Genetic Dissection of Drought and Salt Tolerance Resources in Alfalfa (*Medicago sativa* L.)

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Introduction

☘ Irrigated agriculture consumes 80% of the world's allocable water resources. This consumption level is not sustainable. Enhancing drought/salt resistance and water use efficiency (WUE) of alfalfa are important to meet the challenges of finite available water resource. However, no rapid and precise methods have been developed to identify the resistance resources for the alfalfa breeders. Development of molecular markers associated with the abiotic stressors and improved WUE would be helpful for improving the accuracy in detection, accumulating the major and minor genes, and accelerating the breeding process.

Materials and Methods

☘ 200 alfalfa cultivars and landraces originated worldwide with potential value of drought tolerance were selected from Plant Germplasm Center. Traits associated with drought resistance were evaluated in greenhouse and field in 2013 and 2016. Advanced breeding populations developed by Co-PI Dr. Mike Peel were used for evaluation of salt tolerance.

☘ An integrated framework that merges a QTL mapping approach called "genome-wide association studies (GWAS)" with high- throughput genome sequencing methodologies called "genotyping by sequencing (GBS)" to map traits quickly, efficiently, and in a relatively inexpensive manner. This framework provides a statistical basis for analyzing marker-trait association using linkage disequilibrium.

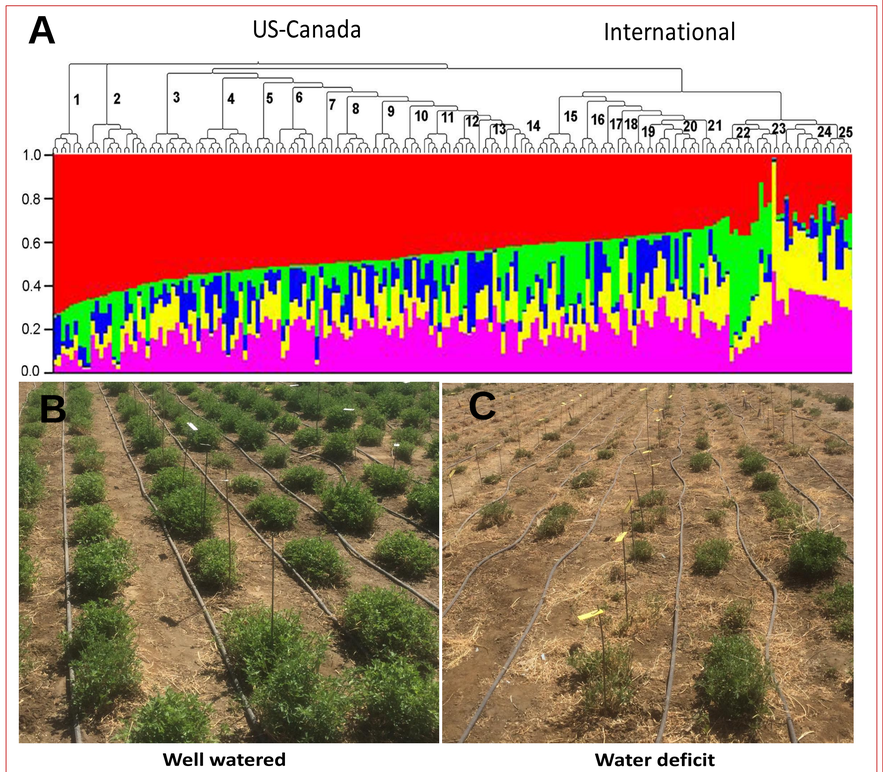

Fig. 1. The population structure of alfalfa germplasm used for this project (A). Drought resistance was evaluated in the field with irrigation treatments (e.g. well watered (B) and water deficit (C).

Results

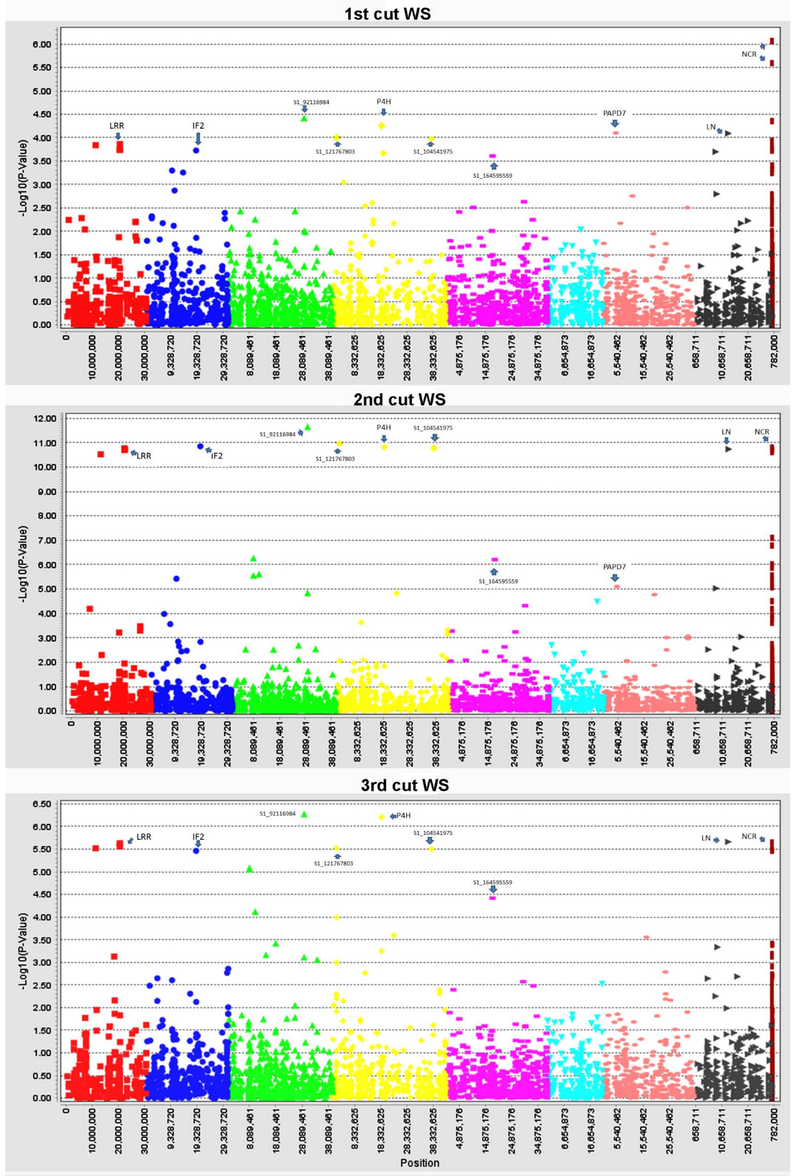

Fig.2 Drought resistance loci identified using GWAS. Biomass yield of three harvests (1st, 2nd and 3rd cuts) was measured under water stress (WS). The chromosome position was based on the reference genome of *M. truncatula* (Mt4.0 v1).

Trait	Marker	SNP	Chr	1st cut		2nd cut		3rd cut	
				p-value	r^2	p-value	r^2	p-value	r^2
Yield/WS	S1_11398797	T/G	1	1.45E-04	0.11	3.03E-11	0.31	3.03E-06	0.17
Yield/WS	S1_20593175	A/T	1	1.82E-04	0.1	1.98E-11	0.3	2.36E-06	0.15
Yield/WS	S1_20593179	C/T	1	1.36E-04	0.1	1.70E-11	0.3	2.75E-06	0.15
Yield/WS	S1_20593180	A/G	1	1.80E-04	0.1	1.95E-11	0.3	2.73E-06	0.15
Yield/WS	S1_20593182	G/T	1	1.82E-04	0.1	1.98E-11	0.3	2.36E-06	0.15
Yield/WS	S1_368369179	T/C	1*	1.56E-04	0.1	1.92E-11	0.3	2.26E-06	0.15
Yield/WS	S1_50692984	T/C	2	1.88E-04	0.1	1.43E-11	0.31	3.45E-06	0.15
Yield/WS	S1_41503160	T/A	2	5.02E-04	0.07	3.82E-06	0.12	NS	NS
Yield/WS	S1_324999216	T/G	2*	4.45E-05	0.12	2.46E-11	0.31	3.32E-06	0.15
Yield/WS	S1_92116984	T/C	3	3.84E-05	0.1	2.33E-12	0.32	5.38E-07	0.15
Yield/WS	S1_71309379	G/C	3	NS	NS	NS	NS	8.34E-06	0.14
Yield/WS	S1_71309383	G/A	3	NS	NS	NS	NS	8.95E-06	0.14
Yield/WS	S1_73439383	A/C	3	NS	NS	NS	NS	7.68E-05	0.12
Yield/WS	S1_276968267	G/C	3*	3.92E-04	0.1	8.09E-08	0.21	3.88E-04	0.1
Yield/WS	S1_276968408	G/A	3*	4.77E-04	0.1	8.09E-08	0.21	NS	NS
Yield/WS	S1_121767803	C/A	4	5.59E-05	0.12	1.52E-11	0.31	6.23E-07	0.17
Yield/WS	S1_104541975	G/T	4	1.00E-04	0.11	1.09E-11	0.32	3.00E-06	0.15
Yield/WS	S1_140803955	C/T	4	1.07E-04	0.1	1.69E-11	0.3	3.14E-06	0.15
Yield/WS	S1_122448799	T/A	4	2.15E-04	0.1	NS	NS	NS	NS
Yield/WS	S1_164595559	G/A	5	2.47E-04	0.09	6.27E-07	0.17	3.83E-05	0.12
Yield/WS	S1_326753692	T/G	6*	8.66E-07	0.15	6.63E-05	0.09	NS	NS
Yield/WS	S1_326753690	T/G	6*	2.56E-06	0.13	1.19E-04	0.08	NS	NS
Yield/WS	S1_323917811	T/C	6*	1.89E-04	0.1	1.98E-11	0.3	3.45E-06	0.15
Yield/WS	S1_212244210	C/A	7	7.95E-05	0.13	7.89E-06	0.2	NS	NS
Yield/WS	S1_352766048	A/G	7*	1.16E-04	0.1	2.10E-11	0.3	3.07E-06	0.15
Yield/WS	S1_255810503	T/C	8	8.12E-05	0.11	1.87E-11	0.31	2.18E-06	0.16
Yield/WS	S1_251014656	T/C	8	2.01E-04	0.1	9.45E-06	0.13	NS	NS
Yield/WS	S1_328564258	A/G	8*	1.69E-04	0.1	1.61E-11	0.3	3.31E-06	0.15

Table 1 Significant markers associated with yield by water stress (WS) in the panel of accessions of alfalfa. Their single nucleotide polymorphism (SNP) and chromosome locations (Chr) were identified by aligning the target sequences to the reference genome (Mt4.0 v1).

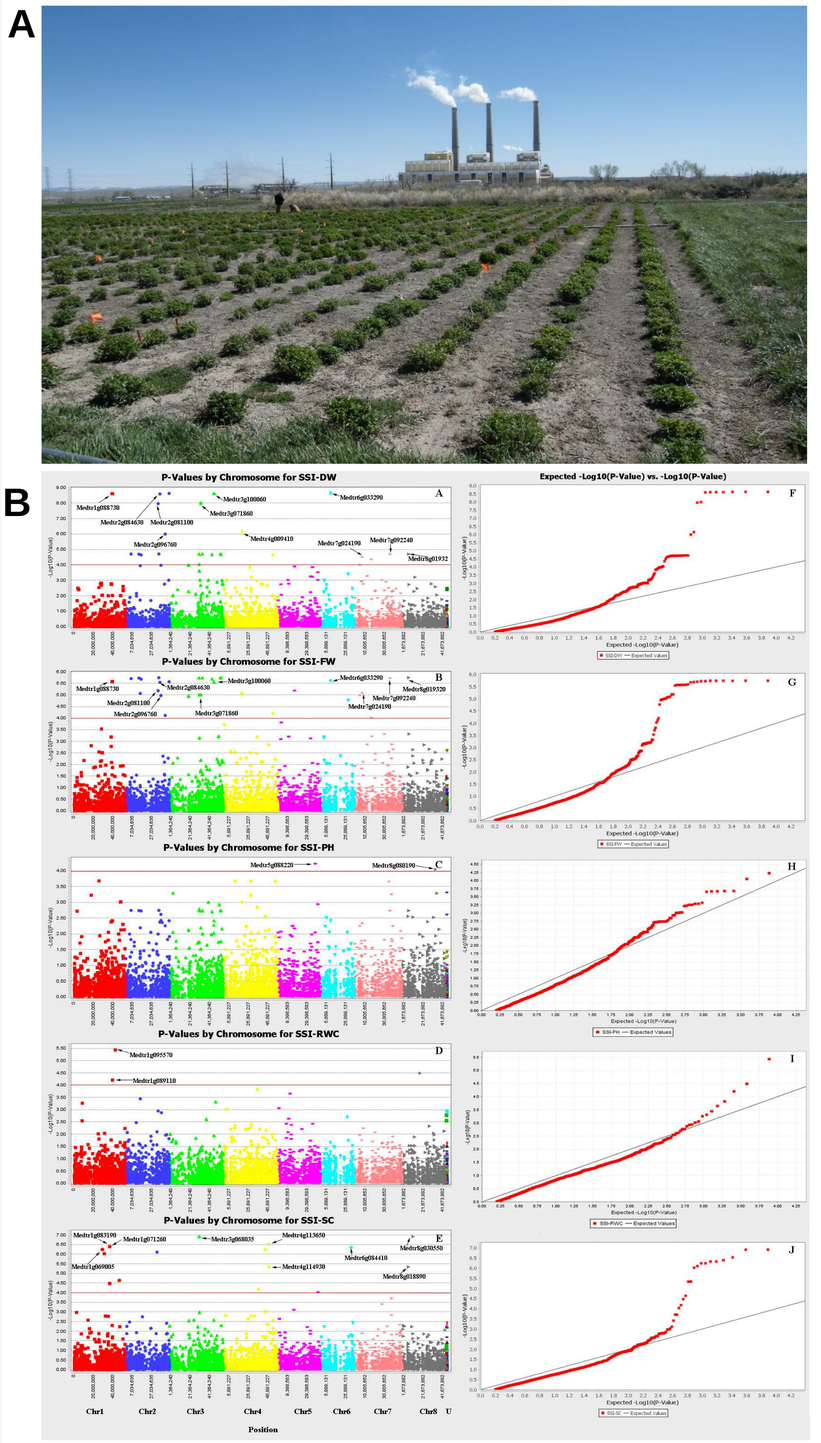

Fig.3 Salt tolerance loci were also identified in the advanced breeding populations developed by Co-PI Mike Peel (A). Dry and fresh weight (DW, FW), plant height (PH), relative water content (RWC) and stomatal conductance (SC) were measured under salt stress and used for GWAS (B). The chromosome positions of the markers were based on the *M. truncatula* genome (Mt4.0 v1).

Conclusion and Future Plans

☘Germplasm and advanced breeding populations for drought and salt tolerance have been used for GWAS.

☘Significant markers associated with drought and salt resistance were identified in the panel of germplasm and breeding populations using GWAS.

☘Resistance loci to drought and salt stresses will be validated in wide range of mapping or breeding populations

☘After validation, the markers closely linked to the resistance loci can be used for MAS in breeding alfalfa with improved drought/salt tolerance.

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