Silica biomineralization in plants alters the structure of lignin

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Supporting information

Supplementary Information 1

Production and isolation of a sorghum double mutant carrying both *bmr6* and *lsi1* mutations

Double mutants were generated by emasculating sorghum *lsi1* [1] mother plants and cross-pollinating them with pollen of sorghum *bmr6* (genetic stock PI639713) obtained from the United States Department of Agriculture (USDA, Griffin, GA, USA). Emasculation and crossing were performed according to House (1985) [2] with some adjustments. Both genotypes were similarly grown in 4 L pots with commercial soil in a greenhouse (Rehovot, Israel) under natural light and temperatures. At ~65 days after emergence, during the afternoon hours, *lsi1* plants with fully exposed panicles were emasculated by holding them upside down and plunging their heads into 42ºC water. After 10 minutes, soaked panicles were tightly covered with a plastic bag overnight. The high humidity trapped in the bag prevents dehiscence, and while anthers emerge from the florets, they do not shed pollen. The following day, each of the emasculated *lsi1* plants was placed alongside a *bmr6* plant and their panicles were bagged together in one paper bag. During the following week, the covered panicles of coupled plants were agitated twice a day to maximize pollen dispersal and improve cross-pollination. F1 seeds were harvested 30 days after emasculation, and planted as described above to generate F2 seeds. Seeds were collected and germinated in growth room for double mutant selection. Leaves of young F2 plants were collected, genomic DNA was extracted using standard methods and genotyping was conducted to identify double mutant plants carrying both *lsi1* and *bmr6* defected genes. The *lsi1* mutation was identified by genotyping with two sets of primers (Table S1), indicating the presence of native or mutated gene [1]. Plants that gave a PCR product for the *lsi1* mutation, but not the wt were chosen for further selection. The CAPS marker for *Bmr6* locus was used to identify *bmr6* mutants, as described before [3]. Shortly, PCR primers were used to amplify a 613-bp fragment of *Bmr6* (Table S1). The amplification products were

digested with the restriction enzyme BsaAI and analysed. Only the mutated and not the wt BMR6 gene carries this BsaAI restriction site, resulting in two fragments of 333 and 280 bp. Double mutant plants were transplanted as described above and the mutations were verified before further use. Seeds were collected from bagged inflorescences to ensure self-fertilization of the homozygote double mutants.

Table S1: PCR primers and conditions for identifying the double mutant plants. *Lsi1* (wt) primer pair amplified only the native *Lsi1* locus, *lsi1* (mut) primer pair amplified only the mutant *lsi1* locus [1]. *Bmr6* primer pair amplified both native and mutated loci. The amplification product was digested by BsaAI only if carried the *bmr6* mutated gene [3].

Product name	Forward	Reverse	Annealing temperature	Product size
Lsi1 (wt)	CTTGCGACGAGGGTTAATTTGG	CCGTTTTCGGCACATCCC	59ºC	230 bp
lsi1 (mut)	CTTGCGACGAGGGTTAATTTGG	ACCAGGTCCATCCATGAGC	59ºC	800 bp
Bmr6/*bmr6*	CACAACCACTCCACTACTGCGAAC	GTCACCACAAGGCATCCATACG	60ºC	613 bp

Supplementary Information 2

Lignin extracted using alkali pretreatment process

Alkali lignin was extracted according to Do et. al (2020) [4], with the following modifications. 1 g of dried biomass was mixed in 15 mL of 1 w/v% aqueous NaOH solution, which was preheated to 60°C. The mixture was stirred at 85°C for 2.5 h, cooled to 40°C, and vacuum filtrated. The obtained extract liquor was acidified to pH 9 by adding 20 w/v% H_2SO_4 and left for 48 h, during which silica precipitated [5]. Silica gel was separated from the liquor by centrifugation and the supernatant was collected. Lignin was then precipitated from the liquor by adjusting the pH to 3 using 20w/v% H_2SO_4 solution. The sample was centrifuged and rinsed 3 times with double distilled water.

Supporting Figure S1

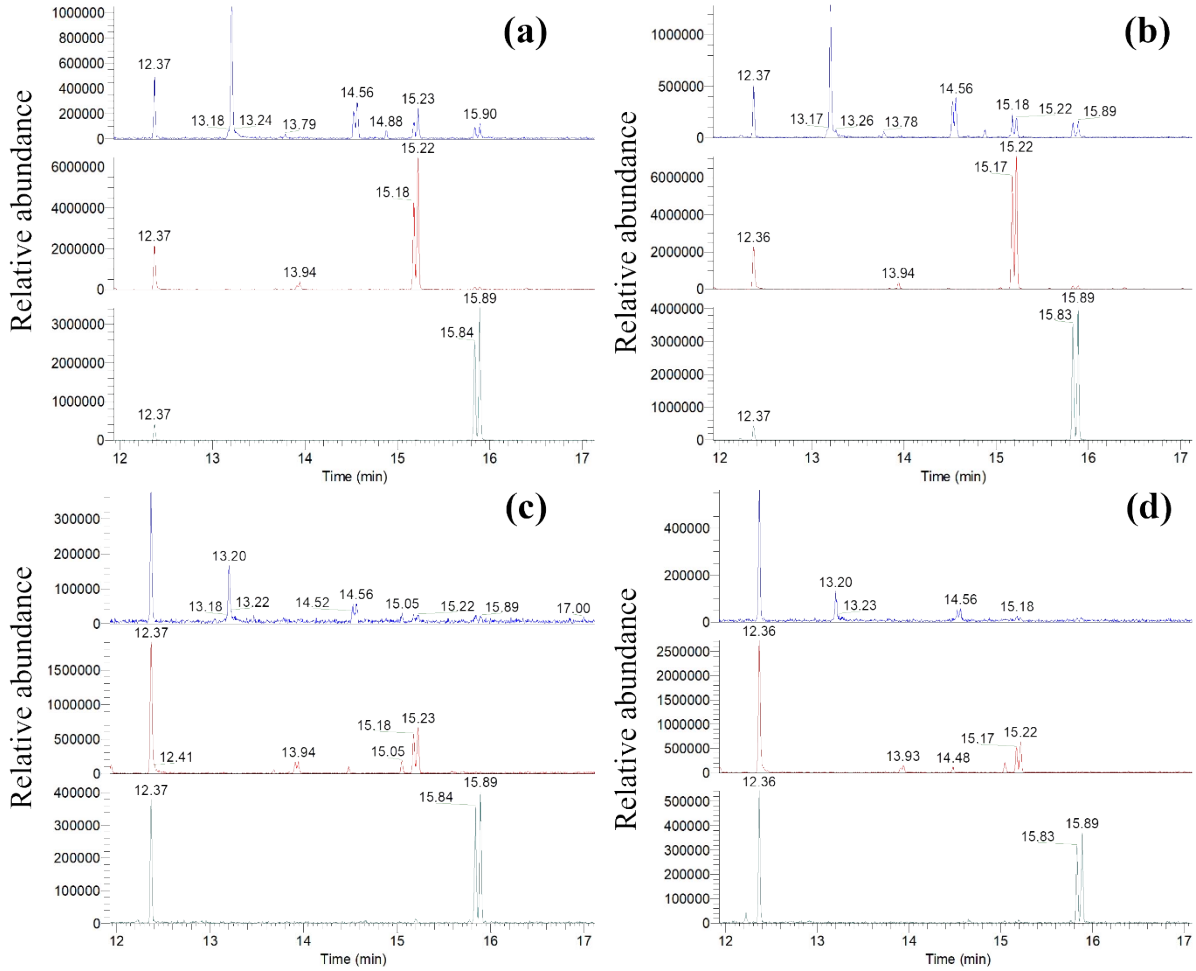

Figure S1. A representative GC-MS chromatogram of trimethyl-silyl (TMS) derivatives of sorghum genotype thioacidolysis products: Extraction of biomass from (a) WT, (b) *lsi1*, (c) *bmr6* and (d) *bmr6*×*lsi1*. The double peaks represent the erythro and threo isomers of p-hydroxyphenyl, guaiacyl and syringyl units released by thioacidolysis. The internal standard (m/z 357.1) appears with retention time (RT) of 12.37 min. H monomers (m/z 239.1) appear with RT of 14.52, 14.56 min (blue). G monomers (m/z 269.1) appear with RT of 15.18, 15.22 min (red). S monomers (m/z 299.1) appear with RT 15.84, 15.89 min (green).

Supporting Figure S2

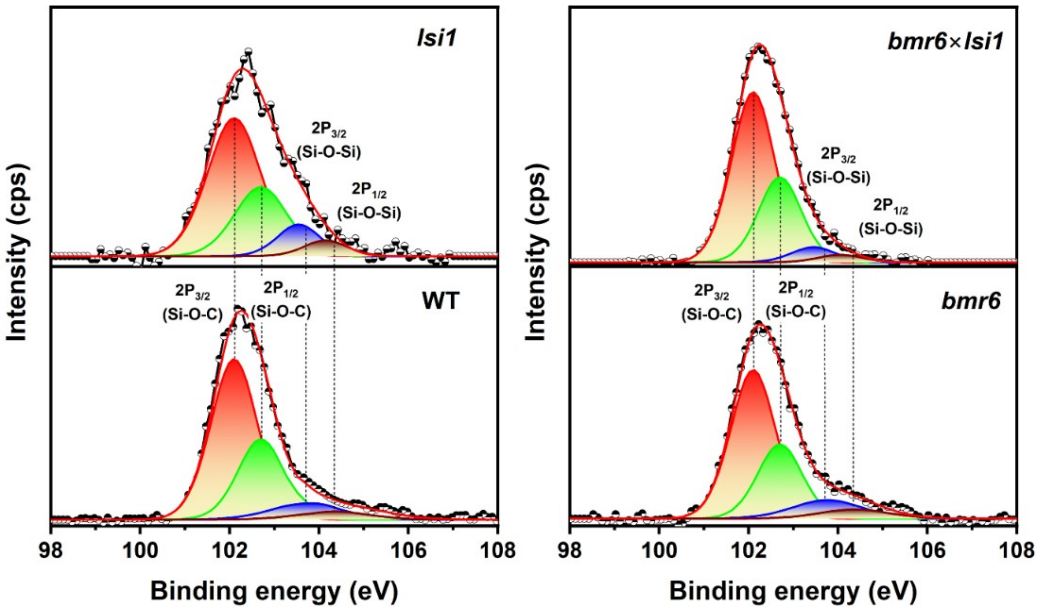

Figure S2. High resolution XPS Si 2p spectra of lignin-silica from WT, *lsi1*, *bmr6* and *bmr6×lsi1* sorghum genotype at silicic acid concentration of 2.5 mM: The curves with filled area are the deconvoluted peaks, the black line plus symbol curves represents the measured intensity, and the red curves represent the cumulative fit.

Supporting Figure S3

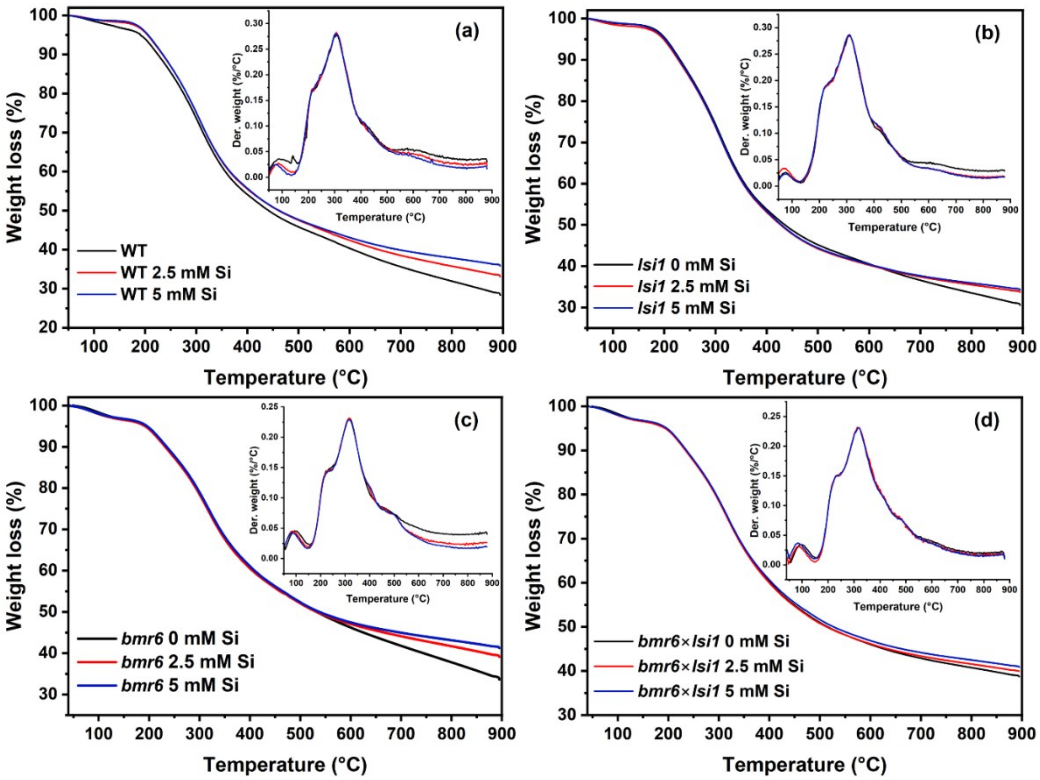

Figure S3. Thermal decomposition behaviour of lignins reacted with 0, 2.5 and 5 mM silicic acid in phosphate buffer solution: (a) WT, (b) *lsi1*, (c) *bmr6* and (d) *bmr6*×*lsi1*.

Supporting Figure S4

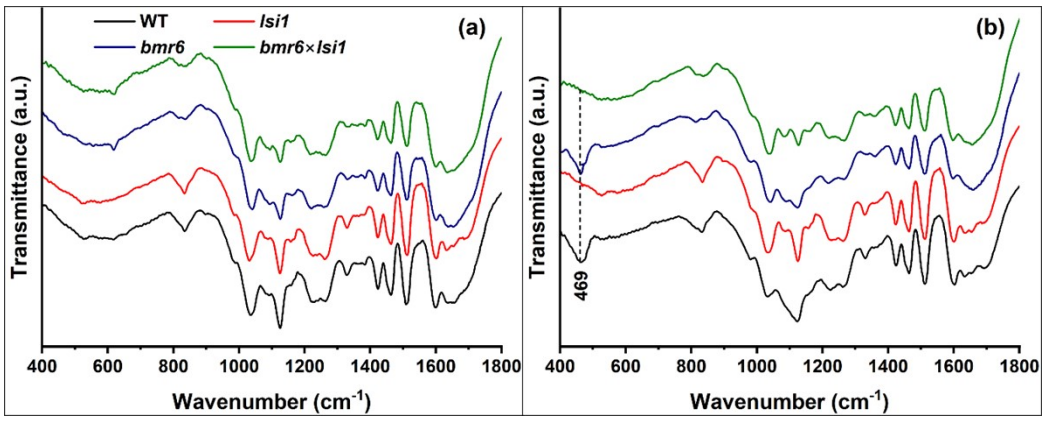

Figure S4. FTIR transmission spectra of lignin extracted using alkali pretreatment process in wavenumber range of 400-1800 cm^{-1}: (a) Lignin extracted in polyethylene beakers. (b) Lignin extracted in glass beakers. A strong Si-O-Si absorption band (469 cm^{-1}) was observed only in WT and *bmr6* and only in lignin extracted in glass beakers.

References

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