

Article title: A role for the gibberellin pathway in biochar-mediated growth promotion.

Journal name: Scientific Reports

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SUPPORTING INFORMATION

Fig S1 Premium biochar (BC) reduces days to flowering and increases number of flowers

Fig S2 Biochar water extracts positively affect seedling growth in *Solanum pennellii*

Fig S3 Gas Chromatography-Mass Spectrometry (GC/MS) of ethyl acetate extracts of Premium biochar (BC)

Table S1 Generalized linear models of the effects of Premium biochar treatment (BC) and gibberellin treatment (GA₄) on shoot weight and length, separated by genotype

Table S2 Generalized linear model of the effects of Arabidopsis genotype and Premium biochar (BC) extract treatment on hypocotyl length

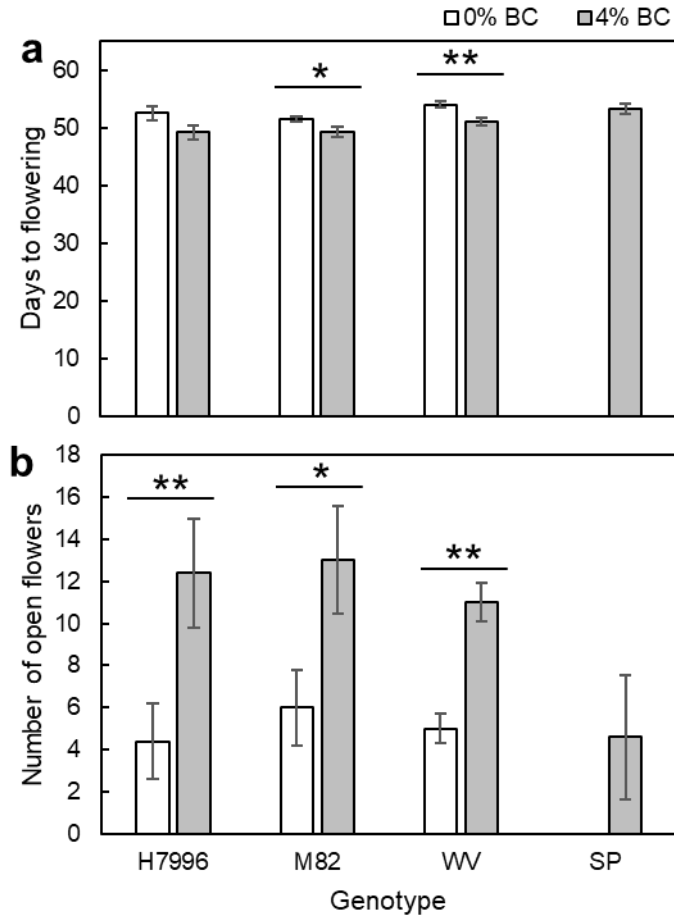


Fig. S1 Premium biochar (BC) reduces days to flowering and increases number of flowers. a) Average number of days to flowering in all 4 genotypes grown in 0% BC or 4% Premium BC. b) Average number of open flowers in all 4 genotypes grown in 0% or 4% Premium BC. Asterisks indicate significance by t-test at the following levels: * $p < 0.1$, ** $p < 0.05$. Error bars represent one standard error. No statistical analysis performed on SP as no control plants had flowered by the end of the experiment (56 days after planting). Abbreviations: H7996 – *Solanum lycopersicum* cv. Hawaii7996. M82 – *S. lycopersicum* cv. M82. WV – *S. pimpinellifolium* accession West Virginia700. SP – *S. pennellii* accession LA0716.

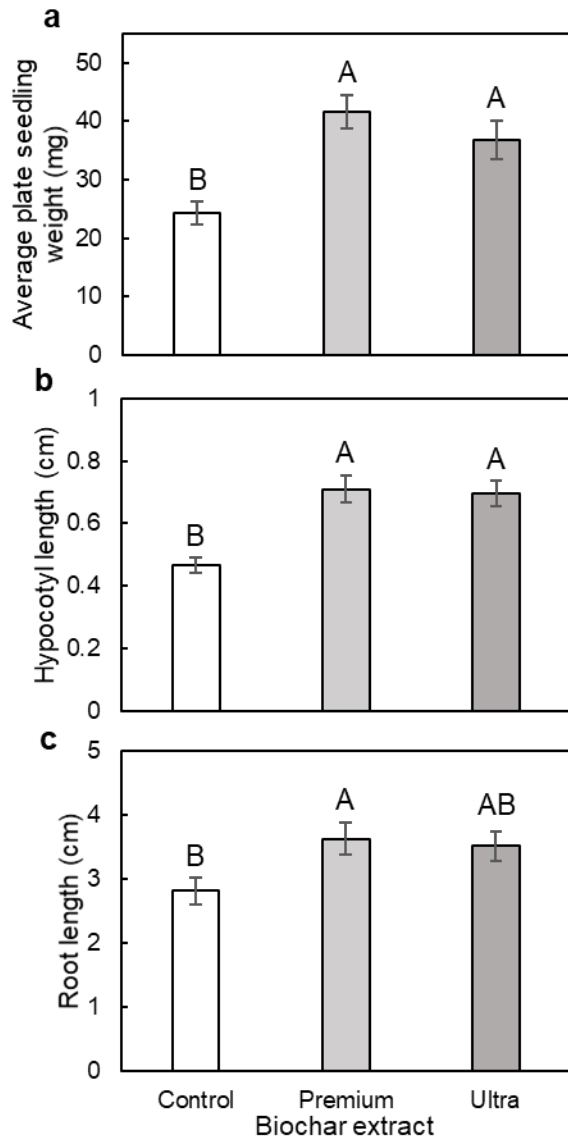


Fig. S2 Biochar water extracts positively affect seedling growth in *Solanum pennellii*. a) average weight of pooled seedlings from each plate in milligrams (mg) b) average hypocotyl length in centimeters (cm) c) average root length in cm and of *S. pennellii* seeds plated with water (Control) or water extracts of the two biochars. Results represent the averages of five plates/treatment of 10 seeds/plate. Data displayed from first biological replicate. Experiment was performed four times with similar results. Significant differences between biochar treatments at $p < 0.05$ as determined by Tukey's honest significant differences indicated by differing letters for a-c. Hypocotyl length values were square root transformed to meet homogeneity of variance assumption. Values displayed are untransformed. Error bars represent one standard error.

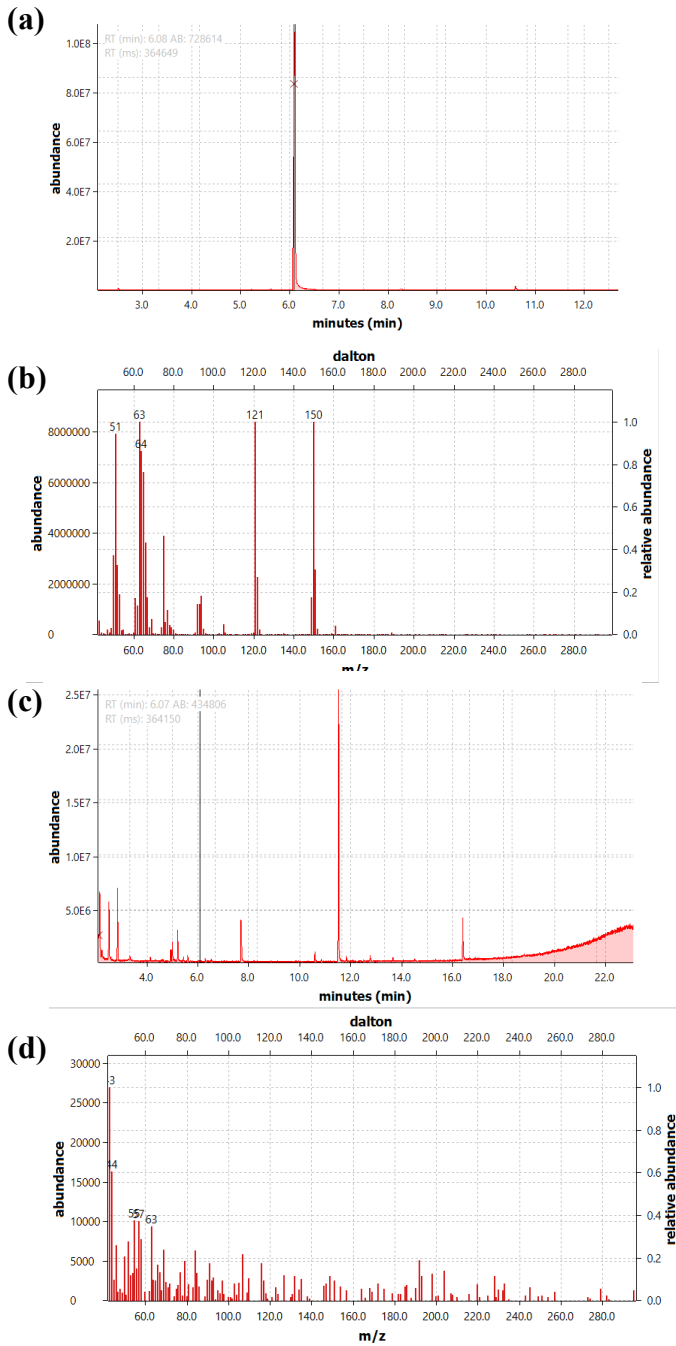


Figure S3: Gas Chromatography-Mass Spectrometry (GC/MS) of ethyl acetate extracts of Premium biochar (BC). a) Chromatogram of 1 mM karrikin 1 (KAR1) in ethyl acetate. Retention time ~6 min. B) Mass spectrum of KAR1 at 6.08 min retention time (marked as X in a). C) Chromatogram of ethyl acetate Premium BC extract. D) Mass spectrum at 6.09 min retention time.

Supplementary Materials and Methods

Solanum pennellii seedling growth on plates.

Control and Premium biochar extract treatments were prepared as for the *Arabidopsis ga3ox1-3* assays. For seedling growth experiments, 10 sterile *S. pennellii* seeds were plated onto treated plates in a single row and placed upright in a growth chamber set to 24 °C, 16-hour day and 8-hour night cycle and an average of 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light. After six days, plates were scanned and measured in ImageJ for root length and hypocotyl length. After scanning, seedlings from each plate were pooled and weighed together. Statistical analysis was performed in JMP12. Statistical differences were tested by ANOVA with treatment as a fixed effect and plate as a random effect, followed by a post-hoc Tukey test with a significance cut-off of $p < 0.05$. No transformations were necessary to meet the homogeneity of variance and normality assumptions. The experiment was repeated four times with similar results.

KAR1 extraction and GC-MS analysis

Premium biochar extraction was performed by first grinding 25 g biochar in a mortar and pestle. After grinding, biochar was stirred at room temperature with 100 ml ethyl acetate for two hours. Then biochar extract was filtered by vacuum filtration with Whatman 42 filter paper. Filtered extracts were then submitted along with 1 mM KAR1 standard to the Purdue Campus-Wide Mass Spectrometry Center. 1 μl samples were analyzed on an Agilent 5975C gas chromatograph-mass spectrometer (GC-MS). Sample separation was done on a DB-5 column 30 m in length, with a 0.25 mm inner diameter and 0.25 μm film thickness. The injection temperature was set to 250 °C. The temperature separation program for the GC was as

follows: 100 °C for 0.1 min, ramped at 10 °C to 320 °C. Mass spectra were scanned in the range of 42-300 amu. Chromatograms were visualized using OpenChrom 1.1.0¹.

Supplementary References

1. Wenig, P. & Odermatt, J. OpenChrom: a cross-platform open source software for the mass spectrometric analysis of chromatographic data. *BMC Bioinformatics* **11**, 405 (2010)

Table S1 Generalized linear models of the effects of Premium biochar treatment (BC) and gibberellin treatment (GA₄) on shoot weight and length, separated by genotype

Type III Tests of Fixed Effects						
Genotype	Response Variable	Effect	Num DF¹	Den DF	F Value	Pr > F
H7996	Shoot Fresh Weight (g) ²	BC	1	372	118.66	<.0001
		GA	1	372	135.26	<.0001
		BC*GA	1	372	0.19	0.6606
	Shoot Length (cm) ³	BC	1	372	118.50	<.0001
		GA	1	372	761.81	<.0001
		BC*GA	1	372	0.99	0.3194
M82	Shoot Fresh Weight (g)	BC	1	384	80.21	<.0001
		GA	1	384	327.67	<.0001
		BC*GA	1	384	11.94	0.0006
	Shoot Length (cm)	BC	1	384	73.37	<.0001
		GA	1	384	1782.15	<.0001
		BC*GA	1	384	11.26	0.0009
WV	Shoot Fresh Weight (g)	BC	1	305	59.30	<.0001
		GA	1	305	145.68	<.0001
		BC*GA	1	305	3.58	0.0595
	Shoot Length (cm)	BC	1	305	36.15	<.0001
		GA	1	305	938.24	<.0001
		BC*GA	1	305	3.24	0.0729
SP	Shoot Fresh Weight (g)	BC	1	266	140.70	<.0001
		GA	1	266	2.42	0.1211
		BC*GA	1	266	1.07	0.3027
	Shoot Length (cm)	BC	1	265	139.82	<.0001
		GA	1	265	75.41	<.0001
		BC*GA	1	265	1.28	0.2589

¹Degrees of Freedom

²Shoot weight - square root transformed for analysis.

³Shoot length - square root transformed for analysis.

Table S2 Generalized linear model of the effects of Arabidopsis genotype and Premium biochar (BC) extract treatment on hypocotyl length

Type III Tests of Fixed Effects					
Response Variable	Effect	Num DF¹	Den DF	F Value	Pr > F
Hypocotyl length (mm)²	Genotype	1	479	544.65	<.0001
	BC	1	479	9.14	0.0026
	Genotype*BC	1	479	25.94	<.0001
	Trial	1	479	82.76	<.0001
	Genotype*Trial	1	479	75.36	<.0001
	BC*Trial	1	479	0.01	0.9038
	Genotype*BC*Trial	1	479	0.02	0.8818

¹Degrees of Freedom

²Hypocotyl length – square root transformed for analysis