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Differential expression of *CmPP16* homologues in pumpkin (*Curcurbita maxima*), winter squash (*C. moschata*) and their interspecific hybrid

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SUMMARY

Grafting is used in vegetable production as an alternative to highly toxic, broad-spectrum soil disinfectants such as methyl bromide and to avoid soil-borne biotic and abiotic stresses. However, deterioration in valuable qualitative characters of the scion occurs in specific graft combinations, such as melon to pumpkin, which makes this environmental-friendly approach less desirable. Understanding the reasons for rootstock-scion incompatibility, and the role of the rootstock, could help to alleviate this problem. We therefore examined the level of expression of CmPP16, a pumpkin (*Cucurbita maxima*) phloem protein that mediates RNA transport from the rootstock into scion tissue, in different pumpkin and squash (*C. moschata*) varieties and in a pumpkin × squash interspecific hybrid. All are currently in use as rootstocks in grafted melon, watermelon and cucumber production. Our data revealed that two different genetic loci, *CmPP16-1* and *CmPP16-2*, which encode CmPP16 proteins, are expressed in pumpkin and squash, but not in melon. Pumpkin stems accumulated much higher levels of *CmPP16* transcripts than squash or interspecific hybrid stems, while in roots *CmPP16*-expression was higher in squash. The pumpkin cultivar with the highest levels of expression of *CmPP16* was the worst rootstock for melon, suggesting a role for CmPP16 in determining scion quality. Our results, if confirmed in additional varieties and species, may provide an effective way to screen for suitable rootstocks in grafted vegetable production.

Most cultivated cucurbits (melon, cucumber and watermelon) are highly susceptible to soil-borne diseases that currently are mainly controlled by highly toxic broad-spectrum soil disinfectants such as methyl bromide. As the use of methyl bromide is to be eliminated during 2005, grafting of good quality but disease-sensitive scions onto resistant rootstocks is an alternative means to improve the agronomic characteristics of crops with a concomitant decrease in chemical use. In addition, automation of grafting (Kurata, 1994) and the combination of micropropagation and micrografting (Sapountzakis and Tsaftaris, 2002; Grigoriadis *et al*., 2005) have been applied successfully to a number of plant species as an efficient way to decrease the production costs of grafted vegetables. Nevertheless, it has been reported that genotypic variation in rootstocks affects growth and productivity of the scion (Zijlstra *et al*., 1994) and that, specifically for pumpkin-melon heterografts, some rootstock-scion combinations caused a significant deterioration in the taste and texture of the fruit (Traka-Mavrona *et al*., 2000).

It seems reasonable to hypothesise that the deterioration in commercially-valuable quality characteristics in the scion in specific grafting combinations is due to signal(s) derived from the rootstock, which are transmitted into the scion, but the nature of these signals remains obscure. According to current evidence (reviewed by Haywood *et al*., 2002), proteins and/or RNAs, particularly siRNAs (Palauqui *et al*., 1997; Yoo *et al*., 2004) that play non-cell-autonomous roles in developmental and physiological processes, could be transported through the plasmodesmata. Experimental results show that specific phloem proteins can be transported from the rootstock to the scion through the heterograft union (Tiedmann and Carstens-Behrens, 1994; Xoconostle-Cázares *et al*., 1999). These proteins are analogous to viral movement proteins (MPs) and mediate an increase in the plasmodesmal size exclusion limit and the trafficking of macromolecules from companion cells to sieve tube elements through plasmodesmata (Balachandran *et al*., 1997). The discovery that one of these proteins, CmPP16, mediates the transport of sense- and antisense-RNAs, as well as other proteins, in phloem sap and is present in stems, roots and especially flowers (Xoconostle-Cázares *et al*., 1999) has drawn our attention to CmPP16 as a candidate molecule that may affect physiological characteristics and fruit quality in melon-pumpkin heterografts. Homologues of CmPP16 have also been found in rice (RPP17 and RPP16), and appear to be present in a variety of other plants (Asano *et al*., 2002).

We report the isolation and characterisation of *CmPP16-1* and *CmPP16-2* homologues from a local

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pumpkin (*C. maxima*) cultivar 'Lefki kolokytha' and a local squash (*C. moschata*) cultivar 'Kalkabaki' as well as a *C. maxima* × *C. moschata* interspecific hybrid 'TZ148'. All three are frequently used as rootstocks in melon, watermelon or cucumber heterografts. We also investigated the expression levels of *CmPP16-1* and *CmPP16-2* mRNAs to determine if quantitative differences in expression correlated with differences observed in rootstock-scion combinations regarding the quality of melon heterografts (Traka-Mavrona *et al*., 2000).

MATERIALS AND METHODS

Plant material

Seeds from the commercial melon (*Cucumis melo*) cultivar 'Galia' (G), the pumpkin (*C. maxima*) cultivar 'Lefki kolokytha' (LK), the winter squash (*C. moschata*) cultivar 'Kalkabaki' (K), and the commercial interspecific hybrid of *C. moschata* × *C. maxima* cultivar 'TZ148' (TZ) were used. Melon seeds were submerged in 75% (v/v) ethanol for 1 min, washed with distilled water then treated with 0.01% (v/v) Triton-X 100 for 15 min. LK, K and TZ seeds were treated with 0.01% (v/v) Triton-X 100 for 30 min. All seeds were then washed thoroughly with autoclaved distilled water and left to germinate for 7 d at room temperature in the dark. The seedlings were planted in compost and transferred to the greenhouse for 6 weeks. At that stage, plant materials (stems, leaves and roots) were collected separately, snap-frozen in liquid nitrogen and stored at –80°C for later analyses.

Isolation of full-length PP16-1 *and* PP16-2 *homologues and phylogenetic analysis*

Total RNA from LK, K and TZ stems was extracted with the Nucleospin® RNA Plant kit (Macherey-Nagel, Dueren, Germany) with on-column DNA digestion during RNA purification using the RNase-free DNase I provided with the kit. Full-length cDNA clones were prepared with an RNA ligase-mediated rapid amplification of cDNA ends (RLM RACE) protocol using the GeneRacer™ Kit (Invitrogen, Paisley, UK) as described. Initially, to estimate the extent of sequence differences between genotypes, we amplified the 5'-termini of the *PP16-1* cDNAs with the gene-specific primer 5'-TGTATAAAGCAACAAGTTTGCAT-3' (PP16-1rev) designed according to the *CmPP16-1* sequence (AF079170) and the 5'-termini of the *PP16-2* cDNAs with the gene-specific primer 5'-CCACTTATTCTTCCTTAATTACC-3' (PP16-2rev) designed according to the *CmPP16-2* sequence (AF079171) of a *C. maxima* genotype (Xoconostle-Cázares *et al*., 1999) and the GeneRacer™ 5' Primer. Amplification was performed in a PTC 200 thermocycler (MJ Research, Waltham, MA, USA) and the programme was: 92°C for 1 min, followed by 35 cycles of 92°C for 30 s, 44°C for 30 s, 72°C for 45 s, then 72°C for 10 min. The amplified cDNA was electrophoresed in 0.8% (w/v) agarose in TAE and recovered from the gel with the NucleoSpin® Extract kit (Macherey-Nagel). The purified 570 bp and 490 bp DNA fragments were then sub-cloned into pGEM-T Easy Vector (Promega, Madison, WI, USA) according to the manufacturer's protocol and ten clones of each genotype were sequenced.

Subsequently, since no extensive differences at the 5'-ends of the cDNAs could be detected, we checked for differences at the 3'-termini of the cDNAs. We designed the primer PP16-5UTR: 5'-TTGACAGACAGAGGCAAC-3' based on the sequence of an identical fragment of the 5'-untranslated region of both the *CmPP16-1* and *-2* sequences, which together with the GeneRacer™ 3'Primer were used to amplify the 3'-terminus of *PP16-1* and *PP16-2* cDNA sequences encompassing the open reading frames (ORFs) of the genes for all genotypes examined. 3'RACE cDNAs were subcloned and ten clones of each genotype were sequenced. Full-length cDNA sequences were compiled using the SeqMan II software (DNASTAR, Madison, WI, U.S.A.) in order to assemble fragment data derived from different clones of the 5'and 3'RACE experiments performed for each genotype. Sequence alignments were performed by the ClustalW method (Thompson *et al*., 1994) using BioEdit software (Hall, 1999). Phylogenetic relationships of these sequences were examined using the Neighbor-Joining Method with *p*-distance correction (Saitou and Nei, 1987). Bootstrap values were derived from 1000 replicate runs. A phylogenetic tree was constructed using the MEGA 2.1 software (Kumar *et al*., 2001)

Southern blotting

Genomic DNA (10 µg) was isolated from leaves of LK using the Nucleospin® Plant kit (Macherey-Nagel), according to the instructions and digested with *Eco* RI, *Eco* RV, *Hin*d III and *Dra* I, (New England Biolabs, Beverly, MA, USA), electrophoresed through a 1% (w/v) agarose gel and transferred onto a positively-charged nylon membrane (Rôche, Mannheim, Germany). Clone *LKPP16-1* served as template for the synthesis of a digoxigenin-labelled *PP16* probe with the PP16-1fw (5'-AGGACCAAATCCATTATGG-3') and PP16-1rev primers using the PCR DIG Probe Synthesis Kit (Rôche). The polymerase chain reaction (PCR) was performed in a PTC 200 thermocycler (MJ Research) and the programme for amplification was: 92°C for 1 min, followed by 35 cycles of 92°C for 1 min, 44°C for 30 s, 72°C for 45 s, then 72°C for 10 min.

Hybridisation was performed as recommended with DIG Easy Hyb buffer (Rôche) at 42°C followed by two stringent washes at 65°C in 0.5X SSC containing 0.1% (w/v) SDS. Detection was performed using the DIG Luminescent Detection Kit (Rôche) according the instructions. Chemiluminescence was detected using the GENEGNOME™ Bio Imaging System (Syngene, Cambridge, UK).

Total RNA preparation for northern hybridisation and analysis

Total RNA was extracted from leaves, stems and roots of LK, K, TZ and G as described in Thompson *et al*. (1983). Total RNA from each sample was fractionated through a 1.2% (w/v) agarose-formaldehyde gel, blotted on a Hybond N-Plus™ nylon membrane (Amersham Pharmacia Biotech, Uppsala, Sweden), then hybridised with DIG-labelled *PP16* probe in DIG Easy Hyb hybridisation buffer (Rôche) at 50°C. Blots were washed twice with 2XSSC containing 0.1% (w/v) SDS at room temperature followed by two washes with 0.5XSSC

containing 0.1% (w/v) SDS at 68°C for 20 min. Anti-DIG antibody binding and detection were performed with the DIG Wash and Block Buffer Set and DIG Northern Starter kit (Rôche) according to the manual. The signal was detected with the CSPD® (Tropix Inc., Bedford, MA, USA) chemiluminescent dye in a GENEGNOME™ (Syngene) bioimager. Analysis of the signal intensities was performed with GeneTools software (Syngene).

Real-time quantitative RT-PCR

DNA-free total RNA (2 µg) from LK, K and TZ stems was reverse transcribed (RT) with 400 U M-MuLV Reverse Transcriptase (New England Biolabs) in the presence of 1 µg oligo-dT_{18} primer and 1 mM dNTPs, in the 1X buffer supplied by the manufacturer. The reaction was carried out at 37°C for 1 h in a final volume of 70 µl.

In real-time quantitative PCR, a 138 bp *PP16-1* sequence was amplified with qPP16-1 (5'-AGGGAGAAATTGAAGTTGGAG-3') and PP16-1rev, and a 132 bp PP16-2 sequence with qPP16-2 (5'-AGGAGTTAGAAAGGGATGGTC-3') and PP16-2rev primers, respectively. The actin reference

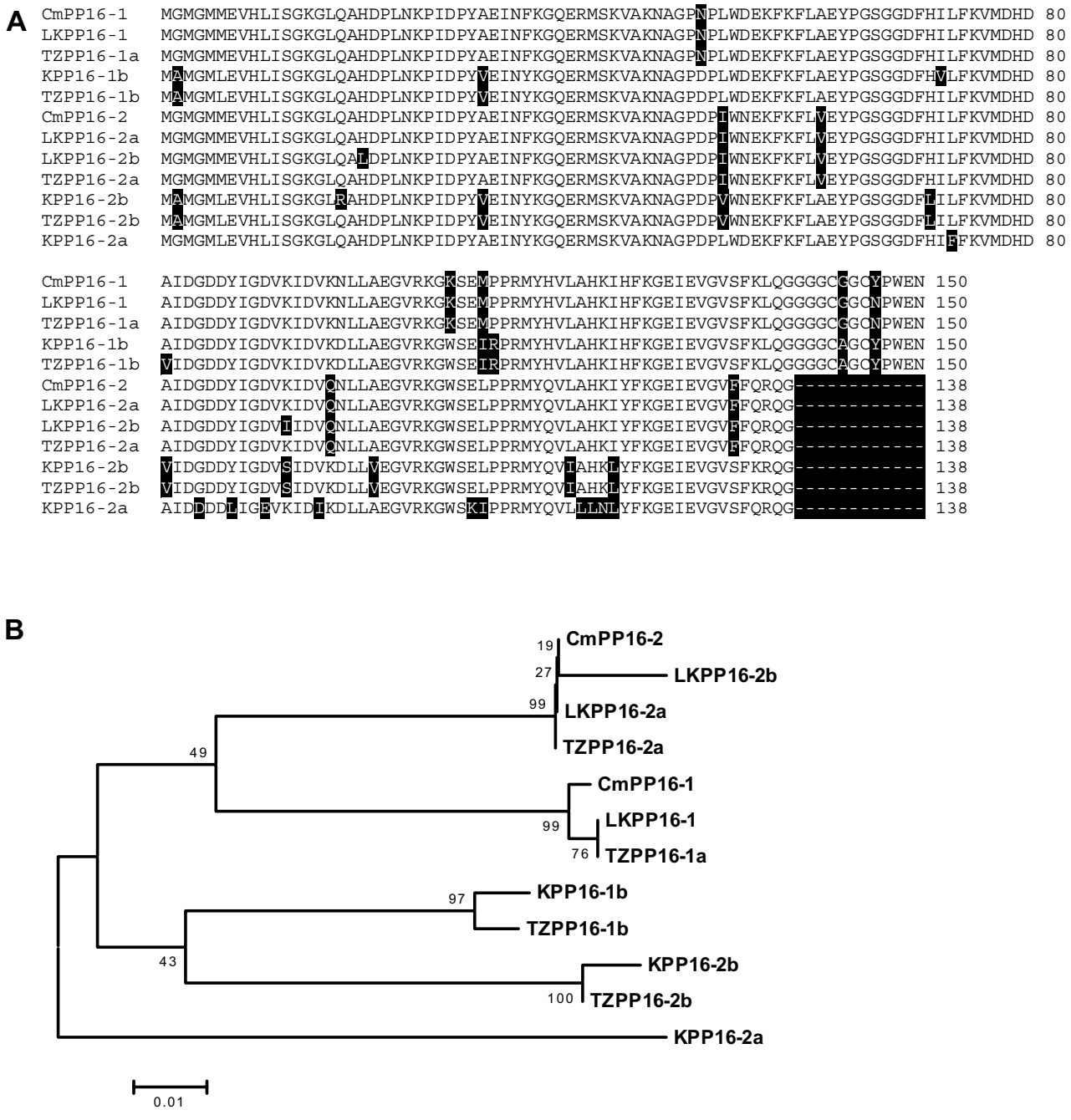

FIG. 1

Panel A, Multiple sequence alignment of the putative translation products derived from the cloned *PP16-1* and *PP16-2* cDNA sequences from the pumpkin (*C. maxima*) cultivar 'Lefki kolokytha' (LK), the winter squash (*C. moschata*) cultivar 'Kalkabaki' (K), and the commercial interspecific hybrid of *C. moschata* × *C. maxima* cultivar 'TZ148' (TZ), using the ClustalW algorithm. Differences between sequences are shown in white letters on a black background. Panel B, Phylogenetic relationships of the protein sequences aligned in Panel A. A phylogenetic tree was generated by the Neighbor-joining method with *p*-distance correction. The scale below the tree indicates amino acid substitutions, and numbers at the nodes are bootstrap values of 1000 replications.

sequence (149 bp) was amplified with the ACTfw (5'-GGGCCAAAAAGATGCCTATGT-3') and ACTrev (5'-GCAACACGAAGCTCATTGTAG-3') primers that were designed according to the published *Cucumis* actin sequences (AB033599 and AB010922). The melting temperature of the products was calculated in an Opticon2 DNA engine (MJ Research) and ranged between 79°–80.5°C. The reaction mix contained 2 µl of cDNA in RT buffer, 0.75 µM of each primer and 1X master mix (DyNAmo™ SYBR® Green qPCR kit; Finnzymes, Espoo, Finland). Each reaction was carried out in quadruplicate in an Opticon2 DNA engine (MJ Research), and the thermocycler conditions were: 95°C for 10 min, then 35 cycles of 92°C for 30 s, 45°C for 20 s, 72°C for 15 s, plate read at 78°C, followed by 72°C for 10 min. The relative amount of RNA in each sample was calculated by the $2^{-\Delta\Delta Ct}$ method using actin as internal control and the lowest relative expression of K as calibrator (Livak and Schmittgen, 2001).

RESULTS

Full-length PP16-1 *and* PP16-2 *sequences from three plant genotypes.*

Three different full-length cDNAs from LK, three from K and four from their interspecific hybrid, TZ were assembled using the SeqMan II software (DNASTAR), based on extensive overlapping of 5'RACE and 3'RACE clones in a region spanning 545 bp for *CmPP16-1* and 450 bp for *CmPP16-2*. Multiple alignment using the nucleotide sequences of these cDNAs along with the sequence of *CmPP16-1* (AF079170) and *CmPP16-2* (AF079171) from another *C. maxima* cultivar (Xoconostle-Cázares *et al*., 1999) produced two distinguishable groups: group *PP16-1* and group *PP16-2*.

Group *PP16-1* consisted of *LKPP16-1* (DQ088371), *KPP16-1* (DQ088368), *TZPP16-1a* (DQ088374) and *TZPP16-1b* (DQ088375) that were 94.2–98.6% identical to *CmPP16-1*. On the other hand, group *PP16-2* consisted of *LKPP16-2a* (DQ088372), *LKPP16-2b* (DQ088373), *KPP16-2a* (DQ088369), *KPP16-2b* (DQ088370), *TZPP16-2a* (DQ088376) and *TZPP16-2b* (DQ088377) that were 91.2–99.6% identical to *CmPP16-2*. Within each group there were striking similarities. Clone *LKPP16-1* differed from *CmPP16-1* by six synonymous nucleotide substitutions and was virtually identical to *TZPP16-1a*. Clone *KPP16-1* differed from *TZPP16-1b* by one synonymous and three conservative nucleotide substitutions. Clone *LKPP16-2a* was virtually identical to *CmPP16-2* and *TZPP16-2a*. Finally, clone *KPP16-2b* differed from *TZPP16-2b* by one conservative amino acid substitution.

Sequence alignment of the putative translated proteins is shown in Figure 1A. Phylogenetic analysis of these proteins (Figure 1B) showed that the sequences formed separate branches according their genotypic origin. All proteins of the PP16-1 group derived from *C. maxima* formed one branch that includes CmPP16-1, LKPP16-1 and TZPP16-1a, while the proteins derived from *C. moschata* formed a separate branch that included KPP16-1b and TZ16-1b. In the same way, PP16-2 group proteins from *C. maxima* fell together in one branch that included CmPP16-2, LKPP16-2a, LKPP16-2b and TZPP16-2a (indicating that this allele originated from the *C. maxima* parent of the interspecific hybrid); while the proteins derived from *C. moschata* formed a separate branch that included KPP16-2b and TZ16-2b (indicating that this allele originated from the *C. moschata* parent of the interspecific hybrid). The KPP16-2a protein was more divergent from the rest of the sequences and its homologue was not isolated from TZ during the course of these experiments. However, its sequence was confirmed since one 5'RACE and three different 3'RACE clones, encompassing the full ORF of the gene, were recovered and the overlapping ORF sequence was identical among them.

CmPP16 *homologues in pumpkin*

A PP16 probe prepared from the sequence of *CmPP16-1* cDNA was used to determine the number of *CmPP16* homologues in pumpkin. The probe was 90% homologous to the *CmPP16-2* cDNA and, under the conditions applied, was able to hybridise with both *CmPP16-1* and *CmPP16-2* genomic sequences. Digestion with *Eco* RI produced two hybridising bands, while digestions with *Eco* RV, *Dra* I and *Hin*d III produced three bands (Figure 2). In each digestion, one

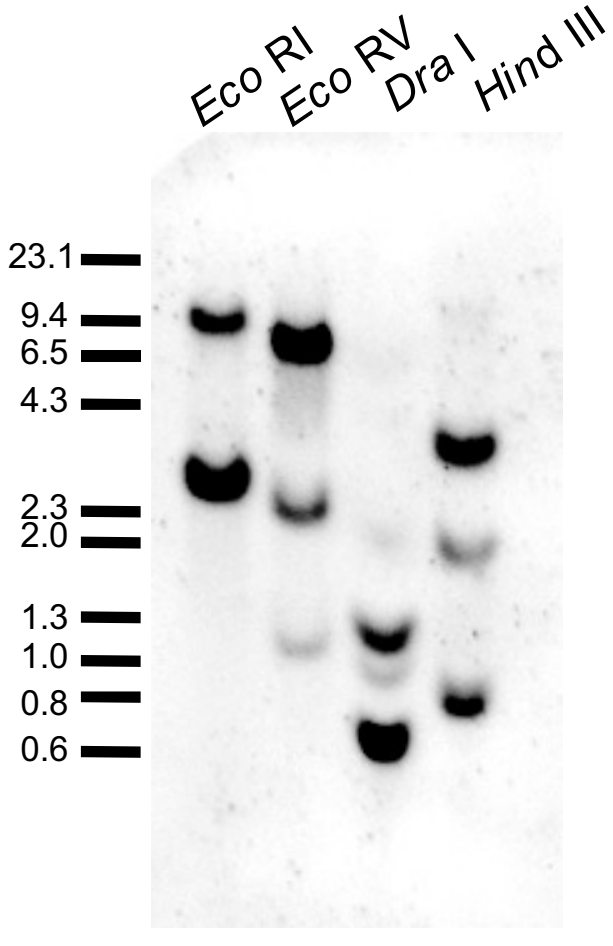

FIG. 2
Southern blot revealing the presence of two *CmPP16* genes in the pumpkin cultivar 'Lefki kolokitha' (LK). Genomic DNA isolated from LK was digested with *Eco* RI, *Eco* RV, *Dra* I or *Hin*d III and hybridised with a *CmPP16-1* probe. These restriction enzyme sites are absent from the *CmPP16-1* and *CmPP16-2* cDNAs. Two bands in the *Eco* RI digestion and three bands from the other enzymes were observed. Since the genomic *CmPP16* sequence is known to be about 1,010 bp, the presence of two *Eco* RI bands of higher size is indicative of the presence of two separate *CmPP16* genes in pumpkin.

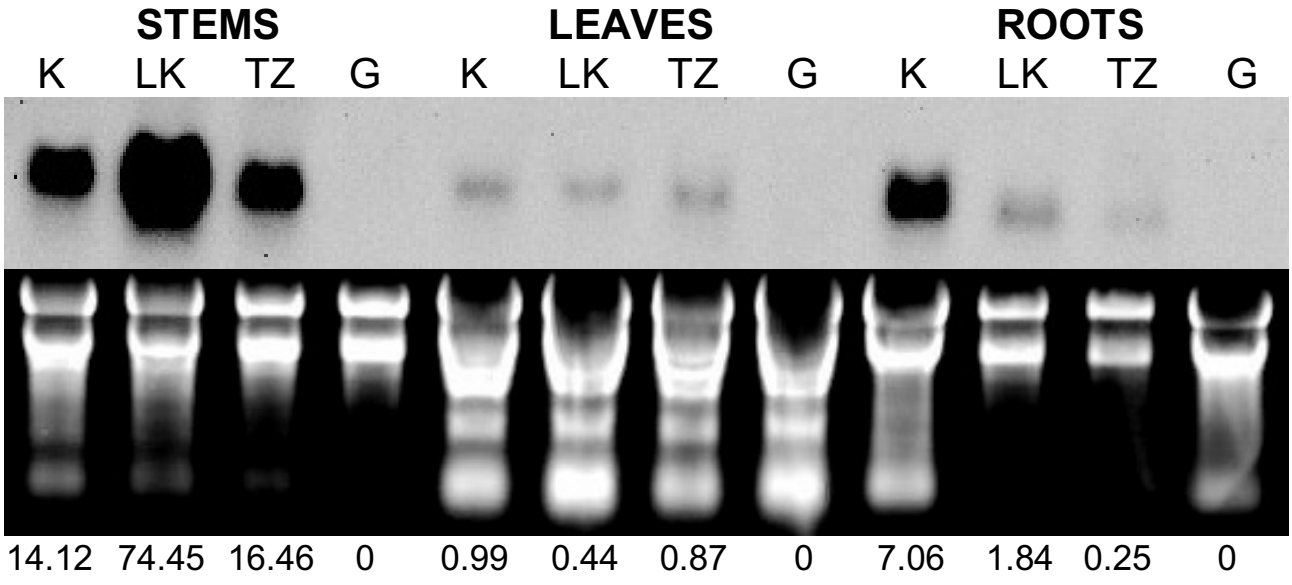

FIG. 3
Northern hybridisation of total RNA from stems, leaves and roots of the commercial melon (*Cucumis melo*) cultivar 'Galia' (G), the pumpkin (*C. maxima*) cultivar 'Lefki kolokytha' (LK), the winter squash (*C. moschata*) cultivar 'Kalkabaki' (K), and the commercial interspecific hybrid of *C. moschata* × *C. maxima* cultivar 'TZ148' (TZ), probed with the *PP16* sequence. Lower panel presents the RNA samples, electrophoresed in a denaturing agarose gel and stained with ethidium bromide, before northern blotting. The numbers at the bottom indicate the relative intensity of each hybridisation signal (upper panel), after correcting for RNA loading.

band was obviously stronger than the others. These restriction enzyme sites were not present in the sequences of *CmPP16-1* and *CmPP16-2* cDNAs. *Eco* RI-digested genomic DNA from another pumpkin cultivar produced a similar hybridisation pattern when hybridised with a full-length *CmPP16-1* cDNA probe, although the weak band was very faint (Xoconostle-Cázares *et al*., 1999; www.sciencemag.org/feature/data/982968.shl). Considering that the genomic *CmPP16* DNA has been reported to be 1010 bp in pumpkin (Xoconostle-Cázares *et al*., 1999), the *Eco* RI-banding pattern indicates that two separate *CmPP16* loci are present in pumpkin.

Northern analyses

Northern blots of total RNAs from K, LK, TZ and G stems, leaves and roots were hybridised with the *PP16* probe. As shown in Figure 3, *PP16* RNAs were present in all pumpkin and squash tissues examined, but were not detectable in melon. Among the tissues studied, *PP16* transcripts were significantly more abundant in stems than in leaves or roots. Comparison of the three stem samples revealed that *PP16* RNA existed in substantially higher levels in LK than in TZ and K. On the other hand, K seems to be comparatively rich in *PP16* RNA in roots.

We also examined the range of variation in the accumulation of *PP16* RNA among individual stems of different LK plants, as well as among individual K plants and observed that it did not exceed a two-fold difference between individual plants of the same species (data not shown). This was well below the five-fold difference detected for *PP16* RNA between LK and K shown in Figure 3.

Real-time quantitative PCR

Real-time quantitative RT-PCR (qPCR) was the method of choice to confirm and further analyse the results from northern hybridisation. qPCR provides both increased sensitivity to discriminate between small differences in *PP16* RNA levels, and the ability to use the subtle differences between the *PP16-1* and *PP16-2* sequences to examine the level of expression of each RNA species separately in the RNA samples.

According to the qPCR analysis (Figure 4), levels of *PP16-1* RNA in the stems of LK were five-fold greater than in K and 2.5-fold greater than in TZ, which was in

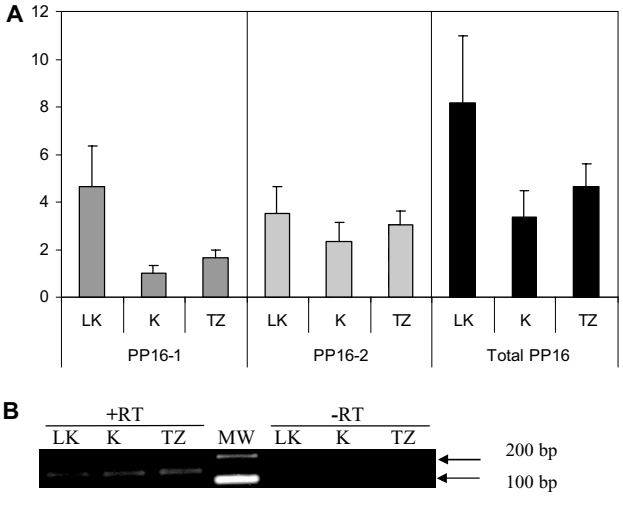

FIG. 4
Real-time quantitative RT-PCR. Panel A, Graphic representation of *PP16-1* and *PP16-2* RNA levels in the stems of the pumpkin (*C. maxima*) cultivar 'Lefki kolokytha' (LK), the winter squash (*C. moschata*) cultivar 'Kalkabaki' (K), and the commercial interspecific hybrid of *C. moschata* × *C. maxima* cultivar 'TZ148' (TZ), estimated with real-time quantitative RT-PCR. Values on the Y-axis are relative to the 'calibrator' (PP16-1 K; the lowest quantity over all samples) that was arbitrarily assigned the value 1. Total *PP16* levels represent the sum of both transcripts in the three genotypes. Panel B, Agarose gel electrophoresis of PCR products showing the absence of genomic DNA-derived amplification products. Reactions were performed with the qPP16-1 and PP16-1rev primers under the same conditions as in real-time quantitative PCR, on reverse-transcribed (+RT) and non-reverse transcribed (–RT) LK, K and TZ total RNA samples. MW are DNA size-markers.

good agreement with the northern analysis. On the other hand, *PP16-2* RNA levels in the three samples did not vary significantly. When *PP16-1* and *PP16-2* levels in the same tissue were compared, it became apparent that although LK did not exhibit significant variation in the levels of the two sequences, *PP16-2* levels in K and TZ were twice as high as *PP16-1* in the same samples.

DISCUSSION

Analysis of ten cDNAs isolated by PCR from *C. maxima*, *C. moschata* and their interspecific hybrid showed that variant alleles of *CmPP16* are present in these genotypes. The presence of two genes in pumpkin was initially suggested by Xoconostle-Cázares *et al.* (1999) and was confirmed by the results in this study (Figure 2). Moreover the presence of *a* and *b* alleles of *LKPP16-2* and *KPP16-2* in the hybrid TZ is strong evidence that *PP16-1* and *PP16-2* are two separate, homologous genes and, while LK and K are homozygous at the *PP16-1* locus, they are both heterozygous at the *PP16-2* locus. The fact that Xoconostle-Cázares *et al.* (1999), did not identify the *PP16-2b* allele in *C. maxima* may reflect the different genotype of plant material used in their study.

It is interesting to observe that, although identifiable, there are few differences in the *PP16* sequences in all species (cultivars), an indication that the events that led to speciation and the divergence of cultivars are rather recent. Nevertheless, no estimations can be made on the time-scale of these events in the absence of a convenient outgroup. Moreover, the fact that even the few mutations that do exist in the nucleotide sequences of these genes are synonymous or conservative (i.e., the new triplet encodes the same amino acid, or one with similar properties that is likely to result in a protein of conserved conformation and properties) shows that the specific characteristics and probably the function of the PP16-1 and PP16-2 proteins have been conserved in the *Cucurbitaceae* through evolution. The most characteristic difference between the two groups of proteins is the 12 amino acid-long sequence present at the carboxyl-terminus of PP16-1 that is missing from the PP16-2 group (Figure 1).

Analysis of *PP16* gene expression in stems, roots and leaves (Figure 3) showed that this transcript could be detected in all pumpkin tissues, but in no melon tissue. Nevertheless, as Asano *et al.* (2002) were able to detect PP16 homologous proteins in melon leaf extracts by immunoblotting, our results may be due to DNA sequence divergence between the pumpkin and melon homologues and might not reflect the lack of a PP16 homologue in melon. Analysis of *PP16* mRNA levels in various pumpkin tissues showed that the *PP16* transcript is highly abundant in stems, less abundant in roots and present at very low levels in leaves, which agrees with the results reported by Xoconostle-Cázares *et al.* (1999).

In more detail, we found that *PP16* RNA levels in leaves were the lowest among all tissues, while in roots only K seemed to have a higher level of the transcript. However, the highest *PP16* mRNA accumulation levels among all pumpkin tissues were observed in stems, and especially in stems of LK. These findings, together with the proposal that the movement protein "signal" is diluted as it travels from cell-to-cell through plasmodesmata (Balachandran *et al.*, 1997) support the idea that the major site of PP16 synthesis could be in the phloem of the stem and it is then transported to and gradually diluted in other parts of the plant. In a more detailed analysis it was observed that, although some variation in expression levels of *PP16* could be detected between different individuals of LK or K (data not shown), it was far lower than that observed between the two different species. It is therefore reasonable to conclude that differences in *PP16* expression between species (cultivars) are not due to random individual polymorphism. The results of real-time quantitative PCR (Figure 4) agree with those from northern hybridisation regarding expression of *PP16* in the plant tissues tested. In addition, they show that most of the differences are due to increased *PP16-1* transcript levels in LK, which on average were five-fold higher than in K, and three-fold higher than in TZ. On the other hand, levels of *PP16-2* RNA did not show such large differences between plants or tissues, although the transcript level in LK was higher than that in K and TZ.

Fruit quality characteristics, such as shape, skin colour, skin or rind smoothness, flesh texture and colour, rind thickness and soluble solids content, are influenced by the rootstock. The nature of the signal(s) originating from the rootstock that could affect these characters remains obscure. Xylem and phloem are the major conduits for the transport of water and the exchange of essential nutrients between distantly located organs, and are the most probable transport routes for the putative fruit quality deterioration signal(s). Xylem conduct water and nutrients obtained by the roots to the plant, and xylem sap may contain substances from the roots that adversely affect fruit quality. Alternatively, or in addition, the phloem not only transfer photosynthates from the leaves to the roots, but also facilitate the transport and delivery of signalling macromolecules (e.g., proteins and ribonucleoprotein complexes) upwards into the plant (Lucas and Lee, 2004). They could be involved in phloem-mediated transport of the putative deterioration signal.

Heterografting experiments with cucumber (as scion) and pumpkin (as rootstock) established that both the CmPP16 protein and its mRNA from the pumpkin rootstock moved within the long-distance phloem translocation stream and had the capacity to influence transcription/translation within the cucumber scion (Xoconostle-Cázares *et al.*, 1999). The fact that we observed the lowest levels of *PP16* transcript in the cultivar K, which also had the lowest adverse impact on the quality of melon heterografts compared to TZ and LK (Traka-Mavrona *et al.*, 2000), points to a possible involvement of PP16 proteins. Recently an additional gene (*CmPSRP1*) coding for the *C. maxima* phloem small RNA binding protein 1 has been proposed to be involved in siRNA trafficking in pumpkin (Yoo *et al.*, 2004). Following a similar approach to the one described in this paper for the *PP16* genes, we have isolated *CmPSRP1*-homologous clones from the three different genomes used here and are currently studying their differential expression (Mermigka and Tsaftaris, unpublished results).

In conclusion, our data show that levels of *CmPP16* gene expression in rootstocks commonly used for melon

heterografts are inversely related to scion fruit quality, thus supporting our hypothesis that CmPP16 protein may be involved in the deterioration of scion quality. Our results, if confirmed in additional varieties and species, may provide an effective way to screen for suitable rootstocks in grafted vegetable production.

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