Isolation and characterization of a cDNA from *Quercus robur* differentially expressed in juvenile-like and mature shoots

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Summary A full-length cDNA clone named *QRCPE* (*Quercus robur* crown preferentially expressed) that is differentially expressed during in vitro culture of mature and juvenile-like shoots of *Quercus robur* L. was identified by differential display. The deduced amino acid sequence showed that the encoded protein is small, contains a predicted N-terminal hydrophobic signal peptide that targets the protein to the cell wall, and is rich in glycine and histidine residues. Accumulation of *QRCPE* mRNA was higher in oak microshoots derived from crown branch shoot cultures than in oak microshoots derived from basal shoot cultures at the end of the multiplication and rooting period. Among organs, the highest accumulation of *QRCPE* transcripts was detected in roots, followed by stems and leaves, with preferential accumulation in specific organs of ontogenetically older shoots. Although *QRCPE* mRNA was abundant in oak zygotic and somatic embryos, almost no *QRCPE* mRNA accumulation was detected in nodular callus cells, suggesting a possible role of this gene in embryonic development. In proliferating shoot cultures of two chestnut (*Castanea sativa* Mill.) clones, the *QRCPE* homolog was preferentially expressed in crown-derived shoots. On the other hand, expression analysis of *QRCPE* in juvenile and mature material from soil-grown oak plants indicated that this gene is expressed from the embryonic to mature phases, but is progressively down-regulated during plant maturation. In vitro culture conditions induced changes in *QRCPE* transcript abundance in both basal and crown shoots in a phase-dependent manner. We conclude that *QRCPE* expression in oak is correlated with the ontogenetic stage of shoots, and thus this gene may be useful as a potential molecular marker for maturation-related characteristics.

Keywords: differential display, gene expression, in vitro shoot cultures, phase change.

Introduction

Phase change or maturation is a developmental process, characterized by the achievement of sexual reproductive competence, that affects the regenerative potential of tissue explants (Poethig 1990). It is generally accepted that a major drawback to cloning a selected tree is the inverse relationship between the attainment of mature characteristics and the ability to propagate adult trees vegetatively. The ability to micropropagate woody plants is also greatly influenced by the maturation stage and, therefore, by the physiological stage of the initial explants (Bonga 1987). McGowran et al. (1998) reported that developmental age of donor trees strongly affects the vigor and morphological traits of in-vitro-cultured shoots of *Quercus robur* L. and *Quercus petraea* ex. Liebl. However, it may be possible to propagate mature trees, because old material exhibiting mature characteristics may coexist in the same tree with ontogenetically younger tissue that retains juvenile characteristics (Hackett 1985).

Previously, we showed that explants taken from crown branches of mature oak and chestnut (*Castanea sativa* Mill.) trees were difficult to culture in vitro compared with explants derived from basal sprouts and epicormic shoots (Sánchez 1991, Sánchez and Vieitez 1991). However, crown shoot cultures were successfully achieved by using explants isolated from oak shoots that flushed on crown branch segments (Vieitez et al. 1994) and stem sections (Evers et al. 1993). We also found that oak shoot cultures derived from basal sprouts exhibited much higher rooting rates than oak shoot cultures derived from crown branches of the same tree (Sánchez et al. 1996).

Maturational changes, which affect reproductive competence, the regenerative potential of tissue and morphological characteristics, are associated with changes in gene expression and hence changes in gene products (Greenwood 1995). Little is known about the molecular events regulating gene expression during phase change in woody plants. Amo-Marco et al. (1993) found two polypeptides in chestnut shoot cultures derived from the crown that were not present in shoot cultures derived from basal shoots, suggesting that maturation is related to the expression of genes involved in the synthesis of mature-phase-specific proteins. Molecular analyses performed on eastern larch (*Larix laricina* (Du Roy) C. Koch) and English ivy (*Hedera helix* L.) revealed that expression of...
genes homologous to the chlorophyll a/b binding protein gene is higher in juvenile tissues than in mature tissues (Hutchison et al. 1990, Woo et al. 1994). Sánchez et al. (1995) found that a cDNA clone designated HW103, encoding a proline-rich protein (PRP), was differentially expressed in juvenile and mature ivy petioles cultured in vitro.

Shoot cultures derived from both basal sprouts and crown branches of the same tree provide an in vitro system suitable for the molecular-level analysis of differences between material of the same genotype, but differing in ontogenetic age and therefore maturation stage. The identification of plant genes involved in phase change may contribute to our understanding of processes such as determination and differentiation, and provide information about the epigenetic basis of phase change and novel molecular markers of maturational processes.

To study the maturation process at the molecular level, we used the differential display technique (Liang and Pardee 1992) to identify plant genes involved in phase change and to find molecular markers for evaluating developmental processes. Here we describe the identification of a cDNA that was preferentially expressed in oak microshoots derived from crown branches and that encoded a putative glycine- and histidine-rich protein. The expression of this gene was also analyzed in chestnut microcuttings as well as in juvenile, juvenile-like and mature tissues of oak plants.

Materials and methods

Plant materials

Stock shoot cultures of oak (Quercus robur L.) were initiated from basal sprouts (BS) and crown branches (C) of a 300-year-old tree (clone Sainza) and maintained in vitro for more than 8 years as described by Vieitez et al. (1994). At the end of the 4-week multiplication cycle, newly developed 2–3-cm-long shoots (1t) were harvested for subculture, rooting or RNA isolation, and the original explant was re-cultured on fresh medium, in a horizontal position, to obtain a second crop of shoots (2t).

Oak rooting experiments were performed as described by Sánchez et al. (1996). Both BS- and C-derived microshoots, 2–3 cm long, and roots developed from indole-3-butyric acid (IBA)-treated BS shoots were harvested after 1 month of root induction treatment with IBA. Roots from C-derived microshoots were excluded because IBA-treated C shoots did not form roots.

Oak somatic embryos, maintained by repetitive somatic embryogenesis (Cuenca et al. 1999), were collected at the cotyledonary stage. Proliferating nodular calli were derived from initial embryogenic calli that had lost embryogenic competence. Plant material was also harvested from 1-month-old plants cultured in vitro from somatic embryos.

To study gene expression in related woody species, whole 3–4-cm-long microshoots (BS and C) were harvested at the end of the 4-week multiplication cycle from stock shoot cultures of two chestnut clones (P1 and P2), previously established in vitro by Sánchez and Vieitez (1991).

To analyze gene expression in juvenile and mature tissues of oak plants, material was harvested from zygotic embryos collected in November, once seed coats were removed, from 2–3-week-old seedlings that had been germinated in a growth chamber, and also from new shoots (2–4-cm long) developed on BS and C cuttings of four mature trees designated A1, A2, A3 and A4, collected in March (just before bud break) and forced to flush in a climate chamber.

Plant material from all sources was harvested, immediately frozen in liquid nitrogen and stored at –70 °C until used for RNA isolation.

Differential display analysis of mRNA

Differential display analysis was performed using the RNAimage™ kit (GenHunter, Nashville, TN) according to the manufacturer’s instructions. Total RNA was isolated as described by Chang et al. (1993). Genomic DNA contamination was removed from the RNA samples by treatment with 3 U of RQ1 RNase-free DNase (Promega, Madison, WI) for 15 min at 37 °C followed by phenol:chloroform extraction and ethanol precipitation. Total RNA was quantified by detecting absorption at 260 and 280 nm and then stored at –70 °C. Total RNA quality was checked by visualization of rRNA in 1.2% agarose gels. Total RNA was isolated from 2–3-cm-long whole shoots (1t) of oak proliferating cultures (BS and C) including both stems and leaves. For first strand cDNA synthesis (RT reaction), 200 ng of total RNA were mixed with 0.2 µM of one-base anchored primer, 20 µM dNTPs and 100 U of MMLV reverse transcriptase (GenHunter) in a 20 µl reaction volume at 37 °C for 60 min. The PCR amplification reactions were performed in a 20 µl final reaction volume containing 2 µl of the cDNA synthesis reaction, 10 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM MgCl2, 2 µM dNTPs, 2.5 µCi [α-32P]-dATP, 1 U of Taq DNA polymerase (Qiagen, Germantown, MD), 0.2 µM anchor primers and 0.2 µM arbitrary primers. Amplifications were performed for 40 cycles at 94 °C for 30 s, 40 °C for 2 min, and 72 °C for 30 s followed by a 5 min extension at 72 °C. To reduce the number of false positives, duplicate RT and PCR reactions were performed for each primer combination, using RNA from two different extractions. The PCR labeled products were separated on 6% denaturing polyacrylamide gels. Bands of interest were excised and eluted from the gel. Four µl of the eluted cDNA was used for reamplification under the same conditions used for the first PCR except that the dNTP concentration was 20 µM and no radiosotope was added. The reamplified products were used as probes in Northern experiments to verify the differential expression of the corresponding cDNA, and they were further cloned.

Northern blot analysis

For Northern blot analysis, total RNA (20 µg) was denatured and subjected to electrophoresis in 1.2% agarose gels containing formaldehyde and then transferred to Bioblot-Nitrocellulose membranes (Costar, Corning-Life Sciences, Acton,
Double-stranded cDNA was synthesized and amplified by PCR with the Advantage Taq Polymerase in the presence of a modified oligo-(dT) primer and the SMART II primer (5′-AACGACATGGTAACAACGCAGAGTACGGG-3′). An S2 specific primer (S2SP) (5′-GGTTGAGAGTT-3′) was deduced from the S2 sequence and purchased from Isogen Bioscience (Maarssen, The Netherlands). Double-stranded cDNA was sequenced by the dideoxy chain termination method in an automated ABI 373 DNA sequencer at the Instituto de Investigaciones Biomédicas (CSIC) Sequencing Facility. At least two different clones per band were sequenced. The resulting cDNA sequence was deposited in the European Molecular Biology Laboratory (EMBL) database (Accession Number AJ271778). Database searches were performed with the National Center for Biotechnology Information network version of BLAST 2.0 (Altschul et al. 1997) (www.ncbi.nlm.nih.gov/BLAST). Global sequence alignments were performed with the CLUSTALW multiple alignment program (Version 1.7, Thompson et al. 1994) (www.clustalw.genome.ad.jp). Signal sequences were predicted by the on-line programs PSORTII (Nakai and Horton 1999) found at http://psort.nibb.ac.jp and SignalP (Version 2.0, Nielsen et al. 1997) found at http://cbs.dtu.dk/services/SignalP.

Results

Identification and sequence analysis of oak QRCPE cDNA

To identify genes differentially expressed in BS- and C-derived microshoots, differential display analysis was carried out on RNA isolated from whole microshoots (including stems and leaves) of oak, collected at the end of the multiplication cycle. Four cDNA fragments corresponding to bands showing different intensities in the polyacrylamide gels were isolated, reamplified and tested by Northern blotting. Only one of these cDNAs hybridized to a differently expressed transcript. This transcript was approximately 590 bases in size and accumulated to about 4-fold higher levels in cultures derived from crown branches than in cultures derived from basal shoots. We designated this gene as QRCPE (Quercus robur crown preferentially expressed).

Sequencing of the partial cDNA clone obtained from the differential display analysis yielded 202 bp with no similarities to sequences deposited in GenBank. We used 5′-RACE to isolate cDNA/PCR products representing the 5′ end of the mRNA and to obtain the full-length cDNA clone. The full-length QRCPE cDNA was found to be 584 bp long, including the poly(A) tail, corresponding to the length of the transcript detected in Northern experiments (approximately 590 bp). The complete nucleotide sequence of QRCPE cDNA and its deduced amino acid sequence are shown in Figure 1. The position of the first potential ATG initiation codon in the sequence was found at position 82, surrounded by the sequence AAAAGGCC, which is highly homologous to the dicot plants consensus translation initiation site (Cavener and Ray 1991). The 266 bp 3′ untranslated region contains a sequence that is identical to the consensus polyadenylation site (AATAAA) located at position 539, 28 residues upstream of an 11 bp poly(A) stretch at the 3′ terminus, indicating that it is a full-length cDNA. The first ATG starts an open reading frame encoding a putative amino acid sequence of 187 residues. The predicted amino acid sequence is shown in Figure 1.

"Figure 1. Nucleotide and deduced amino acid sequences of QRCPE cDNA isolated from oak microshoots. The predicted amino acid sequence is presented in uppercase. The putative signal sequence is denoted by a thin underline. The stop codon is marked with an asterisk. The arrow indicates the primer used for the 5′-RACE. The putative polyadenylation signal is denoted by a thick underline. The DNA sequence numbers are shown at the left and right margins of the figure. The amino acid sequence numbers are shown at the right margin of the figure. The DNA sequence number of QRCPE is AJ271778."
ing at nucleotide 318, and encodes a polypeptide of 79 amino acids with a calculated molecular mass of 8.4 kDa and a pI of 6.13. The predicted polypeptide contains a hydrophobic region of 26 amino acids at the amino terminus with a mean S score of 0.94 (Nielsen et al. 1997), which has the characteristics of a signal peptide. The predicted cleavage site of this putative signal peptide between alanine-26 and arginine-27 obeys von Heijne’s rule -3, -1 (von Heijne 1986). Excluding the signal peptide, the putative mature protein is 53 amino acids long with a flanking non-glycine domain (18 amino acids) and a domain that is moderately rich in glycine (20.8%) and histidine (22.6%) residues, with a calculated molecular mass of 5.67 kDa and a pI of 6.14. The presence of a signal peptide and the absence of an endoplasmic reticulum retention signal suggests that the QRCPE-encoded protein is targeted for the secretory pathway. Moreover, according to the k-NN algorithm (Horton and Nakai 1997) used in the PSORT program, the protein was predicted, with a probability of 77.8%, to be an extracellular or cell-wall protein. Additional analysis based on consensus sequences for conserved functional domains (PROSITE databases) showed that the QRCPE protein contains two potential phosphorylation sites that start at amino acid 25 (casein Kinase II) and a protein Kinase C phosphorylation site.

Comparisons of the QRCPE nucleotide sequence and the predicted amino acid sequence against other known sequences were conducted with BLAST and the latest versions of GenBank, EMBL, GenPep and SwissProt databases. Initial nucleotide sequence analysis revealed that QRCPE has 90% nucleotide identity with a 126 bp sequence (clone MVAX3, Accession L41866) isolated from O. rubus by differential display. Analysis of the QRCPE-deduced amino acid sequence showed significant identity (64 and 53%) with the deduced amino acid sequences of two cDNA clones (ag164 and agNt84), which had been isolated from Alnus glutinosa L. root nodules and which code for two putative glycine- and histidine-rich proteins (Pawlowski et al. 1997) (Figure 2). It was also found that the putative N-terminal signal peptide of QRCPE has high similarity to the signal peptides of these two proteins. The two nodule-specific cDNAs are members of a small gene family in A. glutinosa, expressed in the early stages of actinorhizal nodule development. Significant amino acid sequence identity was also found between QRCPE and two glycine-rich proteins: DC7.1 (41%, Accession P37704) and DC9.1 (39%, Accession P37703), encoded by two cDNAs that were isolated from cell suspensions of Daucus carota L. during the induction of somatic embryogenesis (Aleith and Ritcher 1990). There was also 37% identity with a light-induced glycine-rich protein of Chenopodium rubrum L. (Accession P11898) (Kaldenhoff and Ritcher 1989).

Expression analysis of QRCPE cDNA in juvenile and mature tissues of oak cultured in vitro

The full-length QRCPE cDNA was amplified by PCR and used to probe Northern blots. Hybridization of total RNA from BS-derived and C-derived whole microshoots with 32P-labeled cDNA indicated that QRCPE mRNA abundance was significantly higher in C cultures than in BS cultures harvested at the end of the first (1t) and second crop (2t) of shoots (Figure 3A). Therefore, shoot cultures from the first crop were used in subsequent experiments.

The expression pattern of QRCPE in different shoot tissues was analyzed using total RNA isolated separately from leaves and from stems of BS- and C-derived microshoots harvested at the end of the 4-week multiplication cycle (Figure 3B). We found that QRCPE expression was higher in stems than in leaves, with the highest expression being detected in stems of C-derived microshoots. For a specific tissue (stems or leaves), QRCPE mRNA was more abundant in C-derived tissues than in BS-derived tissues, with the lowest amounts being detected in leaves from BS-derived microshoots.

We also analyzed gene expression in oak microshoots (BS and C) treated with IBA and then cultured for 1 month in rooting medium, and in roots developed from rooted BS shoots. Total RNA isolated from these tissues was subjected to Northern analysis (Figure 3C). The greatest amount of mRNA transcript was detected in roots developed from BS shoots. As was found for proliferating cultures, more mRNA were detected in C shoots than in BS shoots. Thus, the steady-state quantity of QRCPE mRNA is higher in C-derived microshoots than in BS-derived microshoots, at the end of either the multiplication period or the rooting period. These results indicate that this clone can be used as a potential molecular marker to differentiate juvenile-like oak microshoots from those of mature origin. A comparison of mRNA abundance of proliferating shoots (Figure 3A) and shoots growing in rooting medium (Figure 3C) indicated that mRNA accumulation was higher in the auxin-treated shoots than in the proliferating shoots. Thus, QRCPE expression may be induced by auxin, or it may be down-regulated by the cytokinin added to the proliferation medium.

Figure 2. Comparison of the deduced amino acid sequences of QRCPE and two nodule-specific proteins (ag164 and agNt84; Pawlowski et al. 1997) obtained from the analysis of a CLUSTALW alignment with the shading program of GeneDoc. The black shading indicates identical or conserved amino acids found in all three sequences, and the lighter shading indicates homology between two sequences. Dashed lines indicate gaps introduced to allow optimal alignment of the sequence.
Expression of the QRCPE gene was investigated in different oak tissues cultured in vitro at early developmental stages. Northern hybridization analysis showed that the greatest amounts of QRCPE transcript were in somatic embryos, followed by roots and shoots of germinated somatic embryos; however, almost no transcripts were detected in proliferating oak callus (Figure 4). Similar amounts of transcript were observed in juvenile-like shoots (BS) rooted in vitro (Figure 3C) and in juvenile shoots from somatic embryo-derived plants growing in vitro (Figure 4).

**QRCPE expression analysis in chestnut microshoots**

As was previously reported (Sánchez and Vieitez 1991), proliferation rates of chestnut clones P1 and P2 were affected by the origin of the cultures (BS or C). The BS cultures exhibited more vigorous growth and produced more shoots that C cultures (Figure 5). Rooting rates were also strongly influenced by the origin of the shoots.

To examine the expression pattern of the cloned QRCPE cDNA in chestnut microcuttings, total RNA was extracted from BS and C shoots (including stems and leaves) of clones P1 and P2 at the end of the multiplication cycle. When Northern analysis was carried out with the QRCPE insert as a probe, homologous mRNA species were observed to be more abun-
dant in C-derived shoot cultures than in BS-derived shoot cultures in both chestnut clones analyzed (Figure 6). The QRCPE mRNA was more abundant in C shoots of clone P1 than in C shoots of clone P2, probably because of genotypic differences. As observed in oak, the QRCPE gene was differentially expressed in BS and C proliferating microshoots of the two chestnut clones analyzed.

**QRCPE expression analysis in juvenile and mature tissues of oak plants**

To determine if the differential expression of the QRCPE gene in oak microshoots is related to the maturation stage or to the in vitro conditions, we analyzed its expression in tissues from juvenile and mature soil-grown plants. Northern analysis showed that the juvenile (ZS) and juvenile-like (BS) shoots from oak plants exhibited more QRCPE transcript than the mature (C) shoots (Figures 7A and 7B), indicating that QRCPE transcript accumulation is correlated with the ontogenetic stage of the shoots. Large amounts of the QRCPE transcript were detected in zygotic embryos (Figure 7B) and in seedling roots (data not shown). It was also found that QRCPE expression shifts when BS and C shoots are micropropagated, being higher in mature microshoots than in juvenile-like microshoots (Figure 7B), suggesting that its expression is regulated by the in vitro culture conditions.

**Discussion**

We focused on oak genes whose expression changes with transition phase. We successfully combined the techniques of mRNA differential display and 5′-RACE to isolate full-length cDNAs that were differentially expressed in shoot cultures derived from basal shoots and crown branches of a mature oak tree. Changes in gene expression during the maturation process could account for the loss of morphogenetic competence in the mature phase, and may provide clues for understanding the molecular basis of the phase change and for designing strategies to overcome the difficulties associated with micropropagation of mature material.

Of the four cDNAs isolated by differential display, only one, termed QRCPE, was confirmed to represent mRNAs from differentially expressed genes. The QRCPE cDNA was preferentially expressed in cultures derived from crown branches. Sequence analysis of the full-length cDNA showed that an internal region of QRCPE cDNA exhibited high similarity (90%) with a 126 bp clone (MVAX3) isolated from Quercus robur. Although precise details of this study were not published, the MVAX3 sequence was defined as a phase-specific molecular marker. Both sequences might correspond to different polymorphic forms of the same gene. The protein predicted from the coding region of QRCPE cDNA revealed that it is rich in glycine and histidine residues and is highly homologous with several glycine-rich proteins. The highest homology was obtained with the sequences of two nodule-specific agNt84 and ag164 proteins from Alnus glutinosa (Pawlowski et al. 1997), which are reported to be members of a gene family encoding glycine- and histidine-rich proteins. We note that the putative signal peptide of QRCPE shares a high degree of similarity with the signal peptides of agNt84 and ag164. It was also suggested that agNt84 and ag164 are located in the space between the invaginated plasma membrane and the matrix surrounding the endosymbiont. The presence of an optimal hydrophobic signal sequence for transport and subsequent processing of the QRCPE putative protein, as well

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**Figure 6.** Expression of QRCPE in shoot cultures of two chestnut clones (P1 and P2). For each sample, 20 µg of total RNA was isolated from 3–4-cm-long basal shoot cultures (BS) and crown shoot cultures (C) that were harvested at the end of the 4-week multiplication cycle. The RNA was subjected to RNA-blot analysis under the same conditions as described in Figure 3. The ethidium bromide-stained gels indicate RNA integrity and uniform loading before blotting.

**Figure 7.** Comparison of QRCPE expression in oak tissues that were juvenile, juvenile-like, or of mature origin from intact plants and in oak microshoots. (A) Total RNA was isolated from new shoots developed on cuttings of basal shoots (BS) and crown branches (C) of mature oak trees (A1, A2 and A3). (B) Total RNA was isolated from zygotic embryos (ZE), shoots derived from zygotic embryos (ZS), BS shoots and C shoots of the A4 mature tree and proliferating shoot cultures (SC) derived from BS and C shoots. For each sample, 20 µg of total RNA was subjected to Northern analysis under the same conditions as described in Figure 3. Ethidium bromide-stained gels indicate RNA integrity and uniform loading before blotting.
as the results of the PSORT program predicting an extracellular or cell-wall localization for QRCPE, indicate that QRCPE is probably targeted to the cell wall. Although glycine-rich protein (GRP) genes that contain sequences for amino terminal signal peptides are considered to code for structural cell-wall proteins (Cassab 1998), only GRP1.8 has been shown to be located within the cell wall (Ryser and Keller 1992). Additional studies are necessary to determine the specific localization of the QRCPE protein.

Although almost no QRCPE transcripts were detected in dividing cells of callus, high QRCPE expression was detected in somatic embryos growing under the same tissue culture conditions, indicating a cell-type specific regulation. The similarity of QRCPE-deduced protein to two putative proteins encoded by cDNAs that were activated at the onset of somatic embryogenesis in carrot cell suspensions (Aleith and Ritcher 1990), coupled with high QRCPE expression in oak somatic and zygotic embryos, suggest that QRCPE may have a function in embryo development. Other GRP genes encoding putative cell-wall proteins are specifically expressed during the early stages of somatic embryogenesis, and the deduced amino acid sequences suggest an important role as cell-wall proteins in embryogenesis (Sato et al. 1995, Magioli et al. 2001).

The greater accumulation of QRCPE transcripts in mature than in juvenile-like microshoots of chestnut and oak growing under the same in vitro conditions suggests that QRCPE expression is dependent on ontogenetic stage. The accumulation of QRCPE transcripts was highest in roots, followed by stems and leaves, and they also preferentially accumulated in specific organs (stems and leaves) that were derived from ontogenetically older shoots, indicating that QRCPE expression is organ- and phase-dependent. Based on a similar in vitro plant system, Racchi et al. (2001) found no differences between the catalase and superoxide dismutase activity profiles in two lines of oak microcuttings, despite their different ontogenetic origin. However, they found a high level of catalase activity (CAT-2) in rooted microshoots, indicating that this isoform is a protein specifically related to rooting. Because the in vitro rooting capacity of oak and chestnut BS shoots is always higher than C shoots, the higher expression of QRCPE in C microshoots may also be correlated to the reduced rooting ability of these shoots. Preliminary experiments showed different QRCPE expression in BS and C oak shoots at the beginning of the in vitro root induction. Genes coding for putative cell wall proteins are differentially expressed in easy-to-root and difficult-to-root tissues or induced after root induction treatment. Woo et al. (1994) reported the isolation of a PRP gene from ivy that is expressed at higher levels in mature non-root-forming petioles than in juvenile root-forming petioles, after auxin treatment. Moreover, the in situ localization of the PRP mRNA showed an inverse relationship between mRNA abundance in specific cell types involved in root initiation and the ability to form adventitious roots (Sánchez et al. 1995). The mRNA abundance of a member of the α-expansin gene family was increased in hypocotyl cuttings from Pinus taeda L. in response to auxin application during the early stages of induction of adventitious root formation (Hutchison et al. 1999).

The preferential accumulation of QRCPE transcripts in C microshoots of oak and chestnut contrasts with the large amounts of RNA accumulated preferentially in BS shoots of intact oak plants. In vitro culture was found to have a down-regulatory effect on QRCPE transcript abundance in BS shoots as opposed to a slight up-regulatory effect in C shoots. This indicates that QRCPE expression seems to be regulated by in vitro culture conditions in a phase-dependent manner, probably because of a different sensitivity of BS and C oak shoots to a nutrient signal of the culture medium, such as sucrose, inorganic nutrients or the exogenous cytokinin. Watillon et al. (1991) also found that the mRNA accumulation pattern differed between micropropagated plantlets of Malus domestica (L.) Borkh and in vivo grown plants, in response to culture on BA-containing medium. It is possible that QRCPE expression is regulated by cytokinins, because more QRCPE mRNA was detected in oak microshoots growing in the rooting medium without cytokinin than in proliferating cultures. Cytokinins control the expression of many genes in various plants and tissues in a quantitative manner, and this control can be cell-type specific (Smülling et al. 1997). An effect of wounding on QRCPE expression can be discounted, because microshoots developing a callus at their basal ends were excised at the end of the proliferation or the rooting period.

We observed that the QRCPE gene is expressed in all phases: embryonic, juvenile and mature, but its expression appears to be under developmental control and is also regulated by in vitro conditions. The QRCPE encodes a putative glycine- and histidine-rich protein that appears to be localized to the cell wall. It has been shown that gene expression of this class of proteins depends on cell type and developmental stage (Keller 1993, Showalter 1993). The analysis carried out with material from intact oak plants showed that QRCPE expression is progressively down-regulated after seed germination, which could be correlated with the remodeling of plant cells in response to maturation, because the differences observed in RNA accumulation between C and BS shoots seems to be a consequence of their ontogenetic differences.

We conclude that QRCPE may be useful as a molecular marker for the juvenile and mature phases of oak. Further studies are needed to analyze the hormonal regulation of the QRCPE gene as well as to localize the protein and mRNA at the cellular level during the processes of differentiation such as embryogenesis and rooting.

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