Development of High Throughput KASP SNP Markers for Wheat Curl Mite Resistance and their Application in Marker-assisted Breeding

Smit Dhakal[1], Chor Tee Tan[2], Shuyu Liu*[2], Jackie Rudd[2], Qingwu Xue[2], Brock C. Blaser[3], Ravindra Devkota[2], Charlie Rush[2], Maria Pilar Fuentealba[2]

[1]Soil and Crop Science, Texas A&M University, College Station, TX. 77843, [2]Texas A&M AgriLife Research and Extension Center, Texas A&M University, Amarillo, TX. 79106, [3]Plant, Soil and Environmental Science, West Texas A&M University, Canyon, TX. 79016.*Sliu@ag.tamu.edu

INTRODUCTION

- Biotic stresses coupled with abiotic stresses are the major limiting factor for crop production feeding the 9 billion people by 2050.
- Wheat curl mite (*Aceria tosichella* Keifer, WCM) have been a persistent concern to farmers and researchers for at least six decades impacting forage and grain yields, and crop water use efficiency.
- At least 90 plant species hosts have been reported across 34 countries around the world for WCM.
- WCM transmit *Wheat streak mosaic virus* (WSMV), *Wheat mosaic virus* (WMoV), *Triticum mosaic virus* (TriMV), and *Brome streak mosaic virus* (BrSMV) (Navia et al, 2013).
- Yield loss by mite-virus complexes have been reported up to 100% at field level and up to 7% state wide (Appel et al, 2001, Slykhuis, 1976).
- TAM 112, most widely grown hard red winter wheat (HRWW), is noted for resistance to WCM.
- With the application of molecular marker, we are trying to predict the genotype performance and at the same time help to accelerate the breeding process.

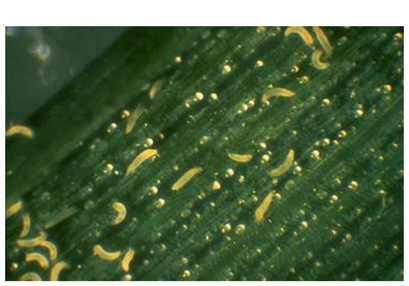

Figure 1: WCM colony seen under microscope

Figure 2: (Left to Right): Susceptible and Resistant line

OBJECTIVES

- Screen the currently grown winter wheat to know their resistance to WCM.
- Map the Wheat curl mite resistance gene in the TAM 112.
- Develop a diagnostic KASP SNP markers for wheat curl mite resistance.

MATERIALS AND METHODS

Phenotyping:

- For screening objective, 40 wheat lines each from Northern Regional Performance Nursery (NRPN), Southern Regional Performance Nursery (SRPN), Texas Elite (TXE) lines, and 52 Cultivar and Elite Breeding (CEB) lines were screened.
- For mapping objective, 124 F7 recombinant inbreed lines from TAM 112/TAM 111 were screened.
- TAM 112 (resistant check), TAM 111, Karl 92 (susceptible check) were also screened.
- A total of 15 plants per line were screened in replicated experiments.
- 24° C day and 20° C night temperature and 14 hrs light and 10 hrs dark period were maintained inside the growth chamber.
- Each plant was infested with WCM at the two-leaf stage .
- Scoring on resistant (R) or susceptible (S), was done on the first and second week after double infestation (Figure 2)

Genotyping and Mapping:

- DNA was extracted from all the lines including parental and check using CTAB protocol.
- DNA samples were sent to the USDA genotyping center, Fargo, ND for 90K SNP genotyping.
- Illumina's iSelect infinium assay platform was used to genotype SNP.
- Fluorescent intensities of A and B allele captured by iScan were analyzed using Genome studio software.
- A total of 90,900 markers including SNPs , DArT, SSR and STS, were screened.
- 7985 polymorphic markers were utilized to construct the genetic map.
- JoinMap version 4.0 was used to create the linkage map.

RESULTS AND DISCUSSIONS

WCM reaction	NRPN	SRPN	TXE	CEB	Total lines
R	3	9	16	17	45
S	34	30	21	33	118
H	3	1	3	2	9
Total	40	40	40	52	172

Table 1: Response of wheat lines across the four tests to wheat curl mite

WCM reaction	SRPN	TXE	CEB	Total lines
R	6	10	4	20
S	0	1	0	1
H	0	2	0	2
Total	6	13	4	23

Table 2: Response of wheat lines with TAM 112 in their pedigree across the four tests to wheat curl mite

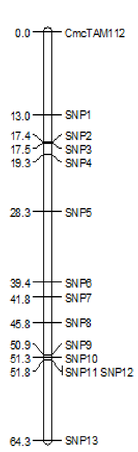

Figure 3: Wheat curl mite resistance gene mapped

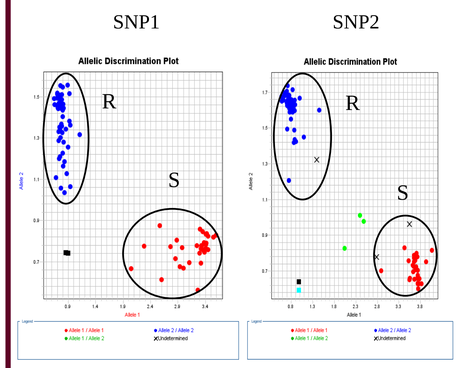

Figure 2:KASPar SNP platform for 96 lines from TAM 112/TAM 111

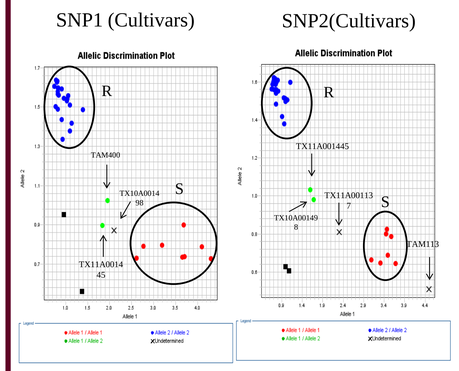

Figure 3:Graph view of screened SNP on cultivar validation

Name	SNP Cmc_{TA} M112 (SNP1)	SNP Cmc_{TAM112} (SNP2	WCM screening	TAM112 or PI268210 pedigree	1AL.1RS
Largo	A	A	R	Yes	No
PI268210	A	A	R	Yes	No
TAM110	B	B	R	Yes	Yes
TAM111	B	B	S	Negative control	No
TAM112	A	A	R	Yes	Yes
TAM113	B	B	S	No	
TAM204	A	A	R	Yes	No
TAM400	H	B	-	No	Yes
TAM401	A	A	S	No	No
TX01M5008	A	A	-	No	-
TX01M5009	A	A	-	No	-
TX01M5009-28	A	A	-	No	-
TX09V7352	A	A	R	Yes	Yes
TX10A001099	B	B	R	Yes	Yes
TX10A001380	A	A	R	No	Yes
TX10A001498	H	H	R	Yes	Yes
TX10A001515	A	A	R	Yes	Yes
TX10A001537	A	A	R	Yes	No
TX10D2063	A	A	R	No	Yes
TX11A001112	B	B	R	No	No
TX11A001137	B	B	R	No	Yes
TX11A001173	A	A	R	No	Yes
TX11A001295	A	A	R	Yes	Yes
TX11A001359	A	A	R	Yes	Yes
TX11A001440	A	A	R	Yes	Yes
TX11A001445	H	H	R	No	Yes
TX11A001549	A	A	R	Yes	No
TX11A001605	A	A	R	Yes	Yes
TX11A001643	A	A	R	Yes	No
TX97V5300	A	A	-	Yes	-
TXGBE273A	B	B	R	Yes	-
TXGBE281C	B	B	R	Yes	-
WB-Cedar	A	A	R	No	Yes

Table 3:Validation of KASPar SNP for Cmc_{TAM112}

I. Screening of Resistance source of WCM present in hard red winter wheat

- Out of 43 resistant lines, 28 have 1AL.1RS translocation containing *Cmc3* gene, while eight lines derived from TAM112 showed resistance even when no 1AL.1RS translocation was present.
- TAM 112 is in the pedigree of 20 resistant lines (Table 2).
- Results suggest that, in addition to 1AL.1RS translocation, there is another gene governing WCM resistance in TAM 112.
- In general line with either 1AL.1RS or TAM112 in its pedigree exhibited a high degree of resistance to WCM.

II. Mapping of the WCM resistant gene in TAM 112

- 99 homozygous resistant: 5 heterozygous: 20 homozygous susceptible.
- TAM 112 was consistently resistant to the WCM colony from Texas collection.
- In addition to the 1AL.1RS translocation, another WCM resistant gene in TAM 112 was mapped (Figure 3).
- The resistance gene present in TAM 112 was designated as ***CmcTAM112***.
- SNP1 was at 13.0 cM away from the Cmc_{TAM112} proximally, among 13 SNP markers mapped onto the same linkage group.

III. Development of KASPar SNP for Cmc_{TAM112}

- Two closely linked array SNPs were converted into Kompetitive allele specific PCR (KASP) SNP for high throughput screening (Figure 2 & 3).
- The KASP SNP s were mapped back onto the regions.
- These two KASP SNP were used to screen a collection of cultivars and elite lines, only two out of 33 did not match between genotypes and phenotypes (Table 3).
- These SNPs are promising for marker-assisted breeding as wheat curl mite screening is very time and money consuming.

CONCLUSIONS

- Resistance in TAM 112 is governed by at least two genes, One is originated from wheat-rye translocation, while the other from *Ae. tauschii*.
- Because of the two resistance genes, TAM 112 was consistently resistant in the screening.
- As both of the genes are single dominant in nature, the resistance can be easily transferred to other adapted wheat cultivars in wheat breeding program.

ACKNOWLEDGEMENTS

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