Evidence of a plant genetic basis for maize roots impacting soil organic matter mineralization

Supplementary materials and methods

A set of 97 elite maize lines from a drought-tolerant maize for Africa (DTMA) panel (Wen et al. 2011; Cairns et al. 2013) and 8 commercial hybrids in Zimbabwe were selected for screening. The hybrids (30G19, SC633, SC513, Pan53, ZAP61, ZAP55, Pristine 601 and PGS61) are widely grown in Zimbabwe. Seeds were imported to Aberdeen, United Kingdom, where they were kept at 4 °C until sowing.

A granitic sandy soil, classified as *Lixisol*, was collected from Domboshawa Research Centre (-17.6091 Lat, 31.13374 Long), Zimbabwe. The soil was sampled from a plot under conventional tillage. Ten soil sub-samples (0-10cm soil depth) were taken at random within the plot. These were mixed into a composite sample and sieved through a 4 mm mesh on-site. The sieved soil was transported in cooler boxes to Aberdeen, United Kingdom, where it was stored at 4 °C until experiment setup. The soil had the following characteristics: pH(H_2O) 4.79, cation exchange capacity 1.01 meq $100g^{-1}$; total C 2.98 mg g^{-1}; total N 0.24 mg g^{-1}; sand 83.7%, silt + clay 16.3%.

For experiment setup, the soil was packed in microcosms (22.5 cm x 5.5 cm diam) to bulk density of 1.44 g cm^{-3} to represent the field bulk density and adjusted to 65% water holding capacity. A 5 cm layer of previously muffle-furnaced sand (0% organic matter) was packed to the bottom of each microcosm before packing the soil. After an initial soil stabilization period of one week, plastic chambers made from syringe tubes (40 ml headspace, with inlet and outlet stopper end tubes for controlled gas flow) were inserted into the middle of microcosms to 2.5 cm depth for trapping CO_2 efflux from soil. Systems were maintained at 22 °C and 70% relative humidity within a plant growth chamber (Mwafulirwa et al. 2016), and each system was sown with one plant including an unplanted control treatment. Plants were grown for 29 days without fertilizer application. Treatments (i.e. maize genotypes) were replicated two to four times in a sequential randomized block design, with two hybrids and the control treatment included in all blocks. A 12-hour daily photoperiod was set with 512 µmol m^{-2} s^{-1} PAR within the plant growth chamber. At the seedling growth stage one week after sowing seeds, a continuous flow of ^{13}C-enriched CO_2 (20 atom% ^{13}C) was passed through the plant growth chamber over the experiment period (Mwafulirwa et al. 2016).

Soil surface CO_2 fluxes were sampled at 16, 23 and 29 days after planting. For this, the gas collection chambers were flushed with CO_2-free air for three minutes, then sealed for 40 minutes

using stopper end tubes to accumulate soil CO_2 efflux in the headspace. Then, 25 ml air was sampled from the headspace with a gas syringe connected to the outlet tubing. The sampled air was used to determine the CO_2 concentration and $^{12}C/^{13}C$ ratios as described in Mwafulirwa et al. (2016). Total C respired was partitioned to two component sources (SOM- and maize root-derived C) based on $\delta^{13}C$ signatures of the air samples, according to Mwafulirwa et al. (2016).

For harvest, shoots were cut at the soil surface level, freeze-dried and then shoot dry weight was recorded. Roots were carefully removed from the soil, washed in deionised water, and stored fresh in 50% ethanol at 4 ^{o}C prior to analysis for morphological traits (i.e. root diameter and length) (Mwafulirwa et al. 2021). For this, fresh roots were carefully spread onto a clear-bottomed reservoir filled with water. Then, the roots were scanned on an Epson Expression 1640XL flatbed scanner (Epson UK, London), and root length and average root diameter were determined using WINrhizo (Regent Instruments, Quebec City, Canada) (George et al. 2014). Thereafter, roots were washed again in deionized water, freeze-dried and root dry weight was recorded.

The statistical software SPSS version 25 (IBM Statistics) was used for correlation, stepwise multiple linear regression and cluster analyses. Correlation and stepwise multiple linear regression analyses were conducted for all genotypes (lines and hybrids) and for lines or hybrids separately. All correlations and regressions were considered significant at $P < 0.05$. Hierarchical cluster analysis (Ward linkage, squared Euclidean distance) was performed for all genotypes.

Analyses of variance for each trait was determined by the restricted maximum likelihood (REML) method using the R program embedded in META-R software (Alvarado et al. 2020). All lines were genotyped with genotyping by sequencing (GBS). Each line were genotyped at the Institute of Biotechnology at Cornell University (http://www.biotech.cornell.edu/brc/genomics-facility), USA (Elshire et al. 2011). Raw GBS data for a total of 955,120 SNP loci was further filtered with the criteria to retain markers with >5% of minor allele frequency (MAF) and <10% of missing data using Trait analysis by association, evolution and linkage (TASSEL v.5.2.24) software (Bradbury et al. 2007). This lead to retain 199,755 high quality single nucleotide polymorphism (SNPs) for the final genome-wide association analyses. Best linear unbiased predictions (BLUPs) calculated for all nine traits were used in GWAS. Trait data were corrected for population structure and kinship (Q + K) using the mixed linear model (MLM). GWAS analysis was done with the R package "FarmCPU – Fixed and random model Circulating Probability Unification" (Liu et al. 2016). Kinship matrix, principal component analyses and linkage disequilibrium were calculated with TASSEL.

Significant associations were declared when the *P* values in independent tests were less than 5×10^{-5}. Manhattan plot, the quantile-quantile (QQ) plots were produced by using TASSEL output in R (R Core Team, 2016). Candidate genes containing or being adjacent to the significant SNPs were obtained from the B73 gene set (version 2.0) in Maize GDB. BLAST searches were performed with 50-bp source sequences of the significantly associated SNPs against the “B73” RefGen_v2. RR-BLUP was used for genomic predictions with five-fold cross validations. RR-BLUP was used to carry out genomic selection (GS) using a five-fold cross validation. Best linear unbiased estimations (BLUEs) for each trait were used for the analysis. A set of high-quality uniformly distributed 5,000 SNPs with no missing values and MAF > 0.05 were used for GS. Cross-validation was repeated for 100 times for sampling of the training and validation sets.

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