## **Supplementary Table and Figures:**

**Appendix A. The primers used in the research** 

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

## Determination of the lethal concentration of glyphosate

Fuzzy seeds were delinted in concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) 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).

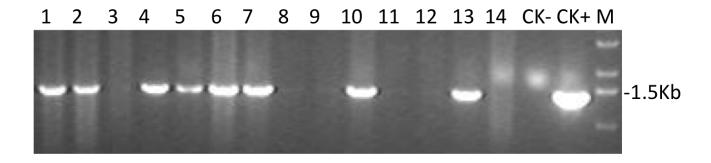
Results indicated that the cotton seedlings could tolerate glyphosate concentrations below 0.2%. When the concentration of glyphosate was ≥ 0.3%, however, all of the cotton seedlings were killed. Based upon these results, the critical lethal concentration was determined to range between 0.2% and 0.3% 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<sup>-</sup>: negative control; CK<sup>+</sup>: positive control (plasmid); M: molecular DNA marker.



## Appendix D. PCR analysis of T<sub>3</sub> 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).

