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

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1. Abstract

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*). By means of genetic mapping, we have generated a genetic map and designed several closely linked markers for *Va*. Black root rot, caused by *Thielaviopsis basicola*, is a common soil-borne fungal disease. It favors cool, wet weather and is common in most Kentucky soils. We are using genotyping by sequencing (GBS) to develop molecular markers for black root rot resistance.

2. Introduction

The conventional tobacco breeding usually identifies genetic variation by visual selection. The process of developing new varieties can take up to 25 years. However, with the advancements in molecular techniques, the duration has been considerably shortened to 5-7 years. One of the important techniques which make it efficient for scientists to select tobacco traits is marker-assisted selection (MAS). One desirable attribute for a user-friendly marker is close linkage with the gene corresponding to phenotype, which significantly precludes false-positive selection due to recombination (or cross-over) events. Other qualities are expected by breeders for genetic markers are: (1) co-dominance (allow distinguishing homozygotes from heterozygotes); (2) a high level of genetic polymorphism (markers can be easily discerned by users); (3) low cost; and (4) high stability (allow the markers to be confirmed and repeated by colleagues).

Potato virus Y (PVY) is a serious disease that occurs in tobacco-growing areas around the world. PVY resistance is conferred by a single recessive factor called *va*. Genetic markers in close association with resistance genes would provide valuable tools for rapid identification of superior resistant lines with high-quality traits. Currently, molecular markers linked to PVY have been proposed. A RAPD marker linked to the ‘‘*va*’’ gene (Noguchi et al. 1999) was obtained. A sequence characterized amplified region (SCAR) marker linked to PVY resistance was also obtained from an AFLP marker (Julio et al. 2006); however the transfer of the RAPD method between laboratories is not always successful. The SCAR marker is a repulsion-phased marker linked to PVY susceptibility. The presence of combination events suggested that the SCAR marker for PVY was not closely linked with the *va* locus. The distance between the SCAR marker and *va* is over 5 cM.

Black root rot, caused *Thielaviopsis basicola*, is a common soil-borne fungal disease. It can be a very severe problem if susceptible tobacco varieties are planted in fields that have not been rotated well. This disease, characterized by black lesions on the roots and hypocotyl, results in

stunt and late-maturing, uneven stands, all of which reduce the yield and quality of tobacco yield. The BRR SCAR marker was also converted from an AFLP marker (Julio et al. 2006). Although it was reported to be closely linked with BRR resistance, this SCAR marker was associated with poor reproducibility. Tobacco breeding for improvement in PVY and BRR resistance is seriously hampered by using these two SCAR markers. User-friendly markers are urgently needed for fighting with disease of PVY and BRR.

3. Material and Methods

3.1 Material An F2 segregating population was constructed by crossing TKF2002 (resistant to both PVY and BRR) with TKF7002 (susceptible to both PVY and BRR). The same F2 population was used to map resistance to PVY and BRR.

3.2 Inoculation with PVY

The symptoms of tobacco vein mottling virus (TVMV), tobacco etch virus (TEV) and potato virus Y (PVY) are very similar, it is usually impossible to distinguish these diseases without doing serological assays. These diseases are often referred to as the PVY complex. Initial symptoms of these diseases may be limited to a slight mottling, yellowing, or other discoloration of the tissue around the veins of the leaves. As the diseases progress, leaf specking and necrosis may occur. These viruses are easily mechanically transmitted. TVMV is used to primarily for selecting the presence or absence of the *va* gene as it confirms the highest level of resistance. Prepare the virus inoculum the day before inoculation. With clean hands, dust 6 week old plant leaves with carborundum. Inoculate dusted leaves by gently rubbing infected leaf sap with a sterile Q-tip. Systemic virus symptoms will develop in 21 days.

Figure 1. Systemic virus symptom of a susceptible plant sample: vein mottling and banding

3.3 Inoculation with BRR

There are two known sources of resistance to BRR 1) single gene resistance from *N. debneyi* and 2) polygenic resistance from *N. tabacum*. Nearly all the commercial burley cultivars grown at present contain the single dominant gene resistance to BRR from *N. debneyi*. This source of resistance offers complete control. The tank test is an excellent way to screen for the single gene resistance from *N. debneyi*. Parental lines and segregating F2 populations were screened in the growth room at KTRDC to identify presence or absence of the resistant gene. Inoculate small seedlings 23-25 days after seeding. Place the tray of tubes containing the small seedlings into the spore suspension. Score the seedlings about two weeks after inoculation.

Figure 2. Difference between roots that are discolored due to colonization of the pathogen: use a scale of 0-5 to rate infection. 0= Healthy root system; 5+ severe necrosis of root system

4. Results and discussion

4.1 PVY marker design

4.1 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 3). 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.

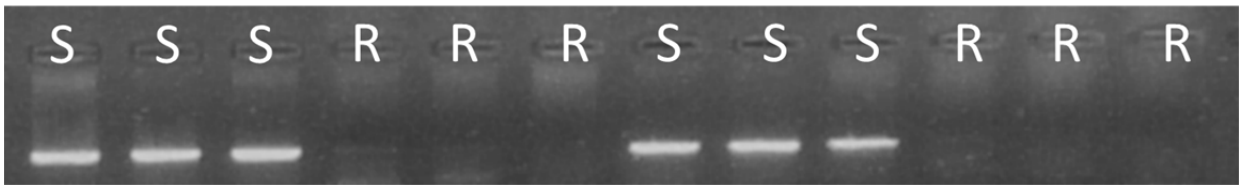

Figure 3. Dominant gene specific marker PVY1, present in susceptible tobacco lines only. S, susceptible; R, resistant.

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) 21 flanked by SSR markers PT60057 and PT60946 (Figure 4). 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 *va* 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.

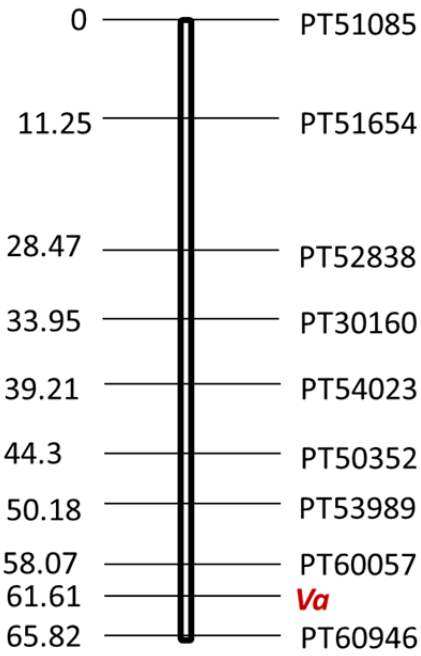

Figure 4. Genetic map of *Va*. *Va* locates at the bottom of LG21 and is flanked by two closely linked dominant markers PT60057 and PT60946.

4.2 BRR marker design

1. We used the 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 543 F2 plants at the end of March, 2015. The disease symptoms were observed and scored. Among the 543 plants, 393 plants were resistant and 150 plants were susceptible. The segregation ration of resistant/susceptible fits 3:1 (χ^2 = 1.88, d*f*=1, P = 0.1), indicating the host susceptibility is controlled by one single dominant gene.

2. We have scored more than 1000 SSR markers, and only 105 are polymorphic between resistant and susceptible parents.

3. We are using genotyping by sequencing (GBS) to develop molecular markers for BRR.

Genotyping by sequencing (GBS) is a rapid and robust approach for reduced-representation sequencing of multiplexed samples that combines genome-wide molecular marker discovery and genotyping. The flexibility and low cost of GBS makes this an excellent tool for many applications and research questions in plant genetics and breeding. One advantage of GBS for mapping single genes in F2 is that the per-sample cost will be low enough that individual samples can be used rather than bulks. This will allow correction or removal of any individuals that were incorrectly phenotyped while confirming segregation of linked markers.

5. Future work

We designed gene specific dominant primers which were referred to as PVY1 for PVY. Dominant markers are unable to distinguish homozygous from heterozygous susceptibility.We will continue to design co-dominant markers for PVY by using the genome sequence information we obtained previously.

We are using genotyping by sequencing (GBS) to develop molecular markers for BRR.

Reference

Julio E, Verrier J-L, de Borne FD (2006) Development of SCAR markers linked to three disease resistances based on AFLP within *Nicotiana tabacum* L. Theor Appl Genet 21:335-346

Noguchi S, Tajima T, Yamamoto Y, Ohno T, Kubo T (1999) Deletion of a large genomic segment in tobacco varieties that are resistant to potato virus Y (PVY). Mol Gen Genet 262: 822-829