Confirmation of QTLs controlling maize grain yield and plant height

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Introduction

Texas, the 12th largest producer of maize within the United States, has not increased grain yield like the Midwestern States in the past decades. Grain yield is a complex quantitative trait, which is positively correlated with plant height in southern and central Texas maize. In this study, we constructed three bi-parental linkage populations (527 lines) using five elite inbreeding lines to validate three SNPs (QTV1 QTV2 and QTV3) identified in a previous genome-wide association (GWAS) study (Barerro et al. 2015), each explaining 3% ~ 5% variation in grain yield in Tx714 test crossed hybrids under drought and irrigated conditions; QTV2 also significantly affected plant height and flowering time (Fig 1.).

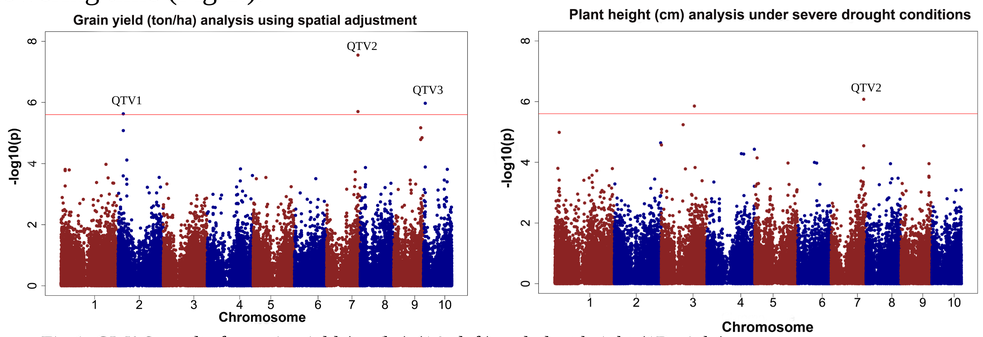

Fig 1. GWAS results for grain yield (ton/ha) (1A. left) and plant height (1B. right) Figure from Barerro et al. 2015.

Materials and Methods

Plant materials and research timeline

- By means of Sanger sequencing, we first tested eleven elite breeding lines and the derived seven existing F_1 lines that were expected to have the polymorphism based on Genotyping By Sequencing (GBS). We identified the specific polymorphism of the three target SNPs (Barerro et al. 2015) within the seven bi-parental linkage populations through alignment (ClustalX 2.1). Finally we selected the three linkage populations used for QTL analysis because they had the most confirmed SNPs and they were relevant from a breeding perspective to derive lines from. These three crosses were Ki3/NC356, Tx740/NC356 and LH82/LAMA2002 (Table1.)

Table 1. The polymorphism of the three SNPs in the parents lines

Pop.	Parent Lines	QTV1	QTV2	QTV3
1	Ki3	A	C	A
1&2	NC356	C	A	G
2	Tx740	A	C	G
3	LH82	C	A	G
3	LAMA2002	A	C	G
	B73 (Ref.)	A	C	G

- The research timeline (Fig 2.):
  - 2014 spring, F_3 growing in College Station;
  - 2014 summer, selfing F_3;
  - 2014 Fall, F_4 growing in Weslaco; selfing F_4 to get F5 and testcrossing with Tx714 to get $F_{3:4}$ testcross hybrids;
  - 2015 spring, F_5 growing in College Station; yield trail on $F_{3:4}$ testcross hybrids and F_5 inbreds;
  - 2015 summer, selfing F_5 to get F_6 and testcrossing with Tx714 to get $F_{4:5}$ testcross hybrids;
  - 2015 fall, yield trails on F_6 inbreds and $F_{4:5}$ testcross hybrids.

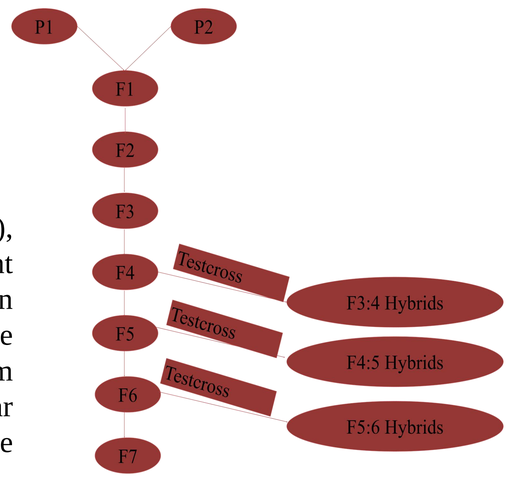

Fig 2. The research timeline in the field

Phenotyping maize height in $F_{4:5}$ progenies

- At the winter nursery (Weslaco, TX, 2014), within each individual plot, average plant height was measured as the distance in inches from the soil line to the top of the tassel; flag leaf height was measured from the soil line to the base of the flag leaf; ear height was measured from the soil line to the base of the top ear node.

Materials and Methods

Genotyping $F_{3:4}$ progenies

- Genomic DNAs were extracted from eight bulked seedlings within each of the $F_{3:4}$ plants using the CTAB method (Chen and Ronald 1999).
- To design the unique markers targeting the candidate SNPs, ~100bp surrounding the candidate SNP on either side were selected to pick the allele-specific primers and allele general primer using BatchPrimer3 v1.0 (Fig 3.) (You et al. 2008).
- KASP® (KBiosciences Allele Specific PCR) assays were used to conduct the genotyping for individual $F_{3:4}$ ear (Fig 4.).

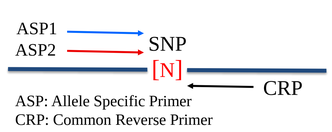

Fig 3. The scheme of primer design

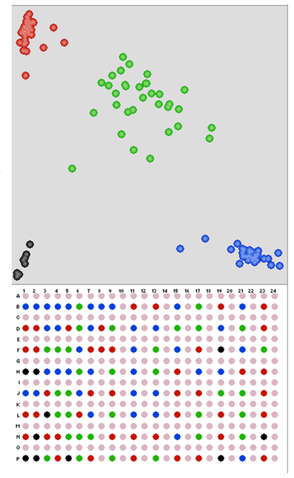

Fig 4.Genotyping plot illustrating three clear cluster. The red data points represent wild genotype (B73), the blue points represent mutants, the green data points represent herterozygous genotype and the black points are the No Template Controls (NTCs).

Preliminary Results and Questions

- Combining the phenotype data collected from $F_{4:5}$ progenies and the genotype data of $F_{3:4}$ progenies, we used JMP (least squares model) to validate each QTV (SNP1, SNP2 and SNP3) within each population. In the population Ki/NC356, one allele of SNP1from parent line Ki3 had significant positive additive effects on the plant height and flag leaf height; the allele of SNP3 from Ki3 had significant negative additive effects on the plant height and flag leaf height and this allele also had significant positive dominant effects on the plant height, flag leaf height and ear height (Table 2.).

Table 2. The JMP results for additive effect and dominant effect of SNP1 and SNP3 in population Ki3/NC356

Trait	SNP	Genetic Variation	Estimate	Std Error	t Ratio	Prob>|t|
Plant Height	SNP1	Additive Effect	1.85	0.52	3.53	0.00*
		Dominance Effect	0.64	0.93	0.7	0.49
	SNP3	Additive Effect	-1.48	0.51	-2.92	0.00*
		Dominance Effect	2.77	0.95	2.91	0.00*
Flag Leaf Height	SNP1	Additive Effect	1.40	0.48	2.89	0.00*
		Dominance Effect	0.35	0.86	0.41	0.68
	SNP3	Additive Effect	-1.27	0.47	-2.72	0.00*
		Dominance Effect	2.00	0.88	2.27	0.02*
Ear Height	SNP3	Additive Effect	0.09	0.37	0.25	0.80
		Dominance Effect	1.85	0.69	2.69	0.00*

- Compared with the results derived from GWAS, we didn't find that SNP2 affected the plant height; however, we did find other SNPs that did. The possible reason is that the phenotype data was collected from the inbred lines in the winter nursery.

Future Work

- We will trial $F_{3:4}$ derived testcross hybrids for yield under drought and irrigation conditions and $F_{3:4}$ inbred yield trials to get more precise phenotypic data(including plant height, flag leaf height, ear height, flowering time, grain yield, aflatoxin content, et al.)
- We will plant $F_{4:5}$ inbred to advance one more generation ($F_{5:6}$) and testcross hybrids ($F_{5:6}$/Tx714)
- We will genotype the $F_{4:5}$ inbred lines to capture residual heterozygosity.

References

1) Farfan, ID Barrero, et al. "Whole genome association study for drought, aflatoxin resistance, and important agronomic traits in maize in a sub-tropical environment." *PLoS ONE*.
2) You, Frank M., et al. "BatchPrimer3: a high throughput web application for PCR and sequencing primer design." *BMC bioinformatics* 9.1 (2008): 253.
3) Larkin, Mark A., et al. "Clustal W and Clustal X version 2.0." *Bioinformatics*23.21 (2007): 2947-2948
4) JMP®, Version <11.0.0>. SAS Institute Inc., Cary, NC, 1989-2007
5) Chen, D-H., and P. C. Ronald. "A rapid DNA mini preparation method suitable for AFLP and other PCR applications." *Plant Molecular Biology Reporter* 17.1 (1999): 53-57

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