Molecular Detection of Jujube Witches’ Broom Phytoplasmas in Micropropagated Jujube Shoots

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Abstract. Jujube (Zizyphus jujuba Mill.) witches’ broom (JWB) is the most important disease in the areas of jujube cultivation in China, where it occurs every year. Micropropagated shoots of the three most important cultivars (‘Lizao’, ‘Junzao’, and ‘Muzao’) in the National Jujube Gene Pool, collected at the Pomology Institute of Shanxi province, were tested for the presence of phytoplasmas. Phytoplasma ribosomal (16Sr) general and specific primer pairs were used in direct or nested polymerase chain reaction (PCR). Positive results were obtained only from symptomatic micropropagated samples of ‘Lizao’ and from phytoplasma controls. Restriction fragment length polymorphism (RFLP) analyses of PCR products with several restriction enzymes revealed that the phytoplasmas infecting the symptomatic plants belong to the 16SrRNA group V subgroup B. The positive correlation between symptoms and the presence of phytoplasmas was verified in tissue culture. Samples from apparently healthy shoots of ‘Junzao’, ‘Muzao’, and ‘Lizao’ were free of phytoplasmas.

In 1997, symptoms of witches’ broom were detected in some ‘Lizao’ jujube plants in an experimental field at the National Jujube Gene Pool (NJGP) at the Pomology Institute of Shanxi Province (PISP), China. Since the disease was associated with the presence of phytoplasma (Zhu et al., 1998), micropropagated 6-month-old shoots of the commercially important cultivars Lizao, Junzao, and Muzao derived from this field were used for DNA extraction to verify the presence of these prokaryotes.

Materials and Methods

Nucleic acids of clover phyllody (CPh) phytoplasma, subgroup 16SrI-C, were obtained from I.-M. Lee (U.S. Dept. of Agriculture, Beltsville, Md). Periwinkle shoots infected with the green valley strain of Western X (GVX) and elm (Ulmus sp.) witches’ broom (ULW) phytoplasmas, groups 16SrIII-A and 16SrV-A, respectively, were obtained from E. Seemüller (BBA Dossenheim, Germany). Shoots infected with elm yellows (EY) phytoplasmas (group 16SrV-A) were obtained from H. Griffiths and W.A. Sinclair (Cornell Univ., Ithaca, N.Y.), and restriction fragment length polymorphism (RFLP) data for the JWB (subgroup 16SrV-B) were obtained from the paper by Lee et al. (1998). Ten micropropagated jujube shoots were selected from ‘Lizao’ L23ZF derived from a symptomatic plant, and from ‘Lizao’ L23, L26, ‘Muzao’, and ‘Junzao’ plants derived from asymptomatic plants. Nucleic acids were extracted from ≈0.5 g of leaves (Fig. 1) and stems per sample following a chloroform/phenol extraction procedure already described (Lee et al., 1991). On these templates, polymerase chain reaction (PCR) experiments were carried out as described by Schaff et al. (1992); primers P1/P7 (Deng and Hiruki, 1991; Schneider et al., 1995), followed in nested PCR by R16F2/R2 (Lee et al., 1995), were employed for amplification of the 16Sr DNA region. Phytoplasma group-specific primer pairs R16(I)F1/R1, R16(III)F2/R1, R16(V)F1/R1, R16(X)F1/R1, and R16(CJ)F1/R1 (Lee et al., 1994, 1995; Zhu et al., 1998) were then used in nested PCR on R16F2/R2-amplified products. Primers R16(CJ)F1/R1 were also employed in direct and in nested PCR using the cycle described by Zhu et al. (1998), with increased annealing temperature to improve specificity. Tubes containing the reaction mixture without DNA templates were included as negative controls. The PCR products were detected by 1% agarose gel electrophoresis, followed by ethidium bromide staining and ultraviolet observation with a transilluminator at 312 nm.

Fig. 1. (A) Shoots of ‘Lizao’ L23ZF jujube plant: small leaves and tiny shoots are typically induced by phytoplasma; (B) and (C) the shoots do not proliferate and have normal-sized leaves. Shoots of healthy plants of ‘Lizao’ L26 and ‘Muzao’, respectively. All shoots are cultured on MS medium.
Results and Discussion

Using primer pairs R16F2/R2 in nested PCR on the PCR products generated with P1/P7, a 1200-bp fragment was obtained from all the ‘Lizao’ L23ZF shoots tested and from the phytoplasma controls (data not shown). The RFLP analyses with HpaII and RsaI on R16F2/R2-amplified fragments showed that the patterns of phytoplasmas infecting jujube L23ZF (Fig. 1) were identical with those of the JWB phytoplasmas previously described (Fig. 1 c and f in Lee et al., 1998), indicating that the phytoplasmas belong to the 16S rRNA group V, subgroup B. From the RFLP pattern of one of the samples digested with RsaI (Fig. 2, arrow), two bands of similar size appear to be present at 527 bp, suggesting that the two 16S genes present in all phytoplasmas could show some differences in strain L23ZF.

Nested PCR with primers specific for some phytoplasma groups [R16(I)F1/R1, R16(III)F2/R1, R16(V)F1/R1, and R16(X)F1/R1] gave no amplification from any of the jujube samples, while bands of the expected lengths were obtained only from corresponding controls (data not shown). Primers R16(CJ)F1/R1 yielded the expected 1100-bp bands from ‘Lizao’ L23ZF samples and from other phytoplasma controls belonging to the 16SrV group. The same primer pair, used in direct or in nested PCR with the cycle reported by Zhu et al. (1998), showed specificity only for samples ‘Lizao’ L23ZF, although the amplified bands were very weak for some of the samples (Fig. 3).

Our results confirm that molecular tests may be used to index micropropagated jujube shoots for phytoplasma infection. ‘Lizao’ jujube appears to be more susceptible to phytoplasma infection than are ‘Muzao’ and ‘Junzao’, which were always negative and in the field showed low percentages of symptomatic plants. ‘Muzao’ and ‘Junzao’ probably carry genetic resistance that could be tapped for incorporation into sensitive commercial varieties of jujube or other fruit plants.

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