Impact of Phenotypic Selection on the Frequencies of SSR-Defined Genomic Regions Associated with Drought Tolerance in Alfalfa

Lovepreet Singh*, Ian Ray and Chris Pierce

Department of Plant and Environmental Sciences, New Mexico State University, MSC 3Q, P.O. Box 30003, Las Cruces, NM.

*E-mail: singhlov@nmsu.edu

Introduction

Drought is a major abiotic stress that limits the productivity of crops worldwide. Alfalfa (*Medicago sativa L.)* is the most economically important forage crop in the U.S., and is the number one cash crop of New Mexico (NM). Large proportions of alfalfa acreage in the western U.S. experience water deficit on a regular basis. The uncertainties in phenotyping under field conditions, and the perennial nature of the crop, significantly reduce the rate of improving drought resilience via conventional plant breeding methods. Marker assisted selection (MAS) for genomic regions associated with drought tolerance could be a promising strategy to overcome these pitfalls. QTLs affecting alfalfa biomass productivity under water-limited conditions have been identified and introgressed into elite genetic backgrounds. Phenotypic selection (PS) imposed upon these materials, after field evaluation is completed, could complement selection gains from MAS by further increasing frequencies of desirable marker alleles and/or decreasing frequencies of unfavorable marker alleles.

Objective

- To determine the impact of phenotypic selection for drought tolerance on marker allele frequencies defining six genomic regions previously shown to be associated with alfalfa biomass production during a drought.

Materials & Methods

Population development

- Marker group populations possessing 1,2 or 3 QTLs associated with high shoot (HS), low shoot (LS), high root (HR) and low root (LR) biomass were crossed with three elite cultivars: NuMex Bill Melton (BM), Malone(Mal) and WL530 (ML).
- Fifteen MAS-derived synthetics and their controls (C_0) were produced and evaluated under deficit irrigation conditions for 3 years near Las Cruces, NM.
- The top 5 performing populations designated as BMHS1, BMHR2, BMC_0, MalHS3 and MLLS2 were identified for advancement.
- 96 Plants with superior vigor were phenotypically selected (PS) from replicated field plots of each population.
- From the BMHS1, BMHR2, BMC_0 and MalHS3 populations, 58, 62, 72 and 62 plants were genotyped, respectively, and were designated as BMHS1_PS, BMHR2_PS, BMC_0 _PS and MalHS3_PS populations.
- For MLLS2, the 96 selected plants were random mated and 224 progeny were genotyped.

Genotyping

- DNA was isolated from leaf tissue using an IBI Scientific Genomic DNA Mini Kit (Plant) and quantified via Pico green assay.
- Multiplex ready PCR[1] was adapted for alfalfa genomic SSRs (see Table 1).
- PCR products labelled with different fluorescent dyes were pooled and analyzed for allele sizes using a CEQ 8000 Genetic Analysis system (Beckman Coulter).
- Some primary marker alleles used in the initial MAS research were subsequently identified in the three cultivars.
- For such cases, additional markers linked to the primary marker were genotyped to search for unique alleles in the targeted genome regions that were not present in the cultivar.

References

1. Hayden et al (2008). Multiplex-ready PCR: a new method for multiplexed SSR and SNP genotyping. *BMC genomics*, *9*(1), 1.
2. Ray et al (2015). Identification of Quantitative Trait Loci for Alfalfa Forage Biomass Productivity during Drought Stress. *Crop Science*, *55*(5), 2012-2033.
3. Li et al (2015). Mapping Fall Dormancy and Winter Injury in Tetraploid Alfalfa. *Crop Science*, *55*(5), 1995-2011.

Table 1. Genome regions affecting alfalfa shoot and root biomass under drought stress and markers used for tracking these regions in the original marker group and PS populations[2].

Linkage group	QTL/genomic region	Biomass effect	Primary marker previously used for selection	Marker used for monitoring response to PS	Genetic distance between primary & alternate marker
1A	qdtfy1A.72	High shoot	AL22	AW199	0.7cM
1B	qdtfy1B.5	Low shoot	BF228	BF214	3.2 cM
1C	qdtfy1C.70	High shoot	AW86	BG249	11.7 cM
3A	pdrt3A.60	Low root	BG57	BG57/ Mt1A07	3.5 cM
5B	qdtfy5B.30	Low shoot	AL29	AL29/ BF28	10.0 cM
8A	pdrt8A.13	Low root	MTIC103	MTIC103	-

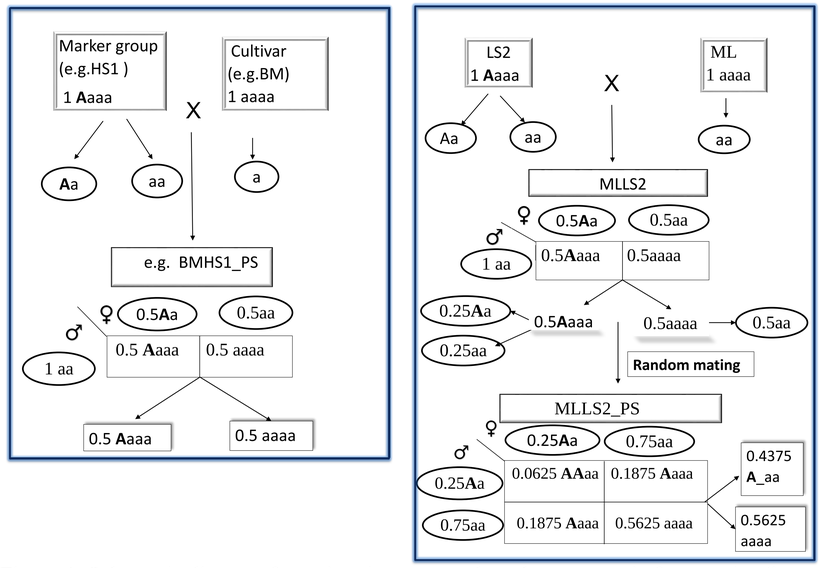

Figure 1. Schematic diagram for estimating expected marker genotypic frequencies (i.e. no phenotypic selection) in BMHS1_PS, BMHR2_PS, BMC_0 _PS and MalHS3_PS (right panel) and MLLS2_PS (left panel). Bold face "**A**" designates marker allele of interest while "a" represents alternative alleles. Presence of a marker allele in a cultivar was also accounted for when estimating the expected genotypic frequencies.

Results

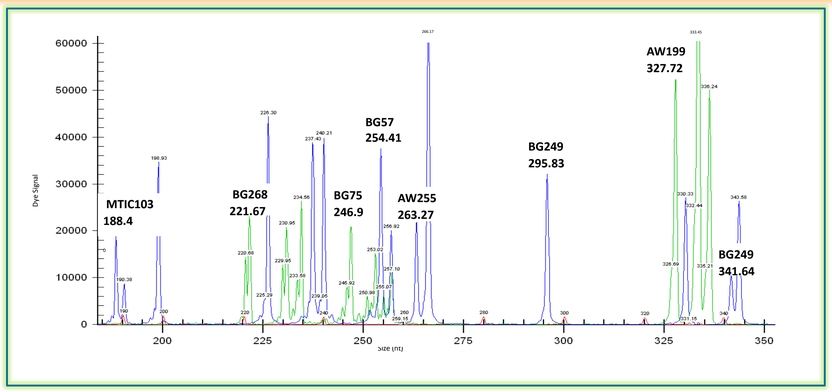

Figure 2. Electropherogram obtained after multiplexing and multi-pooling, enabled scoring of eight marker alleles of interest (bold) amplified by seven different primer pairs.

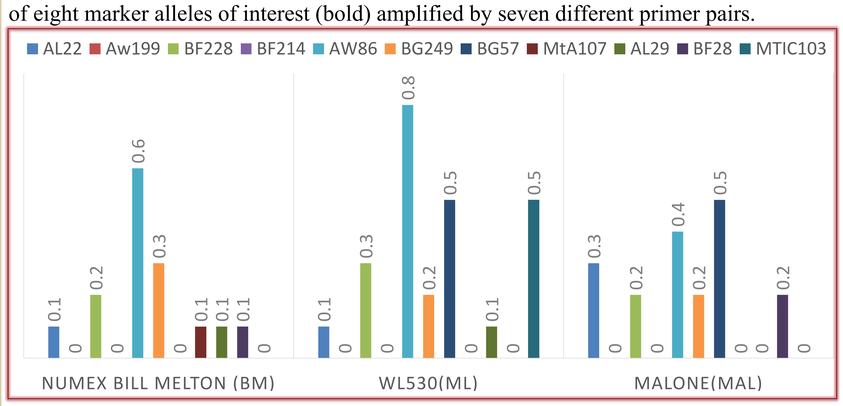

Figure 3. Frequencies of marker alleles of interest in cultivars.

Table 2. Genotypic frequencies of marker alleles for genome regions of interest in five alfalfa MAS-derived populations after phenotypic selection (PS) for drought resilience.

Population	Observed frequency in original marker group population	Estimated expected frequency without PS	Observed frequency after PS	p value
Genomic region: qdtfy1A.72				
MLLS2_PS	0.61	0.28	0.31	0.34
MalHS3_PS	0.99	0.5	0.35	**0.03**†
BMC_0 _PS	0.54	0.27	0.31	0.53
BMHS1_PS	0.5	0.25	0.16	**0.10**
BMHR2_PS	0.47	0.24	0.31	0.20
Genomic region : qdtfy1B.5				
MLLS2_PS	0.97	0.43	0.34	**0.01**
MalHS3_PS	0.57	0.29	0.19	**0.10**
BMC_0 _PS	0.47	0.23	0.22	0.81
BMHS1_PS	0	0	0	
BMHR2_PS	0.5	0.25	0.29	0.48
Genomic region : qdtfy1C.70				
MLLS2_PS	0.34	0.25	0.32	**0.01**
MalHS3_PS	0.56	0.35	0.47	0.12
BMC_0 _PS	0.47	0.35	0.65	**<0.00001**
BMHS1_PS	0.88	0.53	0.6	0.23
BMHR2_PS	0.57	0.39	0.45	0.32
Genomic region : pdrt3A.60				
MLLS2_PS	0.46	0.22	0.23	0.69
MalHS3_PS	0.39	0.2	0.13	0.18
BMC_0 _PS	0.46	0.23	0.14	**0.07**
BMHS1_PS	0.54	0.27	0.19	0.16
BMHR2_PS	0.4	0.2	0.15	0.28
Genomic region : qdtfy5B.30				
MLLS2_PS	0.9	0.4	0.28	**<0.001**
MalHS3_PS	0.46	0.23	0.31	0.16
BMC_0 _PS	0.46	0.27	0.56	**<0.0001**
BMHS1_PS	0.5	0.29	0.31	0.70
BMHR2_PS				
Genomic region : pdrt8A.13				
MLLS2_PS				
MalHS3_PS	0.89	0.44	0.52	0.26
BMC_0 _PS	0.45	0.23	0.14	**0.07**
BMHS1_PS	0.46	0.23	0.24	0.82
BMHR2_PS	0.52	0.26	0.16	**0.08**

†$\alpha = 0.1$.

Summary

- Our results highlight the potential challenges of introgressing target QTL into broad genetic-base alfalfa cultivars using MAS.
- Among eleven significant marker allele responses to phenotypic selection (Table 2), eight were consistent with the expected response, while three were inconsistent.
  - Two of the above inconsistencies involved the qdtfy1A.72 region, where a fall regrowth height QTL was previously mapped in a related population[3]. Consequently, pleiotropic effects could explain the observed responses.
  - The third inconsistency involved the qdtfy5B.30 region in BMC_0_PS and suggests the impact of genetic background on QTL effect.
- Four genomic regions exhibited significant consistent changes in marker allele frequencies in two populations (Table 2), thus validating the response to phenotypic selection.
- These results demonstrated a neutral to positive impact of phenotypic selection on the majority of targeted genome regions previously affiliated with alfalfa productivity in water limited environments.

Acknowledgements

Alfalfa Research Program
Award # 2014-70005-22537
Hatch Project
231557-NM-Ray-I2H

Agricultural Experiment Station