

Supplementary Table and Figures:

Appendix A. The primers used in the research

Primers	sequence(5'-3')	Purposes
CP-F1	ACTCGAGTTAGGCAGCCTTCGTATCGGAGAG	Vector construction for pBI-35SCP4
CP-R1	AGGATCCAACAATGGCACAAATTAACAAC ATGGCACAAG	
CP4-F2	ATGGCACAAATTAACAACATGGCACAAAGGGAT	PCR for CP4-EPSPS
CP4-R2	TCAGGCAGCCTTCGTATCGGAGAGTTCGATCT T	
GhUBQ7-F	GAAGGCATTCCACCTGACCAAC	internal control primer for cotton
GhUBQ7-R	CTTGACCTTCTTCTTCTTGTGCTTG	

Determination of the lethal concentration of glyphosate

Fuzzy seeds were delinted in concentrated sulfuric acid (H₂SO₄) and planted in sterile soil in the greenhouse until the appearance of the first true leaves (7-day). Various concentrations of glyphosate (AppendixB) were subsequently applied as an herbicide stress assay (injury or death) and data were collected ten days after glyphosate treatment (n=50).

Results indicated that the cotton seedlings could tolerate glyphosate concentrations below 0.2%. When the concentration of glyphosate was \geq 0.3%, however, all of the cotton seedlings were killed. Based upon these results, the critical lethal concentration was determined to range between 0.2% and 0.3% glyphosate. In a second assay of cotton seedlings, 0.22% to 0.28% glyphosate sprays were used to more precisely determine the critical concentration. Results indicated that 0.26% glyphosate could effectively kill all plants.

Appendix B. Lethal Glyphosate Concentration Determination

	Glyphosate Application Concentration							
	First Round				Second Round			
	0.1%	0.2%	0.3%	0.4%	0.22%	0.24%	0.26%	0.28%
Seedling #	50	50	50	50	50	50	50	50
10-Day	42	17	0	0	12	6	0	0

Appendix C. PCR analysis of T1 seeds obtained from the initial transformed plants.

Lanes 1-14: 14 seeds randomly selected from seeds of all five transformed lines. CK⁻: negative control; CK⁺: positive control (plasmid); M: molecular DNA marker.



Appendix D. PCR analysis of T₃ cotton plants

Lane 1-20. Each lane represents tissue samples obtained randomly from multiple T₃ glyphosate-resistant cotton lines. CK⁻: negative control; CK⁺: positive control (plasmid).

