Genome Wide Association Studies of Fusarium Wilt Resistance in Wild Spinach

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Introduction

Root symptoms of spinach Fusarium wilt

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *spinaciae* (*Fos*), causes major losses in the primary region of spinach seed production in the USA, western Oregon and Washington. Acid soils in this region are highly conducive to the disease. A majority of spinach parent lines used in hybrid spinach seed crops are very susceptible to *Fos*. Wild spinach, *Spinacia turkestanica*, has been used as a source of resistance to a number of spinach diseases. The potential for *S. turkestanica* accessions to provide sources of resistance to *Fos* remains to be determined.

Objectives

1) Identify potential sources of resistance to *Fos* in *S. turkestanica*.
2) Employ genome wide association studies (GWAS) to map Fusarium wilt resistance found in *S. turkestanica* accessions.

Materials and methods

- *S. turkestanica* (*n* = 74) and *S. oleracea* (*n* = 16) accessions were evaluated for reaction to intermediate (37,500 spores/ml soil) and high (112,500 spores/ml soil) inoculum levels of a mix of three isolates of *Fos* (*Fus*058, *Fus*254, and *Fus*322) compared to non-inoculated soil. The experiment was completed in a greenhouse with a factorial design of genotypes and inoculum levels, and two replicates of six plants per treatment combination.
- A Fusarium wilt severity index (FWSI, 0 to 1) and area under the disease progress curve (AUDPC) were calculated from wilt severity ratings 21, 28, and 35 days after planting (DAP). Fusarium wilt severity was rated on a 0-to-5 ordinal scale (Gatch and du Toit 2015).
- Single nucleotide polymorphism (SNP) markers (*n* = 7,065) were identified with genotype by sequencing (GBS).
- Population structure of the spinach genotypes was investigated using Structure 2.3.4. Structure harvester was employed to deduce the number of *S. turkestanica* groups.
- Marker-trait associations were investigated using TASSEL 5 and GAPID with multiple general and mixed linear models.

Results

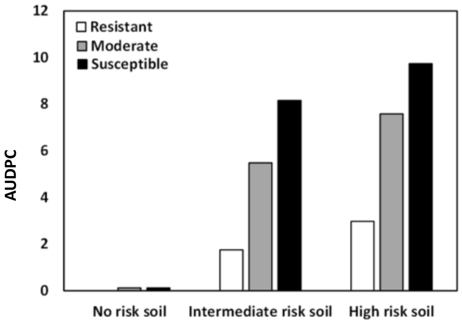

Fig. 1. Area under the disease progress curve (AUDPC) of Fusarium wilt severity index for susceptible, moderate, and partially resistant spinach inbred lines grown in soil with no, intermediate, or high inoculum levels of *Fusarium oxysporum* f. sp. *spinaciae* ($P < 0.05$ among inbred lines).

Results continued

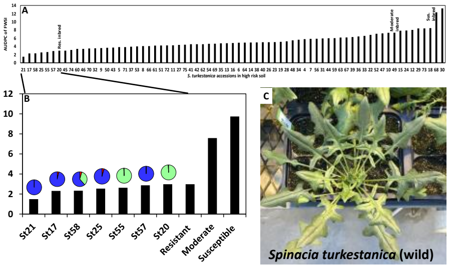

Fig. 2. Area under the disease progress curve (AUDPC) of Fusarium wilt severity index for *Spinacia turkestanica* accessions grown in soil with a high inoculum level ($P < 0.05$).

- Of the 74 *S. turkestanica* accessions, 9 had resistant reactions to the *Fos* isolates, with AUDPC values ≤3.0 at the high inoculum level (Fig. 2A). Likewise, 32 accessions had moderately resistant reactions, with AUDPC values ≤5.0.
- Genotypes St21, St7, St58, St25, St55, St57, and St20 had more resistant reactions (less wilt) than the resistant control inbred (Fig. 2B).

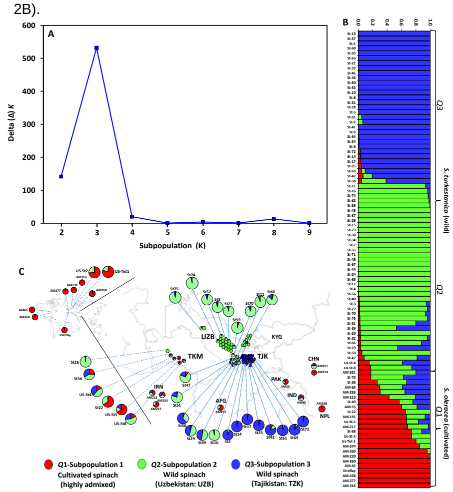

Fig. 3. Population structure of 74 *Spinacia turkestanica* and 16 *S. oleracea* accessions based on 7,065 SNPs. A) Subpopulation $\Delta K = 3$. B) Three subpopulations were identified: *Q1* = *S. oleracea* accessions, *Q2* = *S. turkestanica* accessions, most of which originated from Uzbekistan, and Q3 = *S. turkestanica* accessions, mostly from Tajikistan. C) Geographic distribution of the genotypes [pie charts show proportions of the subpopulations in each accession).

- Three subpopulations were identified: Q1 = *S. oleracea* accessions (10% of accessions), Q2 = *S. turkestanica* accessions (30%), and Q3 = *S. turkestanica* accessions (30%), with the remaining genotypes showing admixture (Fig. 3A and 3B).
- Of the accessions most resistant to Fusarium wilt, genotypes St55 and St20 are in subpopulation *Q2*; accessions St21, St17, St25, and St57 are in *Q3*; and St58 is an admixture of *Q2* and *Q3* (Fig. 2B and 3C).

Table 1. Significant SNPs associated with resistance to spinach Fusarium wilt at high and intermediate inoculum levels, identified using different genome wide association studies (GWAS) models.

SNP	CH	Position	Wilt risk	BLINK	FarmCPU	MLM	MLMM	Super	GLM	T.MLM	T-GLM	SMR
				GWAS model *lod* value								
S1_25951949	1	25951949	Intermediate	2.86	5.11	1.31	2.86	1.55	1.46	1.00	1.12	1.15
S1_29168394	1	29168394	HIGH	3.36	3.36	2.97	3.33	3.06	2.99	2.39	2.71	1.97
S1_44196225	1	44196225	HIGH	3.23	3.23	2.83	3.15	2.86	2.89	2.24	2.55	0.90
S1_48745097	1	48745097	Intermediate	2.17	5.32	1.85	2.17	2.02	2.05	1.73	1.82	3.11
S3_4557414	3	4557414	Intermediate	2.13	5.73	2.39	2.13	2.66	2.62	2.27	**3.09**	0.86
S3_10604391	3	10604391	HIGH	1.52	1.52	1.46	1.51	1.43	1.47	**3.02**	**3.42**	9.67
S3_10604403	3	10604403	HIGH	1.52	1.52	1.46	1.51	1.43	1.47	**3.02**	**3.42**	9.67
S3_10604424	3	10604424	HIGH	1.52	1.52	1.46	1.51	1.43	1.47	**3.02**	**3.42**	9.67
S4_92628754	4	92628754	HIGH	3.20	3.20	2.87	3.19	3.05	2.86	**3.51**	**4.28**	1.40
S6_11538772	6	11538772	HIGH	3.48	3.48	3.05	3.44	3.14	3.08	**3.90**	**4.77**	1.31
S6_35488289	6	35488289	HIGH	3.35	3.35	2.94	3.29	3.13	2.98	2.20	2.42	1.09
S6_38110665	6	38110665	Intermediate	7.91	9.24	4.49	6.58	5.34	5.11	3.64	**5.71**	6.03

SNP = Single nucleotide polymorphism. CH = Chromosome. Position = Physical distance on the chromosome. GWAS models used: BLNK, FarmCPU, MLM, MLMM, Super, GLM, T-MLM, T-GLM, and SMR.

Using multiple GWAS models, 12 significant ($P < 0.001$) SNP markers were associated with mean AUDPC values of Fusarium wilt severity at intermediate and high inoculum levels (Table 1).

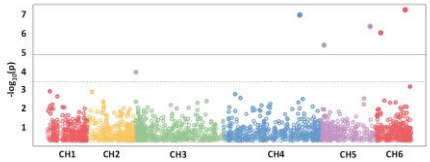

Fig. 4. Manhattan plot showing $-\log_{10}$ of *P* values from a genome wide association study (GWAS) with model BLINK based on the AUDPC values of Fusarium wilt severity at medium risk inoculum level.

A major QTL was identified on chromosome 6H (S6_38110665) in soil with an intermediate inoculum level. The QTL was validated with multiple GWAS models (Table 1 and Fig. 4).

Discussion

- Two distinct sub-populations were identified among the 74 *S. turkestanica* accessions, Q2 and Q3, originating largely from Uzbekistan and Tajikistan, respectively, in central Asia (Gyawali et al. (2021). These subpopulations have implications for selecting accessions with resistance to spinach Fusarium wilt for breeding programs.
- Fusarium wilt resistant *S. turkestanica* accessions may provide new sources of resistance to Fusarium wilt for spinach breeding programs.
- To diversify sources of Fusarium wilt resistance alleles in spinach breeding programs, accessions of *S. turkestanica* with resistance to Fusarium wilt should be selected from both the *Q2* and *Q3* subpopulations.
- A major QTL for Fusarium wilt resistance identified on chromosome 6H in *S. turkestanica* could be introgressed into spinach cultivars with suitable commercial backgrounds.
- The significant SNPs can be used for marker-assisted selection for Fusarium wilt resistance in spinach breeding programs.

Acknowledgements

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