Development of User-friendly Markers for Disease Resistance to Potato Virus Y (PVY) and Black Root Rot (BRR) in Burley Tobacco

The development of multiple disease resistant tobacco varieties through gene pyramiding of resistance to black shank, blue mold, black root rot (BRR), potato virus Y (PVY), and tobacco mosaic virus (TMV) is an important objective of KTTII. Potato virus Y has been classified in the top of the most economically or scientifically important plant viruses. PVY resistance in tobacco is conferred by a single recessive factor called *va*, which is caused by deletion of a large genomic segment containing a eukaryotic translation initiation factor 4E (*eIF4E*). One dominant gene was identified to confer resistance to black root rot, but no closely linked molecular marker was developed. Below are the major progresses we have made.

PVY marker

1. An F2 segregating population was constructed by crossing TKF2002 (resistant) with TKF7002 (susceptible). Of the 860 F2 plants we inoculated with PVY, only 204 individuals were fully resistant. The segregation ratio of susceptible/resistant fits 3:1(χ^2 = 0.75, d*f*=1, P = 0.39), indicating the host susceptibility is controlled by one single dominant gene.

2. Relying on the tobacco *eIF4E* gene sequence, we designed the gene specific primers which were referred to as PVY1 (Figure 1). This dominant marker is coupled with susceptibility and co-segregates with phenotype in the F2 population. It is no wonder that the gene-specific marker is dominant, because genome deletion is the underlying cause for the host resistance. Dominant markers are unable to distinguish homozygous from heterozygous susceptibility; moreover, the reliability for scoring marker could be compromised due to possible unsuccessful PCR amplification.

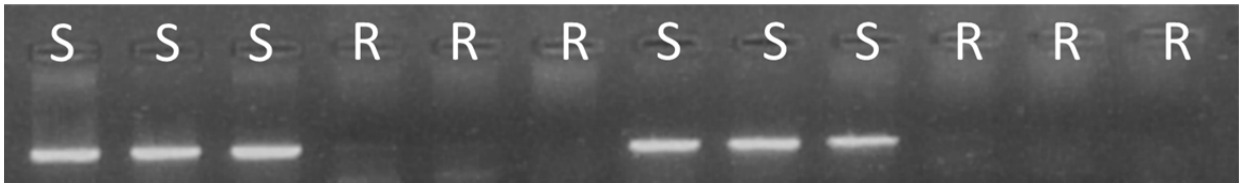

Figure 1. Dominant gene specific marker PVY1, present in susceptible tobacco lines only. S, susceptible; R, resistant.

3. To convert our dominant marker coupling with PVY susceptibility to be co-dominant, we are currently conducting 1) mapping of *va*; and 2) chromosome walking *in silico* to identify the sequences flanking the deleted region in resistant lines. *va* gene locates at the bottom of LG (linkage group) 21flanked by SSR markers PT60057 and PT60946 (Figure 2). Although both markers are closely linked with *va*, they are scored as dominant and missed in resistant TKF2002. Very likely, PT60057 and PT60946 are deleted in resistant lines together with *eIF4E*. Assisted by the tobacco genome sequences released in 2014, we have pinpointed the deleted chromosomal

segment within an about 200 kb region; consisted of more than ten assembled contigs. Unfortunately, we are unable to arrange these contigs in their physical order. However, the sequence information we have obtained is sufficient to design co-dominant markers, we believe.

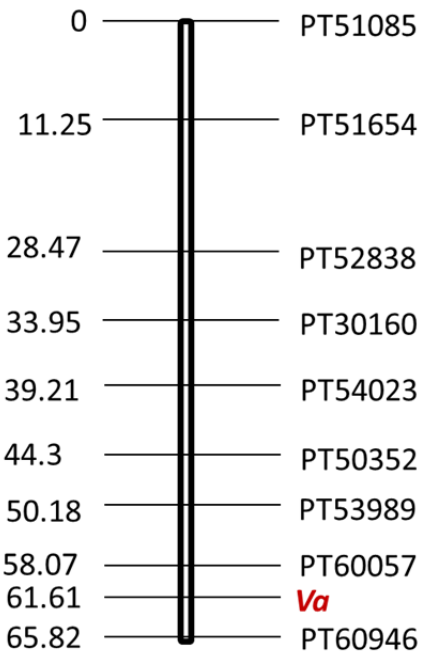

Figure 2. Genetic map of *Va*. *Va* locates at the bottom of LG21and is flanked by two closely linked dominant marker PT60057 and PT60946.

4. GBS (genotyping by sequencing) has been a novel application of NGS (next generation sequencing) protocols for discovering and genotyping SNPs in crop genomes such as tobacco. The GBS approach includes the digestion of genomic DNA with restriction enzymes followed by the ligation of barcode adapter, PCR amplification and sequencing of the amplified DNA pool on a single lane of flow cells. As a cost-effective technique, GBS has emerged as an ultimate MAS (marker-assisted selection) for the modern crop breeding. To find more SNPs (single nucleotide polymorphisms) linked to the *Va* locus, we conducted GBS with 93 F2 plants derived from the cross between TKF2002 and TKF7002. Data mining of GBS results identified several SNPs which are linked to *Va* in coupling repulsion phase. Specific primers were designed for such SNPs. Linkage test for these markers are being tested in my lab.

BRR marker

1. We used same F2 population for mapping PVY resistance to map BRR resistance, because TKF 2002 is resistant whereas TKF 7002 is susceptible to PVY. We inoculated 958 F2 plants at the end of November. Because BRR resistance is conditioned by a QTL (quantitative trait locus), some F2 individuals cannot be clearly scored as resistant or susceptible. At last, we just collected 211 extremely resistant and 197 extremely susceptible plants for linkage mapping.

2. We have scored more than 1000 SSR markers, and only 105 are polymorphic between resistant and susceptible parents (Partial results were present in Figure 3). However, none of these polymeric markers were linked to BRR resistance after we performed linkage analysis.

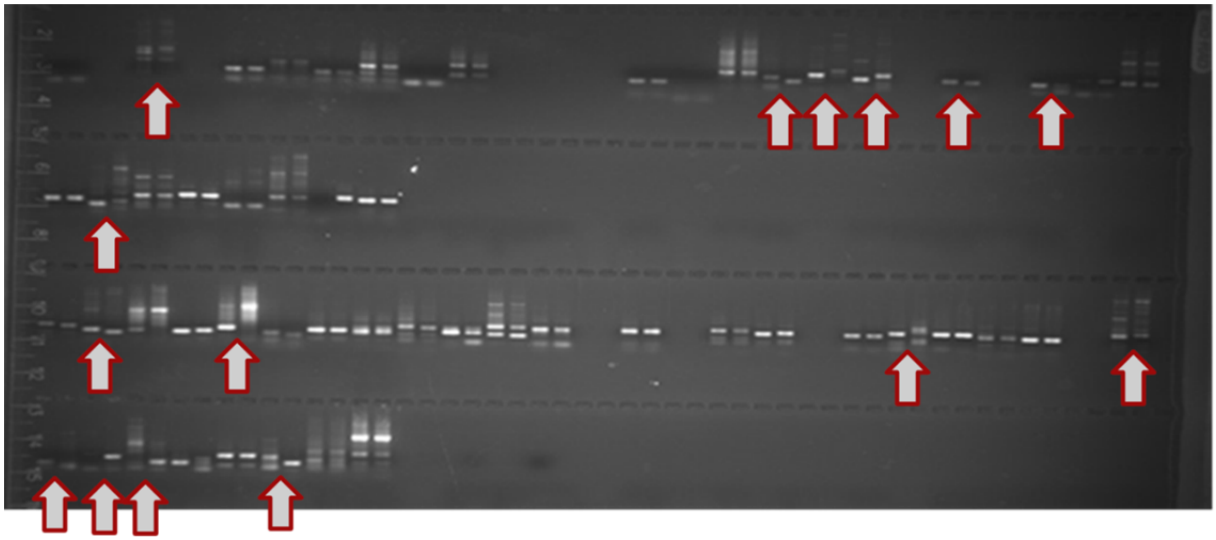

Figure 3. Screening polymorphic markers for genetic mapping of BRR resistance. Arrows indicate the SSR markers which are polymorphic between TKF2002 and TKF 7002.

3. As I mentioned previously, GBS has emerged as an ultimate MAS (marker-assisted selection) for the modern crop breeding. To find SNPs linked to the BRR resistance, we also conducted GBS with another 93-F2 population (45 resistant and 48 susceptible) derived from the cross between TKF2002 and TKF7002. Through data mining, we identified several SNPs which are co-segregated with the disease phenotyping in this segregating population (Figure 4).

Plant	289	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	335	2002	7002
Phenotype	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S
192650-1	H	H	A	A	A	H	A	H	H	A	A	H	H	H	H	A	A	A	H	A	H	H	H	A	H	A	H	A	A	H	A	H	A	H	A	H	A	A	A	H	H	A	A	H	A	A	B
192650-2	H	H	A	A	A	H	A	H	H	A	A	H	H	H	H	A	A	A	H	A	H	H	H	A	H	A	H	A	A	H	A	H	A	H	A	H	A	A	A	H	H	A	A	H	A	A	B
192650-3	H	H	A	A	A	H	A	H	H	A	H	H	H	H	H	A	A	A	H	A	H	H	H	A	H	A	H	A	A	H	A	H	A	H	A	H	A	A	A	H	H	A	A	H	A	A	B
192650-4	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H	A	H	A	H	H	H	H	H	H	H	A	B
219555-1	H	H	H	H	H	A	H	H	H	H	A	A	A	H	H	H	H	H	H	A	H	H	H	H	H	H	H	A	H	H	H	A	A	H	H	H	A	H	H	H	H	H	H	H	A	B
114625-1	B	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H	H	H	H	H	H	H	B	H	H	B	H	A	H	H	H	B	H	H	H	H	A	H	H	H	H	H	A	H	H	A	B

Plant	488	491	492	496	499	501	503	504	506	508	509	511	512	514	517	519	521	522	523	524	527	529	530	532	533	534	535	536	539	540	541	542	543	544	547	549	550	551	555	556	557	558	559	561	562	563	564	565	2002	7002
Phenotype	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S
192650-1	B	B	B	B	B	H	B	B	B	B	B	B	B	B	B	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B
192650-2	A	B	B	B	B	H	B	B	B	B	B	B	B	B	B	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B
192650-3	B	B	B	B	B	H	A	B	B	B	B	B	B	B	B	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B
192650-4	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B
219555-1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B
114625-1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B

Figure 4. Six SNPs (in green cells), which were identified by GBS, linked to the BRR resistance. The genotyping codes for resistant parent TKF2002 and susceptible parent TKF7002 are A (red) and B (blue), respectively. In this selected F2 population (45 resistant and 48 susceptible), the genotype for resistant individuals should be A (red) or H (yellow), and B (blue) for the susceptible. This color map indicated that SNP 192650-4 and 219555-1 are co-segregating with the disease phenotype. R, resistant; S, susceptible.

4. For the next, we will convert these SNPs to PCR-based markers and confirm if they are linked with the BRR resistance.