ORIGINAL ARTICLE

Detection and Characterization of Resistant Gene in Tomato Seeds of PHS165, Hyveg, and Cynus Hybrid Varieties

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Abstract

Background: The demand for tomato is increasing day by day mostly because of the increased per capita fresh fruit consumption. Nonetheless, as a perishable fruit crop, it has relatively short life after ripening thus experiences remarkable post-harvest losses. **Aims:** Our approach in this study is to evaluate the consistency and purity of seeds that are developed to control pest resistance. **Methods:** Touchdown polymerase chain reaction (TD-PCR) and Fourier transform infrared studies are used to test seed genetic purity of three commercial hybrid tomato seeds PHS165, HYVEG, and CYGNUS varieties. TD-PCR is a sensitive and specific method and can be used to detect even low levels of genetic impurities. **Results:** Amplicon of 550bp toward Cry2abresistant gene was found in CYGNUS and HYVEG varieties. Results might suggest that these commercial hybrid tomato seeds may contain genetic material from *Bacillus thuringiensis* that confers resistance to Cry2ab toxin. **Conclusion:** In the present study, the purity of tomato hybrid was assessed by both PCR-based and analytical methods-based screening of commercial tomato hybrids. Further testing and analysis may be necessary to confirm the presence and origin of the Cry2ab-resistant gene in these commercial hybrid tomato seeds and to determine the potential impact on crop yield and pest management strategies.

Key words: CYGNUS, Fourier transform infrared, HYVEG, PHS165, touchdown polymerase chain reaction

INTRODUCTION

Bacterial wilt can indeed be a serious problem for tomato farmers, but several methods have been devised to manage bacterial wilt without efficient bactericides.[1] The use of resistant genes is a promising approach because it can provide long-term protection against the disease.[2] One of the most cultivated vegetables is tomato (*Lycopercicum esculentum*), 274,700 tonnes of tomatoes are farmed in India because of their vitamin and mineral content. Bacterial wilt is the most damaging disease of tomato in India.[3] Pests and diseases, especially *Ralstonia solanacearum*-induced bacterial wilt, contributed the production rate to low yield.[4] Tomato farmers must clean their fields to grow susceptible or resistant varieties. Several methods have been devised to manage bacterial wilt without efficient bactericides that include soil disinfection, grafting, and disease-reducing plants that sterilize the soil and crop rotation with non-host crops.[5-8] The previous studies recommend disease management by introducing resistant varieties, which is economical for farmers and environmentally friendly.[9] Ganiyu *et al*. found bacterial wilt-resistant tomato varieties AVT09803, AVT00201, Tomachiva, and Eyetom to detect resistant genes to be improved for rootstock.[10] Oussou *et al.* found PADMA and platinum resistant to bacterial wilt in nine tomato varieties.[11] Three open-pollinated tomato

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varieties – Sankranthi, Nandi, and Vybhav – now available for cultivation in South India are resistant to tomato leaf curl virus disease.[12] This study evaluates hybrid and local Indian tomato varieties to increase tomato-resistant genes. Our research findings also showed that local or hybrid varieties were equally susceptible to bacterial wilt.[13]

METHODS

Collection of seed material

Bacillus thuringiensis (BT) samples of wild and hybrid varieties were collected from the local market of retailer shop at Guntur, Andhra Pradesh, India. Seeds were washed and cleaned thoroughly for removal of any dust particles. Seeds were soaked in acetone or distilled water for removal of skin and were grinded using either tweeter or pestle. The extract was collected in a vial and spin at 5000 rpm for 2–3 min to collect the fine extract. The extract was stored at lower temperature for further use.[14]

Polymerase chain reaction (PCR) process

The DNA yield was very high ranging from 26 to 50 mg/100 mg of fresh tissue. The A260/A280 was found to be 1.89, suggesting that the isolated DNA was free of proteins. PCR program setup process is carried out as initial denaturation of 95°C for 3 min, denaturation at 94°C for 30 s, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 7 min. Primers for Npt II gene, Forward primer: 5' CGC TAT GTC CTG ATA GCG GTC C; Reverse primer: 5' CAC AAC AGA CAA TCG GCT GCTC. Primers for BT gene, Forward primer: 5' TAC TTG GTG GAG AAC GCA TTG AA; Reverse primer: 5' GAG GTC AAC TAG TCC GAC AAC GAA. Primers for 35S promoter, Forward primer: 5' GCT CCT ACA AAT GCC ATC A; Reverseprimer:5'GATAGTGGGATTGTGCGTCA.[15]

Fourier transform infrared (FTIR) analysis of proteins

Spectra were collected using a Perkin Elmer 27 FTIR spectrometer (Bruker, Germany), with ZnSe crystal cell ATR (PIKE, USA) equipped with a deuterated L-alanine doped triglycine sulfate (TE-DLATGS) detector. Spectra were measured at room temperature, at resolution of 4 cm^{-1}, and 16 scans for each sample in a wavenumber range of 4000 cm^{-1} to 600 cm^{-1}. A background spectrum was scanned before the analysis of each sample to cancel the effect of air components in the spectrum. After each measurement, the crystal was wiped off with laboratory soft tissue wetted by alcohol. Collected spectral data were subjected to a baseline correction (rubber band method) and vector normalization by software named Opus5.5. The final analysis was accomplished by in-house software using Matlab 2014b (Mathworks, Natick MA, USA).[16]

High-performance liquid chromatography (HPLC) analysis of proteins

High-performance liquid chromatography was performed to check the purity of the isolated extract against their standards. A reversed-phase HPL Cusing Schimadzu HPLC-Phenomenox 3000 pump (Schimadzu) Luna 5u C18 column (250 × 4.6 mm) was used. Two buffers were prepared for a gradient type elution; Elution A: 0.1% TFA in 95:5 Water/CAN, Elution B: 0.085% TFA in 75:20:5 ACN/PA/Water. Due to the gradient, a certain ratio for both buffer volumes was maintained and three elution buffers were performed, that is, (A: B)…(80:20) (50:50) (20:80). Acetonitrile washes were done to free the column of any contaminants (if any) followed by equilibration with buffer and loading of the sample. Each buffer was allowed to run for 3 min. After the elution, the column was washed with acetonitrile and the process was repeated for the other sample as well.[17]

RESULTS AND DISCUSSION

DNA quality check

DNA was electrophoresed in gels containing agarose, typically 1% (w/v) in TAE buffer containing 0.5 pg/mL EtBr to allow visualization under UV light. DNA ladder was used as a molecular size marker. Gels were photographed under UV light on a UVI-Tech Bio Imager. Concentration of DNA isolated form hybrid (PHS) is lesser compared to other hybrid tomato plants [Figure 1].

PCR process

DNA gel electrophoresis was used for analysis of selective amplification of DNA fragments through PCR, for controlling DNA fragments after restriction digestion or

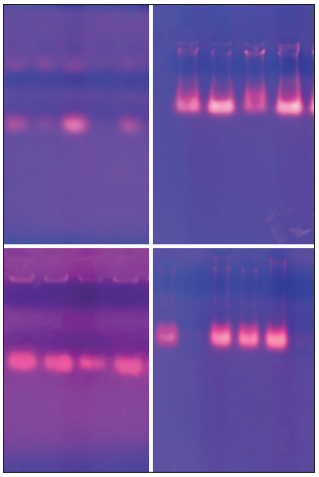

Figure 1: Genomic DNA of wild and hybrid tomato seeds extracted by CTAB method of H1 (PHS), H2(HYVEG), H3(CYGNUS), and Wild

preparation of fragments for ligation or transformation. While working with Ethidium Bromide as fluorescent dye, per gel (150 mL) 1% agarose (MERCK, LEO Agarose), 1 × TAE, and 6 µLEtBr were used. In addition, fluorescent dyes were added to the EtBr running buffer (1XTAE with either 0.02 µL EtBr/mL). For analytical setups, 1–5 µL sample were mixed with ddH2O and 6 × MassRuler® DNA Loading Dye (Fermentas) or 6 × self-made loading dye (2.5 ng/mL Bromphenol blue, 33% [v/v] glycerol) to a total loading volume of 6 µL. For preparative applications, after restriction digestion, the complete 50 µL mix was mixed with 13 µL 6 × loading dye and applied to the gel [Figure 2].

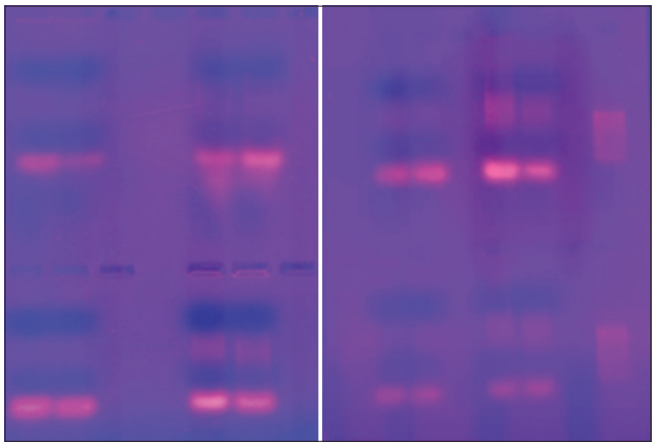

Figure 2: Amplification of CaMV, Cry2ab, and nptII gene loci of H1-3 and wild varieties by polymerase chain reaction

FTIR analysis of proteins

FT-IR spectra of tomato seed samples were analyzed at a resolution of 4 cm^{-1} at 100–150 scans for wild and hybrid seeds. The absorbance spectrum shows bands corresponding to the amide I, amide II, and amide III regions. The spectra were recorded using CaF2 windows fitted with 6 µm tin spacers and the spectrum was recorded at 20°C. The amide II band of proteins consists of many overlapping component bands that represent different structural elements such as a-helices, b-sheets, turns, and non-ordered or irregular structures. The width of the contributing component bands is usually greater than the separation between the maxima of adjacent peaks. Consequently, the individual component bands cannot be resolved in the experimental spectra [Figure 3].

HPLC analysis of proteins

Antibodies were further purified by reversed-phase HPLC using Schimadzu LC20ATHPLC-Phenomenox Luna 5u C18 column (250 × 4.6 mm) in a 10–70% acetonitrile gradient. For this purpose, approximately 20 uL of sample were dissolved in 2 mL 10–30% ACN and 0.1% TFA and injected onto HPLC-pump.

The separation of the peptide from byproducts involved continuous partitioning of the molecules between the mobile phase and the hydrophobic stationary phase. The collected fractions were separated based on their milli absorption

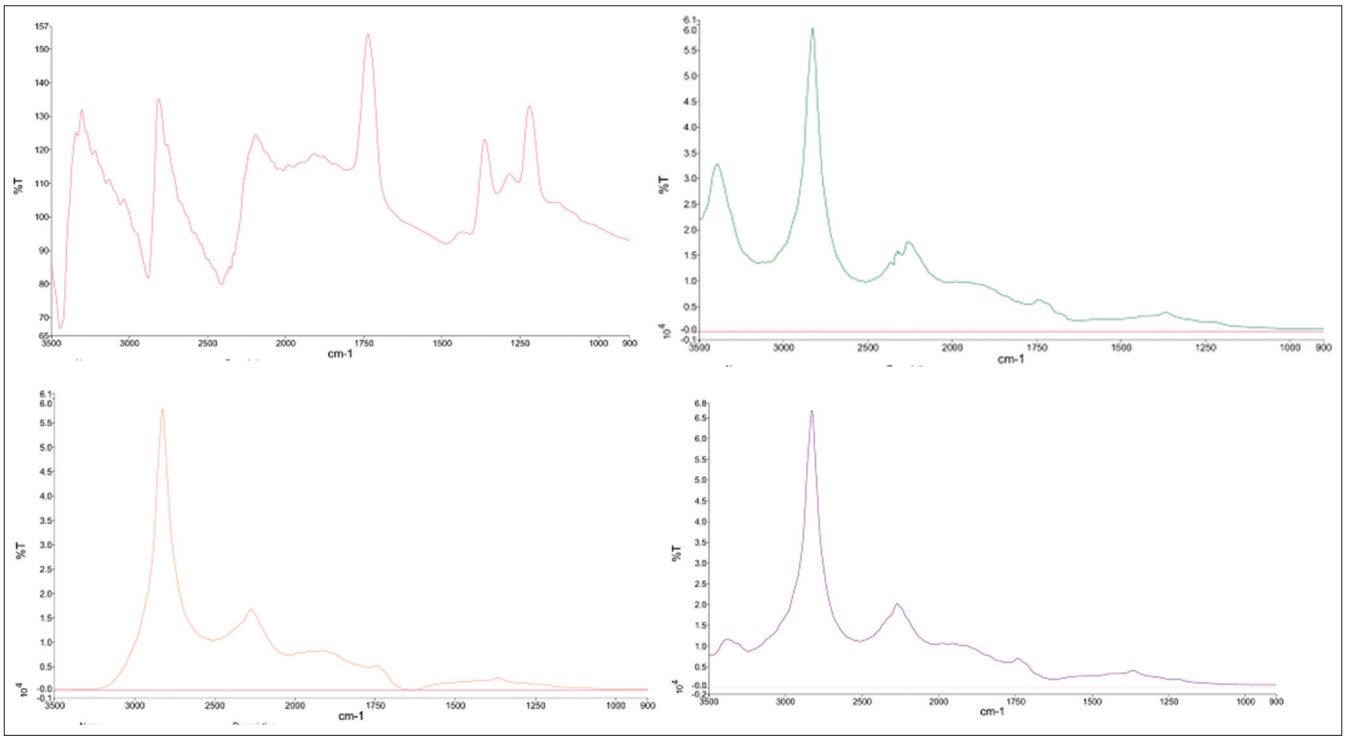

Figure 3: Fourier transform infrared spectra of tomato seed extract. The absorbance spectrum shows bands corresponding to the amides and proteins

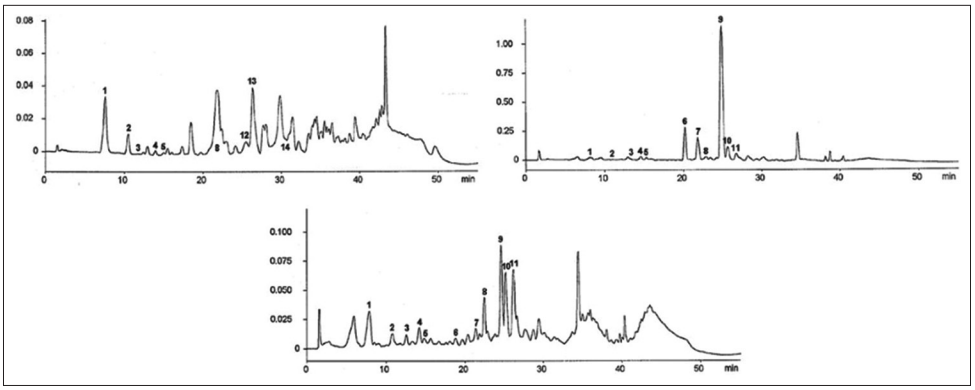

Figure 4: HPLC analysis of hybrid tomato seeds

units and each fraction was then analyzed by tandem mass spectrometer. Fractions containing high-intensity peaks corresponding to the correct molecular weight of the peptides were pooled together and, lyophilized using speed vac lyophilize (Eppendorff, UK) [Figure 4].

CONCLUSION

It can be concluded that both PCR-based and analytical methods-based screening for the purity of commercial Bt tomato hybrids was successful. However, the choice of method depends on the specific needs of the user, as well as the availability of equipment and expertise. The results indicate that the commercial BT tomato seeds sold in the marketplace are not 100% pure. By the above results, it can be concluded that hybrids samples are pure than wild. It can also be concluded that the technology used in the presented study is perfect and can be used for regular screening of BT tomato hybrid seed. This is the first report revealing certain hybrids as moderately resistant variety identified during the present study. Resistant and moderately resistant tomato varieties to bacterial wilt should, when identified, be recommended to farmers, and used to improve the open-pollinated varieties preferred by rural farmers.

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