DETECTION OF TOBACCO NECROSIS VIRUS (TNV) IN PHASEOLUS VULGARIS PLANTS

I. Zitikaitė, J. Staniulis, M. Navalinskienė and V. Kašėta

Abstract

The causal agent of disease was isolated from bean (Phaseolus vulgaris) plants exhibiting virus-like symptoms. Data of host range, symptomology and morphology of particles of the virus isolates from bean revealed close similarity with Tobacco necrosis virus (TNV). The identity of TNV affecting bean plants was confirmed by DAS-ELISA and RT-PCR technique. PCRs revealed that bean crop was affected by TNV similar to ‘Nebraska’ isolate.

Key words: bean, Tobacco necrosis virus, detection, DAS-ELISA, PCR

Introduction

Bean (Phaseolus vulgaris L.) is affected in nature by about 14 viruses (Brunt et al. 1996, Šutic et al. 1999). Two pot viruses were isolated from bean plants in Lithuania (Staniulis 1994). Tobacco necrosis virus (TNV) first discovered in tobacco, causes cucumber necrosis and stipple streaks on bean leaves (Šutic et al. 1999, Bos 1991). TNV is preserved in infected plant parts in the soil, and is transmitted by the fungus Olpidium brassicae (Wor.) Dang and soil water. Particles of TNV are isometric, 26–28 nm in diameter (Kassanis 1970). TNV can infect 298 species in 167 genera of 54 families (Edwardson and Christie 1997). TNV was detected in plum trees, strawberry and raspberry (Staniulis 2003) and ornamental (Navalinskienė 1994) plants in Lithuania.

The objectives of this study were to determine the incidence of virus disease affecting bean crop in Lithuania, and to identify the causal agent.
Materials and methods

Bean plants expressing virus disease symptoms were collected, and after detection of isometric virus particles were investigated. Test plants (Table 1) belonging to the following families: Aizoaceae, Amaranthaceae, Chenopodiaceae, Fabaceae, Solanaceae were inoculated with sap from infected bean leaves. Morphological properties of virus particles from sap extracts of infected plants were examined in negatively stained with 2% UA preparations using a JEM 100S transmission EM (Dijkstra and de Jager 1998). Virus has been identified by DAS-ELISA (Clark and Adams 1977). The IgG and conjugate for detection TNV was used as described (Staniulis et al. 2004). RT-PCR were used for virus detection in experimentally infected tissues of P. vulgaris plants. RNA extraction was carried out according to the instruction of “QuickPrep™ Total RNA Extraction Kit” (Amersham Biosciences, UK). Primer pairs used in RT-PCR were designed using the sequence of TNV ‘Nebraska’ isolate (GenBank accession No. L04261): FNeb (5’-ACAATAGTCTCCAACCTCGAG-3’, nucleotides 910–930) and RNeb (5’-ATCATAACCTGCGTAAGG-3’ complementary to nucleotides 1209–1192), and FD (5’-TCCACCTTCGTAAGG-3’, nucleotides 2885–2906 of TNV D, sequence U62546) and RD (5’-ACAGAATGATTCTATG-3’ complementary to nucleotides 3337–3320) – for D strain. The reaction buffer, RNAse inhibitor, dNTPmix, M-MuLV reverse transcriptase (RT), recombinant Taq polymerase and DNA Ladder were produced by MBIFermentas (Lithuania). The first strand cDNA synthesis, DNA amplification were performed and PCRs products were analysed as described (Staniulis et al. 2004). DNA standard fragment sizes were from top to bottom: 1353, 1078, 872, 692, 310, 281, 271, 234, 194, 118, 72 bp.

Table 1

<table>
<thead>
<tr>
<th>Test plant species</th>
<th>Virus isolates</th>
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<tbody>
<tr>
<td></td>
<td>9809</td>
</tr>
<tr>
<td>Atriplex hortensis L.</td>
<td>L: NL</td>
</tr>
<tr>
<td>Celosia argentea (L.) Kuntze</td>
<td>L: NL</td>
</tr>
<tr>
<td>Chenopodium amaranticolor Coste &amp; Reyn</td>
<td>L: ChIL</td>
</tr>
<tr>
<td>Chenopodium ambrosioides L.</td>
<td>L: NL</td>
</tr>
<tr>
<td>Chenopodium quinoa Willd.</td>
<td>L: ChIL</td>
</tr>
<tr>
<td>Nicotiana rustica L.</td>
<td>L: NL</td>
</tr>
<tr>
<td>Nicotiana tabacum L.</td>
<td>0</td>
</tr>
<tr>
<td>Phaseolus vulgaris L.</td>
<td>L: NL</td>
</tr>
<tr>
<td>Tetragonia expansa Murr.</td>
<td>L: ChIL</td>
</tr>
<tr>
<td>Vigna sinensis (L.) Savi ex Hassk.</td>
<td>L: NL</td>
</tr>
</tbody>
</table>

L – local reaction, NL – necrotic lesions, ChIL – chlorotic lesions, (St) – stunting (uncertain symptom), 0 – no infection, – – not tested.
Results

Naturally infected bean plants showed leaf distortion and mottling followed by necrotic spots and stunted growth. Brown flecks appear on the bean pods. The results of host range study and reaction of main test plant species are summarized in Table 1. Five isolates of the virus always induced local necrotic lesions on inoculated leaves of bean cvs ‘Bataaf’, ‘Red Kidney’, ‘Zlata Saxa’. The inoculated bean cv. ‘Bataaf’ sometimes indicated drastic necrotic reaction, which was followed by wilting of leaves and stunting of plants. Uncertain systemic symptoms of bean cv. ‘Bataaf’ after back inoculation of healthy bean plants likely were not virus nature. The investigated virus isolates induced local chlorotic or necrotic pinpoint on leaves of other inoculated test plants without systemic infection. Isometric particles were readily visualized by the EM in crude sap preparations from naturally infected bean samples (Phot. 1) or from test plants. Samples of inoculated test plants in DAS-ELISA test gave positive reactions with TNV two antisera and confirmed TNV infection in bean. PCR for detection of TNV in bean isolates was successfully used. Specific PCR products were obtained in experimentally infected samples of bean. Specific band was observed in gel analysis at the position corresponding to the expected size of the DNA amplification product of about 280 bp (Phot. 2). The primer pair designed for TNV ‘Nebraska’ isolate on basis of published sequences specifically amplified cDNA templates in RT-PCR of two virus isolates from bean. On the basis of the results of our experiments and literature data these virus isolates could be attributed to TNV from Necrovirus genus.

Phot. 1. TVN particles in crude sap preparation from affected bean isolate (No. 9810) (bar – 100 nm) (photo by I. Zitikaité)

Phot. 2. cDNA amplification products of TNV. Lane 1 – cucumber sample, lanes 2, 5 – bean samples, lanes 3, 4 – tomato samples, lanes 6, 7 – negative and water controls, lane 8 – DNA Ladder (photo by I. Zitikaité)
Discussion

The experimental host range and specific symptoms on all test plants indicated that the virus from bean most closely correspond with the *Tobacco necrosis virus* (Kassanis 1970). Based on particles size and morphology, the virus was considered to be a member of the *Necrovirus* group. RT-PCR data indicated close similarity of investigated isolates from bean with ‘Nebraska’ isolate of TNV, confirming identification obtained by investigation of the host range, symptomology, virus morphology and serology. Products of cDNA amplification using the specific primer pair designed for D strain of TNV were not detected. The ‘Nebraska’ isolate has intermediate characteristics between strains A and D, although serologically related to both strains (Saeki et al. 2001). Positive immunological tests and RT-PCR product size of TNV isolates from bean showed identity with TNV isolates, identified in plums, strawberry and raspberry plants (Staniulis 2003).

Literature

Detection of Tobacco necrosis virus (TNV)...

Authors' address: Dr Irena Zitikaitė,
Dr hab. Juozas Staniulis,
Dr hab. Meletele Navalinskienė,
Vytautas Kašėta,
Institute of Botany,
Žaliųjų Ežerų 49,
08406 Vilnius,
Lithuania
e-mail: izitika@botanika.lt

Accepted for publication: 25.01.2005