



Genetic control of radicle and shoot attributes in a maize (Zea mays L.) double haploid (DH) population under simulated drought

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Introduction

- The so-called spring drought is a serious threat for maize growth as it impedes seedling establishment and causes seedling death.
- The root system strongly responds to drought with growth reduction.
- However, a well-developed root system is crucial for seedling establishment and fitness, especially in drought conditions when reaching deeper, moister soil layers is important for plants.

Objectives

- Identify quantitative trait loci (QTL) for maize seedling radical and shoot growth in simulated drought conditions in the CFD03¹ (F353 x D06) maize DH population.
 Approach
- Establishment of a reliable, fast and accurate root phenotyping method suitable for small maize seedlings.
- Therefore, it is desirable to breed maize for drought resistant seedling root growth.
- This requires identification of genomic regions and alleles associated with improved seedling growth under drought.
- Seedlings growth in 'cigar roll system' in presence and absence of 200 mM sorbitol.
- Measurement of root length (RL), lateral root area (LRA), whole root area (RA) and shoot length (SL) in Fiji.
- Analysis of QTLs and graphical representation of linkage groups in R (version 4.3.3) with package 'R/qtl² and 'LinkageMapView' respectively.

Seedling growth set up





Figure 1: Hydroponic growing system for maize seedlings. Four seeds are placed **F** between two sheets of germination paper and rolled to produce a 'cigar roll'³. If These are placed into 5 L plastic beakers containing water (**mock**) or 200 mM sorbitol solution (**sorb**) to simulate drought. Beakers are placed into a growth cabinet under optimal temperature conditions ($t_{max} = 26$ °C day and $t_{min} = 18$ °C night), 18 hours photoperiod and 60% of relative air humidity. Eight days after sowing, seedlings are harvested. Radicles and shoots are scanned and all images stored and analysed.

Measurement of morphological traits

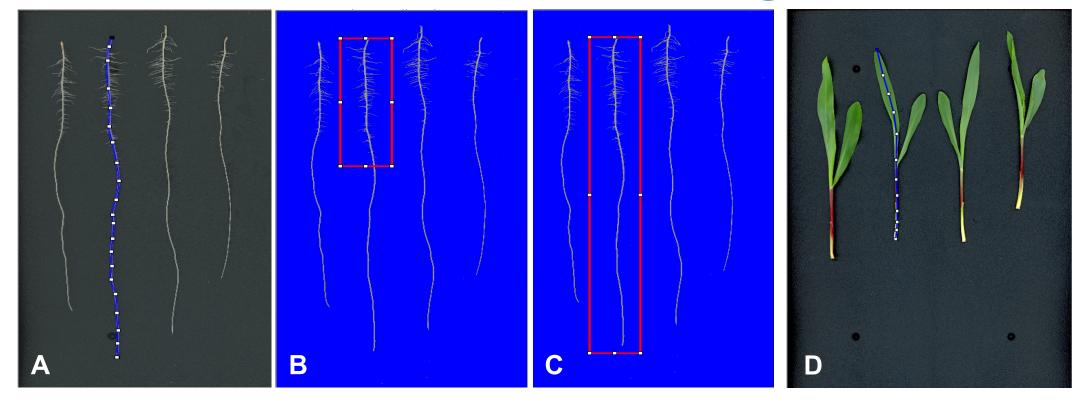


Figure 2: Scanned images of radicles (**A**-**C**) and shoots (**D**) are processed with Fiji Image J⁴ package. Radicle (RL) and shoot length (SL) are measured using the segmented line function (**A** and **D**). The thresholding function is used to separate the radicle image from the background and the white area within the red rectangle is then calculated as in (**B**) for the LRA and in (**C**) for the RA traits.

Indices of growth deficitDELTA = sorb - mockDELTA_{rel} = (sorb - mock)/mock

Results

- In total 24 QTLs were detected. 16 under simulated drought conditions alone and eight for the two indices of growth deficit (i.e. DELTA and DELTA_{rel}).
- No QTL was detected for LRA.
- The percentage of phenotypic variance explained (%PVE), ranges from 2.07 to 11.25 for the detected QTLs.
- Data mining, of genes co-localizing on the B73 maize reference genome assembly, revealed interesting putative: transcription factor, kinase, phosphatase and hormone signaling genes.

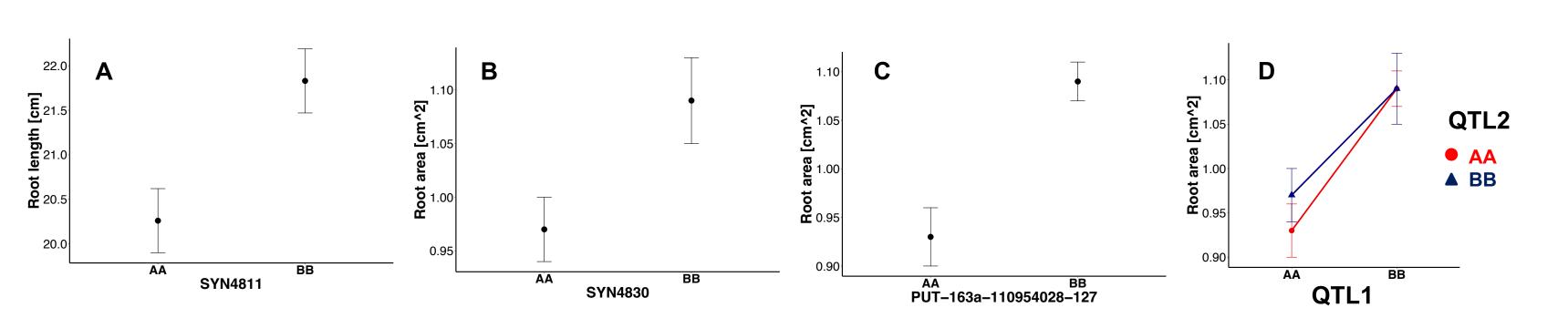


Figure 3: Effect plot (**A-C**) for the most significant marker of each of the three QTLs showing the highest %PVE. For all three markers the 'B' allele is associated with longer radicles (**A**) and larger root system areas (**B** and **C**). For the root area trait (**B** and **C**) the 'B' allele is of maternal origin (i.e. inbred line F353). Effect plot (**D**) showing epistatic effect between two QTLs detected for RA.

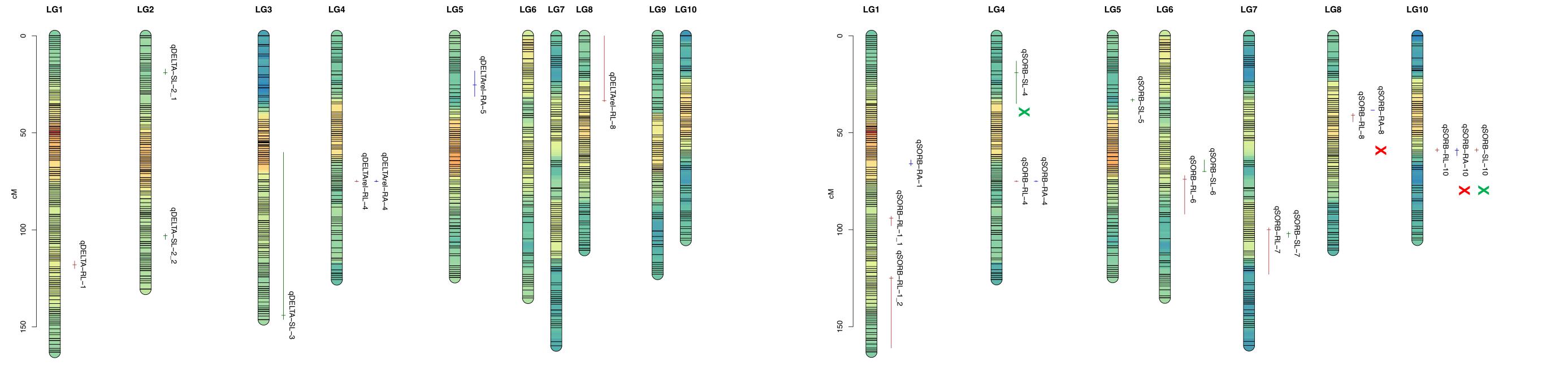


Figure 4: Quantitative trait loci (QTLs) detected for the two indices of growth deficit (DELTA and DELTA_{rel}), reflecting the loss of growth caused by the simulated drought conditions. No QTL mapped on linkage groups (LG) LG6, LG7 and LG9. The same locus on LG4 at position (75.0 cM) and detected for the relativised index of growth deficit (DELTA_{rel}) controls both RL and RA. All detected loci have additive effects, no epistatic effect is detected. QTL and the position of the most significant marker are indicated with a coloured line and a mark respectively; brown for (RL), blue for (RA) and green for (SL).

Figure 5: QTLs detected for three morphological traits (RL, RA and SL) measured under simulated drought stress conditions. No QTLs mapped on linkage groups LG2, LG3 and LG9. Pleiotropy is observed for the locus on LG10 at position (58.9 cM) as this controls the growth of all the three morphological traits. All loci have additive effects. Two pairs of loci show epistatic effects and are indicated by red and green crosses in the graph. QTL and the position of the most significant marker are indicated with a coloured line and a mark respectively; brown for (RL), blue for (RA) and green for (SL).

Conclusion and Outlook

- 24 genomic regions control radicle and shoot growth under simulated drought.
- The 'B' allele, mostly of maternal origin, as associated with less growth loss in response to drought.
- Transcriptomic analysis of roots and shoots will be performed to improve our knowledge about the molecular control of seedling growth under drought stress.
- Validation of the most significant markers for the identified QTLs.

References

¹Bauer E., M. Falque, H. Walter, *et al.*, 2013. Intraspecific variation of recombination rate in maize. *Genome Biol.*, **14**, R103. <u>https://doi.org/10.1186/gb-2013-14-9-r103</u>

²Broman K.W. and S. Sen, 2009. A guide to QTL mapping with R/qtl. *Springer: Verlag*, New York, ISBN: 978-0-387-92124-2
³Zhu J., S.M. Kaeppler and J.P. Lynch, 2005. Mapping QTL controlling root hair length in maize (*Zea Mays* L.) under phosphorous deficiency. *Plant and Soil*, **270**, 299–310. https://doi.org/10.1007/s11104-004-1697-9
⁴Schindelin, J., I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, et al, 2012. Fiji: an open-source platform

for biological-image analysis. *Nature Methods*, **9**(7), 676–682. <u>https://doi:10.2038/nmeth.2019</u>

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