Genomic Analysis of Verticillium Wilt Resistance in Alfalfa (*Medicago sativa* L.)

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Introduction

Alfalfa is the fourth largest crop following corn, soy and wheat in US. Verticillium wilt (VW) of alfalfa is a soilborne disease causes severe yield loss in alfalfa production. Identification of loci associated with VW resistance will facilitate breeding for improving resistance to the disease in alfalfa. In the present investigation, we applied an integrated framework of genome-wide association with high-throughput genotyping by sequencing for identifying VW resistance loci in two alfalfa populations. Phenotyping was done by inoculation of the pathogen to replicated cloned plants of each individual and disease resistance was scored using a standard scale. Marker-trait association by linkage disequilibrium identified a group of SNP markers significantly associated with VW resistance.

Experimental procedures

1)Two alfalfa populations (S & W Seed and Forage Genetics populations) were used. *V. alfalfae* isolates were inoculated to the cloned plants and phenotyped by scoring VW resistance using the standard protocol (NAAIC.org).
2) Individuals were genotyped using genotyping-by-sequencing (GBS), followed by genotype calling using different pipelines (Fig.1).
3) GWAS was performed using linkage disequilibrium.

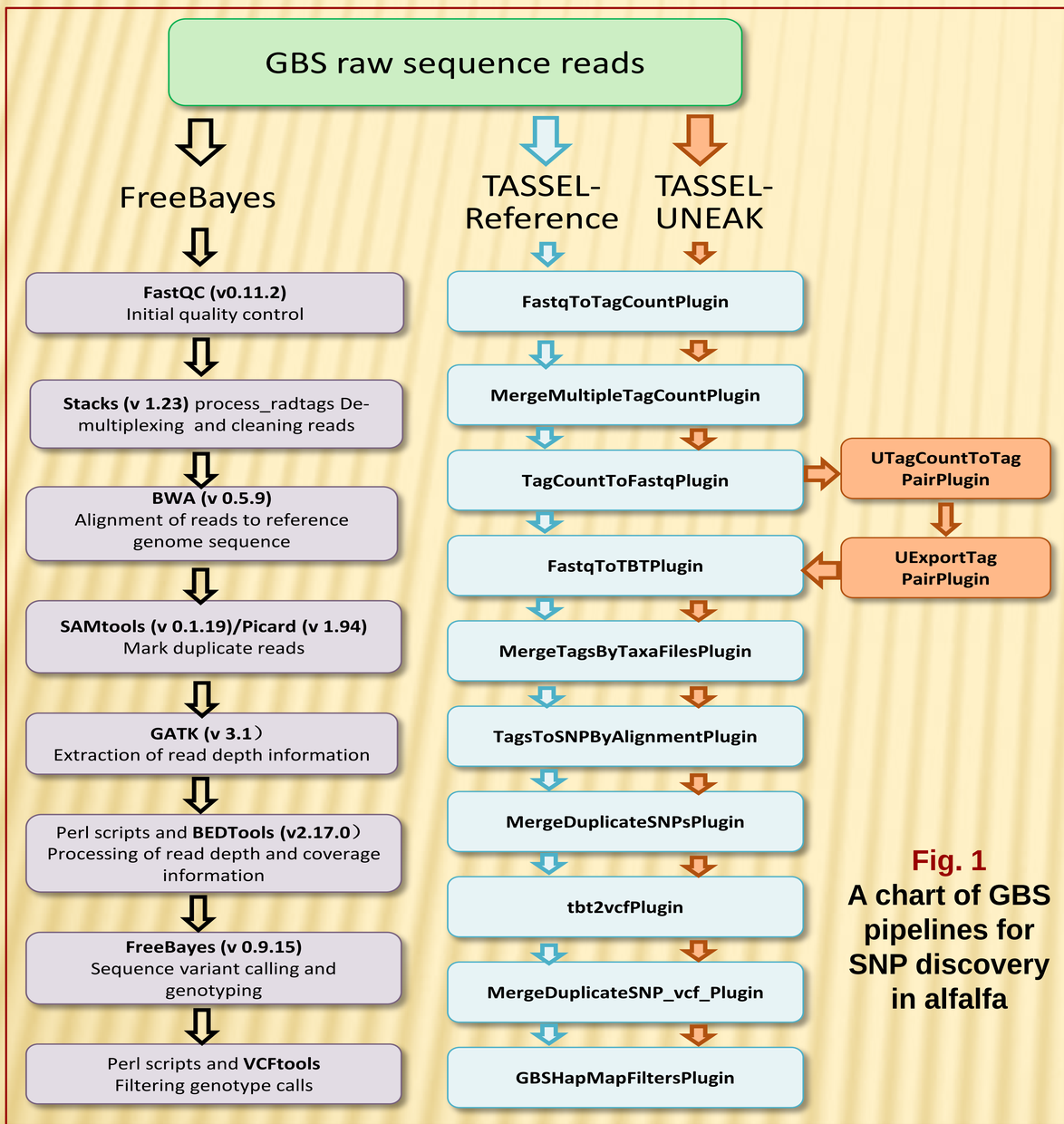

Fig. 1 A chart of GBS pipelines for SNP discovery in alfalfa

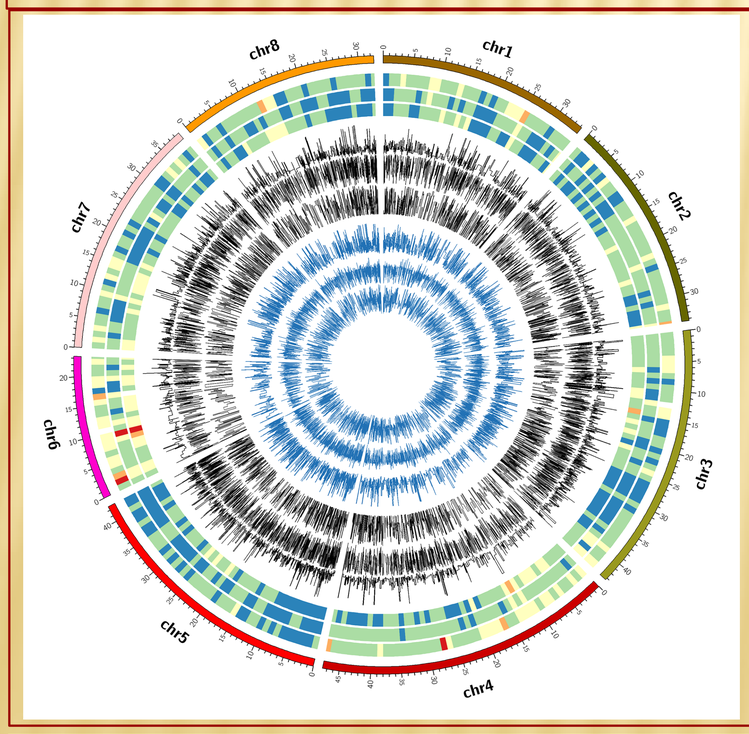

Fig. 2 GBS SNP density, minor allele frequency and heterozygosity of GBS markers generated by different pipelines. From outside to inside circles: chr, chromosome (1). SNP density by FreeBayes (2), Referenced (3) and UNEAK (4) pipelines. Minor allele frequency by FB (5), Ref (6) and UNEAK (7) pipelines. Heterozygosity by FB (8), Ref (9) and UNEAK (10) pipelines.

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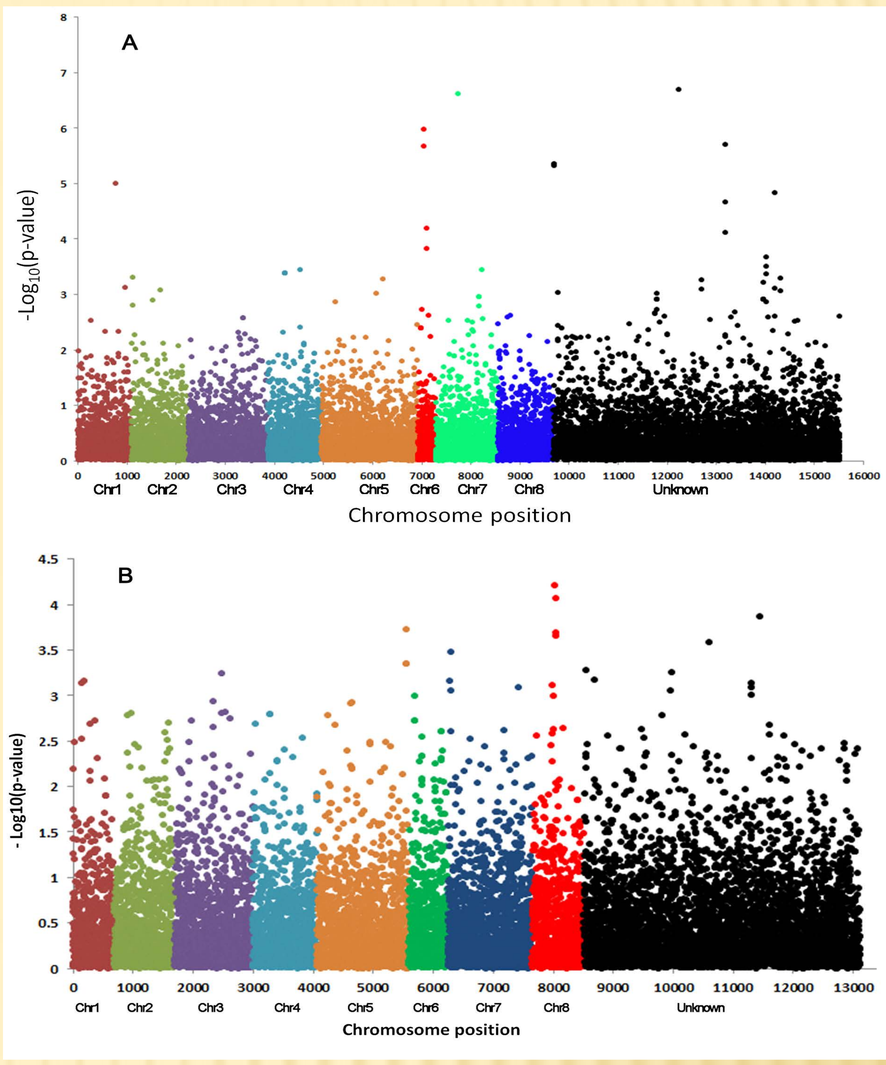

Fig. 3 Manhattan plots of genome-wide association mapping. Showing significant SNP markers associated with alfalfa VW resistance in the S&W Seed (A) and the Forage Genetics (B) populations with different genetic backgrounds. The positions of SNPs were based on the alignment of sequence tags of alfalfa to the *M. truncatula* genome sequence (v4.01)

Table 1. Most significant SNP markers associated with Verticillium wilt resistance in the S & W Seed alfalfa populations

Marker	Reference	Variants	Chr	P-value	R^2	Candidate
S4_64782733	A	C	4	1.25E-08	0.19	
S5_149762654	C	T	5	5.72E-06	0.14	
S6_166044532	G	A	6	6.58E-08	0.19	P450
S6_196781823	C	A	6	1.65E-07	0.15	SPA
S6_330143983	A	G	6	4.13E-06	0.14	CACAATP
S6_189823065	A	C	6	1.95E-05	0.12	
S_191783474	C	T	6	2.86E-05	0.10	PTRP
S6_190311466	T	A	6	9.00E-05	0.10	
S6_357689693	TC	AA	6	1.88E-04	0.10	MTRABCT
S6_288766399	A	G	6	7.14E-05	0.10	MATE

Table 2. Most significant SNP markers associated with Verticillium wilt resistance in the Forage Genetics alfalfa populations

Marker	Reference	Variants	Chr	P-value	R^2	Candidate
S5_40754497	A	C	5	4.47E-04	0.11	TMP
S5_40754542	C	T	5	4.50E-04	0.11	TMP
S5_40754556	AC	GA,AA	5	1.90E-04	0.09	TMP
contig_92284_538	TTTTC	CTCTC,CTTTA,CTTTC	6*	1.34E-04	0.10	TIR-NBS-LRR
contig_67844_1658	AC	GG,AG	6*	2.64E-04	0.10	NCR
S7_708932	TAGA	CAGG,CAGA	7	3.35E-04	0.10	PAT1
S8_15421844	T	C	8	6.15E-05	0.12	TIR-NBS-LRR
S8_15870197	C	T	8	2.20E-04	0.11	LYST
S8_15870215	CTT	CTTT	8	8.59E-05	0.14	LYST
S8_15870225	GGTTAATA	AGTTCATT,AGTTCATA ,AGTTAATA	8	2.05E-04	0.09	LYST

Results and conclusions

1) Among twenty thousands of validate markers, 65% were aligned to the *M tuncatula* genome (Fig. 2). The rests were unknown.

2) Markers significant associated with VW were identified and they located on chromosomes 4, 5 and 6 in the S&W population and 5, 6, 7 and 8 in the Forage Genetics population (Fig. 3, Tables 1 & 2).

3) Overlaps of the resistance loci were found on chromosomes 5 and 6 between the two populations, and between our previous report and the present study (Zhang et al. 2014 PLoS ONE, 9, e115953. doi:10.1371/journal.pone.0115953).

4) BLAST search using the flanking sequences of the resistance loci against *M. truncatula* genome identified candidate genes associated with disease resistance, including TIR-NBS-LRR protein and MDR-ABC transporter. With further investigation, these markers may be used for marker-assisted selection for breeding VW resistant alfalfa.