Discovery of a naturally-occurring allele of eIF4E1.S in Nicotiana tabacum and development of a co-dominant marker

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Abstract Potato virus Y (PVY) is one of the most problematic pathogens affecting tobacco production. Genetic resistance to PVY in cultivated tobacco is mediated by recessive mutations, including the locus designated VAM, and its derived mutant va, both of which were previously shown to contain large chromosomal deletions. Among the genes deleted in lines containing VAM or va is eIF4E1.S, a gene whose protein product has been shown to facilitate infection by certain potyviruses, including PVY. Because the extent of the deletions and the exact nature of their specific breakpoints have not been precisely established for VAM or va, it has not been possible to develop co-dominant markers to facilitate their transfer to elite varieties using molecular breeding technologies. Here, we report the discovery of a novel naturally-occurring allele of eIF4E1.S from the Chinese tobacco landrace Fuquanliuye, designated eIF4E1.Fu, which lacks a genomic region of approximately 27 kb, including the 3’-end of eIF4E1.S. Characterization of the deletion junction enabled the development of a co-dominant PCR-based marker specific for eIF4E1.Fu, which can distinguish eIF4E1.S/eIF4E1.Fu heterozygotes from both homozygous classes. Using this co-dominant marker, we genotyped F2 plants segregating for the mutant eIF4E1.Fu allele and confirmed the correlation between genotype and phenotype. This study describes a novel source of resistance and an ideal

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co-dominant marker that can be applied in breeding for resistance to PVY and other potyviruses.

**Keywords** Nicotiana tabacum · Potato virus Y · Naturally-occurring allele · eIF4E1.S · Co-dominant marker

**Introduction**

Tobacco (Nicotiana tabacum L.) is among the most important commercial and industrial crops in the world. Potato virus Y (PVY) is one of the most economically important pathogens affecting tobacco worldwide, causing severe reductions in yield and leaf quality every year (Tian et al. 2011). Selective breeding and the planting of resistant varieties are the most economical, safe and effective way to control the disease. Resistance sources to PVY in tobacco can be divided into two categories: (1) TI 1406 and its derivatives, with PVY resistance controlled by the recessive loci VAM or va (Koelle 1961; Miller 1987); and (2) dominant resistance genes derived from Nicotiana africana that confer immunity to PVY (Lewis 2005). From a commercial perspective, the va locus is of particular importance as it has been widely used by tobacco breeders for the development of PVY resistant tobacco varieties. In order to improve breeding efficiency, researchers have developed molecular markers associated with va, including Randomly Amplified Polymorphic DNA (RAPD) and Sequence Characterized Amplified Region (SCAR) markers (Noguchi et al. 1999; Julio et al. 2006; Wang et al. 2012). Because these markers are strictly dominant, and the genetic distances between these markers and the va locus are long, their utility in practical breeding applications is limited.

A survey by Noguchi et al. (1999) of 18 PVY resistant tobacco varieties using RAPD markers suggested that in each case the resistance phenotype was associated with a deletion in the same chromosomal region as va, and that the sizes of the deletions varied considerably among the genotypes. Julio et al. (2015) made a comparative analysis of transcripts from PVY resistant and susceptible near-isogenic lines (NILs) of tobacco using next-generation sequencing, and concluded that eIF4E1.S was the tobacco gene responsible for enabling susceptibility to PVY infection in wild type plants. Liu et al. (2015) developed a dominant marker closely linked to the wild-type eIF4E1.S allele based on the sequence information of the eIF4E1.S gene in tobacco, but this marker cannot distinguish heterozygotes (eIF4E1.S/eIF4E1.s) from both homozygous classes, which restricts its practical application in the improvement of PVY resistance using molecular marker-assisted selection. Dluge et al. (2018) made a comparative analysis of transcripts from tobacco genotypes TI 1406 (VAM), K326-va and K326 (wild type) using next-generation sequencing. It was discovered that TI 1406 and K326-va contain large deletions in the region of chromosome 21 where eIF4E1.S is located. Although this study described the nature and extent of the VAM and va chromosomal deletions in considerable detail, the authors ultimately failed to obtain sequence information across a breakpoint, and thus could not develop a co-dominant marker that could be used by breeders for the selection of the VAM or va mutations.

The present study describes the characterization of the molecular basis of PVY resistance in Fuquanliuye, a sun-cured tobacco landrace from Guizhou Province, China that was identified from a large scale screen of tobacco germplasm for resistance to PVY. A genomics-based analysis of Fuquanliuye in the region of eIF4E1.S resulted in the precise characterization of an ~27 kb fragment of the chromosome that was missing in this line. Using this information, we developed a co-dominant marker for the eIF4E1.S gene and its mutant counterpart by designing a set of three allele-specific primers and a corresponding multiplex PCR protocol. The applicability and reliability of the co-dominant marker system was determined by evaluating the degree of concordance between the phenotype and marker genotype in an F2 population of the cross K326 × Fuquanliuye.

**Materials and methods**

Plant materials and DNA extraction

Three flue-cured tobacco varieties, K326, Honghuada-jinyuan (Hongda) and Yunyan87, one sun-cured tobacco variety, Fuquanliuye, three F1 hybrids derived from crosses between the three flue-cured tobacco varieties (as female parents) and Fuquanliuye (as the male parent), and an F2 population derived from a
cross between K326 (female) and Fuquanliuye (male) were used. K326, Hongda and Yunyan87 are the major flue-cured tobacco varieties planted in China, while Fuquanliuye is a PVY-resistant sun-cured tobacco landrace from Guizhou Province, China. All plant materials were collected and identified by the Guizhou Academy of Tobacco Science.

Genomic DNAs of the varieties and their F₁ and F₂ derivatives were isolated from young leaves using the Axyprep™ Multisource Genomic DNA Miniprep Kit (Axygen Scientific, USA). DNA concentrations were determined with a NanoDrop 2000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). All DNA samples were diluted to 80 ng/μl in distilled-deionized water and stored at −20 °C.

Plant inoculations

A necrotic strain of PVY (PVY-NGZ) was isolated from a tobacco field in Guizhou Province, China, and maintained by the Guizhou Academy of Tobacco Sciences. Inoculations were carried out using the high-pressure spray gun method (Dluge et al. 2018). Briefly, inoculum was prepared by grinding systemically infected leaves in phosphate buffer using a mortar and pestle. To facilitate maceration, quartz sand (400 mesh) was added (1% w/v) prior to grinding and subsequently removed by filtration through a 40 mesh Nylon net. Two leaves from young plants at approximately the 7–8 leaf stage were inoculated with the spray gun. Treated plants were examined for symptoms of PVY infection 21 days post-inoculation.

Identification of the deletion mutation of eIF4E1.S in Fuquanliuye tobacco

To characterize the region of the Fuquanliuye genome associated with eIF4E1.S, six different sets of partially overlapping primers (Table 1) spanning from −55-bp to +4423-bp with respect to the translational start site of eIF4E1.S were designed and used for PCR amplifications in both wild type K326 and mutant Fuquanliuye tobacco genotypes. Amplification reactions were performed in a final volume of 20 μl containing 1 x PCR buffer (Mg²⁺ plus), 1.0 U Taq DNA polymerase, 0.2 mM dNTPs, 0.4 μM each of the forward and reverse primers, and 64 ng of genomic DNA. PCR reactions were conducted in a C1000 Touch Thermal Cycler (Bio-rad, Hercules, CA, USA) with an initial denaturation step at 94 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, annealing temperature of 52–58 °C for 30 s and extension 72 °C for 1 min, terminating with the final extension step at 72 °C for 10 min. The amplicons were purified using a PCR Purification Kit (Qiagen) and ligated into the pGEM-T easy vector (Promega) followed by transformation into competent E.coli DH5α cells (TIANGEN). Ten colonies were randomly selected for each PCR product and sequenced by Invitrogen (Shanghai, China). Sequence assembly and analysis were performed using DNAMAN software (Version 6.0). BLAST alignments were carried out using the China Tobacco Genome Database (Version 4.0).

Cloning and verification of the deletion junction in Fuquanliuye

To obtain the junction fragment originating in the 3’ region of eIF4E1.S in Fuquanliuye tobacco, a genome walking approach was initiated using the Genome Walking Kit (TaKaRa, Japan). The junction fragment was amplified by three rounds of thermal asymmetric interlaced PCR (TAIL PCR) using genomic DNA from Fuquanliuye tobacco as the template. Specific primers SP1, SP2, and SP3 (Table 1) were designed for the first, second, and third rounds of genome walking, respectively, corresponding to sequences at the 5’-end of eIF4E1.S located close to the breakpoint. The amplification reactions were performed in 50 μl reaction volumes according to the manufacturer’s protocol, using the specific primers mentioned above and arbitrary degenerate primers provided in the Genome Walking Kit. The amplification products were cloned into the pGEM-T vector and sequenced. The genomic sequence obtained by genome walking was assembled using DNAMAN software, and the specific primer SP4 was designed from the downstream region where the sequence diverged from the wild type genomic DNA. PCR reactions utilizing SP4 together with SP1 were used in PCR reactions to validate the existence of this genomic configuration in Fuquanliuye. The PCR conditions involved an initial denaturation at 94 for 3 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min with a final extension at 72 °C for 10 min. PCR products of expected size were cloned into the pGEM-T vector and sequenced. The resulting fragment was
compared with the reference genome of *N. tabacum* using the BLAST program in the China Tobacco Genome Database (Version 4.0) to identify and characterize the extent of the deletion originating from the *eIF4E1.S* gene region in the Fuquanliuye genome.

Designing and testing of allele-specific primers

The common forward primer (Table 1; FWm) was designed according to a sequence 5’ of the deletion region, namely, a portion of the *eIF4E1.S* gene that is still present in Fuquanliuye. One reverse primer (Table 1; Rm) spans the deletion region to enable amplification of the mutant *eiF4E1.Fu* allele, generating a PCR product of 572 bp; the other reverse primer (Table 1; RW) was designed against a sequence that had been deleted, amplifying a 763 bp product in wild type tobaccos when used with FWm. The amplification reactions were performed in a final volume of 20 µl containing 1 × PCR buffer (Mg²⁺ plus), 1.0 U Taq DNA polymerase, 0.2 mM dNTPs, 0.4 µM each of the forward and reverse primers, and 64 ng of genomic DNA. The PCR cycler conditions included an initial denaturation step at 94 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min, with a final extension step at 72 °C for 10 min. The PCR products were cloned into the pGEM-T vector and validated by DNA sequence analysis.

Tri-primer multiplex PCR assay

To simplify the PCR protocol for use in marker-assisted selection, a tri-primer multiplex PCR assay was designed. PCR amplifications were performed in a 20 µl reaction volumes containing 1 × Premix Taq Version 2.0 plus dye (TaKaRa, Japan), 0.4 µM each of the oligonucleotide primers (FWm/Rm/RW), and 64 ng of template DNA. Thermocycler conditions involved an initial denaturation step at 94 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The PCR products were analyzed by electrophoresis using 2% agarose gels.

### Table 1  Primer sequences used in this study

| Primer name (5’-3’) | Primer sequence (5’-3’) | Purpose |
|---------------------|-------------------------|---------|
| A_F                 | CAAGTACCCTTTTCTACTAATCTCCTACTAACTAAG | Detection of the deletion region |
| A_R                 | TGTTTGATCTGAACAAACAAACAAAGATAAC | |
| B_F                 | GGACCTGAAGAGGAGAATACTGTGAT | |
| B_R                 | CGTCCTAAATTATGTAATGTGTTGTGACTTA | |
| C_F                 | ATTTGGACGAGGACTATCTTG | |
| C_R                 | CTAACTCAAACCGACCACCTAAATAAAGATC | |
| D_F                 | GACCACTATTCTCAAGCTATTCTGATGCTT | |
| D_R                 | CTAAAACATGAAAGTCTGCTCCAC | |
| E_F                 | ATATCAACACACCAAGACAAGTTGATTTG | |
| E_R                 | GGCCACACCTTTGTTATCTTG | |
| F_F                 | TATGCTTATTACAAACATAATTCG | |
| F_R                 | CGTGGCATAGATCCGATTACTG | |
| SP1                 | AGTTTTGTAAGAGTGACGGGTTTAG | Chromosome walking and verification of its result |
| SP2                 | GTGCATTATGCTCTCATATACACAGCTG | |
| SP3                 | CTATTTGATGACGACTAAACGTAGCTG | |
| SP4                 | CCCGAAGGGTTTCCACGACG | |
| FWm                 | CCGATTGTACACCATAGTTGCTTACACG | Marker development |
| Rm                  | GAGATATGTCTAGACGAGTCATCTCTTGACTAC | |
| RW                  | AAACACTGTTTGCAGATTTAAGCAAC | |
Application of co-dominant molecular marker in a segregating F₂ population

An F₂ population was prepared from a cross between K326 and Fuquanliuye. Five hundred F₂ individuals were genotyped using the tri-primer multiplex PCR assay described above. Each F₂ individual was also inoculated with PVY and scored as resistant or susceptible to enable association between marker genotype and resistance phenotype.

Testing marker efficiency across diverse tobacco germplasm

Genomic DNAs were extracted from the 24 diverse tobacco germplasm accessions listed in Supplementary Table 1. PCR amplifications were conducted using the co-dominant marker system described above. Amplification products were resolved by 2% agarose-gel electrophoresis.

Results

Tobacco line Fuquanliuye possesses a partial copy of eIF4E1.S

From a recent screen for PVY resistance from over 900 tobacco germplasm accessions curated by the Guizhou Academy of Tobacco Sciences, nine lines collected in China were identified as resistant (data not shown). As an initial characterization, the nine PVY resistant tobacco accessions were screened for the presence or absence of eIF4E1.S using a combination of whole genome resequencing and PCR. Among the PVY resistant lines surveyed, a variety known as Fuquanliuye was selected for further analysis. To define the nature of eIF4E1.S within the Fuquanliuye genome, six different sets of primers spanning various portions of the gene were designed for PCR analysis of both wild type K326 and mutant Fuquanliuye tobacco genotypes. PCR products were generated in both backgrounds using all six primer pairs, then subsequently cloned, sequenced and aligned to the reference genome sequence of N. tabacum (Hongda). The sequencing results showed that the PCR products amplified from K326 with all primer sets were identical to the corresponding regions of eIF4E1.S, and the PCR products amplified from Fuquanliuye with primer sets A, B and C were also identical to the corresponding regions of eIF4E1.S. Unexpectedly, all PCR products amplified from Fuquanliuye with the primer sets covering regions D, E and F differed completely from the corresponding regions of eIF4E1.S (Fig. 1), and thus appeared to be products of nonspecific amplification. These results suggested that a portion of the eIF4E1.S gene in Fuquanliuye tobacco was deleted, and that the 5' breakpoint of the deletion was located somewhere between bases 2261 and 2854 (Fig. 1).

Cloning and verification of a junction fragment in Fuquanliuye that lacks a portion of eIF4E1.S

To genome walk across the chromosomal region in Fuquanliuye possessing a partial deletion of eIF4E1.S, three rounds of nested-PCR were carried out as described in Materials and Methods. By the third round, a fragment of about 800 bp began to predominate when genomic DNA of Fuquanliuye was used as the template (Fig. 2). Sequence analysis of the cloned fragment revealed that the initial 355 bp from the 5' end of this fragment aligned perfectly with the genomic DNA sequence of eIF4E1.S. Thus, it could be preliminarily concluded that this amplified product was the desired target fragment. An antisense primer designated SP4 was designed against sequences located in the unique 3' end of the amplified fragment. When SP4 was coupled with the sense primer SP1, PCR reactions with genomic DNA from Fuquanliuye yielded the predicted fragment of 1051 bp (Fig. 2). The subsequent cloning and sequencing of the 1051 bp PCR product confirmed that it matched that of the fragment obtained through genome walking. The DNA sequence of the 1051 bp fragment is shown in Fig. 3a.

Alignment of the 1051 bp fragment unique to the Fuquanliuye genome with the current version of the China Tobacco Genome Database revealed that the sequences opposite the deletion junction in Fuquanliuye are located approximately 27 kb downstream in the wild type genome. Close examination of the Fuquanliuye-specific fragment and the reference genome revealed that 26,965 nucleotides (GenBank accession number MW553726) were deleted from the Fuquanliuye genome along with the insertion of the nine nucleotides TTGAAAATT at the breakpoint (Fig. 3b). The deletion event in Fuquanliuye resulted...
in the removal of exons 2 through 5 of eIF4E1.S (Fig. 3b), thus assuring its nonfunction. The novel large deletion mutation in Fuquanliuye that includes the majority of eIF4E1.S was designated eIF4E1.Fu. Analysis of the sequences within the deletion fragment revealed three large reading frames; one predicted to encode a protein greater than 400 amino acids in length and the other two predicted to encode proteins over 1000 amino acids in size (Supplementary Fig. 1). In each case, however, homology searches in public databases revealed no insights with respect to potential function as all homologs were simply annotated as uncharacterized proteins. Nevertheless, given that the chromosomal deletion found in Fuquanliuye is much smaller than the deletions defining the VAM and va loci that span several Mb (Dluge et al. 2018), utilization of eIF4E1.Fu as a source of PVY resistance would be expected to be less disruptive to normal plant growth and development than either of the two traditional sources. The DNA sequence of the region of chromosomal DNA deleted in Fuquanliuye is shown in Supplementary Fig. 1.

Development of a co-dominant marker specific for the PVY resistance allele eIF4E1.Fu

In order to differentiate the null allele eIF4E1.Fu from the wild-type eIF4E1.S allele, we developed a co-dominant PCR-based marker based on the sequence analysis as described above. As shown in Fig. 3b, a common forward primer (FWm) was designed based on the sequence upstream of the 5’ breakpoint, and a reverse primer (Rm) specific to the null allele was designed based on the sequence downstream of the 3’ breakpoint. Another reverse primer (RW) specific to the wild-type allele was designed based on sequences 3’ proximal to the breakpoint, and thus absent in the Fuquanliuye genome. Using primers FWm/RW, a 763-bp fragment specific to the wild-type allele was amplified from homozygous susceptible plants (eIF4E1.S/eIF4E1.S) and heterozygous plants (eIF4E1.S/eIF4E1.Fu), but not from homozygous resistant plants (eIF4E1.Fu/eIF4E1.Fu) (Fig. 4a). Using the FWm/Rm primer pair, a 572-bp fragment specific to the null allele was amplified from homozygous resistant plants and heterozygous plants, but not from homozygous susceptible plants (Fig. 4b). In both reactions, the annealing temperatures of the PCR primers for the two target fragments were identical, so a tri-primer multiplex PCR assay was attempted using the original concentrations of template DNA, primers
and Premix Taq DNA Polymerase, with primers FWm, RW and Rm in equimolar concentration. The tri-primer multiplex PCR was carried out using genomic DNAs from homozygous resistant, homozygous susceptible and heterozygous plants as the template. As can be seen in Fig. 4c, the tri-primer

Fig. 3 Nucleotide sequence and analysis of the deletion mutation in Fuquanliuye. a Cloning and sequence analysis of the junction fragment of the eIF4E1.S gene with a 3’ end deletion. Capital letters represent sequences of the residual 5’ fragment of eIF4E1.S in Fuquanliuye. Lowercase letters show the 3’-junction sequence obtained by genome-walking; the nine additional nucleotides inserted between the breakpoints are shaded grey. Sequences used for PCR primers are highlighted in bold and indicated by arrows. b Precise breakpoint localization of the deletion in eIF4E1.S, including the positions of the three allele-specific primers designed to develop a co-dominant marker for eIF4E1.S and eIF4E1.Fu.
multiplex PCR accurately identified each of the three genotypes.

To further verify the integrity of the assay, the PCR products resulting from the experiments shown in Fig. 4 were subjected to DNA sequence analysis. The sequencing results confirmed that the 763-bp fragment corresponded to the wild-type allele and the 572-bp fragment matched the sequence expected for the mutant eIF4E1.Fu allele.

Validation of the co-dominant molecular marker in an F2 population segregating for PVY resistance

An artificial inoculation test was conducted to identify the degree of PVY resistance in 500 individual plants from an F2 population of the cross K326 × Fuquanliuye. Plant disease resistance/susceptibility was evaluated 21 days after inoculation. Among the 500 F2 plants inoculated, 122 resistant plants and 378 susceptible plants were identified, which is in accordance with the expected 1:3 segregation ratio for a single recessive resistance gene. The same 500 F2 plants were analyzed using the tri-primer multiplex PCR assay. A representative example of the results are presented in Fig. 5, where parent 1 (P1) is Fuquanliuye, parent 2 (P2) is K326, and plants 1–21 are segregating F2 plants. Consistent with expectations, plants 3, 4, 8, 10, 14, 16 and P1 were resistant to PVY and all other plants were susceptible. A 100% correlation between plants homozygous for eIF4E1.Fu and displaying resistance to PVY inoculation was observed across the entire 500 plant population.

Effectiveness of the co-dominant marker across diverse germplasm accessions

To test the efficacy of the co-dominant marker across an array of tobacco genotypes, we used the tri-primer multiplex PCR assay to survey 24 diverse entries from the germplasm collection at Guizhou whose resistance or susceptibility to PVY had been previously established (Supplementary Table 1). Included in this diverse set of tobaccos were flue-cured, sun-cured, burley, and cigar market types. As shown in Fig. 6, all 17 cultivars characterized as being susceptible to PVY amplified a single, robust 763-bp fragment corresponding to the eIF4E1.S gene. With the exception of Fuquanliuye, all other PVY resistant germplasm lines failed to yield an amplification product. Given that previous characterizations of PVY resistance germplasm has shown that the majority of these lines possess exceptionally large chromosomal deletions encompassing not only eIF4E1.S, but numerous other genes as well (Noguchi et al. 1999; Dluge et al. 2018), the failure to detect an amplification product is not surprising. When making crosses with these other PVY resistant materials, the marker developed here would function as a dominant marker similar to that reported by Liu et al. (2015). As expected, DNA extracted from Fuquanliuye produced a single discrete 572-bp fragment.

Discussion

It has been shown that mutations rendering the tobacco gene eIF4E1.S nonfunctional can serve as recessive resistance genes against PVY. Thus, the wild type eIF4E1.S gene can be considered a disease-susceptibility gene for PVY and certain other potyviruses. Liu et al. (2015) developed a dominant DNA marker closely linked to the recessive resistance gene va based on genomic DNA sequence information. Although this marker can distinguish some tobacco varieties resistant to PVY from susceptible varieties, it is not very useful for transferring eIF4E1.S deletion mutations into PVY-susceptible recurrent parents because it can only identify individuals that are homozygous-recessive for the marker allele, and can’t distinguish
between homozygous dominant and heterozygous genotypes. A dominant marker of this nature would require test crosses each generation that would double the time and effort needed to transfer the trait. Furthermore, we found that when we used those primers as markers, amplification of the corresponding specific band was observed in several PVY-resistant sun-cured tobacco landraces identified from different provinces in China by our research team (unpublished results). This is likely the consequence of mutations in \( eIF4E1.S \) in those lines not involving the deletion of the specific chromosomal fragment represented by that marker. Therefore, when screening tobacco germplasm resources for resistance to PVY, it is important to conduct a thorough characterization of the \( eIF4E1.S \) gene in its entirety, as important sources of resistance, such as the \( eIF4E1.Fu \) allele described herein may be missed.

Due to the recent advances in genome-editing technologies, another option for creating new sources of PVY resistance could be through the directed mutagenesis of \( eIF4E1.S \) using these technologies. Though genome editing is undoubtedly a powerful tool, tobacco varieties developed using genome editing may be banned from commercial cultivation in some countries, as currently the European Union has ruled that genome-edited crops must be regulated the same as GMO crops (Lassoued et al. 2019). Therefore, in the current regulatory environment it could be argued that the discovery and exploitation of natural sources of PVY resistance, coupled with easy to use co-dominant markers corresponding to the cognate \( eIF4E1.S \) mutation represents the best strategy for developing new PVY resistant tobaccos varieties.

DNA sequence polymorphism analysis of tobacco \( eIF4E1.S \) alleles of resistant versus susceptible tobacco varieties is the basis for developing co-dominant markers. Studies show that tobacco PVY resistance can be broadly divided into two main types: those controlled by recessive loci such as \( VAM \) and \( va \) (Koelle 1961; Miller 1987), and those which are dominant, conferring an immune resistance to PVY as evidenced in \( Nicotiana africana \) (Lewis 2005). Although the \( va \) locus has been deployed in several commercial tobacco varieties, the transfer of \( va \) to new tobacco varieties using backcross breeding is very difficult due to its recessive phenotype and the fact that all molecular markers targeting this locus that have been described to date are dominant, and thus cannot distinguish between homozygous dominant (VA/VA)
and heterozygous (VA/va) individuals in segregating populations. In addition to the lack of co-dominant markers specific for va, another potential disadvantage of this source of PVY resistance is the size of the deletion that defines this locus. A 2018 study of two PVY-resistant tobacco accessions carrying the VAM and va loci attempted to estimate the extent of the chromosomal regions that are missing at these loci. The VAM deletion was shown to span a minimum of 6.55 Mbp containing 184 predicted genes; the va deletion was characterized as missing at least 5.5 Mbp of chromosomal DNA encoding a minimum of 143 genes (Dluge et al. 2018). Furthermore, the authors of this study were not successful in identifying the location of a deletion breakpoint, information that would have allowed the development of co-dominant markers for these loci. Although a strong linkage drag has already been described for plants possessing the VAM mutation, most notably due to the observation that important trichome exudates are either negligible or completely missing in plants carrying this deletion (Nielsen et al. 1982, 1991), it is also plausible that negative attributes are also associated with the va locus, given the size of the deletion and the number of genes missing in plants carrying va.

The eIF4E1.Fu source of PVY resistance described in this report promises to be a superior source for developing new PVY resistant varieties for several reasons. First, being about 27 Kbp in size, the extent of the chromosomal DNA missing in eIF4E1.Fu is vastly smaller than that associated with VAM (> 6.55 Mbp) or va (> 5.5 Mbp). In addition to the elimination of the majority of eIF4E1.S, we were only able to identify three predicted genes within rest of the sequences deleted in eIF4E1.Fu, all annotated as uncharacterized proteins. Thus, it is very likely that tobacco plants utilizing eIF4E1.Fu as the source of PVY resistance will suffer less linkage drag than plants possessing va. Second, the co-dominant marker specific for eIF4E1.Fu described here represents a powerful tool for tobacco breeders due to its ability to discriminate all three allelic combinations. Finally, as eIF4E1.Fu is a naturally occurring mutation, identified from a collection of sun-cured tobacco varieties in China, it has advantages over mutations that may be produced either via genome editing (regulatory concerns) or using nonspecific mutagens such as EMS that perturb the genome with a host of random mutations.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Dluge KL, Song ZB, Wang BW, Tyler SW, Xiao BG, Li Y, Dewey RE (2018) Characterization of Nicotiana tabacum genotypes possessing deletion mutations that affect potyvirus resistance and the production of trichome exudates. BMC Genomics 19:484
Julio E, Cotucheau J, Decorps C, Volpatti R, Candresse T, Dorlach de Borne T (2015) A eukaryotic translation initiation factor 4e (eif4e) is responsible for the “va” tobacco recessive resistance to potyviruses. Plant Mol Biol Rep 33:609–623
Julio E, Denoyes-Rothan B, Verrier JL, Dorlach de Borne F (2006) Detection of QTLs linked to leaf and smoke properties in Nicotiana tabacum based on a study of 114 recombinant inbred lines. Mol Breeding 18:69–91
Koelle G (1961) Genetic analyse einer Y-virus (Rippenbraune) resistenten mutante der tabaksorte Virgin A. Theor Appl Genet 31:71–72
Lassoued R, Macall DM, Hessel H, Phillips PWB, Smyth SJ (2019) Benefits of genome-edited crops: expert opinion. Transgenic Res 28:247–256
Lewis RS (2005) Transfer of resistance to potato virus Y (PVY) from Nicotiana africana to Nicotiana tabacum: possible influence of tissue culture on the rate of introgression. Theor and Appl Genet 110:678–687
Liu Y, Song ZB, Tong ZJ, Li YP (2015) Molecular marker from recessive gene resistant to potato virus Y of tobacco and its suitability. Acta Tabacaria Sinaca 21:76–81 (in Chinese with English abstract)
Miller RD (1987) Registration of ‘TN 86’ Burley Tobacco. Crop Sci 27:365–366
Nielsen MT, Akers CP, Jarlford VE, Wagner GJ, Berger S (1991) Comparative ultrastructural features of secreting and non-secreting glandular trichomes of two genotypes of Nicotiana tabacum L. Bot Gaz 152:13–22
Nielsen MT, Jones GA, Collins GB (1982) Inheritance pattern for secreting and nonsecreting glandular trichomes in tobacco. Crop Sci 22:1051–1053
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 and Genet 262:822–829
Tian YP, Liu JL, Zhang CL, Liu YY, Wang B, Li XD, Guo ZK, Valkonen JP (2011) Genetic diversity of potato virus Y infecting tobacco crops in China. Phytopathology 101:377–387

© Springer
Wang G, Liu Y, Lu XP, Li KZ (2012) Inheritance of resistance to potato virus Y in tobacco and molecular marker identification. Mol Plant Breed 10:97–103 (in Chinese with English abstract)

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