samples). **(A)** shows GNA standard 1000 ng and 500 ng: lanes 2 and 3, respectively, and triple-promoter *pUBD5-1:GNA* line 1-2T: lane 10. **(B)** shows GNA standard 1000 ng and 500 ng: lanes 2 and 3, respectively, and triple-promoter *pUBD5-1:GNA* line 5-1G: lane 10. **(C)** shows GNA standard 250 ng and 125 ng: lanes 4 and 5, respectively, and *pUBD5-1:GNA* line 5-3C: lane 11.



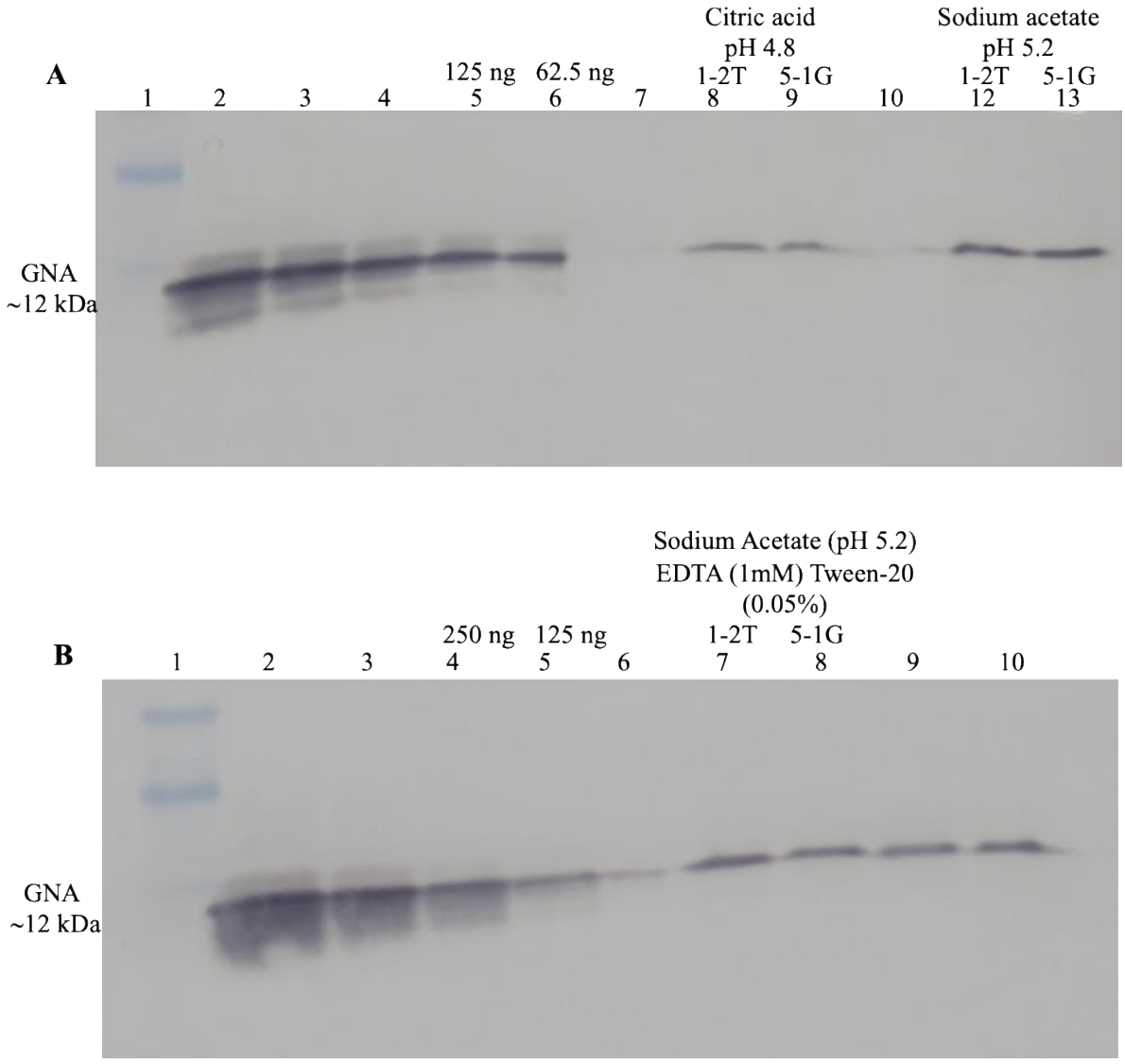

**Supplementary Figure S6. (A), (B)** and (**C**) Full-length uncropped immunoblot membranes used to prepare FIGURE 2E (Immunoblot for culm samples). **(A)** shows GNA standard 250 ng and 125 ng: lanes 4 and 5, respectively, and triple-promoter *pUBD5-1:GNA* line 1-2T: lane 7. **(B)** shows GNA standard 500 ng and 250 ng: lanes 2 and 3; respectively, triple-promoter *pUBD5-1:GNA* line 5-1G: lane 10. **(C)** shows GNA standard 250 ng and 125 ng: lanes 2 and 3; respectively, and triple-promoter *pUBD5-1:GNA* line 5-3C: lane 10.



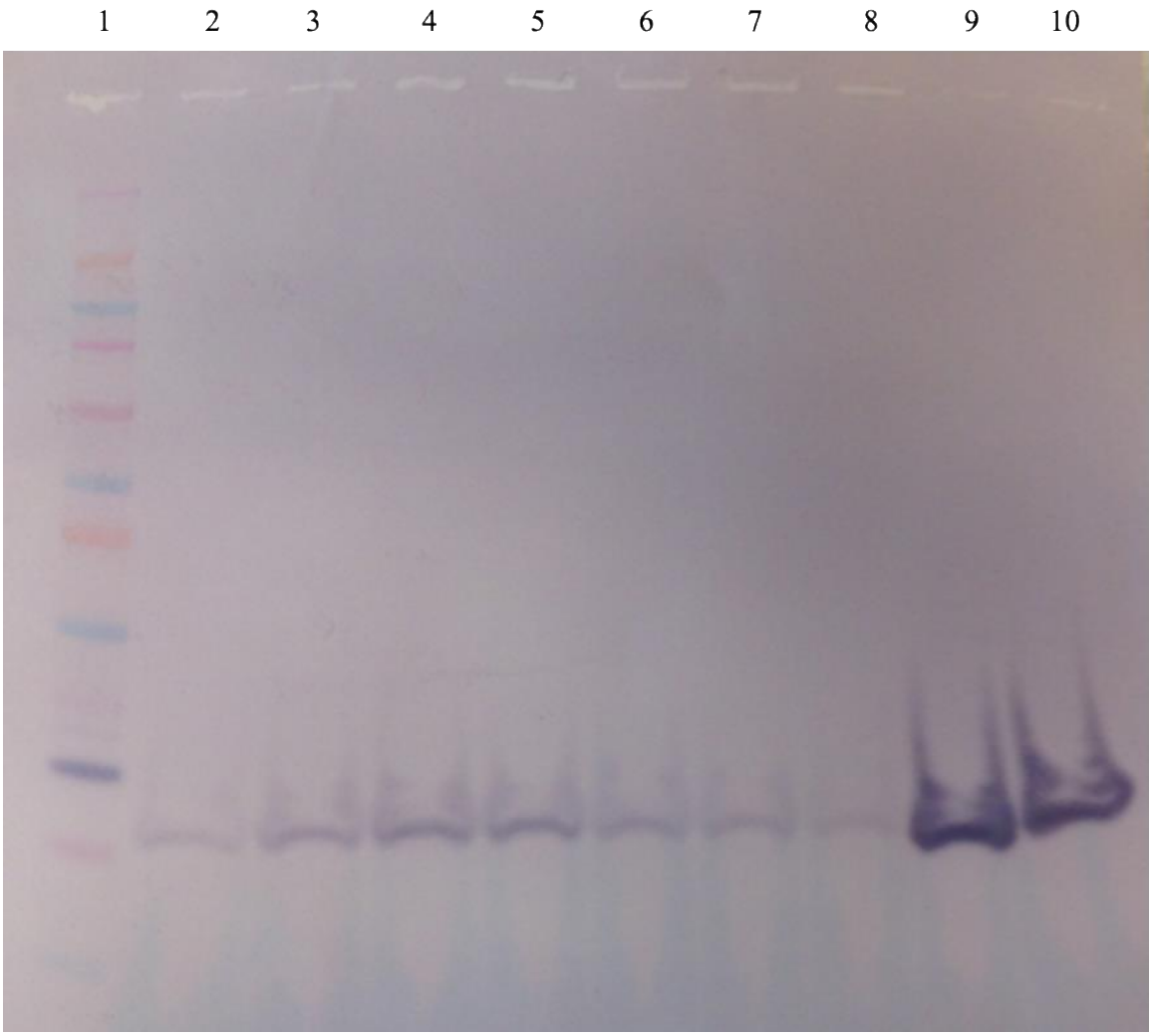

**Supplementary Figure S7.** Full-length uncropped immunoblot membrane used to prepare FIGURE 3. Lanes 2 and 3 show single-promoter *pUbi:GNA* line 1G-1 before and after salicylic acid treatment, respectively; lanes 10 and 12 show triple-promoter *pUBD5-1:GNA* line 1-2T before and after salicylic acid treatment, respectively; lane 13 shows 125 ng of GNA standard.



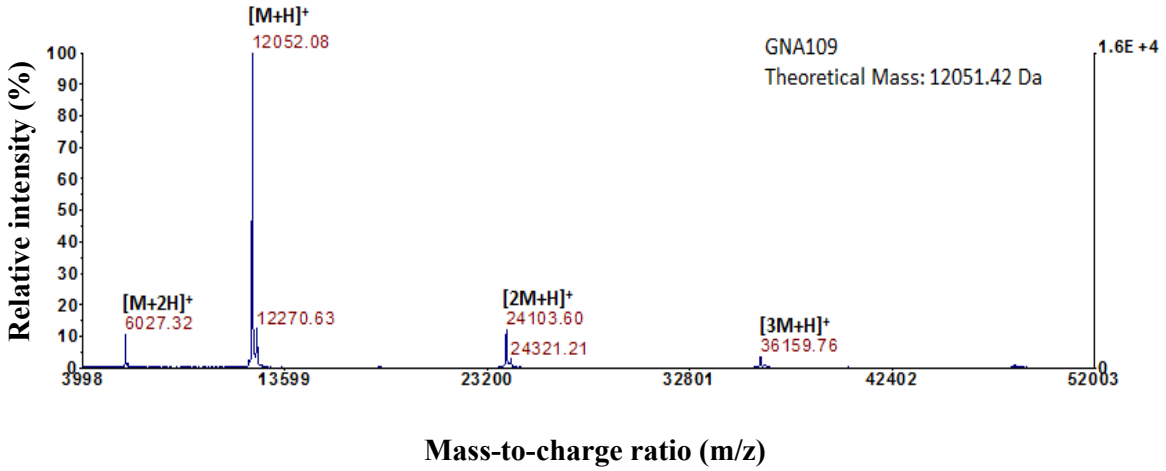

**Supplementary Figure S8.** Flowchart of the bench-scale extraction of recombinant GNA from transgenic sugarcane and energy cane culms.




**Supplementary Figure S9. (A)** and (**B**) Full-length uncropped immunoblot membrane used to prepare FIGURE 4. **(A)** shows the following: GNA standard 125 ng and 62.5 ng: lanes 5 and 6, respectively; triple-promoter *pUBD5-1:GNA* lines 1-2T and 5-1G total soluble protein (TSP) extraction with citric acid pH 4.8: lanes 8 and 9, respectively; TSP extraction of the same lines with sodium acetate pH 5.2: lanes 12 and 13, respectively. **(B)** shows GNA standard 250 ng and 125 ng: lanes 4 and 5, respectively, and triple promoter *pUBD5-1:GNA* lines 1-2T and 5-1G TSP extractions with sodium acetate (pH5.2)/EDTA/Tween-20: lanes 7 and 8.




**Supplementary Figure S10.** Full-length uncropped immunoblot membrane used to prepare FIGURE 5A2. Lanes 1 to 7 represent elution fractions 1.A.7, 1.A.8, 1.A.9, 1.A.10., 1.A.11, 1.A.12 and 1.B.1, respectively.




**Supplementary Figure S11.** Analysis of recombinant $GNA_{109}$ under non-reduced conditions using Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-TOF MS). The MS spectra of $GNA_{109}$ display the molecular weights of *m/z* 12,052.08 $[M+H]^+$, *m/z* 24,103.60 $[2M+H]^+$ and *m/z* 36,159.76 $[3M+H]^+$, representing the monomer, dimer and trimer of $GNA_{109}$, respectively.

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LEC2GNA     MAKASLLILAAIFLGVITPSCLSDNILYSGETLSTGEFLNYGSFVFIMQEDCNLVLYDVD
1NIV_A      ----------------------DNILYSGETLSTGEFLNYGSFVFIMQEDCNLVLYDVD
                                   ************************************

LEC2GNA     KPIWATNTGGLSRSCFLSMQTDGNLVVYNPSNKPIWASNTGGQNGNYVCILQKDRNVVIY
1NIV_A      KPIWATNTGGLSRSCFLSMQTDGNLVVYNPSNKPIWASNTGGQNGNYVCILQKDRNVVIY
            ************************************************************

LEC2GNA     GTDRWATGTHTGLVGIPASPPSEKYPTAGKIKLVTAK
1NIV_A      GTDRWATGTHTG------------------------
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**Supplementary Figure S12.** Multiple alignment of amino-acid sequences of the translated GNA peptide transformed into sugarcane (*Galanthus nivalis* lectin; LECGNA2, Protein Bank accession number AAA33346) and the mature snowdrop-bulb native GNA (Vector Labs) (*Galanthus nivalis* agglutinin; 1NIV, Protein Data Bank accession number 1NIV_A). The consensus sequence motif QXDXNXVXY (QEDCNLVLY), involved in recognition of the α-D-mannose substrate, is boxed in red. Multiple amino-acid sequence alignment was performed using ClustalW 2.0 program (www.ebi.ac.uk/clustalw).

**Supplementary TABLE S1** Primers used for determination of promoter:*GNA* cassette integration in staked triple promoter:*GNA* sugarcane lines.

| **Primer** | **Primer sequence (5'-3')** |
|---|---|
| *pUbi*-F | TGTGCATGTGTTCTCCTTTTT |
| *pSHDIR5-1*-F | TGTGGGCGACAGCTTGATAC GTGT |
| *pSCBV21*-F | CAGATGCTTGTGCAACTGGT |
| *pSHEF1α*-F | CACTTGTTCCCTTGCTGGTT |
| *35ST*-R | GCTCAACACATGAGCGAAAC |
| *GNA127*-R | TCCCCTGTAGAGAGAGTC |