DNA Marker Analysis of Biomass Production under Drought Stress in Tetraploid Alfalfa

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Introduction

Fig.1: Alfalfa genotypes used in this study (left-right) MF, F_1 & CH

Alfalfa (*Medicago sativa*) is the most important cultivated forage in the U.S. and the No.1 cash crop in New Mexico. It is a cross-pollinated perennial crop. Alfalfa has an autotetraploid (2n=4x=32) genome with a size close to 1 billion bases.

Water deficit is the most significant environmental stress factor limiting U.S. crop production (Boyer 1996). Water use efficiency (WUE) and drought tolerance are physiologically and genetically complex, and are controlled by quantitative trait loci (QTL).

DNA markers, such as simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs), are widely used to identify the location of QTL influencing the traits of interest.

Objectives

- Collect forage yield data from ~900 field research plots from an alfalfa backcross mapping population under drought stress for 3 years.
- Develop SNP markers for 50-100 stress-related candidate genes and genotype 95 BC_1F_1 individuals in the mapping populations.
- Associate the phenotypic data with available EST-SSR markers (Sledge 2005) and SNP markers that are under development to detect QTL influencing forage yield under limited irrigation.

Phenotyping

Mapping populations development (fig. 2)

- Parents: *M. sativa* subsp. *sativa* var. 'Chilean' (CH) & *M. sativa* subsp. *falcata* var 'Wisfal' (MF) with relative high degree of DNA polymorphism (Seqovia-Lerma 2003) and contrasting phenotypes (fig. 1).
- One vigorous F_1 plant from crosses of CH & MF was backcrossed to the CH parent to generate a BC_1F_1 population, CHBC, in 2000.
- BC_1F_1 plants were crossed to *M. sativa* var Peruvian in 2002 & 2003 to produce enough half-sib seeds for replicated field trials.

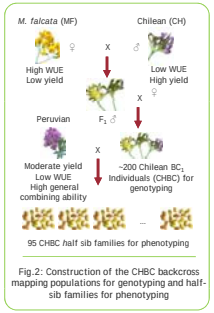

Fig.2: Construction of the CHBC backcross mapping populations for genotyping and half-sib families for phenotyping

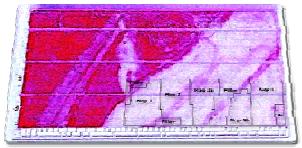

Fig.3: Aerial infrared photo of spring wheat 32 DAI showing heterogeneous soil and the area of study

Experimental design

- Early 2004, spring wheat was planted in the experimental field and drought-stressed. Infrared image taken 32 days after irrigation (DAI) showed heterogeneous soil water holding capacity (fig.3).
- Half-sib families of the BC population with check plots of the cultivar 'Wilson' as covariates (fig. 4) were seeded on most homogeneous areas available following randomized complete block design (RCBD) with 4 replications (fig. 5) in Oct. 2004.

Fig.5: Aerial photo of alfalfa experimental field under normal irrigation in spring 2007

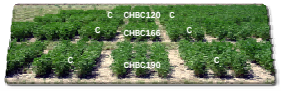

Fig.4: Half sib families planted as 3-row plots (3'X5') with alternative check ('Wilson') rows

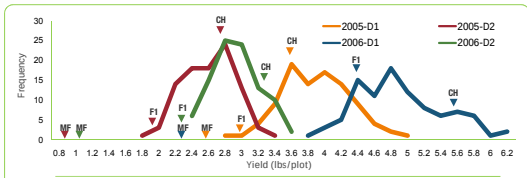

Fig.6: Distributions of fresh yield of CHBC half-sib families and parents for 4 drought cycles

Phenotypic data collection and results (Shown only 2005 and 2006 data)

- Fresh forage yield (lbs/plot) for CH, MF, F_1, 95 CHBC half-sib families, and 'Wilson' checks were collected using a flail harvest during 4 drought cycles in 2005 and 2006. Figure 6 shows the distributions of adjusted means.
- Many BC families (12.5% genome from exotic *falcata* parent) in every harvest numerically outperformed the elite CH parent (fig. 6).
- Several BC families performed as good as commercial variety 'Wilson' (data not shown).
- RCBD Split-Split Plot Analysis of Covariate (table 1).
  - BC_1 half-sib families yielded significantly differently under drought stress.
  - Covariate (check yield) accounted for a significant portion of residual error and reduced block effect.
  - Entries showed significant interactions with years and harvests, harvests should therefore be analyzed individually for mark-trait association.

Table1: ANCOVA table for fresh yield of half-sib families for 2 years & 2 harvest per year

Source	DF	MS
Blocks	3	0.07
Entries	94	1.65**
Years	1	41.42**
Year*Entry	94	0.39**
Harvests	1	23.87**
Harvest*Entry	94	0.29**
Harvest*Year	1	0.56*
Harv*Yr*Ent	94	0.14
Check Yield	1	140.45**
Error	569	0.18

*P<0.1 **P<0.001

Genotyping

- Sources of candidate genes for drought tolerance: 1) genes differently expressed under drought stress based on previous microarray studies, 2) genes with known related function, 3) genes identified in other stress studies.
- Tentative consensus (TC) sequences from *Medicago truncatula* (*Mt*) gene index and *Mt* genomic sequences from NCBI were retrieved and aligned to design gene specific primer pairs (GSPs).
- PCR products of CH, MF and F1 sequenced to identify single-dose (SD) SNPs (fig 7) . Multiplex SNP primer extension reactions (SPE) used for genotyping on Beckman Coulter CEQ8000 capillary sequencer.
- ~500 candidate genes under consideration.
- ~350 GSPs developed, ~200 GSPs sequenced.
- 30 SD-SNP markers discovered and genotyped.
- 29 SNP markers and 209 EST-SSR markers used to build a linkage map using JoinMap and map drawn by MapChart (fig. 8).

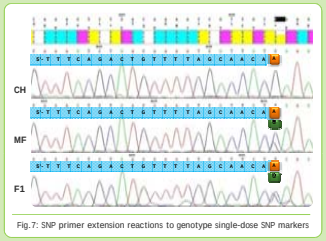

Fig.7: SNP primer extension reactions to genotype single-dose SNP markers

Marker-Trait Association

- Single-factor ANOVA in SAS to test association of the fresh yield data of half-sib families for 4 drought harvests and DNA marker data for BC_1F_1 individuals. Most significant effects on any of the 4 harvests marked on the map (fig. 8).
- Several marker interactions (table 2) showed greater associations with and effects on the yields.
- EST-SSR marker AL22 accounts for 14% of phenotypic variation and most significant 3-marker interaction AL22+BE137+BE140 accounts for 44% of the variation.

Table2: ANOVA analysis for fresh yield and SSR marker

SSR MF Allele	Linkage Group	Highest R^2	Highest Yield Effect (%)
AL22	1A	0.14	+8*
228/90/214	1A-2	0.13	-8*
AL22 + BE140	1A 8B	0.26	+16*
AL22 + BE137	1A 6A	0.34	+12*
22+137+140	1A 6A 8B	0.44	+19*

*P<0.01

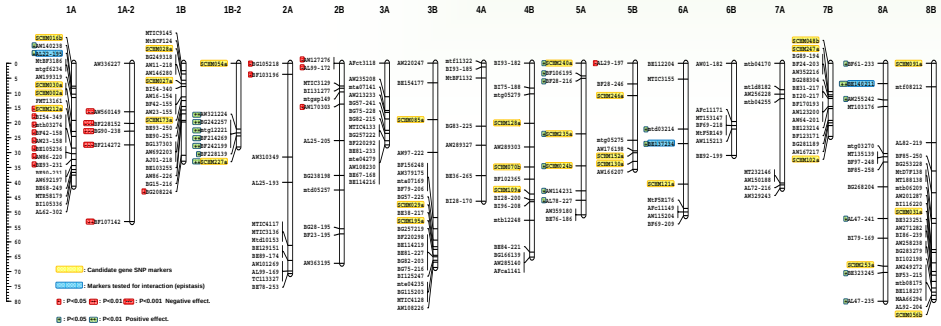

Fig.8: Alfalfa linkage map consists of 29 SNP and 209 EST-SSR markers with significant mark-trait association regions and potential marker interactions indicated.

Summary

- Forage yield data collected for 3 years. Data available for 7 normal, 4 drought , and 3 recovery cycles.
- ~350 candidate gene specific primer pairs developed. ~200 PCR products of 3 parents sequenced.
- 29 single-dose SNP markers genotyped and assigned to linkage maps with 209 EST-SSR markers.
- Simple statistical analyses identified significant marker-trait association (candidate QTL).

Ongoing and Future Work

- Biomass for 2007 drought cycles and underground mass (roots and crowns) collected and under analysis.
- More SNP markers for candidate genes under development to genotype the mapping population.
- Saturate current linkage maps with new SNP markers. Map-based QTL detection with additional traits (drought recovery yield, yield difference between drought and normal cycles, underground mass, etc.).
- Compare QTL detection efficiency between using EST-SSR markers and candidate gene markers.

Significance and Application

- Study mimicked commercial breeding or production conditions with seeded plots, elite parents, & similar field/drought conditions. Superior BC genotypes may be used in future variety breeding programs.
- Extensively utilized genomic information from model plants, e.g. *Medicago truncatula*.
- Complex experimental design and field layout based on soil water holding capacity to minimize errors.
- Long term project: continuously add markers to the linkage map and detect QTL for desired traits, use QTL markers to screen germplasm collection in search for additional allelic variation for breeding purpose.

References: - Boyer JS. 1996. Advances in drought tolerance in plants. *Adv in Agron.* 56:187-219.
- Seqovia-Lerma A, et al. 2003. AFLP-based assessment of genetic diversity among nine alfalfa germplasms using bulk DNA templates. *Genome.* 46(1):51-8.
- Sledge MK, et al. 2005. An expressed sequence tag SSR map of tetraploid alfalfa (*Medicago sativa* L.). *Theor Appl Genet.* 111(5):980-92.

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