

Developing Molecular Markers for Enhancing Resistance to Drought and High Salinity in Alfalfa (*Medicago sativa* L.)

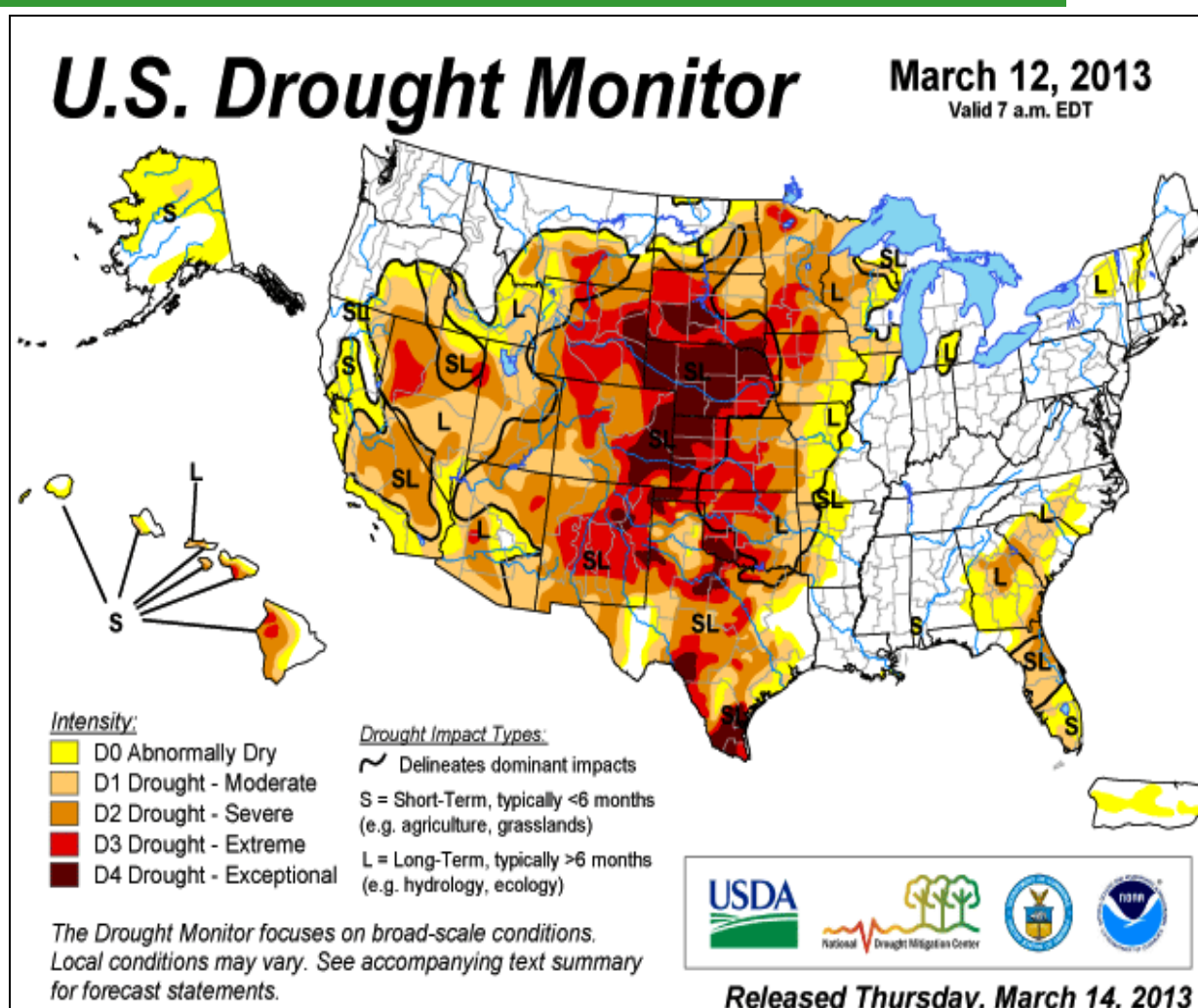
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Introduction

Irrigated agriculture consumes 80% of the world's allocable water resources. This consumption level is not sustainable.

Enhancing drought resistance (DR) 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 DR and improved WUE would be helpful for improving the accuracy in detection, accumulating the major and minor genes, and accelerating the breeding process.



Results

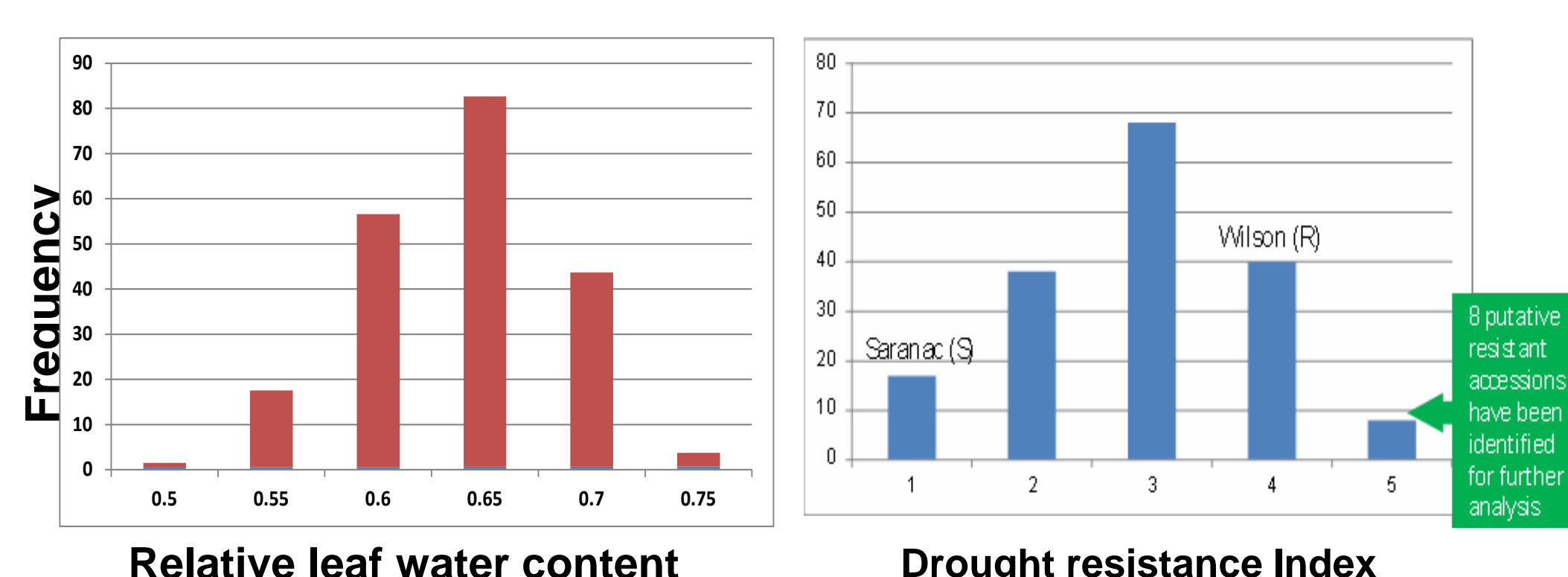


Fig. 2 The frequency statistics for relative leaf water content (RWC), drought resistance index (DRI, 1 to 5 = S to R) in the panel of accessions

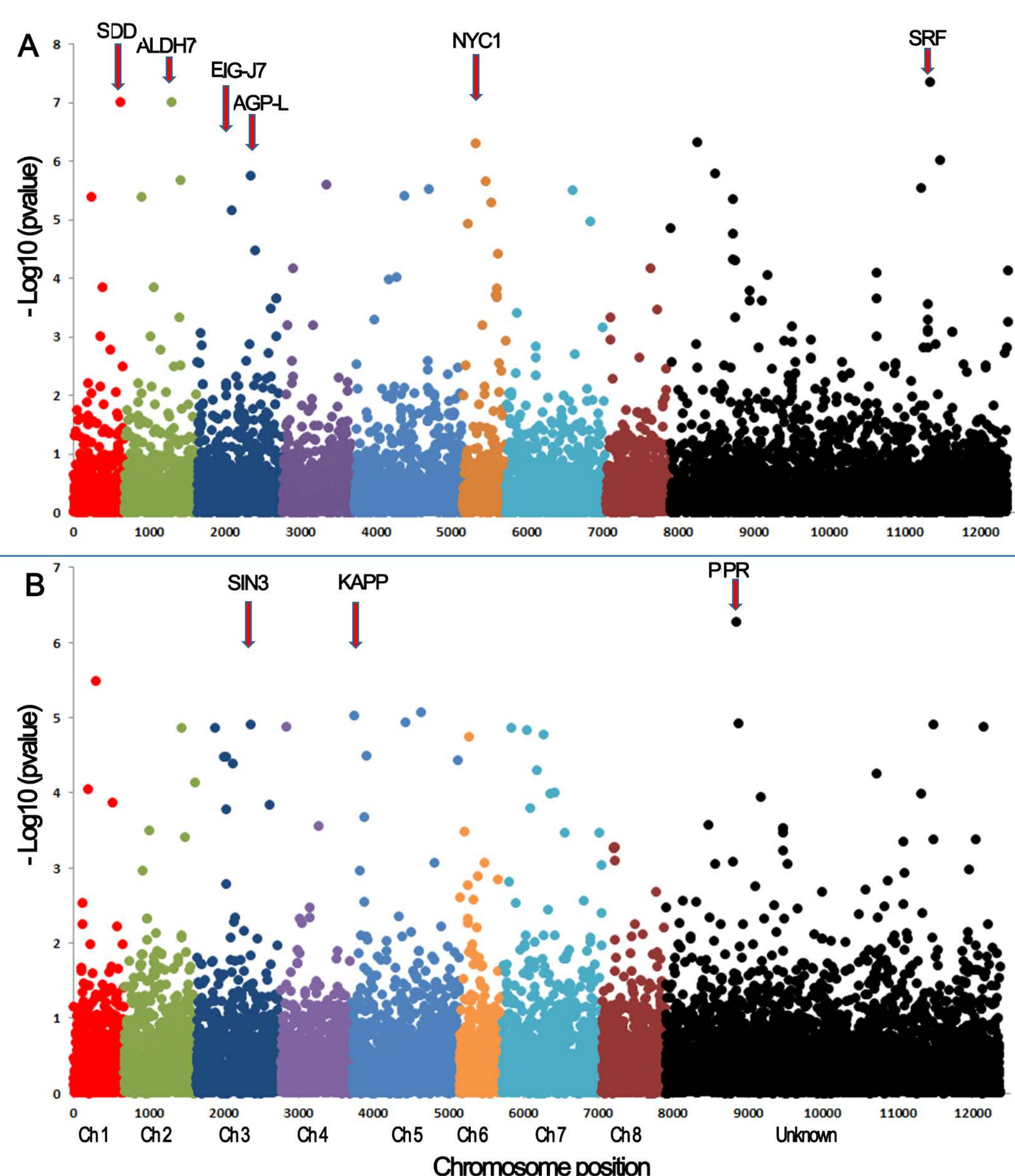


Fig.3 Manhattan plots resulting from GWAS for drought resistance index (A) and leaf relative water content (B) in alfalfa. The chromosome position was based on the reference genome of *M. truncatula* (Mt4.0 v1). The abbreviations at the top of plots are putative candidate genes and the arrows indicate their genetic positions in the reference genome. SDD, subtilisin-like protease; ALDH7A1, Aldehyde dehydrogenase family 7 member A1; EIG-J7, Elicitor inducible protein EIG-J7; AGP-L, Glucose-1-phosphate.

Table 1 Significant markers associated with DRI and RWC in the panel of accessions ("U", unknown chromosome).

Markers associated with drought resistance index						
Trait	Marker	Ref allele	Variant	Chr	p-Value	Effect
DRI	1_19125550	T	A,C	1	9.59E-08	-2.12
DRI	1_19681672	TCT	CCG,TTG,TCA,TCG	1	3.94E-06	0.48
DRI	2_12216981	T	C,G	2	2.03E-06	-0.57
DRI	2_15380249	TAAT	CATT,CATC,TCAT,CAAT	2	7.73E-06	-2.61
DRI	2_5751832	TAAG	TGAG,TAAT	2	2.05E-06	0.08
DRI	contig_113898_480	TCAAA	CCAAG,TCAAG	2*	1.60E-06	-2.63
DRI	3_28938985	CACTA	GACTG,CACTAACTCTA,CACTA,CACTG	3	6.71E-06	-2.64
DRI	3_42400246	CGGAT	TGGAC,CGGAC	3	1.76E-06	-2.62
DRI	4_44343064	AGGG	GGGA,GGGG	4	2.44E-06	-2.61
DRI	contig_102202_110	GG	AA,GA	4*	4.74E-07	-2.63
DRI	5_21015344	G	A,T	5	3.81E-06	0.24
DRI	5_7439536	TG	CA,CT,CG	5	2.91E-06	-2.62
DRI	6_18926195	GAG	AAC,AAG,GAC	6	2.18E-06	-0.14
DRI	6_19756214	TTTGTGT	TTTGTGTGTGT,TTTCATTGT,CTTGTGT	6	4.84E-07	-2.64
DRI	6_21240052	ATTT	GTCT,GTTC,GTTT	6	4.99E-06	-2.64
DRI	7_11861405	CAA	CA,CAG	7	3.06E-06	-2.63
DRI	contig_66405_1600	TCCT	CCA,TCTG,TCCA,TCCG	8*	2.81E-06	0.14
DRI	contig_69188_111	C	A	8*	4.47E-08	-2.65
DRI	contig_72424_3165	CATTTTG	CGTTTA,CGTTTTA,CATTCTA,CATTGTG,CATTTTA	U	9.46E-07	-0.29
DRI	contig_122638_109	TTT	ATA,ATC,CCT,CTT	U	4.39E-06	-2.63
Markers associated with relative leaf water content						
Trait	Marker	Ref allele	Variant	Chr	p-Value	Effect
RWC	1_25872745	ACTA	GCTG,ACTG	1	3.25E-06	0.18
RWC	contig_94352_776	CTTT	TTTC,CCTT,ATTT,TTTT	1*	1.30E-05	0.13
RWC	2_18311315	T	C,G	2	1.38E-05	0.16
RWC	3_28142935	C	T	3	1.34E-05	-0.14
RWC	3_4235727	CG	TA,CA,GG,TG	3	1.24E-05	0.22
RWC	4_11161035	TGTA	AGTG,AGTA	4	1.32E-05	0.19
RWC	5_28225009	AAAT	CAAC,GAAT,AAAA,AAAC	5	8.55E-06	0.03
RWC	5_3579590	TGTA	TTGT,GAGA,TTGA	5	9.32E-06	0.16
RWC	5_396392	T	C	5	1.15E-05	0.16
RWC	contig_131800_116	ATA	GTG,GTA	6*	5.21E-07	0.14
RWC	7_11951427	TGTT	CGTA,TTTT,TGCT,TGTA	7	1.37E-05	0.45
RWC	7_2982089	C	G,T	7	1.67E-05	0.14
RWC	7_31140861	AC	GA,GC,TC	7	1.47E-05	0.17
RWC	contig_72424_3154	GCAAT	ACAC,ATAT,ACAT	U	1.24E-05	0.46
RWC	contig_134721_180	GGG	TGA,TGG	U	1.18E-05	0.12

Table 2. List of accessions with highest scores of drought resistance.

PI number	Genus	Species	Subsp.	Cultivar name	Country	Develop	Score
PI211610	Medicago	sativa	sativa	NA	Afghanistan	Cultivar	5.00
PI452483	Medicago	sativa	sativa	PEACE	Canada	Cultivar	5.00
PI452501	Medicago	sativa	sativa	LADAK	Canada	Cultivar	5.00
PI452470	Medicago	sativa	sativa	CN37529	Canada	Cultivar	4.89
PI255962	Medicago	sativa	sativa	Rambler	Canada	Cultivar	4.78
PI346817	Medicago	sativa	sativa	Sc. Mass 3651	Canada	Cultivar	4.67
PI452478	Medicago	sativa	sativa	CN37536	Canada	Cultivar	4.67
PI467985	Medicago	sativa	varia	GRIMM	Canada	Cultivar	4.67
PI468014	Medicago	sativa	sativa	CN39577	Canada	Cultivar	4.67
PI452480	Medicago	sativa	sativa	CN37538	Canada	Cultivar	4.61
PI468028	Medicago	sativa	varia	GRIMM	Canada	Cultivar	4.56
PI511303	Medicago	sativa	sativa	NA	China	NA	5.00
PI244674	Medicago	sativa	sativa	E.C. 772	India	Cultivar	4.78
PI247006	Medicago	sativa	sativa	NA	Lebanon	Cultivar	5.00
PI452494	Medicago	sativa	sativa	ORENBERG	Montana	Cultivar	5.00
PI452497	Medicago	sativa	sativa	CN37554	Montana	Cultivar	5.00
PI467966	Medicago	sativa	sativa	CN39529	Montana	Cultivar	5.00
PI467982	Medicago	sativa	sativa	CN39545	Montana	Cultivar	5.00
PI467983	Medicago	sativa	sativa	CN39546	Montana	Cultivar	5.00
PI452496	Medicago	sativa	sativa	CN37553	Montana	Cultivar	4.89
PI467967	Medicago	sativa	sativa	GRIMM	Montana	Cultivar	4.78
PI467961	Medicago	sativa	sativa	CN39524	Montana	Cultivar	4.56
PI467885	Medicago	sativa	sativa	CN 39451	N Dakota	Cultivar	4.56
PI467895	Medicago	sativa	sativa	CN 39459	N Dakota	Cultivar	4.56
PI467907	Medicago	sativa	sativa	52	S Dakota	Cultivar	4.89
PI467978	Medicago	sativa	sativa	CN39541	S Dakota	Cultivar	4.56
PI234481	Medicago	sativa	sativa	NA	Spain	Cultivar	5.00

Conclusion and Future Plans

Great genetic variations were found in 200 alfalfa cultivars and landraces evaluated for drought resistance in greenhouse and field.

Twenty seven accessions with high resistance scores were selected and may carry resistance gene and have potential value for developing populations for mapping QTLs and breeding for drought resistance in alfalfa.

Significant markers associated with DRI and RWC were identified in the panel of accessions using genome-wide association. After validation.

The same panel of accessions is being used for identifying loci associated with salt tolerance.

Additional populations for drought and salt tolerance are under development. 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.

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 2014.

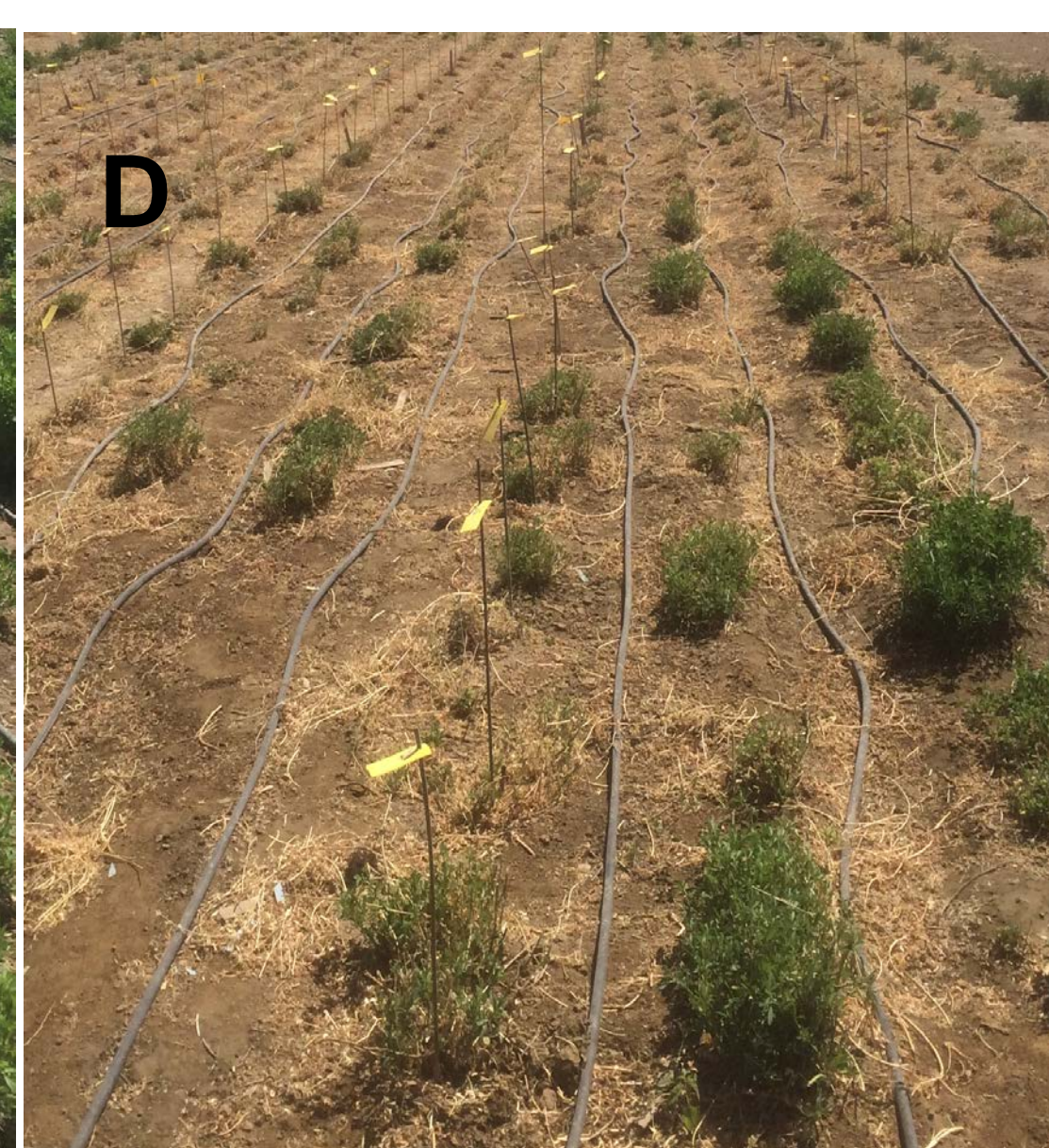
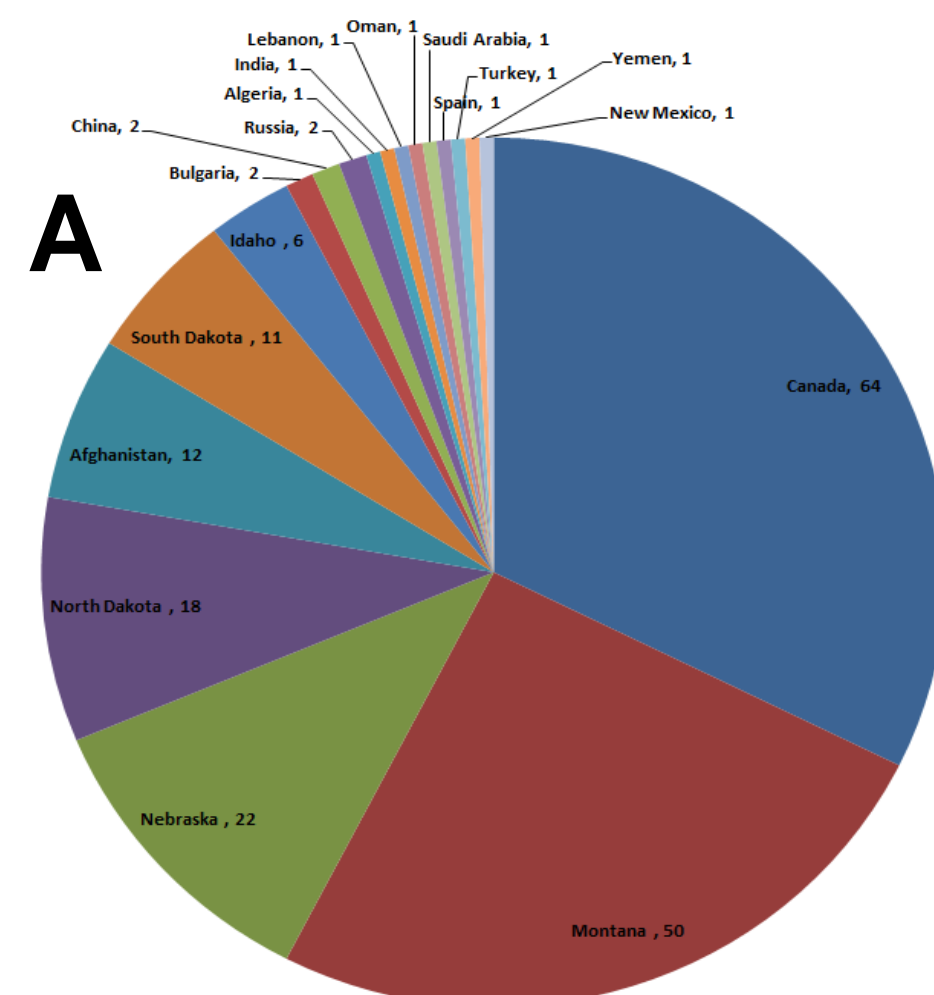


Fig. 1. The origin of germplasm used for this project (A). Drought resistance was evaluated in greenhouse (B), and field with irrigation treatments (e.g. well watered (C) and water deficit (D)).

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.